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Contribution au développement d'une filière éco-innovante de phytomanagement de sols pollués par les éléments traces : culture de plantes aromatiques et production d'huiles essentielles

Robin Raveau

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Contribution au développement d'une filière éco-innovante de phytomanagement de sols pollués par les éléments traces : culture de plantes aromatiques et production d'huiles essentielles

Par

Robin RAVEAU

Soutenue le 17 décembre 2020 devant le jury :

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Liste des abréviations

ADN : Acide Désoxyribonucléique

ASV : Amplicon Sequence Variant – Variant de Séquence d’Amplicon

ARN : Acide Ribonucléique

CE₅₀ : Concentration effectrice médiane

CI₅₀ : Concentration inhibitrice médiane

CMA : Champignon mycorhizien à arbuscules

β-CD : β-cyclodextrine

DMSO : Diméthylsulfoxyde

dNTP : Désoxyribonucléotides triphosphate

DO : Densité optique

ERO : Espèces réactives de l’oxygène

ET : Eléments traces

GC : Gas Chromatography – Chromatographie en phase gazeuse

GC-MS : Gas Chromatography-Mass Spectrometry – Chromatographie en phase gazeuse couplée à la spectrométrie de masse

HE : Huile essentielle

PCR : Polymerase Chain Reaction

PPAM : Plantes à parfum, aromatiques et médicinales

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Introduction générale

Introduction générale

La pollution des sols, héritage de plusieurs décennies d'activités industrielle, minière et agricole, est un enjeu sociétal mondial. Elle représente des dangers majeurs, notamment sanitaires et environnementaux. Plus de 10 millions de sites pollués sont ainsi répertoriés à l'échelle mondiale et constituent une préoccupation prioritaire (Gong et al., 2018; Khalid et al., 2017). Au niveau européen, les estimations indiquent 2,5 millions de sites potentiellement pollués, dont 300 000 nécessiteraient une réhabilitation urgente (Mench et al., 2018). Parmi les polluants les plus fréquemment retrouvés, les éléments traces (ET) occupent une place importante (37 % des sites pollués en Europe - Mench et al., 2018; Panagos et al., 2013).

Si les ET sont naturellement présents dans le fonds géochimique des sols, leur enrichissement est lié à des phénomènes géologiques et climatiques (éruptions volcaniques, altération de la roche mère) et en grande majorité à des activités anthropiques (Ali et al., 2019; Antoniadis et al., 2017; Khalid et al., 2017). Les activités industrielles, minières, agricoles et les transports ont notamment contribué à la pollution en ET de divers compartiments des écosystèmes terrestres et/ou aquatiques, principalement à cause des retombées atmosphériques (Antoniadis et al., 2017; Khalid et al., 2017; Mahar et al., 2016). L'utilisation intensive d'intrants contenant des ET (boues de stations d'épurations des eaux usées, produits phytosanitaires, etc) a aussi participé à long terme à l'enrichissement ainsi qu'à la pollution des sols par ces éléments (Antoniadis et al., 2017). Connus pour être non dégradables, les ET s'accumulent dans les différents compartiments de l'environnement dont les sols, ainsi qu'au sein des organismes vivants, pour lesquels ils peuvent présenter une toxicité (Antoniadis et al., 2017; Gong et al., 2018). Les plus fréquemment retrouvés dans les sols pollués sont le plomb (Pb), le chrome (Cr), le cuivre (Cu), l'arsenic (As), le zinc (Zn), le nickel (Ni), le cadmium (Cd) et le mercure (Hg) (Basol, 2020), qui sont à l'origine, en cas d'exposition répétée ou excessive, de nombreuses perturbations physiologiques et pathologies sévères chez l'Homme : saturnisme, affections neurologiques, anémie, sclérose en plaques, etc (Ali et al., 2013; Järup, 2003; Khalid et al., 2017; World Health Organisation, 2007). Certains éléments, comme le Cd, le Hg ou le Pb, sont également connus pour être cancérigènes, mutagènes ainsi que reprotoxiques (Järup, 2003; World Health Organisation, 2007). La problématique de la pollution des sols par les ET menace donc non seulement la santé humaine et l'équilibre des écosystèmes, mais pose également des problèmes majeurs en termes de durabilité des systèmes agricoles et de sécurité alimentaire, en réduisant les rendements des cultures et en les rendant impropres à la consommation humaine ou animale.

A l'échelle nationale, plus de 7200 sites pollués sont recensés (Basol, 2020). De par son passé industriel et sa forte densité de population, la région Hauts-de-France est particulièrement touchée par cette problématique et compte à ce jour près de 1000 sites pollués (13.5 % du patrimoine national), en particulier dans l'ancien bassin minier (Basol, 2020). Parmi les secteurs d'activités de la région, l'exploitation minière, la sidérurgie et la métallurgie ont contribué pour beaucoup à la pollution des sols par les ET (Douay et al., 2013; Sterckeman et al., 2002). C'est notamment le cas de l'ancienne fonderie de plomb Metaleurop Nord, située à Noyelles-Godault (62). En activité pendant plus d'un siècle, jusqu'à sa fermeture en 2003, elle a généré des quantités considérables de poussières chargées en ET, qui, par retombées atmosphériques, ont contribué à la pollution des sols alentours (Douay et al., 2013; Sterckeman et al., 2002). Le Cd, le Pb ainsi que le Zn en sont les polluants majeurs (Sterckeman et al., 2002). Outre les fortes teneurs de ces éléments dans le périmètre avoisinant l'ancienne fonderie, la pollution est d'autant plus problématique qu'elle s'étend sur une large surface

de terres agricoles (730 ha) et représente un danger pour près de 60 000 habitants dans le secteur. En effet, les ET peuvent être sujet aux phénomènes naturels d'érosion des sols sous l'effet du vent (envols de poussières) ou de l'eau (ruissellements de surface, lessivage en profondeur) et ainsi représenter un danger par exposition directe ou par une contamination de la chaîne alimentaire pour les populations environnantes (Kumpiene et al., 2019; Lydakis-Simantiris et al., 2016). De plus, les sols pollués présentent un réel danger en fonction des usages, de la topographie et de l'exposition du site. Notamment, un transfert de Cd et de Zn des sols vers les productions végétales agricoles, potagères ou encore vers la végétation herbacée ou ligneuse a été constaté (Bidar et al., 2007; Douay et al., 2008b, 2008a, 2013; Roussel et al., 2010).

Par ailleurs, la zone Metaleurop (site atelier du réseau SAFIR, Sites Ateliers Français pour l'Innovation et la Recherche – Figure 1) fait également l'objet de politiques territoriales, sous la forme d'arrêtés préfectoraux successifs depuis 2015, prévoyant un encadrement des activités et productions, en particulier agricoles, en fonction du degré de pollution du sol.



Figure 1: Cartographie des sites du réseau SAFIR incluant le site Metaleurop (★) (Crabos, 2012).

Toute activité agricole est ainsi exclue dans la zone 1, la plus polluée (> 1000 ppm de Pb ou > 20 ppm de Cd – Figure 2), regroupant le site Metaleurop et la zone urbaine autour de celui-ci. Au sein de la zone 2 (entre 500 et 1000 ppm de Pb ou entre 10 et 20 ppm de Cd) et de la zone 3 (entre 200 et 500 ppm de Pb ou entre 4 et 10 ppm de Cd) plus élargie, couvrant près de 700 ha de terres agricoles, un encadrement sanitaire des productions agricoles a été mis en place par un système d'analyses libératoires (vérification de conformité) avant leur mise sur le marché. Les productions ne répondant pas aux critères de qualité attendus et présentant notamment des concentrations élevées en ET sont déclassées ou détruites.

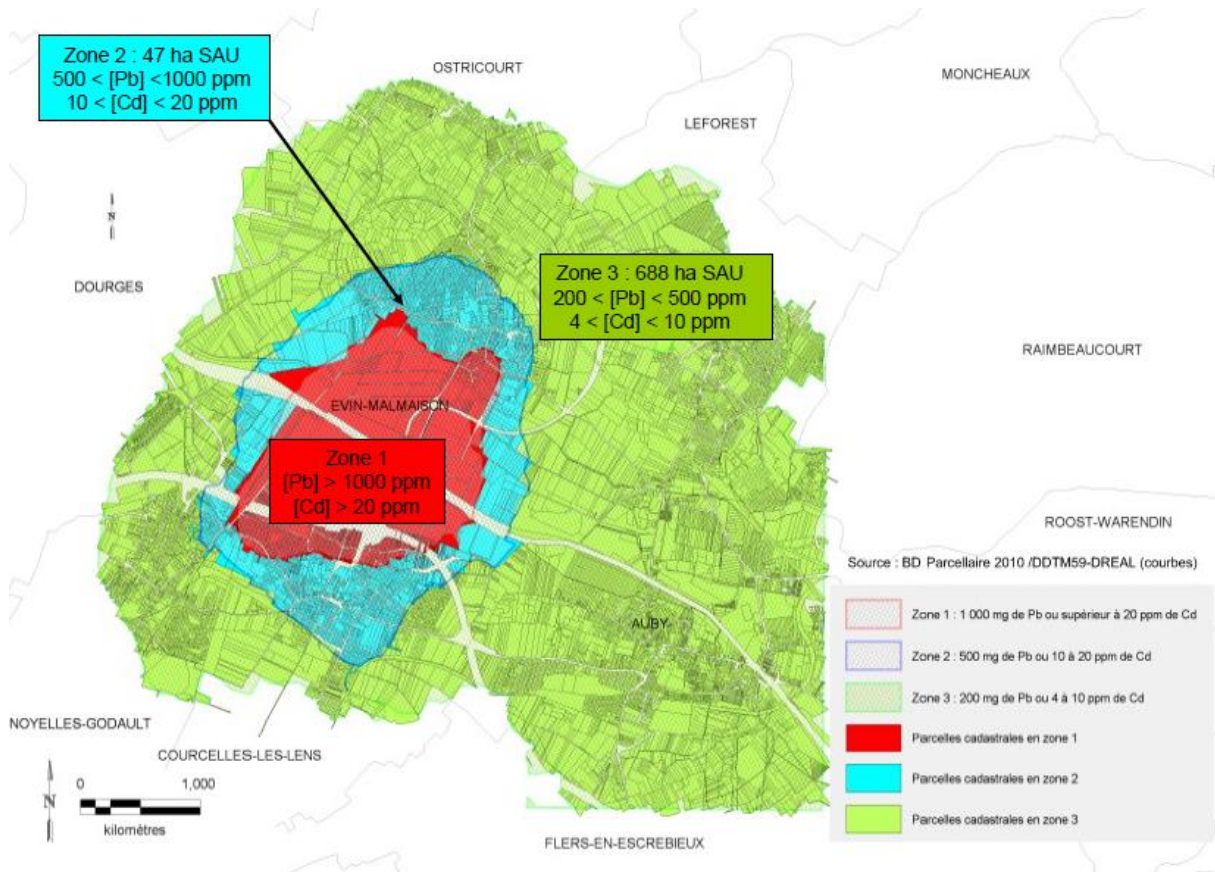


Figure 2: Délimitation parcellaire du périmètre du site Metaleurop (DRAAF Hauts-de-France, 2017).

Il est à noter également que les analyses étaient jusqu'alors prises en charge par l'état, mais sont désormais à la charge du producteur. Ces considérations représentent donc un enjeu économique majeur, en plus de la problématique sanitaire, pour les agriculteurs du secteur. Ainsi, dans le contexte actuel de forte pression démographique et de raréfaction des sols, notamment à vocation agricole, la question de la gestion et de la requalification des sites et sols pollués par les ET, en milieu rural et urbain, représente un enjeu majeur.

Selon la nature et le degré de la pollution ainsi que les usages futurs du site, différentes méthodes de requalification existent. Outre les techniques physico-chimiques, qui sont à l'heure actuelle les méthodes de remédiation des sols les plus fréquemment employées, les phytotechnologies, basées sur l'utilisation de plantes et de leurs microorganismes associés, peuvent constituer une alternative intéressante d'un point de vue technique, environnemental et socio-économique (Burgès et al., 2018; Gong et al., 2018). Ces dernières, visant à remédier ou à réduire le risque lié à la présence des polluants dans le sol, sont plus respectueuses de l'environnement, relativement peu coûteuses, applicables *in situ* à de grandes superficies et bien acceptées par l'opinion publique (Burgès et al., 2018; Gong et al., 2018). De plus, l'installation ou la restauration d'un couvert végétal sur le sol pollué présente l'avantage non négligeable de limiter les phénomènes d'érosion éolienne ou hydrique, de réduire les transferts de polluants entre compartiments de l'environnement et de contribuer à la refonctionnalisation des sols et à leur reconquête (Bert et al., 2017; Kidd et al., 2015; Mench et al., 2010). Parmi ces phytotechnologies, la phytostabilisation semble appropriée pour la gestion de grandes surfaces, telles que les terres agricoles polluées par les ET. Basée sur l'utilisation de plantes capables de limiter les transferts d'ET du sol vers leurs parties aériennes, elle a pour finalité de réduire

la mobilité des polluants en les stabilisant au niveau de la rhizosphère (Bert et al., 2017; Burges et al., 2018; Mench et al., 2010). L'une des clés du succès des phytotechnologies repose donc sur la capacité des plantes à se développer dans des conditions toxiques pour la majorité des végétaux et à établir un couvert végétal. Afin de favoriser l'installation du couvert végétal et la croissance des plantes ou de contribuer à l'immobilisation des ET dans la rhizosphère et d'ainsi en réduire la biodisponibilité et la toxicité, la phytostabilisation est souvent assistée par l'utilisation d'amendements minéraux, organiques ou biologiques (Deng and Cao, 2017; Gómez-Sagasti et al., 2018; Gong et al., 2018; Kumpiene et al., 2019). Parmi les amendements biologiques, l'utilisation d'inoculum à base de champignons mycorhiziens à arbuscules (CMA) se développe de plus en plus. Ces champignons sont notamment capables d'établir une relation symbiotique avec la majorité des espèces végétales (80 %) et sont connus pour améliorer la croissance de leur hôte végétal par une meilleure nutrition minérale et hydrique, en échange de substrats carbonés fournis par la plante (Begum et al., 2019; Smith and Read, 2008; Smith et al., 2011). Il a également été démontré que la symbiose mycorhizienne pouvait améliorer la tolérance des plantes au stress généré par la présence des ET dans le sol (Ferrol et al., 2016; Garg et al., 2017; Hause and Fester, 2005) et contribuer à leur immobilisation au niveau de la rhizosphère (Begum et al., 2019; Garg et al., 2017; Kumpiene et al., 2019), ce qui dans le cadre d'une stratégie de phytostabilisation aidée pourrait s'avérer particulièrement précieux.

Cependant, la rentabilité socio-économique de cette phytotechnologie constitue l'un des principaux freins à son développement (Kumpiene et al., 2019). L'intégration de la démarche de phytostabilisation aidée dans une approche plus globale de phytomanagement des sols pollués semble donc indispensable. En effet, le phytomanagement intègre la réduction du risque lié à la présence des ET dans le sol à une réflexion plus large portant sur la mise en place d'un usage durable et viable d'un point de vue économique du site pollué (Burges et al., 2018; Evangelou et al., 2015; Mench et al., 2018). Ainsi, en minimisant les coûts de mise en place et de gestion et/ou en valorisant la biomasse végétale produite, les bénéfices pourront être d'ordre économique mais également écologique en favorisant notamment la restauration des écosystèmes dégradés à travers la refunctionalisation de ces sols (Burges et al., 2018; Mench et al., 2018).

Le développement de filières à hautes valeurs ajoutées, par la production de substances biosourcées issues de la biomasse végétale récoltée sur des parcelles polluées impropres à l'agriculture nourricière, permettrait d'abaisser les coûts globaux de gestion et de requalification de ces sites et valoriserait des ressources en sol par ailleurs délaissées, en répondant aux attentes environnementales, économiques et sociales. A l'heure actuelle, la majorité des filières de valorisation de biomasse issue de sites pollués repose sur l'utilisation de cultures à vocation bioénergétique, telles le miscanthus, le maïs, le colza ou encore des espèces ligneuses comme le saule ou le peuplier, ou sur la production d'écomatériaux (Bernal et al., 2019; Bert et al., 2017; Chalot et al., 2020; Mench et al., 2010; Zgorelec et al., 2020). Parmi les filières de valorisation qui pourraient présenter une rentabilité importante, la culture de plantes à parfums aromatiques et médicinales (PPAM) productrices d'huiles essentielles (HE) peut être proposée comme une option novatrice et prometteuse. En effet, le marché mondial des produits biosourcés d'origine végétale tels que les HE est en constante augmentation et pourrait dépasser les 15 milliards de dollars en 2026 (Allied Market Research, 2020 ; CBI, 2020). Pour répondre à cette demande, la relocalisation d'une partie de la production sur des sites pollués constitue une piste d'étude. De plus, les HE et leurs composés aromatiques principaux trouvent leur utilité dans divers domaines à vocation non-alimentaire, tels que les industries cosmétiques, pharmaceutiques et phytosanitaires (De Clerck et al., 2020; Muchembled et al., 2017).

Mon projet de thèse s'inscrit dans ce contexte général et entre dans le cadre du Projet PhytEO (Phytostabilization and Essential Oils - <https://phyteo.univ-littoral.fr>), financé par l'ADEME (APR Graine 2016), qui avait pour principale ambition de développer une filière innovante de valorisation non alimentaire de biomasse produite sur des sols agricoles pollués par des ET, au sein du site atelier de Metaleurop. Ce projet de recherche a pour objectif d'évaluer *in situ* l'intérêt d'un mode de gestion combinant la phytostabilisation aidée et la production d'HE distillées à partir de trois PPAM (saugé sclarée, coriandre, angélique) cultivées *in situ* et ceci via une démarche intégrant les aspects techniques, environnementaux et socio-économiques. Il repose sur un partenariat transdisciplinaire universitaire (UCEIV-ULCO, TVES-ULCO, INERIS), institutionnel (DRAAF, Chambre d'Agriculture des Hauts de France, Association des agriculteurs...) et privé (Ferrant PHE, producteur d'HE). Ma contribution au projet PhytEO a consisté à participer à la mise en place du dispositif expérimental sur deux parcelles agricoles polluée ou non (démonstrateur *in situ* à l'échelle de l'hectare), à caractériser le site notamment d'un point de vue biologique, à choisir et à suivre la croissance de différentes espèces de PPAM productrices d'HE sur un sol pollué, à étudier la composition des HE distillées, à évaluer leur qualité, à déterminer certaines de leurs propriétés biologiques, en vue d'usages en protection des cultures ou en santé humaine et à étudier l'impact de la culture de PPAM sur le microbiote rhizosphérique. L'intérêt de l'ajout d'un amendement biologique à base de CMA a été également évalué dans ce contexte de phytomanagement. Enfin, une étude de viabilité économique de la filière PPAM-HE a été suggérée.

Après une **introduction générale**, mettant en lumière le contexte de ce projet de thèse, le manuscrit et l'articulation des différentes parties qui le compose s'organiseront comme suit :

- **La première partie** de ce manuscrit consiste en une **synthèse bibliographique** structurée autour des points suivants, incluant notamment un chapitre d'ouvrage (soumis) et une revue déjà parue :
 - Les ET dans l'environnement
 - La phytostabilisation aidée des sols pollués par les ETCes deux derniers points sont abordés en totalité dans le cadre du chapitre d'ouvrage suivant : « Chapter 8 : Recent advances in microbial aided phytostabilisation of trace element contaminated soils », par Robin Raveau, Anissa Lounès-Hadj Sahraoui et Joël Fontaine, dans *Advances in Microbe-assisted Phytoremediation of Polluted Sites*, eds. K. Buddh et Y. Ma (Elsevier, 2021).
 - La biosynthèse des métabolites entrant dans la composition des HE ainsi que les méthodes d'obtention des HE
 - La production d'HE à activités biologiquesCette partie a été traitée dans le cadre d'une revue, « Essential Oils as Potential Alternative Biocontrol Products against Plant Pathogens and Weeds: A Review », par Robin Raveau, Joël Fontaine et Anissa Lounès-Hadj Sahraoui, *publiée dans Foods* (2020).
 - Le microbiote des sols pollués
 - Les PPAM utilisées dans le cadre de cette étude
- **La problématique** ainsi que les **principales questions scientifiques posées**, auxquelles ce projet de thèse tentera d'apporter des réponses, sont ensuite abordées.
- **La partie 2** décrit en détails l'ensemble des **matériels et méthodes** utilisés lors de ce travail.

- **La partie 3** présente les **résultats** obtenus et leurs **discussions**, organisés en 4 publications :
 - **Publication 1 : Robin Raveau, Joël Fontaine, Valérie Bert, Alexandre Perlein, Benoit Tisserant, Anissa Lounès-Hadj Sahraoui** « *In situ* cultivation of aromatic plant species for the phytomanagement of an aged-trace element contaminated soil: plant biomass improvement options and techno-economic assessment of the essential oil production channel », à soumettre dans *Science of the Total Environment*.
 - **Publication 2 : Robin Raveau, Joël Fontaine, Anthony Verdin, Loris Mistrulli, Patrice Ferrant, Frédéric Laruelle, Sophie Fourmentin, Anissa Lounès-Hadj Sahraoui** « Biological activities of clary sage and coriander essential oils: *in vitro* screening towards their potential use as crop protection and healthcare products », soumise dans *Industrial crops and products*.
 - **Publication 3 : Robin Raveau, Joël Fontaine, Mohamed Hijri, Anissa Lounès-Hadj Sahraoui** « The aromatic plant clary sage shaped bacterial communities in the roots and in the trace element-contaminated soil more than mycorrhizal inoculation – a two-year monitoring field trial », publiée dans *Frontiers in Microbiology* (2020).
 - **Publication 4 : Robin Raveau, Anissa Lounès-Hadj Sahraoui, Mohamed Hijri, Joël Fontaine** « Clary sage cultivation and mycorrhizal inoculation influence rhizosphere fungal community structure over time in a trace-element polluted site », soumise dans *Mycorrhiza*.
- **La partie 4** constitue une **discussion générale** qui met en relation et discute les principaux résultats obtenus dans les différentes expériences tout en ouvrant sur les **perspectives** envisagées pour la poursuite de ce travail.
- Enfin ce manuscrit s'achève avec des **conclusions générales**.

Les **références bibliographiques** citées dans la synthèse bibliographique ainsi que dans la discussion générale sont citées à la fin du manuscrit.

Les différentes valorisations et communications auxquelles j'ai participé durant mes années de thèse seront également présentées en **annexe**.

Partie 1 :

Synthèse bibliographique

- I. Les éléments traces dans l'environnement (*Revue 1, Raveau et al, 2021, soumise*)
- II. La phytostabilisation aidée des sols pollués par les ET (*Revue 1, Raveau et al, 2021, soumise*)

Parmi les nombreux polluants détectés dans les sites et sols pollués, les ET font l'objet d'une attention particulière en raison de leurs effets néfastes sur l'environnement mais également sur la santé humaine. La requalification des sites contaminés par les ET représente donc un enjeu majeur à l'échelle mondiale.

Les phytotechnologies et la phytostabilisation en particulier, bien acceptées par le grand public, durables, applicables à de grandes surfaces et présentées comme plus respectueuses de l'environnement et moins coûteuses que leurs homologues physico-chimiques, font partie des méthodes émergentes de gestion des sites pollués (Burges et al., 2018; Kumpiene et al., 2019). La phytostabilisation repose sur l'utilisation de plantes et de leurs microorganismes associés pour stabiliser et immobiliser les polluants présents dans les sols au niveau de la rhizosphère (Burges et al., 2018; Kidd et al., 2015; Mench et al., 2010). Le succès de la phytostabilisation repose en premier lieu sur l'établissement des plantes dans des conditions environnementales souvent défavorables de par la phytotoxicité des ET pour la majorité des végétaux supérieurs et le manque de fertilité de certains sols, et également sur l'immobilisation des polluants du sol. Dans ce but et afin d'améliorer le processus de phytostabilisation et l'installation du couvert végétal, l'apport d'amendements, de natures minérale, organique ou biologique, a été mis en avant. En particulier, l'utilisation d'amendements biologiques à base de microorganismes, contribuant à l'immobilisation des ET dans le sol et favorisant le développement des plantes (Deng and Cao, 2017; Gong et al., 2018; Kumpiene et al., 2019), pourrait être particulièrement intéressant.

A l'heure actuelle, les enjeux majeurs conditionnant le développement de ces phytotechnologies portent sur la démonstration de leurs performances (cas des multi-pollutions, efficacité des amendements, transferts et suivi des polluants dans la plante, durée de traitement, etc), l'optimisation des itinéraires techniques (du semis à la récolte) et le développement de filières de valorisation de la biomasse produite sur ces sols pollués ou fortement anthropisés. Initialement assimilée à un déchet, cette biomasse est désormais utilisée comme une ressource valorisable, permet d'abaisser les coûts globaux de gestion et/ou de requalification des sites et redonne un intérêt économique à des sites en attente de requalification ou d'aménagement. Dans ce cadre, la combinaison des phytotechnologies avec une méthode de valorisation de la biomasse produite sur ces sols contaminés, pourrait permettre un usage durable du site, contribuer à sa reconversion économique et à la restauration de services écosystémiques (Dongmei and Changqun, 2008; Kidd et al., 2015; Kim et al., 2018).

Ainsi, après un bref rappel sur les propriétés physico-chimiques et la biodisponibilité des ET dans les sols, ce chapitre (*Revue 1, Raveau et al, 2021, soumise*) s'attachera à explorer les points suivants :

- Sur quels critères est basé le choix des espèces végétales utilisées dans le cadre de la phytostabilisation de sols pollués par les ET ?
- Quels sont les bénéfices apportés par les amendements microbiens dans le cadre d'une stratégie de phytostabilisation aidée ?

Synthèse bibliographique

- A l'heure actuelle, quels sont les freins au développement de la phytostabilisation aidée et quelles solutions/perspectives existent, notamment en vue de valoriser la biomasse produite ?

Cette revue couvre la totalité des sections *I. Les éléments traces dans l'environnement* et *II. La phytostabilisation aidée des sols pollués par les ET* de la synthèse bibliographique.

Title: Advances in Microbe-assisted Phytoremediation of Polluted Sites
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Chapter 8: Recent advances in microbial aided phytostabilisation of trace element contaminated soils

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Abstract

Soil contamination by trace elements (TE) has been a major concern worldwide, regarding human health and environmental quality. Phytotechnologies are nowadays offering cost-effective and eco-friendly risk-management and remediation methods towards TE-contaminated soils. Phytostabilisation in particular, relying on the ability of plants and their associated microorganisms to immobilize soil contaminants is of great interest. Additionally, it may be greatly enhanced by the addition of microbial amendments, susceptible to improve TE immobilization and to promote vegetation establishment and growth. The combination of this green technology with a profitable site management, besides the contamination risk-management and the restoration of ecosystem services, provides economic value to the site. Thus, the aim of this chapter is to highlight the key criteria towards a successful aided phytostabilisation, yet taking into account the current limitations that this phytotechnology is facing. Additionally, suitable valorisation channel for biomass produced on contaminated sites will be further investigated.

Keywords: aided phytostabilisation – microbial amendments – trace elements – biomass valorization

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Introduction

From more than 10 million contaminated sites listed worldwide, Trace Elements (TE) appear as the most frequent soil contaminants, with more than 50% of the previously mentioned sites that are contaminated with TE (Gong *et al.*, 2018; Khalid *et al.*, 2017). Additionally, the majority of the TE-contaminated sites are found in developed countries (Khalid *et al.*, 2017). In Europe, TE are the most frequent soil contaminants listed (35%), originating from both natural and anthropogenic sources and estimated to exceed threshold concentrations in almost 30% of the European soil surface (Panagos *et al.*, 2013; Tóth *et al.*, 2016). Natural origins may include terrestrial sources, in the form of volcanic eruptions and TE-containing parent rock outcropping and weathering (Ali *et al.*, 2019; Gong *et al.*, 2018; Khalid *et al.*, 2017; Kidd *et al.*, 2015). TE anthropogenic sources mainly result from industrial activities, including atmospheric deposition from smelting and fossil fuels combustion, mining and agricultural practices or from human-induced soil erosion (Antoniadis *et al.*, 2017; Karbowska, 2016; Mahar *et al.*, 2016).

Due to their non-biodegradability, TE are a major threat to both human health and environmental quality. One of the major issues is the TE transportation, resulting mainly from soil wind and water erosion and leaching to groundwaters and sediments, causing eutrophication and possible human exposure through drinkable water. Considering their biological role, TE are classified as either essential or non-essential elements for plant growth. Several TE, such as arsenic (As), cobalt, copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) are necessary for an optimal plant growth and development and hence regarded as biologically essential, whereas cadmium (Cd), lead (Pb) or mercury are for instance biologically non-essential (Ali *et al.*, 2019; Arif *et al.*, 2016). Another major concern is the toxicity resulting from TE accumulation in living organisms and bioavailability in soils, as it is in fact likely to affect biodiversity as well as soil ecosystem services. Moreover, TE are also susceptible to harm plants, affecting both growth and crop yields. Eventually, TE are posing significant risks towards animals and human beings, either by direct exposure or contamination of the food chain (Antoniadis *et al.*, 2017; Gong *et al.*, 2018).

For the past decades, physicochemical (soil excavation and/or replacement, vitrification, electrokinetic remediation, thermal treatment, chemical immobilization or stabilization or soil washing – Gong *et al.*, 2018) as well as biological remediation means (phytotechnologies and bioremediation by microorganisms) have been investigated for their potential to remediate or mitigate the environmental and health risks bound to the presence of TE. However, the physicochemical conventional methods, which are currently the most widely used techniques, are often expensive and extremely energy consuming, may lead to an irreversible degradation of the soil ecosystem and are not suitable for the remediation of wide contaminated areas (Burgess *et al.*, 2018; Gong *et al.*, 2018). One of the existing options to tackle these issues, considered as more “environmental-friendly”, is notably the use of plants and their associated microorganisms, relying on their natural ability to sequester or immobilize TE in the rhizosphere, involving mechanisms such as complexation, precipitation or root adsorption (Antoniadis *et al.*, 2017; Burgess *et al.*, 2018; Kidd *et al.*, 2015; Gong *et al.*, 2018). It could potentially be aided through the use of microbial, organic or mineral amendments to either immobilize soil contaminants, such as TE, hence reducing their mobility and availability or to facilitate plant’s establishment and growth. This process is referred to as “aided phytostabilization” or “aided phytoimmobilization”. A major strength of aided phytostabilisation is the reduction of the risk induced by the presence of one or several soil contaminants, by reducing their mobility and availability in the soil and water as well as avoiding a potential additional risk due to the transportation of the excavated soil for further *ex situ* processing (Kumpiene *et al.*, 2019). The presence of the vegetation cover allows to reduce leaching and surface runoff phenomena and to control wind erosion. Additionally, the revegetation on marginal sites such as mine tailings provides aesthetic and ecological values (Bolan *et al.*, 2011; Dominguez *et al.*, 2008; Dominguez *et al.*, 2010; Mench *et al.*, 2010; Robinson *et al.*, 2009). Furthermore, aided phytostabilisation is considered as less invasive regarding

soil structure and function and ecosystem services compared to the physicochemical remediation techniques (Kim *et al.*, 2018), and may contribute to their restoration, by influencing microbial communities and biodiversity (Dongmei and Changqun, 2008; González *et al.*, 2017; Kidd *et al.*, 2015). Based on phytotechnologies, aided phytostabilisation is also well accepted by general public as it is regarded as environmentally friendly. It is an emerging and growing technology with a high potential, considering its relatively low cost and cost effectiveness, sustainability, environmental compatibility and aestheticism in comparison with the conventional remediation technologies (Kumpiene *et al.*, 2019; Malik *et al.*, 2015; Schwitzguébel *et al.*, 2002). It however currently faces several obstacles, in particular because of the time scale of the method and the lack of scientific feedback, slowing down its development (Kumpiene *et al.*, 2019).

In this chapter, we focus on the effectiveness of microbial amendments to further investigate and discuss their benefits in aided phytostabilisation, especially on the enhancement of the TE immobilization as well as on their effects on plant's growth. We also discuss the advantages and limitations of aided phytostabilization and the benefits coming from its integration in a wider phytomanagement approach, embracing considerations towards ecological aspects and biomass valorisation and the potentially resulting economic, social or environmental benefits, which could hence constitute a viable option for the management and use of contaminated soils.

Part I. Phytostabilisation

TE behaviour in soils - speciation and mobility

In practice, the determination of the TE total content is not sufficient to explain their mobility and bioavailability in soil. The speciation of a TE, which refers to the existing forms and possible associations of a specific element and aims at identifying the feasible combinations between anions and cations in solution or the feasible combinations between anions and cations and their localisation on or in the matrix when in the solid phase, is then of great interest (Bourrelie and Berthelie, 1998). TE in soils are split between liquid and solid phases, under various chemical forms. This distribution is determined by (1) the form in which the TE were initially introduced into the soil, (2) the soil physicochemical properties and (3) the other "species" in presence, that is the chemical form in which other TE and chemical elements are present and the chemical bonds they are able to establish (ADEME and APCA, 2005; Bourrelie and Berthelie, 1998). Most of the time, TE are mainly concentrated in the solid phase, as the amount present in the soil solution represents only a limited fraction of the element. Depending on their location and on the involved reactions, TE are more or less mobile (Figure 1).

In particular, TE may be retained in the soil solid phase, as most of the organic compounds (especially humic acids in the organic matter) and clay minerals in soil bear either positive or negative surface charges, involved in ion exchange or sorption reactions (Ahmed *et al.*, 2019; Citeau *et al.*, 2008). Specifically, sorption may happen in two distinct ways: by ion exchange and by surface complexation (Citeau *et al.*, 2008).

In the liquid phase (namely the soil solution), TE may be found in the form of cations, hydroxides or oxyanions, depending on their electric charge, their electronegativity and/or their ionic radius. As oxygen is the prevailing anion in both water and solid soil constituent (oxides, hydroxides, carbonates, phosphates, *etc*), TE speciation is mostly assessed through interactions with oxygen, either by hydration or hydroxylation (Bourrelie and Berthelie, 1998).

Additionally, complexation may occur in the presence of organic compounds in the soil solution, such as organic acids (in particular low molecular-weight ones – citric, oxalic, humic and fulvic acids), peptides or proteins, or other organic ligands such as siderophores, originating from exudation and microbial activity in the plant's rhizosphere (Ahmed *et al.*, 2019). Inorganic soil constituents, including carbonates, chlorides, nitrites and nitrates, sulfates or phosphates, whose amounts will vary according

to parent rock, climate conditions and the use of soil amendments or agricultural inputs, may as well be involved in TE complexation (Citeau *et al.*, 2008; Montalvo *et al.*, 2016).

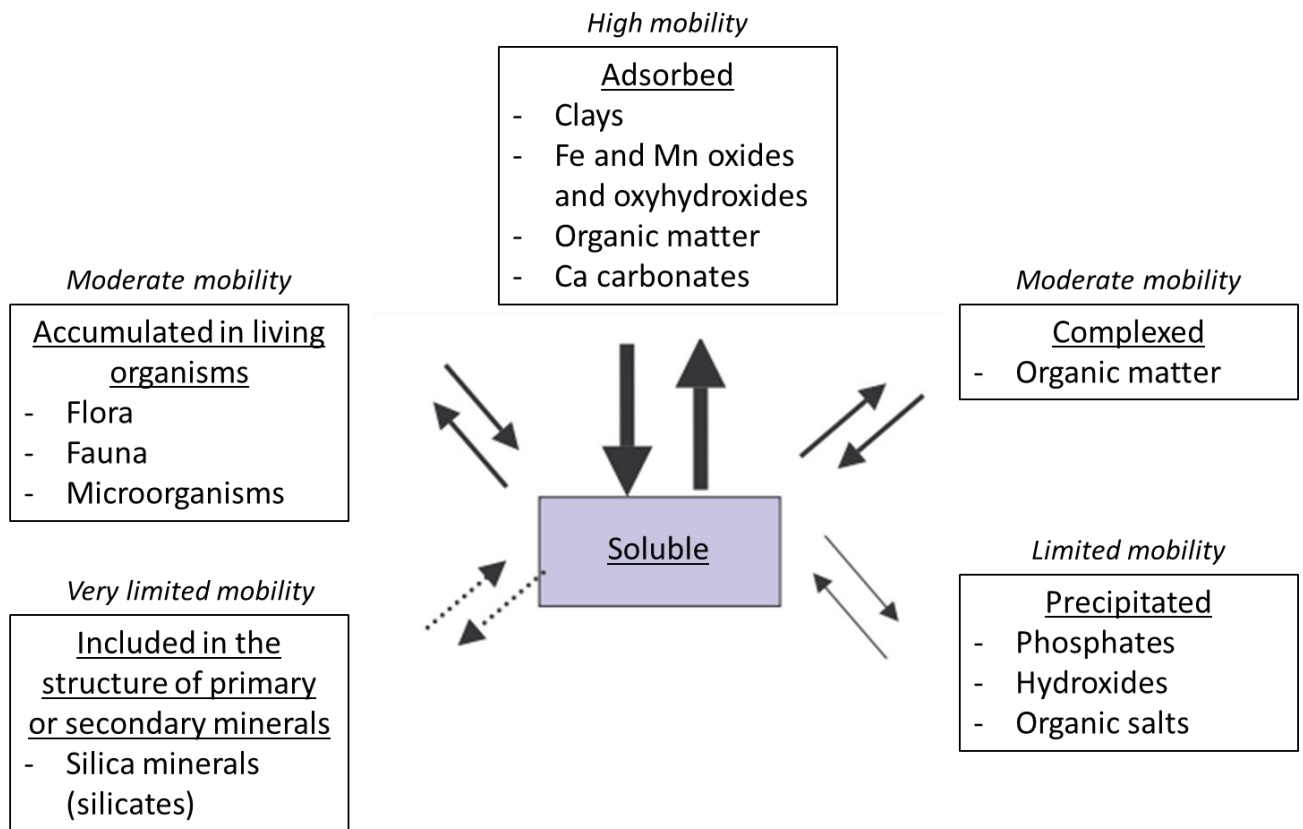


Figure 1: TE speciation and mobility in soil (adapted from ADEME and APCA, 2005)

Several parameters have been evidenced to directly affect TE mobility, especially between the liquid and solid phases, and availability in soil. The most frequently highlighted physicochemical parameters are temperature, oxidation/reduction potential (redox potential) and pH. This latter is arguably the most prevailing factor explaining TE mobility in soils. In fact, in most cases, a low pH has been demonstrated to favour TE availability by proton exchange or disruption of the retention phase, while on the contrary, a high pH mainly results in a TE immobilization, thanks to an increase of the cation exchange capacity, the formation of insoluble salts and the complexation with dissolved organic matter (Caporale and Violante, 2016; Citeau *et al.*, 2008; Montalvo *et al.*, 2016). Additionally, when TE are stable under their cationic form (Cd, Zn, Mn, Pb, Al, *etc.*), their solubility decreases with an increased pH, and conversely for the TE stable under their anionic form (As, Sb, S). Redox potential is also likely to affect TE speciation, by (1) affecting their oxidation level, (2) affecting the oxidation level of the complexing compounds or (3) creating or dissolving TE carrier phases (Bourrelier and Berthelin, 1998). Eventually, temperature effects are diverse: it may directly facilitate the dissolution of the compounds containing a TE, hence increase its availability and mobility, while an indirect effect has also been demonstrated on TE speciation, by modifying soil microbial activity, hence affecting organic matter degradation and resulting in either the production of acid (decreasing pH) or complexing compounds (ADEME and APCA, 2005).

It should be kept in mind that parameters influencing TE speciation are not fixed in time and are likely to change, with a modification of soil and climate conditions. This may induce radical changes regarding TE mobility and bioavailability (Bolan *et al.*, 2011). In that matter, phytotechnologies aiming at introducing a sustainable vegetation cover, besides limiting erosion and runoff phenomena, are

modulating for example temperature variations at the ground level and are hence valuable regarding soil function (Bolan *et al.*, 2011; Lozano-Parra *et al.*, 2018).

TE uptake and transfer in plant tissues

When cultivated on TE contaminated soils, plants, because of the potential accumulation of TE in their tissues, are presenting a risk for both human and animal health. The crop yields may also be significantly affected. These risks are closely related to the TE bioavailability for plants that is the ability of TE to penetrate into the plant tissues and to accumulate during the plant's development (Ali *et al.*, 2019; Bourrelier and Berthelin, 1998; Seshadri *et al.*, 2015). The TE bioavailability for plants results from several consecutive phenomena, contributing to the transfer of the TE from the soil solid phase to the plant tissues. Specifically, it first requires the transfer of the TE from the soil solid phase to the soil solution, followed by its diffusion and/or advection transportation through the rhizosphere to the root surface and eventually its uptake by root cells and transportation through the root to the plant tissues (Bourrelier and Berthelin, 1998). TE speciation, soil composition (presence of clay, organic matter content, pH, redox potential), climate conditions (temperature, humidity) and plant characteristics (behaviour regarding TE, species, variety) are numerous parameters that may modulate TE bioavailability and transfer to plant tissues (Bourrelier and Berthelin, 1998). In particular, it has previously been shown that plants able to grow on soils with high TE concentrations, are restricting a potential uptake in their aboveground tissues, by accumulating TE especially in the root system (Baker, 1981; Peterson, 1971, 1975). Based on the plant's metal uptake pattern, three different behaviours can be categorised as follows and as displayed on Figure 2:

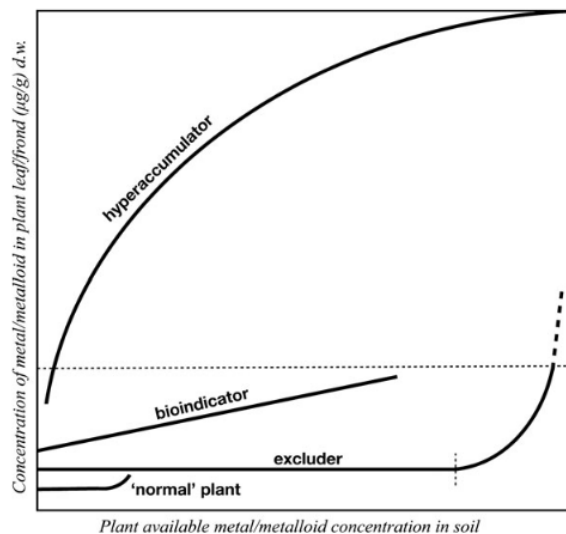


Figure 2: Plants' behaviour regarding TE concentrations in soil - from van der Ent *et al.*, 2012

- Accumulators and hyperaccumulators exhibit an active metal uptake and accumulation in their aboveground parts, which may be specific to one or several TE. Hyperaccumulators have specialized mechanisms that allow them to accumulate high amounts of TE up to 10% of their dry weight, regardless of the soil concentration (Baker, 1981; van der Ent *et al.*, 2012). *Arabidopsis halleri*, *Pteris vittata* L. and *Solanum nigrum* are some examples of Cd-hyperaccumulating species previously reported (Li *et al.*, 2006; van der Ent *et al.*, 2012). Similarly, *Noccaea caerulea* was described as a Cd, Pb and Zn hyperaccumulator (Bert *et al.*, 2003; van der Ent *et al.*, 2012), while *Alyssum murale* is a Ni-hyperaccumulator (Massoura *et al.*, 2004).
- Indicators are able to regulate both metal uptake and transfer to the shoots, reaching amounts that are indicative of the soil concentration (Garg *et al.*, 2017). *Trifolium pratense* L. was

previously reported as a Ni-indicator (Massoura *et al.*, 2004). Similarly, *Larrea divaricata* and *Prosopis alba* were reported as Cd, Ni and Zn-indicators (Garg *et al.*, 2017).

- Excluders are able to either restricting a potential metal entry into the root or accumulating TE in the root system to avoid a transfer to the shoots, with really low amounts of TE transported to the aboveground parts. Yet, the amounts of TE present in their roots may be elevated (Baker *et al.*, 2000; Burges *et al.*, 2018; Massoura *et al.*, 2004; Tang *et al.*, 2019). *Anthyllis vulneraria* and *Biscutella laevigata* are two examples of Cu-excluding species (Baker, 1981), while *Triticum aestivum* L. was highlighted as Ni-excluder (Massoura *et al.*, 2004). Similarly, *Agrostis stolonifera* L., *Pinus radiata* or *Silene maritime* were reported as excluders (Garg *et al.*, 2017).

Additionally, two indexes are commonly measured to highlight the plant's behaviour regarding TE. First, Bioconcentration Factor (BCF) is described as the ability of plants to accumulate TE from the soil, especially in their roots (Mishra and Pandey, 2019). It may be expressed using the following equation:

$$\text{BCF} = \frac{\text{TE concentration (in root)}}{\text{TE concentration (in soil)}}$$

Translocation factor (TF) is another important index used to assess a plant's potential for phytoremediation purposes. It is calculated from the ratio of the TE presence in the plant's shoots compared to that in the plant's roots (Mishra and Pandey, 2019) using the equation:

$$\text{TF} = \frac{\text{TE concentration (in aerial parts)}}{\text{TE concentration (in roots)}}$$

In particular, plant species with a BCF value lower than 1 are considered excluders, whereas those with a BCF value equal to or above 1 are considered accumulators (Baker, 1981). Additionally, plants displaying a BCF value equal to or above 10 are classified as potential hyperaccumulators (Ma *et al.*, 2001).

To cope with TE stress and toxicity, many higher plants species have evolved several defence mechanisms aiming at either reducing TE uptake into roots and shoots, sequestering TE into vacuoles, binding it to specific proteins such as phytochelatins and metallothioneins, or activating antioxidative pathways (Shahid *et al.*, 2015; Tiwari and Lata, 2018). In the case of accumulators or hyperaccumulators, tolerance mechanisms are exacerbated and in particular the transfer of TE from the roots to the aboveground parts of the plant are enabled (Fahr *et al.*, 2013; Rascio and Navari-Izzo, 2011). As the aim of this chapter is to provide insights on aided phytostabilisation, accumulators and hyperaccumulators which are more suitable for phytoextraction purposes won't be further covered.

Plant tolerance to TE toxicity

Stress factors can severely affect both establishment of cultivated plants and their performance in the remediation approach. Limiting biotic and abiotic factors, in addition to TE phytotoxicity, are likely to compromise the remediation's success. In particular, TE, when they exceed a certain threshold, may become toxic for living organisms and depending on the plant species, they may cause direct and indirect damages (Ali *et al.*, 2019; Arif *et al.*, 2016). From a cellular and molecular perspective, TE toxicity harms plants in various ways, from the germination of the seeds to the plant development, altering the key biological and physiological processes. They are able to affect pigment synthesis (e.g. chlorophyll and anthocyanins, respectively involved in plant photosynthesis and protection against abiotic stresses (Boonyapookana *et al.*, 2002; Landi *et al.*, 2015), photosynthesis (affecting both CO₂ fixation and uptake and the chloroplast membrane's structure – Prasad and Strzałka, 1999; Sharma and Agrawal, 2005), respiration (involving mitochondrial damages – Keunen *et al.*, 2011), hormonal balance (in particular stimulating the synthesis of ethylene – Arteca and Arteca, 2007; Wani *et al.*, 2012), nutrient assimilation (by reducing nutrient availability and uptake – Sharma and Agrawal, 2005;

Wani *et al.*, 2012), DNA replication (due to the presence of oxidative compounds, damaging DNA molecules among others and hence compromising DNA replication and transcription – Kamaludeen *et al.*, 2003; Wani *et al.*, 2012) and overall protein synthesis (by inactivating it and by modulating the gene expression, hence stimulating the synthesis of stress-induced proteins – Ahmad *et al.*, 2012; Singh *et al.*, 2016).

In particular, TE are exerting toxicity throughout three groups of mechanisms (Singh *et al.*, 2016):

- Certain TE, such as As or Cd share similarities with nutrients (respectively phosphorus and Zn for that example) and signalling-involved ions (such as Ca^{2+}), resulting in an alteration of the calcium-dependent signalling as well as a competitive binding and absorption in the root (DalCorso *et al.*, 2013; Vetterlein *et al.*, 2007).
- TE can inhibit functional proteins, by interfering in their structure, interacting with sulfhydryl groups and thus altering their function (Ahsan *et al.*, 2010; Li *et al.*, 2006; Singh *et al.*, 2009). Additionally, this enzyme functional loss, may be due to a substitution and displacement of essential cations on specific binding sites, thus reducing or eliminating their activity (DalCorso *et al.*, 2008; DalCorso *et al.*, 2013; Sharma and Sharma, 1996).
- TE are also responsible for the stimulation of reactive oxygen species (ROS) overproduction, such as superoxide anion radical (O_2^-), hydroxyl radicals ($\cdot OH$) or hydrogen peroxide (H_2O_2), known to affect and disrupt macromolecules, phytohormones, membranes and signalling pathways and exert geno- and cytotoxicity (Dutta *et al.*, 2018; Sharma and Dietz, 2009; Singh and Shah, 2014).

In the past decades, the use of several “omic” approaches at different structural levels (metabolomic, ionic, transcriptomic and proteomic) helped to clearly identify tolerance mechanisms to TE stress. First, the induction of several genes in the case of TE stress has been highlighted. In particular, induced genes can be gathered in two groups: the regulatory genes and the functional genes (Tran *et al.*, 2010). For the regulatory ones, transcriptomics used for *Arabidopsis*, *Brassica* and *Lycopersicon* species exposed the implication of several transcription factors such as *bHLH*, *bZIP*, *AP2/ERF* and *DREB* when a TE stress occurred, able to modulate the expression of genes involved in stress-response (LeDuc *et al.*, 2006; Shameer *et al.*, 2009; Singh *et al.*, 2016; Tiwari and Lata, 2018). Additionally, genes from the functional group, are responsible for the encoding of various metabolites and proteins, including amines, phenols and sugars, involved in TE tolerance mechanisms (Singh *et al.*, 2016).

TE avoidance or tolerance mechanisms, such as the creation of a physical barrier through the synthesis of callose or the sequestration of the TE into the vacuoles, take place in plant roots (Singh *et al.*, 2016). Several mechanisms involving TE immobilization and compartmentation may be valuable regarding phytostabilization potential and may also be evaluated in terms of plant tolerance to TE-stress and can be gathered under 3 main strategies:

- Extracellular chelation and precipitation and cell wall binding:

The contribution of root exudates has been identified to reduce the TE uptake into the root by binding them in the rhizosphere, hence reducing both their bioavailability and mobility (Dong *et al.*, 2007; Guo *et al.*, 2006; Luo *et al.*, 2014). In particular, low molecular weight organic acids are behaving as TE chelators (Chiang *et al.*, 2011; Montiel-Rozas *et al.*, 2016) and are susceptible to rely on soil acidification to also influence the TE availability and mobility (Montiel-Rozas *et al.*, 2016; Seshadri *et al.*, 2015; Zhixin *et al.*, 2013). The intracellular concentration of TE may be modulated by immobilization phenomena involving in particular binding to the cell walls. Cell wall is a key compartment where TE sorption occurs, thanks to the presence of polysaccharides, proteins, amino acids and phenolic compounds, able to bind TE, but which is also likely to suffer damages from TE if the concentration is too high (Andosch *et al.*, 2015; Krzesłowska, 2011; Lang and Wernitznig, 2013; Williams *et al.*, 2000).

- Intracellular TE-sequestration and translocation

The role of numerous transporters has been highlighted in the tolerance mechanisms regarding TE toxicity. First ATPases, also referred to as Heavy Metal ATPases (HMAs), are TE transporters activated by the hydrolysis of ATP molecules, whose role is to translocate TE out of the cytoplasm, across membranes, either to the extracellular space or into specific organelles (vesicles and vacuoles). They are therefore involved in transport, exclusion and compartmentation of the TE (Grennan, 2009; Liu *et al.*, 2017; Singh *et al.*, 2016; Williams *et al.*, 2000). Then, Metal Tolerance Proteins (MTPs) also called Cation Diffusion Facilitators, are another group of membrane transporters, involved in either the efflux of metallic cations from the cytoplasm to the extracellular compartment or in their vacuolar or vesicular storage and in their homeostasis (Haney *et al.*, 2005; Ricachenevsky *et al.*, 2013; Zhang and Liu, 2017). And finally, Zinc-iron permease (ZIP) and associated macrophage protein (NRAMP) are two other prominent families of membrane transporters involved in the regulation of the intracellular concentration of TE (Jalmi *et al.*, 2018; Nevo and Nelson, 2006; Singh *et al.*, 2016).

Under stress conditions, plants are also able to induce the synthesis of two groups of proteins clearly involved in the chelation of TE. First, phytochelatins (PCs), which are glutathione oligomers, are bearing thiol groups involved in the chelation of metallic ions and in the formation of complexes (Cobbett, 2000; Cobbett and Goldsbrough, 2002). The whole process involving PCs relies on their biosynthesis, the complexation with the TE, the transportation of the TE-PC complex towards vacuoles and finally its sequestration into vacuoles, leading to a detoxification (Jain *et al.*, 2018). Secondly, metallothioneins (MTs) are another group of intracellular low molecular weight proteins, bearing cysteine-rich peptides able to bind TE with a high affinity, whose synthesis is induced in their presence (Coyle *et al.*, 2002; Ruttkay-Nedecky *et al.*, 2013; Zimeri *et al.*, 2005). They are acting as chelating agents and are also involved in the scavenging of TE or ROS (Chen *et al.*, 2014). Additionally, sequestration and compartmentation of TE into specific organelles such as vesicles or vacuoles has been evidenced (Peng and Gong, 2014; Sharma *et al.*, 2016).

Several amino acids and their derivatives as well as amine-containing molecules have also been found to be able to chelate TE, hence reducing their availability and toxicity for the plant. In particular, proline and histidine have been reported to chelate metallic ions under TE stress, in both cells and xylem sap (Rai, 2002; Sharma and Dietz, 2006; Talanova *et al.*, 2000; Yusuf *et al.*, 2012). The induction and synthesis of several phenolic compounds, containing hydroxyl and carboxyl groups susceptible to bind to metallic ions, has also been found increased under TE stress, in particular in the presence of Fe and Cu (Jun *et al.*, 2003; Winkel-Shirley, 2002) or aluminium (Díaz *et al.*, 2001).

- Reducing the oxidative stress resulting from the presence of TE:

First, the activation of the antioxidant system, induced by the presence of ROS, and involving several enzymes such as catalases (peroxides detoxifying - Morita *et al.*, 1994; Lin and Kao, 2000), superoxide dismutases (superoxide radicals dismutating – Fridovich, 1989) and several peroxidases and reductases (scavenging, detoxifying and signalling roles - Asada, 1992; Asada, 1996 ; Foyer *et al.*, 1997; Mittler, 2002), allows to avoid cell damages caused by certain ROS.

Additionally, the role of two metabolites, namely glutathione and alpha-tocopherol, has also been highlighted: the former is a tripeptide, substrate for an enzyme (Glutathione reductase) involved in the antioxidant system (Kumar *et al.*, 2012; Singh *et al.*, 2015). The latter is synthesized in higher plants and found to be involved in scavenging ROS and lipid peroxyl radicals (Collin *et al.*, 2008; Fritsche *et al.*, 2017; Munne-Bosch, 2005; Wani *et al.*, 2012). Other non-enzymatic metabolites such as vitamins B6, C or E, ascorbate, alkaloids, proline and polyphenols are also involved in the antioxidant system (Dutta *et al.*, 2018; Fritsche *et al.*, 2017).

Plant's selection

One of the key criteria in a phytotechnology aiming at reducing TE availability is the consideration of plants' behaviour regarding TE toxicity, so as to select plants that are able to grow in conditions that are toxic to most of the higher plants. From that perspective, the use of metal tolerant plants, also called metallophytes, could be relevant. Owing to the biological mechanisms that they have developed over time, allowing them to resist high TE concentrations, they are indeed adapted to grow in TE-contaminated conditions (Baker, 1981; Burges *et al.*, 2018; Ginocchio and Baker, 2004; Tang *et al.*, 2019).

Pioneer species, plants that have evolved to successfully establish, grow and colonize on disturbed area, are often considered as a good choice (Dongmei and Changqun, 2008; Guadarrama *et al.*, 2007; Kidd *et al.*, 2015). But the careful selection should also depend on the chosen strategy as previously mentioned (Burges *et al.*, 2018; Kidd *et al.*, 2015). Thus, excluders will be favoured for aided phytostabilisation. In addition, fast growing species, able to develop a consistent vegetation cover, as well as perennial species, may be valuable. Moreover, considerations regarding seeds' availability, the choice of cultivated or revegetating plants and the valorisation channel intended for the produced biomass are several other criteria which deserve to be highlighted in a global approach. Table 1 gathers the main plant species previously studied in phytostabilisation approaches. The use of herbaceous species, notably *Poaceae*, seems to be dominant, especially for revegetation purposes. Many of the listed studies are in fact investigating the revegetation of mining sites and the ability of herbaceous species to grow and quickly develop a vegetation cover is of great interest. Additionally, tree species such as *Populus* spp or *Salix* spp., able to produce a consequent amount of biomass and to reach deeper soil contamination in comparison with herbaceous species, as well as allowing to manage the contaminated site on the long term are also studied.

Great attention must however be given to invasive or potentially invasive species, during the plant's choice, so as to avoid unnecessary introduction and proliferation of these undesirable species. Indeed, plant species that could be relevant, owing to their presence on a TE-contaminated site or their phytostabilisation potential, may be registered as invasive ones (*Cyperus agrostis*, *Phytolacca americana*, *Senecio inaequidens* and *Sporobolus indicus*), based on existing results (Bes *et al.*, 2010; Kidd *et al.*, 2015). Japanese knotweed (*Fallopia japonica*) is another well-known example of a highly TE-tolerant species, widely spread worldwide even on TE contaminated area, and yet listed as a highly invasive species (Kidd *et al.*, 2015). On another note, native plant species, such as metallophytes, might be well adapted to the specific and potentially extreme conditions observed in a studied contaminated area and could be efficient, without any addition (Bidar *et al.*, 2007; Burges *et al.*, 2016). *Silene paradoxa* and *Rosmarinus officinalis* L. are two among many examples of metallophytes that have been studied for phytostabilisation purposes (Affholder *et al.*, 2020; Colzi *et al.*, 2014).

The characteristics of the site are another great concern. In addition to the high concentrations of TE in metalliferous area, soils conditions may be extreme and result in a wide variety of stresses, in terms of nutrient and organic matter content, acidity, alkalinity or salinity, water availability, soil structure and biotic quality of the soil, affecting the establishment of the plant species (Burges *et al.*, 2016, 2018; Kidd *et al.*, 2015; Wang *et al.*, 2017; Wu *et al.*, 2013). Mine tailings are one among many examples of area where revegetation is subject to extreme conditions, often characterized by high concentrations of TE, high acidity and/or salinity and poor physical structure (Burges *et al.*, 2016; Li *et al.*, 2018). The introduction of amendments to mine soil during phytostabilization has then frequently been investigated in order to facilitate plant colonization and survival (Burges *et al.*, 2016) and will be further discussed in the following section.

Synthèse bibliographique

Table 1 : Plant species used in TE phytostabilisation

Type of plant	Family	Plants species	Immobilized TE	References
Herbaceous	Poaceae	<i>Festuca rubra</i>	Cd and Zn	Burges <i>et al.</i> , 2016
			Zn and Pb	Galende <i>et al.</i> , 2014
			Cu	Radziemska <i>et al.</i> , 2017
			Cu, Ni and Cd	Radziemska <i>et al.</i> , 2019
			Zn, Cd, Pb and Cu	Vangronsveld <i>et al.</i> , 1996
		<i>Lolium perenne</i>	Cd, Pb, Zn	Alvarenga <i>et al.</i> , 2009
			Cd, Pb, Zn	Epelde <i>et al.</i> , 2009
			Cu, Pb and Zn	Arienzo <i>et al.</i> 2004
			Cd, Pb and Zn	Bidar <i>et al.</i> , 2007
			Ni and Cu	Radziemska <i>et al.</i> , 2018
	<i>Agrostis capillaris</i>	Cu	Touceda-González <i>et al.</i> , 2017	
		Zn, Cd, Pb and Cu	Vangronsveld <i>et al.</i> , 1996	
	<i>Agrostis stolonifera</i>	Cd, Pb, Zn, As and Cu	Pérez-de-Mora <i>et al.</i> , 2006	
		<i>Pennisetum americanum</i> × <i>purpureum</i>	Cd, Pb and Zn	Zhang <i>et al.</i> , 2016
			<i>Pennisetum purpureum</i> Schum.	Cd, Cu, Pb and Zn
		<i>Cynodon dactylon</i> L.	Cd, Cu, Pb and Zn	Yang <i>et al.</i> , 2016a
		<i>Festuca arundinacea</i>	Cu, Pb and Zn	Pierzynski <i>et al.</i> , 2002
		<i>Lygeum spartum</i>	Cd, Pb and Zn	Martinez-Martinez <i>et al.</i> , 2017
		<i>Miscanthus sinensis</i> × <i>giganteus</i>	Cd, Pb and Zn	Pavel <i>et al.</i> , 2014
		<i>Neyraudia reynaudiana</i> (Kunth.) Keng.	Cd, Cu, Pb and Zn	Yang <i>et al.</i> , 2016a
		<i>Panicum repens</i> L.	Cd, Cu, Pb and Zn	Yang <i>et al.</i> , 2016
		<i>Phalaris arundinacea</i>	Ni	Korzeniowska and Stanislawska-Glubiak, 2019
	<i>Piptatherum miliaceum</i>	Cd, Pb and Zn	Martinez-Martinez <i>et al.</i> , 2017	
	<i>Sorghum dochna</i>	Cd, Pb and Zn	Zhang <i>et al.</i> , 2016	
	<i>Zea mays</i>	Ni	Korzeniowska and Stanislawska-Glubiak, 2019	
	Fabaceae	<i>Lupinus luteus</i>	As	Dary <i>et al.</i> , 2010
		<i>Trifolium repens</i>	Cd, Pb, Zn	Bidar <i>et al.</i> , 2007
<i>Vicia faba</i>		Cu	Fatnassi <i>et al.</i> , 2013	
Lamiaceae	<i>Rosmarinus officinalis</i>	Cd and Zn	Madejon <i>et al.</i> , 2009	
Juncaceae	<i>Juncus effusus</i> L.	Pb	Najeeb <i>et al.</i> , 2014	
Dennstaedtiaceae	<i>Pteridium aquilinum</i>	Cd, Cu, Pb and Zn	Lee <i>et al.</i> , 2014	
Crassulaceae	<i>Hylotelephium spectabile</i>	Zn and Pb	Gu <i>et al.</i> , 2017	
Brassicaceae	<i>Brassica juncea</i>	Cr, Cu and Zn	Radziemska <i>et al.</i> , 2017	
Athyriaceae	<i>Athyrium wardii</i>	Pb	Zhao <i>et al.</i> , 2016a	
Shrub	Amaranthaceae	<i>Atriplex lentiformis</i>	Zn and Pb	Eissa, 2015
			Zn, Pb, Cu and As	de-Bashan <i>et al.</i> , 2010
		<i>Atriplex undulata</i>	Zn and Pb	Eissa, 2015
Tree	Salicaceae	<i>Populus spp.</i>	As, Pb, Cu, Ni	French <i>et al.</i> , 2006
			Cu	Touceda-González <i>et al.</i> , 2017
			Cu and Zn	Brunner <i>et al.</i> , 2007
	<i>Salix spp.</i>	As, Pb, Cu, Ni	French <i>et al.</i> 2006	
		Ni	Korzeniowska and Stanislawska-Glubiak, 2019	
		Cu	Touceda-González <i>et al.</i> , 2017	
		Cu and Zn	Brunner <i>et al.</i> , 2007	
Pinaceae	<i>Picea abies</i>	Cu and Zn	Brunner <i>et al.</i> , 2007	
Myrtaceae	<i>Eucalyptus robusta</i>	Cd, Cu, Pb and Zn	Yang <i>et al.</i> , 2016a	
Betulaceae	<i>Alnus spp.</i>	As, Pb, Cu and Ni	French <i>et al.</i> , 2006	

Part II. Aided phytostabilisation

The use of amendments in the context of phytostabilisation has been widely studied for decades, especially aiming at reducing the mobility and availability of TE in soils (Kumpiene *et al.*, 2019). In a wider perspective, amendments have been demonstrated to be useful for the reduction of the TE mobility, availability and toxicity (Gong *et al.*, 2018; Kumpiene *et al.*, 2019), but also for the improvement of the soil properties, from both physico-chemical and microbial considerations (Gomez-Sagasti *et al.*, 2018; Hijbeek *et al.*, 2017) and finally for the enhancement of the plant growth and tolerance to TE (Deng and Cao, 2017; Ferrol *et al.*, 2016; Gong *et al.*, 2018). From various natures, amendments can be gathered into 3 main groups: the mineral amendments (including clay and liming materials, ashes, phosphate compounds and metal oxides – Gong *et al.*, 2018), the organic ones (biosolids including biochar, and organic matter-based ones, such as composts, animal and green manures and crop residues – Gomez-Sagasti *et al.*, 2018), and microbial ones (plant associated microorganisms [PAM] – Deng and Cao, 2017; Ferrol *et al.*, 2016; Gong *et al.*, 2018). In particular, microbial amendments consist of various microbial communities (fungi, bacteria - Naik *et al.*, 2019), which are located in the rhizosphere, either in the root, on the root surface or in close proximity and associated with the root (Basu *et al.*, 2017; Ferreira *et al.*, 2019; Klopper and Schroth, 1978). These PAM (namely bacterial or fungal endophytes, endo- or ectomycorrhizal fungi, actinomycetes, yeasts and rhizosphere bacteria – Naik *et al.*, 2019; Rajkumar *et al.*, 2012; Tiwari and Lata, 2018) are widely studied for agricultural applications, as inoculants, in particular in terms of plant growth, crop yield and stress tolerance, but are also relevant in an aided phytostabilisation approach as they are likely to reduce TE availability and mobility in soil and to increase plant biomass, thus improving both the success rate of the channel and the biomass valorization perspectives (Basu *et al.*, 2017; Kumar *et al.*, 2019b; Naik *et al.*, 2019).

This section will put a focus on microbial amendments' use in aided phytostabilisation, to further explore their benefits on TE immobilisation and reduced bioavailability, as well as their effects on plant's growth and development.

Effect of microbial amendments on soil properties

In an aided phytostabilisation approach, the restoration of a vegetation cap on a TE-disturbed soil has shown positive outcomes regarding soil properties (Kohler *et al.*, 2014; Yang *et al.*, 2017). PAM have been evidenced to play a role in other ecosystem services, in particular in soil structure and aggregation (Gianinazzi *et al.*, 2010; Lehmann *et al.*, 2017; Ma *et al.*, 2016; Rillig *et al.*, 2006). Arbuscular mycorrhizal fungi (AMF) are widespread and multifunctional members of the soil biota which play a key role in soil aggregate formation and stabilization. Their role may be considered at different scales, namely macro- and micro-aggregation and is attributable to several biophysical (direct interaction with the soil particles and aggregates), biochemical phenomena and biological interactions (Lehmann *et al.*, 2017). A comprehensive review by Lehmann *et al.*, 2017, summarizes and further explains the underlying mechanisms (direct interaction of fungal mycelium with soil particles or aggregates). In particular, the soil stability has been demonstrated often correlated with the extension of the fungal mycelium, playing a key structuring and binding role (Gianinazzi *et al.*, 2010; Lehman *et al.*, 2017), hence reducing the availability of TE due to the presence of binding agents in microaggregates (Lehmann *et al.*, 2017). The secretion of bacterial and fungal products (organic acids, polysaccharides, hydrophobines and glycoproteins, including AMF's glomalin) has additionally been evidenced as impacting the attraction and fixation of the soil particles and aggregates and stabilizing the latter (Gianinazzi *et al.*, 2010; Lehmann *et al.*, 2017; Rillig *et al.*, 2007). Finally, PAM have been found influencing the structure and evolution of the microbial communities associated with the plant roots or in the rhizosphere (Andrade *et al.*, 1997; 1998; Cavagnaro *et al.*, 2006; Rillig *et al.*, 2007), which are likely to affect soil stability as well (Barea *et al.*, 2005). Evidence have shown the role of hyphal-associated bacteria in soil aggregation by affecting particle alignment (Caesar-Tonthat, 2002).

Additionally, studies have demonstrated that the AMF in combination with the soil biota could increase water stability of macroaggregates, but these considerations are species and context dependent (Lehman *et al.*, 2017; Rillig *et al.*, 2005).

A modification of the soil pH is another factor that PAM can modulate. In particular AMF and cyanobacteria have been demonstrated able to increase soil pH (Ma *et al.*, 2016), hence reducing the availability and mobility of the TE in the soil and for the plant (Bago *et al.*, 1998; Etesami, 2018; Navarro-Pedreño *et al.*, 2018).

Microbial amendment's effect on TE immobilization

In aided phytostabilisation, the fact that PAM may be able to reduce TE mobility and availability could be particularly relevant. In particular, several AMF and bacteria species have been studied during the past decades for aided phytostabilization purposes (Tables 2 and 3). *Funneliformis mosseae* and *Rhizophagus irregularis* are the two most studied AMF species in that matter (Table 2), whereas *Pseudomonas* is the most widely studied bacteria genera (Table 3).

The immobilization of TE may occur in two different compartments:

- Extracellular chelation and immobilization of TE:

First, the chelation of TE in the rhizosphere is a key point towards a reduced TE availability (Begum *et al.*, 2019; Ferrol *et al.*, 2016; González-Guerrero *et al.*, 2008). As mentioned in the previous subsection, PAM are likely to affect the soil properties and hence modifying the mobility and availability of the TE for the plant. glomalin-related soil proteins among other fungal exudates (including organic acids as well – Garg *et al.*, 2017) are able to chelate the TE (Wang *et al.*, 2019) and hence reducing their availability in the soil (Cornejo *et al.*, 2008; Gil-Cardesa *et al.*, 2014; González-Chávez *et al.*, 2004). Bacteria genera such as *Staphylococcus aureus*, *Micrococcus luteus*, and *Azotobacter* spp. have also been demonstrated as able to produce extracellular compounds (i.e. polysaccharides, carbohydrates, nucleic and fatty acids), negatively charged and acting as metal binders (Maier *et al.*, 2009).

Additionally, the role of various microorganisms in the transformation of TE through redox reactions has been well studied (Chatterjee *et al.*, 2009; Majumder *et al.*, 2013; Olegario *et al.*, 2010; Oves *et al.*, 2013). By altering the speciation of TE, PAM may affect their solubility and mobility in soil and toxicity towards living organisms (Ma *et al.*, 2016). In particular, *Pseudomonas aeruginosa* has been demonstrated to reduce through enzymatic action chromium from its Cr⁶⁺ to its Cr³⁺ form, which is less mobile and non-toxic (Oves *et al.*, 2013). On the contrary, *Bacillus* sp. and *Geobacillus* sp. were able to oxidize As from As³⁺ to As⁵⁺, for the same benefits as previously stated (Majumder *et al.*, 2013). From a wider perspective, Cu and Hg have been pointed out as more soluble with low oxidation levels, while TE such as As, Cr or Se are more mobile and toxic with higher levels of oxidation (Ma *et al.*, 2016; Ross, 1994).

Biosurfactants, which are amphiphilic compounds either produced on microbial cell surface or excreted in the extracellular medium, are able to complex with TE, reducing their availability in soil but facilitating the acquisition for the microorganism (Ma *et al.*, 2016; Sheng *et al.*, 2008). Biosurfactants are mostly produced by bacteria (*Bacillus*, *Torulopsis* or *Pseudomonas* – non exhaustive list) and constituted of either one or several compounds, namely mycolic acids, polysaccharides or of lipidic nature (Ma *et al.*, 2016). Siderophores are another group of low molecular weight microbial metabolites, produced by both bacteria (in particular *Pseudomonas* and *Burkholderia* genera and cyanobacteria - Banerjee *et al.*, 2018; Etesami, 2018) and fungi (Haselwandter, 2008; Sherrington *et al.*, 2018) under iron-limiting conditions, which are able through functional groups to chelate iron-containing compounds with a high affinity (Banerjee *et al.*, 2018; Etesami, 2018; Ma *et al.*, 2016). Siderophores are classified according to the nature of the functional groups and of the binding site with the ferric ions (namely α -carboxylates, catecholates and hydroxamates – Shilev *et al.*, 2011; Winkelmann, 2002).

Synthèse bibliographique

Table 2: Arbuscular mycorrhizal fungi (AMF) and plant species in aided phytostabilisation approaches

Tested fungi	Host plant	Main results regarding MTE stabilisation	References
<i>Funneliformis mosseae</i>	<i>Cajanus cajan</i> L.	Reduction of Cd uptake by roots and subsequent translocation into leaves	Garg and Chandel, 2012
	<i>Festuca arundinacea</i>	Decreased Pb and Cd concentrations in shoots and Cd and Zn in roots	Gu <i>et al.</i> , 2013b
	<i>Festuca arundinacea</i>	Decreased Cd concentration in roots and shoots, increased Cu and Pb concentrations in roots	Gu <i>et al.</i> , 2017
	<i>Hylotelephium spectabile</i>	Higher MTE retention in roots, lower accumulation in shoots, regarding Cd, Cu, Pb and Zn	Gu <i>et al.</i> , 2017
	<i>Lolium perenne</i>		Gu <i>et al.</i> , 2017
	<i>Medicago sativa</i>	Increased accumulation of Cu in roots and Cd in shoots / Lower accumulation of Pb and Cd in roots and of Cu and Pb in shoots	Lin <i>et al.</i> , 2007
	<i>Ricinus communis</i>	Decreased Pb soil availability	González-Chávez <i>et al.</i> , 2019
	<i>Robinia pseudoacacia</i> L.	Increased Pb accumulation in roots	Huang <i>et al.</i> , 2019
	<i>Sedum spectabile</i>	Increased uptake of Cu, Cd, Zn and Pb in roots	Gu <i>et al.</i> , 2013a
	<i>Sesbania cannabina</i>	Increased accumulation of Cu and Zn in roots and of Cd in shoots, lower accumulation of Cu and Pb in shoots	Lin <i>et al.</i> , 2007
	<i>Sesbania rostrata</i>	Decreased accumulation of Cu in shoots and of Cu and Zn in roots	Lin <i>et al.</i> , 2007
	<i>Tetraclinis articulata</i>	No direct effect of the AMF on Cr, Ni, As and Pb content in roots and shoots	Curaqueo <i>et al.</i> , 2014
	<i>Tradescantia pallida</i>	No effect on MTE uptake in both roots and shoots	Gu <i>et al.</i> , 2017
	<i>Trifolium pratense</i> L.	Increased Zn uptake in roots, decreased translocation in shoots	Chen <i>et al.</i> , 2003
<i>Zea mays</i> var. Karaj	Cd stabilization in soil and roots	Malekzadeh <i>et al.</i> , 2011	
<i>Rhizophagus irregularis</i>	<i>Cajanus cajan</i> L.	Reduced Cd and Zn uptake to roots and decreased transfer from roots to leaves	Garg and Singh, 2017
	<i>Festuca rubra</i>	No evaluation of Zn mobility and concentrations in plants	Orlowska <i>et al.</i> , 2005
	<i>Nicotiana tabacum</i>	Enhanced Cd immobilization in soil	Janoušková <i>et al.</i> , 2006
	<i>Plantago lanceolata</i>	No evaluation of Zn mobility and concentrations in plants	Orlowska <i>et al.</i> , 2005
	<i>Robinia pseudoacacia</i> L.	No effect on Pb stabilization in comparison with non inoculated plants	Yang <i>et al.</i> , 2016b
<i>Glomus etunicatum</i>	<i>Festuca arundinacea</i>	No evidence of MTE stabilization by the AMF	Pierzynski <i>et al.</i> , 2002
	<i>Festuca rubra</i>		
<i>Glomus claroideum</i>	<i>Plantago lanceolata</i>	No evaluation of Zn mobility and concentrations in plants	Orlowska <i>et al.</i> , 2005
	<i>Festuca rubra</i>		
<i>Acaulospora</i> sp.	<i>Ricinus communis</i>	Reduced Pb accumulation in leaves	González-Chávez <i>et al.</i> , 2019
	<i>Trigonella foenum-graecum</i> L.	Slightly reduced Cd uptake in shoots, enhanced in roots	Abdelhameed and Rahab, 2019
<i>Glomus clarum</i>	<i>Festuca arundinacea</i>	No evidence of MTE stabilization by the AMF	Pierzynski <i>et al.</i> , 2002
	<i>Trigonella foenum-graecum</i> L.	Slightly reduced Cd accumulation in shoots, enhanced in roots	Abdelhameed and Rahab, 2019
<i>Glomus geosporum</i>	<i>Festuca rubra</i>	No evaluation of Zn mobility and concentrations in plants	Orlowska <i>et al.</i> , 2005
	<i>Plantago lanceolata</i>		
<i>Claroideoglomus etunicatum</i>	<i>Mimosa caesalpiniaefolia</i> Benth.	Increased Mn retention in roots, lower transfer in shoots	Garcia <i>et al.</i> , 2018
<i>Gigaspora gigantea</i>	<i>Ricinus communis</i>	Limited effects on Pb stabilization	González-Chávez <i>et al.</i> , 2019
<i>Gigaspora nigra</i>	<i>Trigonella foenum-graecum</i> L.	Slightly reduced Cd uptake in shoots, enhanced in roots	Abdelhameed and Rahab, 2019
<i>Glomus macrocarpum</i>	<i>Zea mays</i> L.	Increased Cd uptake in roots	de Andrade and Silveira, 2008
<i>Glomus monosporum</i>	<i>Trigonella foenum-graecum</i> L.	Slightly reduced Cd uptake in shoots, enhanced in roots	Abdelhameed and Rahab, 2019
<i>Rhizophagus clarus</i>	<i>Mimosa caesalpiniaefolia</i> Benth.	No effect on Mn stabilization	Garcia <i>et al.</i> , 2018

Beside their iron-chelating property, siderophores are likely to form complexes with other TE sharing chemical similarities with iron as well, hence reducing their bioavailability in the extracellular medium and toxicity towards other living organisms. Additionally, the complexation of TE with siderophores may facilitate their precipitation and affect their speciation (Banerjee *et al.*, 2018; Etesami, 2018; Shilev *et al.*, 2011).

Table 3: Bacterial and plant species in aided phytostabilisation approaches

Tested bacteria	Host plant	Main results regarding TE phytostabilisation	References
<i>Pseudomonas aeruginosa</i>	Chickpea	Decreased Cr uptake in roots, shoots and seeds	Oves <i>et al.</i> , 2013
	<i>Lycopersicon esculentum</i>	Alleviated Cd accumulation in seedlings	Khanna <i>et al.</i> , 2019
<i>Pseudomonas putida</i>	<i>Brassica chinensis</i>	Reduced Cd uptake and accumulation in plant	Xu <i>et al.</i> , 2012
<i>Pseudomonas</i> sp.	<i>Festuca rubra</i>	Significantly lower shoots concentrations of Cd and Zn	Burges <i>et al.</i> , 2016
	Chickpea	Ni reduced uptake in plant	Tank and Saraf, 2009
	Corn	Reduced Zn accumulation in shoots	Hamidpour <i>et al.</i> , 2019
<i>Bradyrhizobium</i> sp.	<i>Lolium multiflorum</i>	Reduced Cd uptake and accumulation in plant	Guo and Chi, 2014
	<i>Glycine max</i>	Reduced Cd uptake and accumulation in plant	Guo and Chi, 2014
<i>Burkholderia gladioli</i>	<i>Lycopersicon esculentum</i>	Alleviated Cd accumulation in seedlings	Khanna <i>et al.</i> , 2019
<i>Burkholderia</i> sp.	<i>Salix caprea</i>	Reduced Cd uptake and accumulation in plant	Kuffner <i>et al.</i> , 2010
<i>Azotobacter</i> spp.	<i>Triticum aestivum</i>	Decreased Cd and Cr uptake by reducing their mobility	Joshi and Juwarkar, 2009
<i>Cellulosimicrobium cellulans</i>	<i>Green chili</i>	Cr reduced uptake in both shoots and roots	Chatterjee <i>et al.</i> , 2009
<i>Pantoea</i> sp.	<i>Guinea grass</i>	Reduced Cu uptake and accumulation in plant	Huo <i>et al.</i> , 2012
<i>Ralstonia eutropha</i>	<i>Zea mays</i>	Reduced Cd uptake and accumulation in plant	Moreira <i>et al.</i> , 2014
<i>Serratia</i> sp.	<i>Lupinus luteus</i>	Reduced transfer of As, Cd and Cu from roots to shoots	Aafi <i>et al.</i> , 2012

Eventually, PAM reportedly adsorbate TE on their cell walls, in particular in hyphae (de Andrade and Silveira, 2008; Garg and Chandel, 2012) and spores (Gonzalez-Chavez *et al.*, 2002; González-Guerrero *et al.*, 2008; Joner *et al.*, 2000) thanks to the structure of the fungal walls, containing chitin and glucans and negatively charged (Anahid *et al.*, 2011; Bellion *et al.*, 2006; González-Guerrero *et al.*, 2008), or on bacterial anionic cell surface, involving the presence of carboxylic, amino, thio, hydroxo, and hydroxycarboxylic functional groups adsorbing TE (Banerjee *et al.*, 2018).

- Intracellular immobilization and compartmentation of TE:

The PAM also tend to chelate and sequester TE in both cytosol of the microbial cells (in particular in spores and hyphae for fungi) and in organelles, namely vesicles and vacuoles, under increasing concentrations of TE (Ferrol *et al.*, 2016; Garg *et al.*, 2017; González-Guerrero *et al.*, 2008), involving MTs, PCs, amino and organic acids in the cytosol (Cobbett and Goldsbrough, 2002; Garg *et al.*, 2017; González-Guerrero *et al.*, 2008; Hall, 2002). The compartmentalization in vacuoles is achieved through specific ABC and ZnT1 transporters evidenced for AMF (Ferrol *et al.*, 2016; Garg *et al.*, 2017) and the storage of metallic cations further involves MTs or polyphosphates (Ferrol *et al.*, 2016; González-Guerrero *et al.*, 2008; Hijikata *et al.*, 2010). Under high concentrations of TE, a longitudinal transport of the TE-rich vacuoles, directed to the fungal spores, has been evidenced for AMF (Ashford, 2002; Darrah *et al.*, 2006; Garg *et al.*, 2017; Uetake *et al.*, 2002). The previous mechanisms, in the case of elevated TE concentrations, contribute to a reduction of the intracellular availability of the TE and hence to a decreased potent toxicity, towards the microorganism but also towards the host plant as the uptake is regulated by the PAM, as well as a reduction in the mobile pool of TE in soil. In particular, it has been shown in mycorrhized plants that the distribution of the metal transporters was affected (Ouziad *et al.*, 2005) and that the genes MtZIP2 (*Medicago truncatula* – Burleigh *et al.*, 2003) and LeNramp1 and LeNramp3 (Tomato – Ouziad *et al.*, 2005), coding for TE-transporters, were down-regulated. Numerous studies have also shown an alleviated TE uptake in the presence of AMF. For

example, a significantly reduced cadmium uptake in both roots and shoots was shown in *Lotus japonicus* inoculated with *R. irregularis* (Chen *et al.*, 2018). Similarly, *Lonicera japonica* and *Robinia pseudoaccacia*, respectively inoculated with *G. versiforme* and *F. mosseae*, were demonstrated to limit Cd uptake and transfer (Jiang *et al.*, 2016) and to sequester Pb in the roots, significantly decreasing its translocation in aerial parts (Huang *et al.*, 2019). Finally, *Tagetes erecta*, when inoculated with *G. constrictum*, limited zinc, cobalt, manganese and copper uptake from sewage water irrigation, enhancing biomass production, yield, nutrient acquisition and mycorrhizal colonization rates as well (Elhindi *et al.*, 2018).

Microbial amendment's effect on plant growth and development

As previously stated, TE may have noxious effects on plants' growth and development, hence compromising the success of the aided phytostabilisation. Better plant growth, as further investigated in the current section, may result in better performances, even under stressful conditions, and result in a better tolerance regarding metallic stress as well as a better establishment and sustainability of the vegetation cover, hence improving the global performances of the aided phytostabilisation by indirect means. Additionally, this might lead to a higher amount of biomass, which might be valuable in terms of biomass valorisation. Many microbial communities among PAM are known to be able to stimulate directly and indirectly the plant growth by different means, such as the production of valuable metabolites and the improvement of the nutrient availability, the soil properties and the plant health and stress tolerance (Ferreira *et al.*, 2019; Gupta *et al.*, 2018; Naik *et al.*, 2019). These communities are used and widely studied for their benefits in cropping systems, not only for the improved plant performances through enhanced water and nutrient uptake, but also for their contribution to an increased tolerance towards biotic and abiotic environmental stresses (drought, salinity, TE-toxicity – Begum *et al.*, 2019; Kumar *et al.*, 2019a; Smith and Read, 2008) and their benefits could be translated in aided phytostabilisation as well.

Microbial amendment's effect on nutrient solubilisation and acquisition

More specifically, PAM are able to facilitate the nutrient uptake, through various mechanisms. For example, AMF are able to enhance nutrient uptake, through a larger exploration of the soil, thanks to the external hyphae network allowing access to nutrients and water out of the depletion zone located close to the root (Begum *et al.*, 2019; Ferrol *et al.*, 2016). AMF are also able to promote phosphorus uptake, even in low supply conditions (Ferrol *et al.*, 2018; Smith and Read, 2008), involving specific high-affinity Pi transporters and a stimulation of the gene expression encoding these transporters (Smith *et al.*, 2011), as well as facilitating the uptake by plants through the formation of fungal structures (arbuscules) and the expression of the host-plant genes regarding phosphorus uptake through transporters (Kobae, 2019; Pumplin *et al.*, 2012). However, high availability and concentrations in phosphorus are often an obstacle for the establishment of the mycorrhizal symbiosis (Begum *et al.*, 2019; Kobae, 2019).

Phosphorus, which is an essential nutrient for plant, exclusively found in the form of phosphates and most of the time under an insoluble form not bioavailable for plants, is dissolved and solubilised by several bacteria genera, including *Azotobacter* (Kannapiran and Ramkumar, 2011), *Burkholderia*, *Enterobacter* (Mamta *et al.*, 2010), *Bacillus* and *Pseudomonas* (Sharma *et al.*, 2013), hence enhancing its availability for the plants (Sharma *et al.*, 2013).

Additionally, potassium is mainly found in soils as a non-exchangeable mineral and is susceptible to be dissolved, mainly by *Bacillus spp.* This particular trait enhances potassium availability for plants in the form of K⁺ cations (Sattar *et al.*, 2019). Eventually, nitrogen which is mostly present in the atmosphere and poorly available for plants in this form, and hence a limiting factor, is fixed and supplied to plants by several bacteria genera (*Azotobacter*, *Azospirillum*, *Azorhizobium* and *Rhizobium*, non-exhaustive list - Cocking, 2003; Kraepiel *et al.*, 2009; Rueda *et al.*, 2016; Steenhoudt and Vanderleyden, 2000) in the form of ammonium (Ferreira *et al.*, 2019). Nitrogen absorption and transfer to the host-plant has

additionally been evidenced for AMF (Battini *et al.*, 2017; Hodge and Storer, 2015; Turrini *et al.*, 2018), as well as the role of AMF in the facilitated uptake of micro-elements (Begum *et al.*, 2019; Ferrol *et al.*, 2016).

Microbial amendment's effect on phytohormones production

Many plant physiological processes (such as growth, tissue differentiation and stomatal movement – Davies, 2013), are stimulated or regulated through the synthesis of phytohormones, including auxins and ethylene (Ferreira *et al.*, 2019; Kumar *et al.*, 2019a). One of the highlighted mechanisms for the growth stimulation is the ability of beneficial microbes (fungi and bacteria) and not only plants, to synthesize the previously mentioned hormones (Duca *et al.*, 2014; Ferreira *et al.*, 2019; Kumar *et al.*, 2019a). Indole-acetic-acid is the most common hormone of the auxin class, involved in several key metabolic pathways: indole-3-pyruvic acid, indole-3-acetamide and indole-3-acetonitrile based pathways (Duca *et al.*, 2014; Ferreira *et al.*, 2019) and has been found synthesised by several bacteria genera (*Agrobacterium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Rhizobium* and *Streptomyces* - Ahmad *et al.*, 2008; Farajzadeh *et al.*, 2012; Gopalakrishnan *et al.*, 2014; Mehta *et al.*, 2010; Perrig *et al.*, 2007; Rashid *et al.*, 2012; Shailesh *et al.*, 2011; Sitbon *et al.*, 1992; Tan *et al.*, 2014; Torres-Rubio *et al.*, 2000; Viruel *et al.*, 2011).

Another key regulator for the growth of roots and aerial parts of the plants is ethylene: an excess of ethylene results in an inhibited growth (Dubois *et al.*, 2018). In addition, ethylene production is stimulated under stress, including TE-stress and pathogen infection (Dubois *et al.*, 2018; Ferreira *et al.*, 2019). In particular, bacteria such as *Azotobacter*, *Bacillus* and *Pseudomonas* have been demonstrated to produce ACC deaminase, an enzyme able to reduce the potent ethylene toxicity by cleaving it (Belimov *et al.*, 2001; Dubey *et al.*, 2012; Farajzadeh *et al.*, 2012; Kamran *et al.*, 2016; Sandhya *et al.*, 2010).

Microbial amendment's effect on stress mediation

PAM are considered as playing a key role in the mitigation of the noxious effects attributable to the presence of TE in the soil, especially when the concentrations exceed the plant bearable levels (Ferrol *et al.*, 2016). An important consideration is the fact that under TE-stress, the reduction of the TE toxicity is not always linked with a reduced TE uptake (de Andrade *et al.*, 2008; Carvalho *et al.*, 2006; Joner and Leyval, 2001; de Souza *et al.*, 2012). From a global point of view, the host-plant metabolism in the presence of PAM and in particular of the arbuscular mycorrhizal symbiosis has been found reprogrammed, from a transcriptomic consideration, modifying the plant responses to biotic and abiotic stresses and its primary and secondary metabolism (Ferrol *et al.*, 2016; Harrison, 1999; Hause and Fester, 2005), suggesting that the plant may save energy for other purposes, including growth. As an example, antioxidant enzymes as well as MTs and PCs transcription were found significantly reduced in mycorrhizal plants such as tomato or *Medicago truncatula* (Aloui *et al.*, 2009; Ferrol *et al.*, 2016; Ouziad *et al.*, 2005).

As in the plants, TE-stress can induce an oxidative response in the microbial cells, likely to generate ROS and causing cell alterations. To avoid and repair the potential damages, the PAM are able to modulate the expression of several of their own genes, encoding for various metabolites of their antioxidant system, in the presence of TE (Ferrol *et al.*, 2016; Garg *et al.*, 2017). This trait has not a direct effect on the host-plant, but PAM with a non-compromised cell integrity are more likely to mitigate efficiently the effect of the TE-stress throughout the different highlighted mechanisms. In particular, the expression of superoxide dismutases (González-Guerrero *et al.*, 2008; 2010) and glutathione S-transferases (Hildebrandt *et al.*, 2007) was highlighted for *R. irregularis*, induced in the presence of elevated amounts of Cu and Zn. Similarly, genes coding for antioxidant metabolites including vitamins (B6, C and E – Benabdellah *et al.*, 2009; Burns *et al.*, 2005; Lin *et al.*, 2011; Ferrol *et al.*, 2016) and glutathione (Garg and Singla, 2012; Pallara *et al.*, 2013) were evidenced for several microorganisms such as *R. irregularis*, *G. mosseae*, *Bacillus subtilis*, *E. coli* or *Streptomyces* spp.

(Benabdellah *et al.*, 2009; Burns *et al.*, 2005; Lin *et al.*, 2011; Pallara *et al.*, 2013), to cope with the ROS by scavenging or preventing the formation of RO species (Ferrol *et al.*, 2016; Garg *et al.*, 2017; González-Guerrero *et al.*, 2010).

Considering biotic stressors, PAM are also able to confer protection to their host plants against some pathogenic fungi or bacteria or against some phytophagous insects (Backer *et al.*, 2018; Beneduzi *et al.*, 2012; Ferreira *et al.*, 2019; Pozo *et al.*, 2010). Several protection mechanisms have been brought forward, including nutritional aspects, but also the activation of the plant defence system and global changes in the plant architecture, notably the root system morphology (Azcón-Aguilar and Barea 1996; Pozo *et al.*, 2010; Whipps, 2004). More specifically, this plant protection effect against biotic stress, can mainly be achieved through inducing and producing plant-defence enzymes and lytic enzymes, such as peroxidase, phenylalanine ammonia-lyase or glucanase, regulating the ethylene's level (Beneduzi *et al.*, 2012; Kumar *et al.*, 2019a), competing for available nutrients and space (Backer *et al.*, 2018; Ferreira *et al.*, 2019) or producing antibiotic compounds (Compant *et al.*, 2005; Doumbou *et al.*, 2001; Fravel, 1988; Lugtenberg and Kamilova, 2009). Beside their antagonistic effect, several PAM (*Pseudomonas* spp. or *Streptomyces* spp. – Duponnois and Planchette, 2003; Franco-correa *et al.*, 2010; Labbé *et al.*, 2014) have been found to exert a positive effect on AMF and to stimulate the establishment of mycorrhizal symbiosis (Mycorrhiza Helper Bacteria), resulting in a tripartite interaction (Deveau and Labbé, 2016; Garg *et al.*, 2017; Labbé *et al.*, 2014).

Combined use of amendments

On another note, very little is known about the combined effects of the amendments, as they are most of the time studied apart, despite the benefits observed with each amendment separately. In fact, in a context of soil contamination, a combination of the beneficial outcomes from several amendments might be worthwhile. As demonstrated by Curaqueo *et al.* (2014), the combination of composted olive wastes with the inoculation by *G. mosseae* resulted in a consistent improvement of the aided phytostabilisation of a mine tailing, by enhancing both nutrients uptake and soil microbial function. In other words, by limiting the availability of TE – which are likely to affect the development of bacteria or AMF as well (high TE concentrations have indeed been demonstrated to inhibit spore germination, root colonization or mycelium growth – Del Val *et al.*, 1999; Göhre and Paszkowski, 2006) – or enhancing soil properties, by adding either mineral, organic amendments or synergistic microbial amendments, the contribution of the microorganisms regarding plant growth and TE-stress tolerance may be increased.

In that regard, several studies have demonstrated a positive outcome when AMF and bacteria (mycorrhiza helper bacteria) were applied together. Synergistic effects could occur between these bacteria and AMF (Deveau and Labbé, 2016; Garg *et al.*, 2017; Iffis *et al.*, 2014; Labbé *et al.*, 2014), resulting in an enhanced efficiency in alleviating the environmental stresses as well as in the growth of the host-plant (Barea *et al.*, 2005). More specifically, the mycorrhizal colonization of the roots of *Populus deltoides* has been demonstrated higher for *Laccaria bicolor* (ectomycorrhizal fungi) in the presence of several *Pseudomonas* strains (Labbé *et al.*, 2014). Positive correlations have also been evidenced in the presence of co-applied AMF and bacteria strain: *Trifolium repens* inoculated with both Cd-adapted strains of *G. mosseae* and *Brevibacillus* sp. produced higher amounts of biomass, through an enhanced nutrient acquisition, and presented a higher number of symbiotic structures, while accumulating lesser amounts of Cd (Vivas *et al.*, 2003). Similar results have been obtained with *Trifolium repens* in the presence of Ni, inoculated with Ni-tolerant strains (Vivas *et al.*, 2006a) or Zn (Vivas *et al.*, 2006b), for the co-inoculation of *Zea mays* L. by the Gram-positive bacteria *Micrococcus roseus* and a native AMF under elevated Cd concentrations (Malekzadeh *et al.*, 2011) and for *Zea mays* L. in the presence of *Glomus* spp. and *Pseudomonas* sp. (Dhawi *et al.*, 2015).

From a wider perspective, aided phytostabilisation would benefit from these associations. It should then, not only focus on reducing the TE risk, but also on enhancing the soil quality regarding both

microbial and physico-chemical properties (Epelde *et al.*, 2009; Wasilkowski *et al.*, 2017), to improve the efficiency of such methods. In the next section, the limitations affecting aided phytostabilization will be further investigated, in order to provide insights towards feasibility and appropriate valorisation channels.

Part III. Limitations and future scope

Limitations of aided phytostabilisation

Examples of recent successfully *in situ* applied aided phytostabilisation are numerous, mainly focusing on the revegetation or rehabilitation of acidic mine tailings (Burges *et al.*, 2016; Dary *et al.*, 2014; Galende *et al.*, 2014; Garaiurrebaso *et al.*, 2017; Kumpiene *et al.*, 2009; Li *et al.*, 2018; Sylvain *et al.*, 2016; Yang *et al.*, 2016).

However, this kind of approach is sometimes introduced as relevant only when other remediation technologies are not applicable or suitable (Mench *et al.*, 2010). Despite the relatively low setting-up cost compared to other soil remediation technologies (Bolan *et al.*, 2011; Cundy *et al.*, 2016; Miller and Miller, 2007; Robinson *et al.*, 2009), additional expenses may be mandatory, particularly regarding the initial site assessment so as to choose the most suitable options in terms of amendments and plant species, as well as the time-monitoring of the vegetative cover and the inherent expenditures (Bolan *et al.*, 2011). One of the major downsides of the technology is the fact that this is not a cleaning-up method, rather a risk management, involving that the contaminant remains on site (Bolan *et al.*, 2011; Burges *et al.*, 2018; Mench *et al.*, 2010; Robinson *et al.*, 2009). The limitation of the possible future usages of the site is one of the possible long-term consequences, as the contamination persists (Bolan *et al.*, 2011). Additionally, in the context of global warming, extreme climatic phenomenon and soil properties alteration may occur, resulting in an increased TE bioavailability and mobility. One should note that extreme weather conditions may affect the sustainability of the vegetation cover and the soil properties as well, which might induce a migration or a partial release of the contamination, hence threatening the success of the phytostabilisation (Bolan *et al.*, 2011; Burges *et al.*, 2018).

In addition, even though the aim of the technology is to reduce the bioavailability of the TE, they may still enter the food chain at different levels (herbivores – food products), resulting in a potential human exposure (Bolan *et al.*, 2011; Dominguez *et al.*, 2010; Evangelou *et al.*, 2015; Robinson *et al.*, 2009).

Finally, the economic rentability of the technology if the vegetation cover is not valued (Burges *et al.*, 2018; Vigil *et al.*, 2015) and the extended timescale of that kind of technologies (Burges *et al.*, 2018; Jan *et al.*, 2016) are often highlighted as weaknesses.

Future scope: Phytomanagement of TE-contaminated soils

Nonetheless, by considering the aided phytostabilisation in a wider approach, in a phytomanagement channel, there are several suitable options to include the valorisation of the vegetation cover in the method. The phytomanagement of a contaminated site, involves the use of phytotechnologies and aims at identifying and setting-up the most suitable method for the mitigation of the contamination, while either minimizing the setting-up and ongoing costs inherent to the technology or identifying the best valorization option for the biomass (Bert *et al.*, 2017; Evangelou *et al.*, 2015; Robinson *et al.*, 2009). The latter can either be economic in nature, by generating an income through the production of valuable biomass products from the vegetation cover or of ecological value, notably through the restoration of degraded ecosystems (Bolan *et al.*, 2011; Burges *et al.*, 2018; Evangelou *et al.*, 2015; Robinson *et al.*, 2009). While phytostabilisation aims at providing risk mitigation, phytomanagement encourages the use of phytotechnologies as part of an integrated site management (Burges *et al.*, 2018). A promising outcome is to choose plant species that can provide an added value so as to generate an economic benefit (Bernal *et al.*, 2019; Burges *et al.*, 2018; Evangelou *et al.*, 2015).

If biomass valorisation appears mandatory (Evangelou *et al.*, 2015), particular attention should be paid to the sustainability of the vegetation cover, to not compromise the approach (especially during upkeep and harvesting). In that wider approach, as for an aided phytostabilisation, there should be considerations towards (Evangelou *et al.*, 2015; Mench *et al.*, 2010; Robinson *et al.*, 2009):

- the choice of the plant species,
- the compatibility between selected plant species and microorganisms,
- suitable amendments,
- the introduction of non-native species in opposition to the use of endemic ones
- an adequate cropping system, potentially involving perennial crops, as the site needs to be continuously vegetated

Beside the ecological value (namely increasing biodiversity, creating an ecological niche for rare species, restoring soil structure and function, avoiding or restricting erosion, leaching and run-off phenomenon and providing ecosystem services – Bolan *et al.*, 2011; Burges *et al.*, 2018; Cundy *et al.*, 2016; Evangelou *et al.*, 2015; Kidd *et al.*, 2015; Robinson *et al.*, 2009) occurring during the phytomanagement of contaminated areas, valorisation channels for economic benefits are also studied (Burges *et al.*, 2018; Evangelou *et al.*, 2015; Robinson *et al.*, 2009). Local outcomes, thanks to previously established installations (biogas plant, EO distiller, combustion plant, *etc.*), may constitute driving factors for these valorisation channels. Based on the data gathered from aided phytostabilisation studies, these valorisation channels can be declined in two main fields:

- Bioenergy production

This channel is probably the most investigated and developed one, with many outcomes. It refers to the production of renewable energy from biological sources that can be used for heat, electricity or fuel purposes. The vegetation cover may find uses directly converted into heat (aerial biomass from plants, wood-energy, straw, coproducts), processed into gases (organic wastes decomposition, methanation), into liquids (including ethanol & biofuel, originating from maize (*Zea mays*), sugarcane (*Saccharum officinarum*), wheat (*Triticum spp.*), rapeseed (*Brassica napus*), and soy (*Glycine max*) or from lignocellulosic material – Evangelou *et al.*, 2015) or used for co-generation (Bernal *et al.*, 2019; Evangelou *et al.*, 2015; Robinson *et al.*, 2009; Vigil *et al.*, 2015). In that matter, inoculation with several PAM (*Burkholderia sp.* GL12, *Bacillus megaterium* JL35 and *Sphingomonas sp.* YM22) was found to significantly increase (from 33% to 56%) maize's above-ground tissue dry weights (Sheng *et al.*, 2012). Similarly, the inoculation of two poplar clones (*Populus trichocarpa* x *P. maximowiczii* section Tacahamaca and *P. deltoides* x *P. nigra* section Aigeiros) with different mixtures of PAM resulted in an increase of the biomass production as well as in a reduced accumulation of Zn, Cu, Pb and Cr in poplar's leaves (Ciadamidaro *et al.*, 2017; Phanthavongsa *et al.*, 2017). Finally, the presence of fungal endophytes in poplar, cultivated on TE-contaminated soils, exhibited an increase of the shoot biomass, hence depicting a higher potential of the tree and its associated microorganisms to be valuable in both remediation of the contaminated site and biomass production for bioenergy purposes (Lacercat-Didier *et al.*, 2016).

This outcome has the advantage, over other renewable energies, to be the only source of fixed carbon in the biomass, suitable for the production of transport fuels (Evangelou *et al.*, 2015). Additionally, it has a real economic potential, owing to the rapid expansion of China but is highly dependent on the oil price on the world market (Evangelou *et al.*, 2015) and requires that the risk emanating from the potential presence of TE in the biomass is mitigated (e.g. retain the volatile TE during the biomass combustion or avoid the contamination of other area due to the presence of TE in the digestate after biogas or bioethanol production – Bernal *et al.*, 2019; Evangelou *et al.*, 2015).

Additionally, as previously stated, the support of previously established valorisation channels could help for the setting-up and the sustainability of the method.

- Bio-sourced materials

Biochar, among other biomaterials is investigated as a valorisation channel: produced by pyrolysis, it is commonly used to amend agricultural soils and to sequester carbon from organic matter, in addition to the heat production during the pyrolysis process (Bernal *et al.*, 2019; Evangelou *et al.*, 2015; Ippolito *et al.*, 2012; Liu *et al.*, 2019; Sun *et al.*, 2017). Nonetheless, the application of biochar is not without its own risk. Even if the biomass contains low amounts of TE, their mobility and availability when trapped in the biochar may change over time, affected by microbial activity, soil pH and other interactions – including organic acids – with the biochar, hence potentially hazardous (Evangelou *et al.*, 2015; Liu *et al.*, 2019). Nevertheless, synergistic effects of biochar and AMF applied together have been demonstrated in Cd-contaminated conditions, resulting in a better nutrient (N, P, K and Ca) supply and a reduced Cd accumulation in *Medicago sativa* shoots (Zhang *et al.*, 2019). Similar effects have been demonstrated on maize in Cd-contaminated conditions, resulting in an enhanced maize's growth and an alleviated Cd toxicity (Liu *et al.*, 2018).

The distillation of essential oils (EO) from aromatic plants grown on TE contaminated sites is another possibility to obtain high added-value products (Bolan *et al.*, 2011; Lydakakis-Simantiris *et al.*, 2015; Zheljazkov *et al.*, 2008a). Thanks to the distillation process, TE are not transferred into the EO or in very limited amounts, making the EO marketable, provided that the production costs and the EO prices are profitable (Zheljazkov *et al.*, 2008a; Zheljazkov *et al.*, 2008b). However, great care should be observed for the valorisation of the distillation residues, as they may be concentrated mixtures containing the TE present in the distilled biomass (Zheljazkov *et al.*, 2008a; Zheljazkov *et al.*, 2008b). Additionally, in some specific cases, root colonization by AMF has been brought forward as a way to significantly increase the EO concentration in plant. In particular, root colonization by AMF is suspected to have an effect on both qualitative and quantitative patterns of secondary metabolites, including EO (Khaosaad *et al.*, 2006). An augmentation (up to 78%) of EO concentration was for example observed with a *Glomus fasciculatum* inoculum on *Foeniculum vulgare* (Kapoor *et al.*, 2004). Similarly, coriander inoculated with *G. mosseae* and *G. fasciculatum* displayed increased EO contents of respectively 28 and 43% (Kapoor *et al.*, 2002). As EO are present in relatively small proportions in plants and display low distillation yields, increasing the amount of plant biomass or the EO concentration in the plant, even by a slight amount, may be of great interest (Khaosaad *et al.*, 2006). However, the installation costs for a distillation unit are quite high, representing a curb for the technology. Once again, it could be a driving factor to identify a local distiller to reduce the biomass transport expenses and to avoid the installation costs.

Finally, timber or fibres productions are another alternative as well as other end-products, including textile production, vegetal concrete and in a broader way renewable material (Bolan *et al.*, 2011; Burges *et al.*, 2018; Cundy *et al.*, 2016; Jeannin *et al.*, 2020; Yang *et al.*, 2010).

Conclusion

Based on the revegetation of contaminated soils and the introduction of amendments, aided phytostabilisation is considered a minimally invasive remediation technique that may simultaneously help stabilising the contaminants and improving soil functions and services. Through the selection of plants, cropping schemes and soil amendments, aided phytostabilisation may be adapted to different metal contaminants and soil types, including strongly anthropized soils, which are sometimes difficult to remediate. Indeed, these contaminated sites generally present unfavorable cultural conditions due to pH, soil structure, salinity, low fertility or the presence of several types of toxic substances. It is therefore important to adapt this environmentally friendly "green" technology to each contaminated site. Considering the physicochemical and climatic constraints of each site as well as the TE mobility in the soil, the plant's needs regarding its growth and the potential future biomass valorisation, it could be relevant to introduce complementary amendments in a reasoned way, to further evaluate their potential synergistic effects. As mentioned in this chapter, limiting the availability of TE by using

mineral, organic or synergistic microbial amendments may significantly raise the contribution of the microorganisms regarding plant's growth and TE-stress tolerance.

In addition, during the last few years, phytotechnologies have progressively shifted to phytomanagement. This concept is based on the cultivation of non-food crops on contaminated soils to source biomasses for various bioeconomy sectors. Several well-established valorisation channels for the biomass produced on contaminated sites have been identified and could provide a significant economic benefit. However, one of the current challenges that phytotechnologies are facing resides in identifying, assigning and quantifying the private and social benefits of these methods and in evaluating the monetary value to the ecosystem services that they render in the context of land management.

In a general way, the feedbacks (feasibility studies, constraints, costs and benefits) from a variety of field experiments in demonstration projects will be required to develop models and decision-support tools, towards the optimization and the increase of public acceptance of these phytotechnologies as well as to convince policy makers.

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III. Les huiles essentielles (HE)

1. Biosynthèse des composés des HE

Les HE sont composées de métabolites secondaires, majoritairement des terpénoïdes et des composés phénoliques, notamment les phénylpropanoïdes.

1.1. Biosynthèse des terpénoïdes

La biosynthèse de ces composés s'appuie sur deux précurseurs isopréniques, l'isopentényl pyrophosphate ainsi que son isomère, le diméthylallyl pyrophosphate. Deux voies de biosynthèse conduisent à ces unités à cinq carbones (Hemmerlin *et al.*, 2012) :

- La voie de l'acide mévalonique (MVA)
- La voie du méthylérythritol 4-phosphate (MEP)

La première, cytoplasmique est rencontrée chez les eucaryotes et la plupart des bactéries, et prend son origine au niveau de l'acétyl-coenzyme A, produit de la glycolyse. Le mécanisme réactionnel conduit à l'obtention de l'acide mévalonique et par la suite aux deux précurseurs isopréniques, l'isopentényl pyrophosphate et le diméthylallyl pyrophosphate. Ceux-ci réagissent enfin entre eux pour former les différents précurseurs des mono-, di- et sesquiterpènes (Figure 3). La seconde est, elle, spécifique aux végétaux et se déroule au niveau des plastides, conduisant à la formation du méthylérythritol 4-phosphate, un composé intermédiaire en C₅ (Figure 3). Plusieurs catalyses enzymatiques successives permettent ensuite d'aboutir aux isomères isopentényl pyrophosphate et diméthylallyl pyrophosphate, donnant enfin lieu aux précurseurs des mono- et tétraterpènes en particulier, respectivement le géranyl pyrophosphate et le géranyl-farnésyl pyrophosphate (Jiang *et al.*, 2006 ; Hemmerlin *et al.*, 2012).

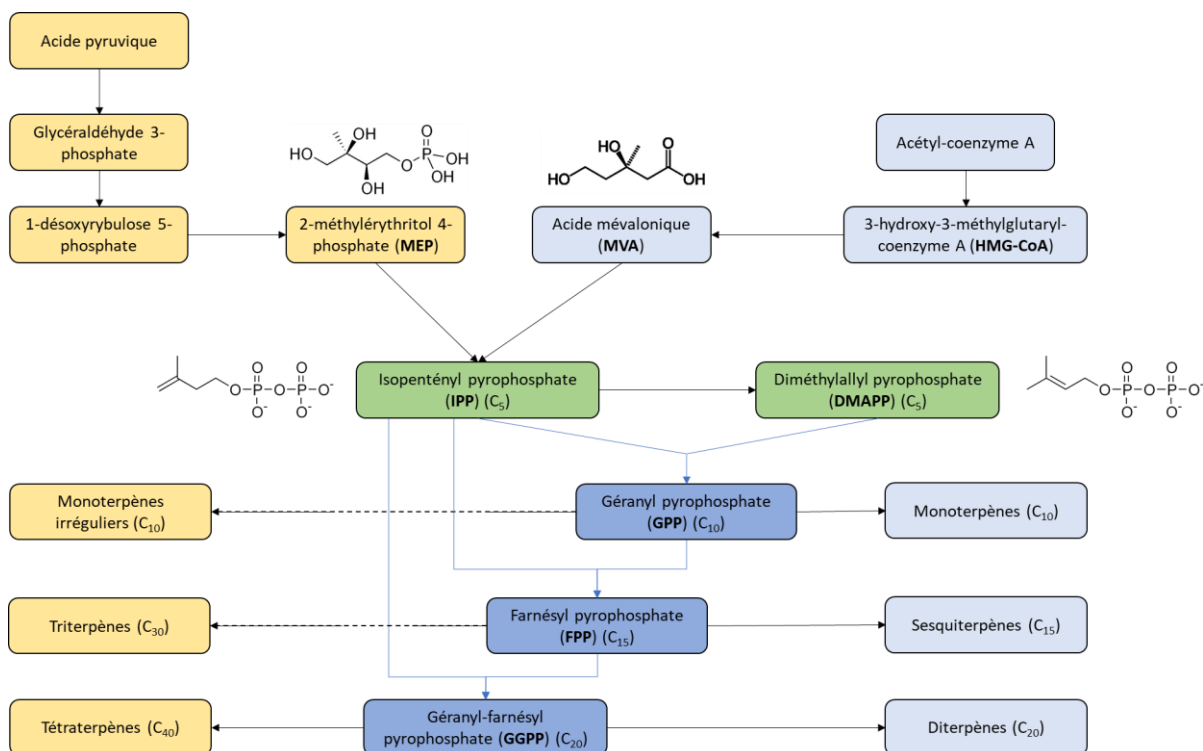


Figure 3: Biosynthèse des terpénoïdes par les voies du méthylérythritol phosphate (en jaune, à gauche) et de l'acide mévalonique (en bleu clair, à droite) (adapté de Hemmerlin *et al.*, 2012; Jiang *et al.*, 2006).

Par ailleurs, la classification des terpénoïdes est basée sur la longueur de la chaîne carbonée et en particulier sur le nombre d'unités isopréniques incorporées dans la chaîne.

1.2. Biosynthèse des phénylpropanoïdes

La biosynthèse de l'ensemble des phénylpropanoïdes se fait par l'intermédiaire de la phénylalanine. Cette dernière provient notamment de l'acide shikimique, obtenu par la voie des pentoses phosphates, et est à l'origine de l'acide trans-cinnamique, constituant un intermédiaire majeur dans la voie de biosynthèse des phénylpropanoïdes (Vogt, 2010). Ceux-ci dérivent en effet tous de l'acide trans-cinnamique, obtenu à partir de la phénylalanine par une réaction de désamination catalysée par la phénylalanine ammonia-lyase et possèdent donc au minimum un noyau aromatique (Figure 4). Une voie alternative est également observée, notamment chez les monocotylédones, impliquant une activité tyrosine ammonia-lyase de la phénylalanine ammonia-lyase, qui par désamination de la tyrosine permet l'obtention directe de l'acide *p*-coumarique. La phénylalanine ammonia-lyase joue donc un rôle clé dans le métabolisme des phénylpropanoïdes, car de son activité dépend la régulation du flux de carbone passant du métabolisme primaire à celui des phénylpropanoïdes (Ferrer *et al.*, 2008 ; Vogt, 2010).

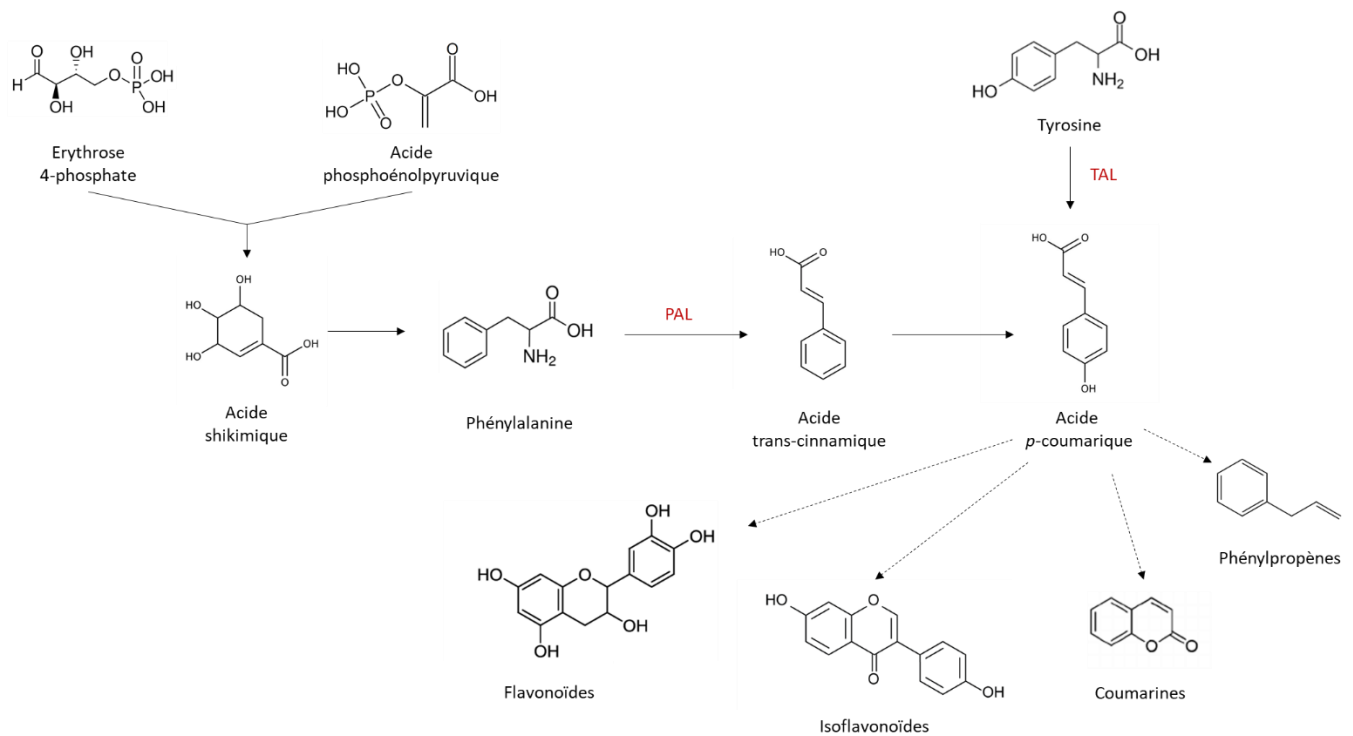


Figure 4: Voie de biosynthèse des principaux phénylpropanoïdes présents dans les huiles essentielles (adapté de Vogt, 2010). PAL : Phénylalanine ammonia-lyase ; TAL : Tyrosine ammonia-lyase.

2. Procédés d'obtention des HE ou de leurs composés

De nombreuses techniques conduisent à l'obtention des HE à partir de la biomasse végétale. Il est à noter que la distillation ou l'extraction de celles-ci à partir des plantes aromatiques conduit à l'obtention d'un produit complexe. D'autres techniques d'extraction, de purification ou de synthèse chimique sont donc nécessaires à l'obtention des composés majoritaires purs. Par ailleurs, les rendements de distillation sont généralement bas, en raison des faibles teneurs en HE dans la matière végétale, elles-mêmes sujettes à des contraintes liées au développement de la plante (contraintes

climatiques, nature et fertilité du sol, santé de la plante - (Kumar et al., 2013; Li et al., 2014). Les techniques d'extraction des HE sont donc en constante évolution, dans le but d'améliorer les rendements d'obtention ainsi que la qualité du produit obtenu. Il est également intéressant de noter que le choix de la technique de distillation est conditionné par la localisation de l'HE dans les tissus végétaux.

2.1. Techniques d'extraction conventionnelles

2.1.1. L'hydrodistillation

Mise au point dès le IX^e siècle, l'hydrodistillation est la technique de distillation la plus ancienne et la plus répandue et constitue la méthode standard de distillation d'HE à partir d'une biomasse végétale. Cette méthode implique l'immersion de la matière végétale dans de l'eau portée à ébullition. La vapeur d'eau formée entraîne avec elle les constituants volatils de l'HE, puis est recondensée dans un réfrigérant. Après décantation du distillat obtenu, la phase aqueuse est éliminée et l'HE est ainsi recueillie dans un essencier (Ferhat et al., 2007; Li et al., 2014; Moradalizadeh et al., 2013; Tongnuanchan and Benjakul, 2014).

2.1.2. L'entraînement à la vapeur

Cette technique traditionnelle de distillation se distingue de la précédente dans la mesure où l'eau dans son état liquide et la biomasse végétale ne sont pas en contact direct. Une source de chaleur – dans la majorité des pilotes industriels une chaudière – permet de porter l'eau à ébullition, puis c'est l'action de la vapeur d'eau, circulant à travers la matière végétale par courant ascendant, qui va engendrer des déformations et ruptures au niveau de la structure cellulaire et permettre la libération des composés volatils. Le mélange « eau-HE » qui en résulte sera par la suite recondensé au niveau d'un réfrigérant, puis laissé à décanter pour séparer la phase aqueuse et l'HE. L'absence de contact direct entre l'eau et la matière végétale permet d'éviter des phénomènes d'hydrolyse et de dégradation des composés de l'HE et d'en altérer la qualité (Li et al., 2014; Tongnuanchan and Benjakul, 2014). La vapeur d'eau peut également être injectée par le haut du système, donnant lieu à de l'hydrodiffusion, avec une circulation de la vapeur par courant descendant. Cette méthode permet un temps de réaction moindre et consomme donc moins d'énergie (Bernard et al., 1988; Tongnuanchan and Benjakul, 2014).

2.1.3. L'enfleurage

Méthode utilisée traditionnellement en parfumerie pour l'extraction d'HE à partir de fleurs fraîches (jasmin, violette), l'enfleurage peut être réalisée à froid ou à chaud. Elle requiert l'ajout de graisses animale ou végétale inodores, capable d'adsorber les composés volatils aromatiques. Les graisses saturées par les composés aromatiques sont ensuite rincées avec de l'alcool pour séparer les deux phases et en récupérer les extraits végétaux (Salomé-Abarca et al., 2015).

2.1.4. Extraction par solvants

L'utilisation de solvants organiques permet l'extraction des HE ou de certains de leurs composés spécifiquement. Le solvant choisi, ne devant pas réagir chimiquement avec l'extrait, est d'abord mélangé avec la matière végétale, puis le système est chauffé avant une étape de filtration. Le filtrat obtenu est enfin concentré par évaporation du solvant. Le concentrat obtenu se présente sous la forme d'une résine ou d'une « concrète », mélange cireux d'HE. Cette dernière est extraite par l'emploi d'alcool absolu lors d'une distillation à basse température, puis concentrée par évaporation de l'alcool.

En revanche, cette technique présente des inconvénients : le procédé est relativement long et du fait de l'emploi des solvants, des traces de solvants peuvent persister et sont souvent difficiles à éliminer (Bernard et al., 1988). Le choix du ou des solvants employés est donc délicat. L'acétone, l'hexane, l'éther de pétrole, le méthanol ou l'éthanol sont communément employés (Salomé-Abarca et al., 2015; Tongnuanchan and Benjakul, 2014).

2.1.5. Pressage

Le principe de ce procédé s'appuie sur la rupture mécanique par pression à froid, des cellules sécrétrices riches en HE. L'HE ainsi libérée est ensuite entraînée par un flux d'eau, formant une émulsion constituée d'eau et d'HE. Cette dernière sera enfin isolée par décantation ou par centrifugation. Ce procédé est notamment utilisé pour les agrumes (Ferhat et al., 2007; Tongnuanchan and Benjakul, 2014).

2.2. Techniques innovantes d'extraction

Si l'hydrodistillation et l'entraînement à la vapeur restent les méthodes de distillation les plus fréquemment utilisées à l'heure actuelle, les techniques traditionnelles d'extraction ont montré une certaine limite en termes de productivité, de rentabilité et de qualité du produit final. En effet, les relativement faibles rendements obtenus, la dégradation de composés thermolabiles ainsi que la présence résiduelle de solvants organiques potentiellement toxiques sont autant de points critiques que les nouvelles techniques d'extraction tentent d'améliorer.

2.2.1. Extraction aux fluides supercritiques

Les fluides supercritiques ont été étudiés comme une méthode alternative d'extraction des HE. Un fluide est considéré comme supercritique lorsqu'il se présente dans un état intermédiaire entre le gaz et le liquide. Dans des conditions de température et de pression bien définies, le fluide possède une faible viscosité, une forte diffusivité et une densité proche de celle de l'état liquide. De ce fait, il présente la particularité d'extraire les molécules aromatiques à partir de la matière végétale. Le CO₂ est le fluide supercritique le plus couramment employé, du fait de ses caractéristiques critiques « modérées » ($T_c = 31,2 \text{ °C}$; $P_c = 72,9 \text{ bar}$ – Bernard et al., 1988 ; Zhao and Zhang, 2014). Il est également chimiquement inerte, non inflammable, non toxique et est disponible à un coût relativement faible pour un haut niveau de pureté. Un autre avantage de cette méthode est la possibilité d'éliminer le solvant par simple dépression et de pouvoir le recycler pour le réintroduire directement dans le système (Santoyo et al., 2014; Zhao and Zhang, 2014).

2.2.2. Extraction assistée par ultrasons

Le principe de l'extraction assistée par ultrasons est d'exposer la matière végétale en solution aqueuse à des ondes sonores comprises entre 20 kHz et 10 MHz. Pendant la sonication, les ondes traversent les parois végétales, induisant des vibrations mécaniques et conduisant au phénomène de cavitation. L'éclatement des bulles de cavitation ainsi formées permet de fracturer les cellules sécrétrices et de libérer les HE. La température, la fréquence et l'intensité choisies ainsi que le temps d'exposition sont des paramètres importants du système (Chemat et al., 2011). En plus d'améliorer les rendements d'extraction, cette méthode permet de réduire significativement les temps d'extraction, l'énergie consommée ainsi que les quantités de solvant nécessaires. Par ailleurs, les HE produites sont souvent de très haute qualité, avec peu de phénomènes de dégradation thermique (Chemat et al., 2011).

2.2.3. Extraction assistée par micro-ondes

L'utilisation des micro-ondes, ondes électromagnétiques dont la fréquence varie entre 300 MHz et 300 GHz et la longueur d'onde entre 1 mm et 1 m, constitue une méthode innovante de chimie verte. Dans ce procédé, la matière végétale en solution aqueuse ou en présence d'un ou plusieurs solvants organiques est placée dans un réacteur micro-ondes. La circulation des ondes à travers la matière permet un chauffage plus sélectif et plus rapide que l'eau et induit des changements dans la structure cellulaire, permettant d'une part d'accélérer le procédé, de réduire l'énergie nécessaire au système ainsi que les quantités de solvants nécessaires et d'autre part d'élever les rendements d'extractions (Chemat et al., 2009; Filly et al., 2014; Moradalizadeh et al., 2013). Afin d'éviter d'éventuelles interférences avec d'autres systèmes de communication notamment, 0,915 et 2,45 MHz sont les fréquences les plus fréquemment autorisées pour des applications médicales, industrielles ou scientifiques (Chemat et al., 2009; Filly et al., 2014).

Il est intéressant de noter également que les techniques présentées ici comme des méthodes innovantes sont souvent combinées à des techniques classiques pour en améliorer les effets. Ainsi, l'utilisation de micro-ondes ou d'ultrasons est souvent complémentaire des méthodes d'hydrodistillation, d'entraînement à la vapeur ou encore d'extraction par solvants.

3. Le marché des HE

3.1. Production mondiale d'HE

Le marché mondial des HE est en plein essor, porté notamment par le développement de l'aromathérapie dans de nombreux pays sur les continents américain et asiatique, l'utilisation croissante d'HE dans les produits cosmétiques et par le dynamisme des marchés occidentaux.

En volume, les huiles essentielles d'agrumes (dominées par l'orange douce et le citron) et de menthes représentent l'essentiel des flux mondiaux, auxquels s'ajoutent les HE d'eucalyptus, de lavandin et lavande, *etc* (Tableau 1). Une multitude d'autres HE, présentant une forte valeur mais souvent échangées sous des tonnages plus modestes, sont également présentes sur le marché mondial mais plus difficiles à estimer en raison de la nomenclature douanière parfois peu précise (France Agrimer, 2018). A noter que les prix des HE sont fortement influencés par la part des échanges réalisés sous contrat, par la qualité de la production ainsi que par les tonnages produits et échangés sur les marchés nationaux et mondiaux, et enfin par l'importance de la concurrence sur ces mêmes marchés. Les mécanismes de régulation du marché sont encore peu nombreux et contribuent à laisser libre cours à une fluctuation importante du prix des HE.

3.2. Marché français

Si la France est un acteur important sur le marché mondial des HE, elle reste néanmoins un petit producteur en termes de volumes et se démarque notamment grâce à ses productions spécifiques : le lavandin, la lavande, la sauge sclarée ainsi que l'hélichryse italienne. La production en HE de lavandin à l'échelle mondiale est couverte à plus de 90 % par la France, ainsi que près de 33 % de la production mondiale en HE de lavande. La concurrence étrangère est néanmoins forte (plantations massives d'hélichryse dans les pays de l'Est et de lavande, *etc*). Une AOP a notamment été créée (1981) pour protéger la lavande de Haute Provence, ainsi qu'une IGP en 1996 (AOP lavande de Haute Provence, <https://www.lavande-aop.fr>).

Tableau 1: Principales productions d'HE à l'échelle mondiale exprimées en tonnage et estimées en termes de valeur marchande (Agrimer 2020).

HE	Production en tonnes (chiffres 2019)	Valeur estimée (M€)	Principaux pays producteurs (Liste non exhaustive)
Orange douce	49000	294	Argentine, Brésil, Espagne, Israël, Italie, USA
Menthe des champs	42000	840	Brésil, Chine, Inde
Citron	9000	225	Argentine, Brésil, Israël, Italie, USA
Eucalyptus	4000	88	Australie, Brésil, Chine, Inde
Menthe poivrée	3500	112	Inde, USA
Citronnelle	3000	81	Chine, Inde, Indonésie
Clou de girofle	2500	52	Inde, Indonésie, Madagascar, Sri Lanka, Tanzanie
Menthe douce	2000	58	Chine, Inde, USA
Cèdre	2000	?	Chine, Inde, Maroc, USA
Lavandin	1550	42	Espagne, France

En 2018, la surface du territoire français occupée par les PPAM était de plus de 53 000 ha, surface en constante augmentation sur les dernières années (20 % de progression depuis 2014), pour plus de 5000 producteurs (France Agrimer, 2018).

Les plantes médicinales sont principalement représentées par le pavot (13 000 ha), tandis que les plantes à parfum occupent la majeure partie (près de 30 000 ha) des surfaces cultivées pour la production française et sont principalement représentées par trois espèces : le lavandin, la lavande (25 620 ha en surfaces cumulées pour les deux espèces) ainsi que la sauge sclarée (3400 ha – France Agrimer, 2018). Ces dernières totalisent notamment près de 95 % de la superficie totale de culture des PPAM du Sud-est. Ces spécialités sont de forts marqueurs de l'identité régionale (Provence, Corse), mais la France est aussi riche d'autres productions (Figure 5), dont certaines sont en croissance forte (thym, menthe, camomille, mélisse, etc), face à une demande importante de l'industrie.

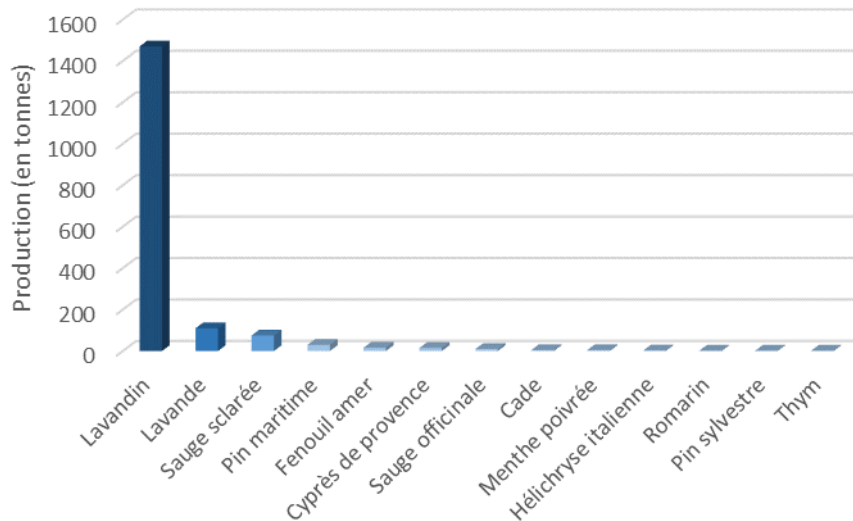


Figure 5: Quantités moyennes des principales HE produites en France (chiffres 2017, estimation France Agrimer).

A noter que les bassins de production à l'échelle nationale sont très spécialisés, avec la production de plantes à parfum traditionnellement concentrée dans le Sud-Est (Figure 6), notamment en raison des conditions climatiques et du bassin naturel d'implantation de plusieurs de ces espèces (saugé sclarée, lavande, thym, romarin, etc). Toutefois, malgré une identité territoriale très forte, la culture des PPAM se retrouve sur l'ensemble du territoire français métropolitain.

Dans la région des Hauts-de-France, par exemple, la surface totale dédiée à la culture des PPAM est inférieure à 1000 ha, majoritairement destinée à la culture de plantes aromatiques (frais ou sec) et médicinales. La société Ferrant PHE, localisée dans le Pas-de-Calais, est l'un des seuls acteurs de la filière présent dans le département, mais constitue l'un des leaders européens sur le marché des HE provenant d'espèces de la famille des Alliées. Elle est notamment la seule à proposer des HE provenant de l'échalote, de l'oignon ou de la ciboulette sur le marché, et produit également 80 % des volumes mondiaux en HE de poireau (Ferrant PHE, <https://www.ferrantphe.com/> ; région Hauts-de-France, <https://www.hautsdefrance.fr/huiles-essentielles-ferrant/>).

En termes de cours des HE, concernant les espèces à parfum majoritaires précédemment citées, le prix du lavandin se situe entre 27 et 40 €/kg selon les variétés cultivées, celui de la lavande entre 115 et 140 €/kg et celui de la saugé sclarée entre 100 et 150 €/kg (France Agrimer, 2018).

A noter également que la balance commerciale liée au marché global français des HE est légèrement excédentaire en termes de valeur mais déficitaire en termes de quantité, indiquant que les HE exportées possèdent une valeur ajoutée supérieure à celles importées. Le volume des imports représente 8919 tonnes d'HE pour une valeur de 395 M€, tandis que le volume des exports représente 5422 tonnes pour une valeur totale de 404 M€ (France Agrimer, 2018). Le chiffre d'affaires rapporté à la production agricole est estimé à 150 M€, tandis qu'en termes de produits finis, la production française estimée en 2010 était de 4 milliards d'euros et l'activité générait plus de 30 000 emplois, directs et indirects (Ministère de l'agriculture, 2017).

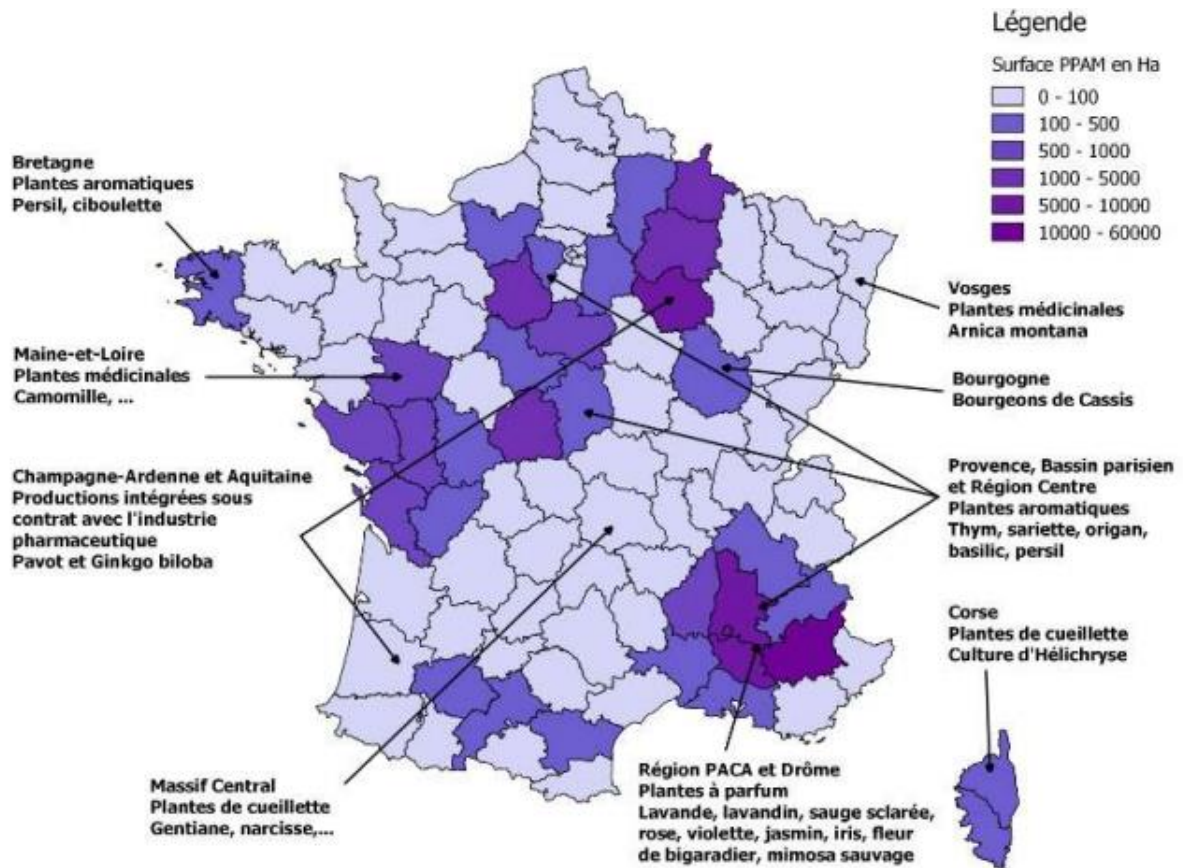


Figure 6: Répartition des surfaces cultivées en PPAM à l'échelle de la France métropolitaine (France agrimer, 2018).

3.3. Débouchés pour la filière HE

Les PPAM ont des usages très variés. Les principaux débouchés de la filière PPAM et de la production d'HE sont l'agro-alimentaire, la parfumerie, la cosmétique, l'aromathérapie, les produits d'entretien et les produits pharmaceutiques (Figure 7 – France Agrimer, 2020).

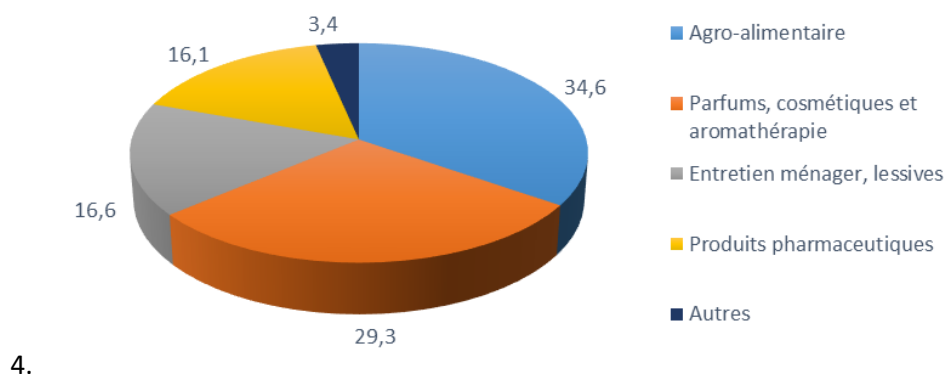


Figure 7: Principaux secteurs de débouché des HE (chiffres France Agrimer 2020).

En France, le marché des cosmétiques naturels, incluant les cosmétiques à base d'HE a par exemple connu une croissance de +18 % entre 2017 et 2018, pour atteindre une valeur globale estimée à 757 M€, ce qui représente environ 6 % du marché global des cosmétiques (France Agrimer, 2018).

Néanmoins, de nouveaux usages tels que les compléments alimentaires, l'alimentation animale et les usages vétérinaires ainsi que la protection des cultures représentent des possibilités de

développement futures pour la filière PPAM (Ministère de l'agriculture, 2017). De nombreuses études sont par exemple menées pour l'utilisation d'HE en tant que produits de protection des plantes, à vocation herbicide, fongicide ou encore insecticide (Raveau et al., 2020). Certaines HE (orange douce, menthe verte, citronnelle ou encore clou de girofle) sont d'ailleurs homologuées et peuvent être utilisées à ces égards, en France ou en Europe (E-phy, <https://ephy.anses.fr> ; EU pesticide database, https://ec.europa.eu/food/plant/pesticides/eu-pesticides-db_en).

4. Les propriétés biologiques des HE en protection des cultures (*Revue 2 : Raveau et al., 2020*)

Grâce à leur richesse en principes actifs, les HE possèdent des propriétés biologiques variées dont l'intérêt est reconnu depuis plusieurs siècles en particulier dans les domaines de la médecine, de l'industrie alimentaire, de la parfumerie et des cosmétiques (Bakkali et al., 2008; Bassolé and Juliani, 2012).

Dans le contexte actuel, malgré leurs bénéfices en termes de sécurité alimentaire et de gain de rendements, l'utilisation des produits phytosanitaires pour la protection des cultures est controversée, en raison de leurs effets directs ou indirects sur l'environnement et la santé humaine (Barzman et al., 2015; Lamichhane et al., 2016). Par ailleurs, l'usage intensif et non raisonné de ces produits conduit aussi à l'apparition de phénomènes de résistance chez les microorganismes responsables des maladies des cultures mais également chez les espèces adventices (Lamichhane et al., 2016). Une réduction du recours aux produits conventionnels est ainsi visée par des directives à l'échelle européenne (91/414/EEC, 1993 et 1107/2009/EC, 2011) mais également mondiale. La France s'est elle engagée, dans le cadre du plan Ecophyto II+, à réduire l'usage des pesticides de 25% d'ici à 2020, puis de 50% d'ici à 2025 (Ecophyto, <http://agriculture.gouv.fr/ecophyto>). Malgré cette volonté de diminuer l'usage des pesticides, il est nécessaire de maintenir les rendements cultureux et la qualité des productions agricoles, afin de garantir la sécurité alimentaire.

L'orientation vers des méthodes de lutte alternatives est donc fortement encouragée et la recherche scientifique se tourne à l'heure actuelle vers les produits d'origine naturelle et notamment vers les extraits végétaux, à vocation de biocontrôle, c'est-à-dire reposant sur l'utilisation de mécanismes naturels (Agriculture.gouv.fr ; IBMA France). Le marché du biocontrôle, s'il ne représente à l'heure actuelle qu'une faible proportion du marché des produits phytosanitaire (11 % en 2020 - IBMA France) est aujourd'hui en plein essor avec notamment la transition agroécologique incluant la protection intégrée des cultures qui constitue un élément moteur. Récemment, les HE ont fait l'objet d'un intérêt croissant, suite à la publication de nombreuses études relatives à leurs propriétés antifongiques, insecticides et herbicides (Benelli et al., 2017; Pavela and Benelli, 2016). Malgré cela, leur utilisation en protection des cultures demeure étonnamment restreinte.

Dans le cadre de cette revue (*Revue 2, Raveau et al., 2020*), nous nous intéresserons, après un bref rappel des spécificités et de la composition chimique des HE, aux aspects suivants :

- Quels sont les principaux usages auxquels se prêtent les HE dans le cadre de la protection des cultures ?
- Quels sont les principaux mécanismes d'actions identifiés par rapport aux propriétés antimicrobiennes (antifongiques et antibactériennes) et herbicides des HE ?
- Quelles sont les limitations actuelles à l'utilisation des HE en protection des cultures ?
- Par quels moyens innovants est-il possible d'améliorer l'efficacité et la fiabilité des HE et ainsi de promouvoir leur utilisation ?

Review

Essential Oils as Potential Alternative Biocontrol Products against Plant Pathogens and Weeds: A Review

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Abstract: Naturally produced by aromatic plants, essential oils (EO) contain a wide range of volatile molecules, including mostly secondary metabolites, which possess several biological activities. Essential oils properties such as antioxidant, antimicrobial and anti-inflammatory activities are known for a long time and hence widely used in traditional medicines, cosmetics and food industries. However, despite their effects against many phytopathogenic fungi, oomycetes and bacteria as well as weeds, their use in agriculture remains surprisingly scarce. The purpose of the present review is to gather and discuss up-to-date biological activities of EO against weeds, plant pathogenic fungi, oomycetes and bacteria, reported in the scientific literature. Innovative methods, potentially valuable to improve the efficiency and reliability of EO, have been investigated. In particular, their use towards a more sustainable agriculture has been discussed, aiming at encouraging the use of alternative products to substitute synthetic pesticides to control weeds and plant diseases, without significantly affecting crop yields. An overview of the market and the recent advances on the regulation of these products as well as future challenges to promote their development and wider use in disease management programs is described. Because of several recent reviews on EO insecticidal properties, this topic is not covered in the present review.

Keywords: essential oils; biological properties; crop protection; sustainable agriculture

1. Introduction

Plants are naturally able to produce a wide range of molecules, especially secondary metabolites, which are known to perform a function in protecting plants against pathogens, owing to their biological properties [1]. Among these molecules, more than 3000 essential oils (EO), which are complex mixtures mostly constituted of secondary metabolites, are identified and known [2]. Many of the EO are known for centuries for their anti-septic, antioxidant and anaesthetic properties and a lot among them have been reported for their use in traditional medicine. Essential oils constitute an important source of biologically active compounds—antibacterial, insecticidal, fungicidal, nematocidal, herbicidal, antioxidant and anti-inflammatory [2–5]. Three hundred of them are commercialised and frequently used in cosmetics and flavours as well as in the food industries [2,6]. They are also used in the food sector as spices or to prepare beverages [7].

Biological control is not a new concept and it is gaining a lot of interest recently, for the integrated management of crop pests. Biocontrol products are classified into four main classes, including macro-organisms, microorganisms, semiochemical products and natural substances originating from plant, algae, microorganisms, animal or mineral sources [8]. Essential oils are found amongst all

natural substances of vegetable origin and therefore considered as potential biocontrol products. The current overuse of synthetic pesticides, causing environment and human health negative effects and pesticide-resistant biotypes, the emergence of resistance phenomena and the pesticides' withdrawal and restrictions (Directive 91/414/EEC, July 1993 and Regulation 1107/2009/EC, 2011) on a European but also on a worldwide scale, are encouraging a reduction in pesticides' use and the need for alternative control methods and integrated pest management (IPM) systems [9].

With a better acknowledgement of IPM approaches [10], biocontrol products and especially EO have a significant interest as they are bio-sourced products regarded as more ecological and alternative solutions in comparison with synthetic pesticides which show greater environmental and human health risks [11,12]. In that matter, the use of biocontrol agents and EO as substitutive solutions to synthetic pesticides is greatly encouraged in Europe by the directive 2009/128/CE, which aims to reduce the application of pesticides and promote the introduction of molecules and agricultural inputs more in line with sustainable development (<http://data.europa.eu/eli/dir/2009/128/oj>). Nonetheless, parameters influencing EO activity or efficiency need to be investigated, in order to legitimate their use as alternative methods to pesticides, alone or in addition to other molecules.

Thus, the current review focuses on gathering information and results from various studies on EO properties—with a focus on biological activities against weeds and plant pathogens, affecting crops pre- or post-harvest, in particular fungi, oomycetes and bacteria. An overview of the market and the recent advances on the regulation of these products as well as future challenges to promote their development and wider use in disease management programs is described. As insecticide use has been the most reviewed biological property, in comparison with other ones against plant pathogens, with the recent contribution of several authors on that subject [13–19], as well as in several book chapters [20–22], it is not covered in the current paper.

1.1. Specificities of Essential Oils

Naturally produced by aromatic plants and commonly obtained by hydrodistillation or steam distillation, EO are synthesized by all aromatic plant organs, flowers, buds, leaves, seeds, fruits, roots and rhizomes, wood and bark in relatively small amounts. They are located and stored in secretory cells, cavities or canals, epidermic cells or glandular hairs [3,4].

Either colourless or with a colour ranging from pale yellow to brown, these oils are commonly liquid at room temperature, but densities may be very different, and some oils can be resinous or even solid [23]. Poorly soluble in water but highly soluble in organic solvents, they are classified as fat-soluble [24].

Essential oils are usually rich in various compounds, comprising 20 to 60 active substances, and in many cases, can be characterized by up to three major components, at a relatively high concentration compared to other compounds present in trace amounts [2,3]. For example, linalool (68%) is found in *Coriander sativum* EO, limonene (54%) and α and β -pinene (respectively 7 and 3.5%) in *Pinus pinea* EO, carvacrol (65%) and thymol (15%) in *Origanum heracleoticum* EO and menthol (59%) and menthone (19%) are found in *Mentha x piperita* EO [3,25,26]. The major components found in EO are often responsible for their biological properties and can be gathered in two main groups:

- Terpene hydrocarbons, constituted of monoterpenes and sesquiterpenes. Monoterpenes represent 80% of the EO's composition [27,28].
- Oxygenated compounds, constituted mostly of alcohols, phenols, aldehydes and esters. The aromatic and oxygenated compounds occur less in EO than terpenes but are yet frequent [3,29].

The chemical composition of the EO varies, depending on the organ the EO is extracted from [29–31]. As an example, EO from *Salvia officinalis* displayed a significantly different composition, whether it was distilled from leaves, stems or flowers. In fact, α -thujone was the major identified compound, respectively representing 30, 55 and 18% of the EO compositions. Similarly, camphor which was identified in the EO distilled from the three different organs, varied from 19.5 to 3.5% (respectively in

the EO from leaves and flowers [31]). In addition, for a same plant species, EO's yield and chemical composition are wildly variable under the influence of several parameters, depending on growth and development conditions of the plant they originate from, climatic conditions (temperature, rainfall, humidity, light intensity), culture site (soil composition, acidity, pollution and mineral nutrition availability), harvesting time [30–32] and the root colonisation by symbiotic microorganisms, in particular arbuscular mycorrhizal fungi [33,34]. Differences in terms of chemical composition also appear between plant species of the same genus and more precisely between varieties of the same plant species, especially regarding the main compounds' proportions [35,36].

Owing mostly to their volatile nature and to the thermolability of their components, EO are very susceptible to degradation [5,37]. First, because of the close structural relationship between molecules, they may easily convert into each other through different processes, triggered by various factors which may affect them during storage or use, causing their degradation [5,38]. This occasional degradation is possible to assess through several chemical indexes (peroxide index, acid index, etc.), physical measurements (refraction index, density, ethanol miscibility, etc.) or chromatographic analyses [5,37].

Among all the degradation ways known, oxidation, isomerisation, polymerisation and dehydrogenation are the most frequent ones [5]. In practical terms, EO's degradation is affected by several chemical and environmental factors, influencing first the likelihood of EO to be altered and then the reaction's process. External factors including temperature, light and oxygen availability and the presence of impurities in EO as well as the nature of EO compounds and their structure may be determinant regarding EO's stability [5].

Chemical molecules are most of the time very susceptible to temperature variations. In lemon EO an increased temperature leads to a drop in geranial, neral and β -phellandrene concentrations, whereas an increase in *p*-cymene, limonene oxide and geranic acid amounts [39]. Besides volatilization, oxidation reactions may occur under thermic stresses. These reactions are divided into different categories: oxidative cleavage of carbon-carbon double bonds, dehydrogenation leading to aromatic cycle formation, epoxide formation and allylic oxidation resulting in alcohols, ketones and aldehydes apparition [40]. As an example, terpenoids are known to be both volatile and heat sensitive and may either be easily oxidized or hydrolysed, based on their structure [5].

Essential oils are also very sensitive to light radiation. More specifically, it has been shown that changes in EO composition occurred in light (in comparison with a storage in dark conditions), especially an oxidation of major compounds such as monoterpenes in EO from laurel and fennel. Oxidation occurs even in the dark, but at a relatively slower rate [41].

Isomerisation process is favoured by light radiations on EO as well. A modification in the composition of anise, clove or cinnamon EO, with the transformation of *trans*-anethole into *cis*-anethole as a striking feature, results in a highly increased toxicity and an unpleasant smell [42]. It is notable that for the same concentration, two aromatic molecules may have very different properties, especially olfactory ones (depending on volatility and molecular structure); if the perception threshold of the altered molecule is consequently lower for an organism, compared to the unaffected one, this might be sufficient to deteriorate the product and its efficiency [38,42].

The impact of light and temperature in presence of atmospheric oxygen has been investigated [28]. Even at low temperature, it has been shown that EO oxidation could occur and result in the formation of peroxide radicals and hydroperoxides. In fact, oxygen solubility in the EO increases with a decreased temperature (Henry's Law). For example, in rosemary, pine, lavender and thyme EO, higher amounts of peroxides were detected at low temperature [28].

According to the previous observations, it appears necessary to find optimal conditions for EO storage. Processing EO with a non-reactive gas has been investigated, but optimal storage conditions remain unclear and only a few EO or volatiles have been subject to storage experiments so far. Nonetheless, a storage at room temperature, in the absence of both oxygen and light are highly recommended [5,37]. In addition to the three external factors presented so far, EO are also susceptible to react with the packaging material or with impurities present in the EO's mix. Humidity rate and some

metal contaminations may result in oxidation reactions, with the prior presence of hydroperoxides in the EO [43].

One should keep in mind that because of their potential degradation, EO properties may be severely affected [44]. There are numerous examples of flavouring agents losing their organoleptic properties and going through viscosity change, because of the alteration of the EO's main compounds [5].

To summarise, a specific molecule may be affected in many ways and get altered through several degradation processes, which may eventually result in the apparition of various degradation compounds. To illustrate that observation, degradation of lemon EO at 40 °C, in presence of oxygen and copper oxide can lead to the apparition of the following compounds [39]: *p*-cymene, limonene oxide, α -terpineol and geranic acid. It is important to mention that attention should be paid to storage conditions so as to avoid unwanted degradation, that may alter the biological properties of the EO as well as exerting a potent toxicity due to the presence of alteration compounds.

1.2. Essential Oil's Use in Agriculture, against Plant Pathogens and Weeds

An increasing number of EO has shown an interesting activity from an agricultural consideration, against a broad spectrum of micro-organisms *in vitro* and *in planta* and against weeds and bioindicator plants.

1.2.1. Antifungal and Anti-Oomycete Properties

Phytopathogenic fungi are responsible for nearly 30% of all crop diseases [45,46] and may have a high impact on crops, affecting them during cultivation or post-harvest, during storage. From an economic concern, they can cause high yield losses by damaging host plant, whereas on a sanitary aspect, some of the fungi (*Aspergillus* sp., *Fusarium* sp., etc.) are known to produce mycotoxins, responsible for pneumopathies or containing carcinogenic compounds. Previous studies reported EO activities against plant pathogenic fungi and major phytopathogenic fungi from the previous decade until 2010 [47–49]. The present manuscript focuses on the most recent contributions to the field. In fact, effects of a consequent number of different EO have been investigated toward a wide range of phytopathogenic fungi and oomycetes in the past decade (Table 1). The complexity in comparing the different results resides in the different methods used for the fungicidal assays, with their results expressed in different ways with either *in vitro* or *in planta* assessments (IC₅₀, MIC and MFC—respectively half maximal inhibitory concentration, minimal inhibitory concentration and minimum fungicidal concentration—inhibition zone, etc.). Among all the phytopathogenic fungi targeted by the described works, *Alternaria*, *Botrytis*, *Fusarium*, *Penicillium* and *Rhizoctonia* are the most studied ones (Table 1). It has been demonstrated through many studies that the response of a specific phytopathogenic fungus in contact with EO was highly variable from one EO to another: *Botrytis cinerea* is inhibited by EO from black caraway and fennel, but not from peppermint [50]. One should note that the presence of either phenolic (fennel EO) or aromatic compounds (black caraway) seem to exert a higher antifungal activity against *B. cinerea*. Similarly, *Aspergillus* sp. was shown susceptible to EO from lemongrass, clove, oregano and thyme but not susceptible to cinnamon and ginger EO [51] and *Penicillium digitatum* highly affected by thyme and summer savory EO, less by fennel and sweet basil ones [52].

The same kind of pattern has been observed with EO. *Mentha x piperita* has been demonstrated efficient against *Rhizoctonia solani* and *Macrophomina phaseolina*, showing a lower MIC than *Bunium persicum* and *Thymus vulgaris* EO [53], but less efficient in the management of *Fusarium oxysporum* [54] and *Penicillium verrucosum* [55], nevertheless expressing an antifungal activity. Furthermore, EO from Lemongrass (*Cymbopogon citratus*) was demonstrated efficient against *Colletotrichum gloeosporioides* [56] and *Aspergillus* spp. [51], exerting a high antifungal activity, but less efficient against *F. oxysporum* requiring relatively high inhibitory concentrations [57].

Table 1. Antifungal properties of essential oils (EO) against phytopathogenic fungi and oomycetes studied during the last decade.

Target Organism	Disease Caused by the Pathogen	Essential Oil Distilled from	References	
<i>Fungi</i>	<i>Alternaria alternata</i>	<i>Carum carvi</i> L., <i>Carum opticum</i> L. and <i>Foeniculum vulgare</i> L.	[58]	
		18 egyptian plants	[59]	
		<i>Echinophora platyloba</i> (seed)	[60]	
		<i>Thuja plicata</i> , <i>Eugenia caryophyllata</i> L., <i>Lavandula angustifolia</i> , <i>Origanum vulgare</i> L., <i>Salvia sclarea</i> and <i>Thymus vulgaris</i> L.	[61]	
		<i>Thymus zygii</i> s	[62]	
		<i>Laurus nobilis</i>	[63]	
	<i>Alternaria humicola</i>	Alternariose	<i>Asarum heterotropoides</i>	[64]
	<i>Alternaria solani</i>	Early blight	<i>Angelica archangelica</i>	[65]
	<i>Alternaria spp.</i>	Alternariose	<i>Pinus pinea</i>	[25]
			<i>Genista quadriflora</i>	[66]
			<i>Pulicaria mauritanica</i>	[67]
			<i>Warionia saharae</i>	[68]
	<i>Aspergillus carbonarius</i>	Ochratoxin producer	<i>Citrus x limon</i> L.	[69]
	<i>Aspergillus flavus</i>	Rot and mould, aflatoxins production, aspergillois	<i>Mentha x piperita</i> , <i>Origanum spp.</i> , <i>Rosmarinus officinalis</i> L., <i>Schinus mole</i> L. and <i>Tagetes minuta</i> L.	[70]
			<i>Eucalyptus</i> sp., <i>Ferula galbaniflua</i> , <i>Thymus capitatus</i> and <i>Syzygium aromaticum</i>	[71]
			<i>Curcuma longa</i>	[72]
			<i>Angelica glauca</i> , <i>Plectranthus rugosus</i> , <i>Valeriana wallichii</i>	[73]
			<i>Mentha spicata</i>	[74]
			<i>Michelia alba</i>	[75]
			<i>Ocimum basilicum</i> and <i>Vetiveria zizanioides</i>	[76]
<i>Artemisia nilagirica</i>			[77]	
<i>Santolina chamaecyparissus</i>			[78]	
<i>Thuja plicata</i> , <i>Eugenia caryophyllata</i> L., <i>Lavandula angustifolia</i> , <i>Origanum vulgare</i> L., <i>Salvia sclarea</i> and <i>Thymus vulgaris</i> L.			[61]	
<i>Aspergillus fumigatus</i>	Rot and mould, aflatoxins production, aspergillois	<i>Ocimum basilicum</i> L.	[79]	
		<i>Genista quadriflora</i>	[66]	
		<i>Ocimum basilicum</i> L.	[80]	
		<i>Lallemantia royleana</i>	[81]	
		<i>Artemisia nilagirica</i>	[77]	
		<i>Ocimum basilicum</i> and <i>Vetiveria zizanioides</i>	[76]	
		<i>Solidago canadensis</i> L.	[82]	
<i>Aspergillus niger</i>	Mould	<i>Marrubium vulgare</i>	[83]	
		<i>Artemisia nilagirica</i>	[77]	
		<i>Ocimum basilicum</i> and <i>Vetiveria zizanioides</i>	[76]	
		<i>Solidago canadensis</i> L.	[82]	
		<i>Marrubium vulgare</i>	[83]	
<i>Aspergillus ochraceus</i>	Ochratoxin producer	<i>Artemisia nilagirica</i>	[77]	
<i>Aspergillus parasiticus</i>	Mould	<i>Citrus x limon</i> L.	[69]	
<i>Aspergillus spp.</i>		<i>Cinnamomum zeylanicum</i> , <i>Thymus vulgaris</i> L., <i>Origanum vulgare</i> L., <i>Syzygium aromaticum</i> L.), <i>Cymbopogon citratus</i> and <i>Zingiber officinale</i> Rosc.	[51]	
		14 different botanical plant species	[72]	
<i>Bipolaris oryzae</i>	Brown spot	<i>Piper sarmentosum</i>	[73]	

Table 1. Cont.

Target Organism	Disease Caused by the Pathogen	Essential Oil Distilled from	References			
<i>Fungi</i>	<i>Bipolaris sorokiniana</i>	<i>Pinus pinea</i>	[25]			
		<i>Eucalyptus erythrocorys</i>	[84]			
	<i>Biscogniauxia mediterranea</i>	Charcoal disease	<i>Eucalyptus</i> spp.	[54]		
	<i>Botryotinia fuckeliana</i>	Grey mould	<i>Thymus zygis</i>	[62]		
			<i>Cestrum nocturnum</i>	[85]		
			<i>Carum carvi</i> L., <i>Foeniculum vulgare</i> L. and <i>Mentha x piperita</i>	[50]		
			18 egyptian plants	[59]		
			<i>Mentha pulegium</i>	[86]		
			<i>Metasequoia glyptostroboides</i>	[87]		
			<i>Origanum heracleoticum</i>	[88]		
			<i>Botrytis cinerea</i>	Grey mould	<i>Origanum majorana</i>	[84]
					<i>Eucalyptus erythrocorys</i>	[84]
					<i>Tetraclinis articulata</i>	[89]
	<i>Thymus</i> spp.	[90]				
	<i>Melissa officinalis</i>	[91]				
	<i>Cinnamomum cassia</i>	[92]				
	<i>Angelica archangelica</i>	[65]				
	<i>Solidago canadensis</i> L.	[82]				
	<i>Melaleuca alternifolia</i>	[93]				
	<i>Tetraclinis articulata</i>	[94]				
<i>Marrubium vulgare</i>	[83]					
<i>Choanephora cucurbitarum</i>	Fruit and blossom rot	<i>Cinnamomum camphora</i>	[95]			
		<i>Syzygium cumini</i>	[96]			
<i>Cladosporium cladosporioides</i>	Rot	<i>Citrus x limon</i> L.	[69]			
		<i>Thuja plicata</i> , <i>Eugenia caryophyllata</i> L., <i>Lavandula angustifolia</i> , <i>Origanum vulgare</i> L., <i>Salvia sclarea</i> and <i>Thymus vulgaris</i> L.	[61]			
<i>Colletotrichum capsici</i>	Leaf spot	<i>Cestrum nocturnum</i>	[85]			
		<i>Metasequoia glyptostroboides</i>	[87]			
		<i>Piper chaba</i>	[97]			
<i>Colletotrichum gloeosporioides</i>	Leaf spot	<i>Cymbopogon</i> sp.	[98]			
		<i>Asarum heterotropoides</i>	[64]			
<i>Colletotrichum tricbellum</i>	Leaf spot					
<i>Curvularia fallax</i>	Black sheath spot —Leaf spot	<i>Echinophora platyloba</i> (seed)	[60]			
<i>Cytospora sacchari</i>	Stem canker on sugarcane					
<i>Eurotium herbariorum</i>	Mould	<i>Citrus x limon</i> L.	[69]			
<i>Fusarium avenaceum</i>	Ear blight and root rot of cereals	<i>Eucalyptus erythrocorys</i>	[84]			
		18 egyptian plant species	[59]			
		<i>Metasequoia glyptostroboides</i>	[87]			
<i>Fusarium oxysporum</i>	Fusarium wilt (vascular disease)	<i>Eucalyptus erythrocorys</i>	[84]			

Table 1. Cont.

Target Organism	Disease Caused by the Pathogen	Essential Oil Distilled from	References	
Fungi	<i>Fusarium oxysporum</i>	<i>Genista quadriflora</i>	[66]	
		<i>Echinophora platyloba</i> (seed)	[60]	
		<i>Piper chaba</i>	[97]	
		<i>Syzygium aromaticum</i> , <i>Eucalyptus globulus</i> , <i>Cymbopogon citratus</i> and <i>Mentha x piperita</i>	[56]	
		<i>Mikania scandens</i>	[57]	
		<i>Salmea scandens</i>	[99]	
	<i>Fusarium solani</i>	Root rot, soft rot of plant tissues	18 egyptian plant species	[59]
			<i>Metasequoia glyptostroboides</i>	[87]
			<i>Eucalyptus erythrocorys</i>	[84]
			<i>Asarum heterotropoides</i>	[64]
			<i>Angelica glauca</i> , <i>Plectranthus rugosus</i> , <i>Valeriana wallichii</i>	[73]
			<i>Piper chaba</i>	[97]
	<i>Fusarium spp.</i>		<i>Marrubium vulgare</i>	[83]
			<i>Cestrum nocturnum</i>	[85]
<i>Pinus pinea</i>			[25]	
<i>Rosmarinus officinalis</i>			[100]	
<i>Tetraclinis articulata</i>			[90]	
<i>Angelica archangelica</i>			[65]	
		14 different botanical plant species	[101]	
<i>Fusarium sulphureum</i>	Dry rot	<i>Zanthoxylum bungeanum</i>	[102]	
<i>Fusarium verticillioides</i>	Ear rot on maize	<i>Curcuma longa</i>	[103]	
<i>Geotrichum citri-aurantii</i>	Sour rot (post-harvest)	<i>Thymus spp.</i>	[104]	
<i>Lasioidiplodia theobromae</i>	Rot and dieback (forest species)	<i>Myrcia lundiana</i>	[105]	
<i>Macrophomina phaseolina</i>	Damping-off, seedling blight, rot	<i>Mentha x piperita</i> and <i>Ocimum basilicum</i>	[106]	
		<i>Echinophora platyloba</i> (seed)	[60]	
<i>Microdochium nivale</i>	Patch lawn disease	<i>Pinus pinea</i>	[25]	
<i>Monilinia fructicola</i>	Brown rot	<i>Mentha pulegium</i>	[86]	
		<i>Solidago canadensis</i> L.	[82]	
<i>Penicillium digitatum</i>	Green mould (post-harvest)	<i>Carum carvi</i> L., <i>Carum opticum</i> L. and <i>Foeniculum vulgare</i> L.	[58]	
		<i>Foeniculum vulgare</i> Mill., <i>Satureja hortensis</i> L., <i>Ocimum basilicum</i> L. and <i>Thymus vulgaris</i> L.	[53]	
		<i>Thymus spp.</i>	[104]	
		<i>Marrubium vulgare</i>	[83]	
<i>Penicillium expansum</i>	Post-harvest mould	<i>Melissa officinalis</i>	[91]	
		<i>Pulicaria mauritanica</i>	[67]	
		<i>Solidago canadensis</i> L.	[82]	
		<i>Warionia saharae</i>	[68]	

Table 1. Cont.

Target Organism	Disease Caused by the Pathogen	Essential Oil Distilled from	References
<i>Penicillium italicum</i>	Blue mould	<i>Thymus</i> spp.	[104]
		<i>Rosmarinus officinalis</i>	[100]
<i>Penicillium</i> spp.		<i>Mentha x piperita</i> , <i>Origanum</i> spp., <i>Rosmarinus officinalis</i> L., <i>Schinus mole</i> L. and <i>Tagetes minuta</i> L.	[70]
		<i>Citrus x limon</i> L.	[69]
		<i>Ocimum basilicum</i>	[79]
		<i>Ocimum basilicum</i>	[80]
		<i>Ocimum basilicum</i> and <i>Vetiveria zizanioides</i>	[76]
<i>Penicillium verrucosum</i>	Ochratoxin producer	Residues of <i>Lamiceae</i> species	[52]
		<i>Allium sativum</i> L., <i>Mentha x piperita</i> , <i>Origanum onites</i> L. and <i>Salvia officinalis</i> L.	[107]
Fungi	<i>Rhizoctonia solani</i>	<i>Cestrum nocturnum</i>	[85]
		<i>Metasequoia glyptostroboides</i>	[87]
		<i>Asarum heterotropoides</i>	[64]
		<i>Angelica archangelica</i>	[65]
		<i>Bunium persicum</i> , <i>Foeniculum vulgare</i> , <i>Juniperus polycarpus</i> , <i>Mentha</i> spp., <i>Ocimum</i> <i>basilicum</i> , <i>Thymus vulgaris</i> and <i>Zingiber officinale</i>	[106]
		<i>Piper chaba</i>	[97]
		<i>Syzygium cumini</i>	[96]
		<i>Thymus</i> spp.	[90]
		<i>Mikania scandens</i>	[57]
		<i>Piper sarmentosum</i>	[108]
<i>Rhizoctonia</i> sp.		<i>Pinus pinea</i>	[25]
<i>Rhizopus microsporus</i>	Rice seedling blight, various head, grain and ear rots	<i>Ocimum basilicum</i> and <i>Vetiveria zizanioides</i>	[76]
<i>Rhizopus stolonifer</i>	Storage/post-harvest rot	<i>Foeniculum vulgare</i> Mill., <i>Satureja hortensis</i> L., <i>Ocimum basilicum</i> L. and <i>Thymus vulgaris</i> L.	[53]
		<i>Melissa officinalis</i>	[91]
		<i>Pulicaria mauritanica</i>	[67]
<i>Sclerotinia sclerotiorum</i>	White mould	<i>Warionia saharae</i>	[68]
		<i>Cestrum nocturnum</i>	[85]
		<i>Metasequoia glyptostroboides</i>	[87]
		<i>Ziziphora clinopodioides</i>	[109]
<i>Verticillium dahliae</i>	Verticillium wilt	35 plant's botanical species	[110]
<i>Villosiclava virens</i>	Rice false smut	18 plant's botanical species	[111]
<i>Phytophthora cactorum</i>	Root rot	<i>Asarum heterotropoides</i>	[64]
		<i>Cestrum nocturnum</i>	[85]
Oomycetes	Blight	<i>Metasequoia glyptostroboides</i>	[87]
		<i>Piper chaba</i>	[97]
		<i>Salmea scandens</i>	[99]
<i>Phytophthora infestans</i>	Late blight	<i>Citrus sinensis</i> Cadenera, <i>Citrus limon</i> Eureka and <i>Citrus bergamia</i> Castagnaro	[112]
		<i>Thymus</i> spp.	[90]
		<i>Origanum majorana</i> L.	[113]

Table 1. Cont.

Target Organism	Disease Caused by the Pathogen	Essential Oil Distilled from	References
<i>Oomycetes</i>	<i>Phytophthora megakarya</i>	Black pod disease	<i>Syzygium aromaticum</i> and <i>Zanthoxylum xanthoxyloides</i> [55]
	<i>Pythium</i> spp.	Root rot	<i>Thymus</i> spp. [90]
			<i>Mikania scandens</i> [57]

In addition, the results from these studies indicate that EO have the potential to target fungi affecting plants both during the cultivation or causing diseases occurring during the storage (post-harvest diseases), including in particular several species from *Penicillium* genus (*P. digitatum*, *P. expansum*, *P. italicum*), *Geotrichum citri-aurantii* or *Rhizopus stolonifer*.

1.2.2. Bactericidal Properties

Bacteria causing diseases on plants may have a considerable economic impact. As an example, bacterial diseases caused by *Xanthomonas* spp. affect a wide range of host plants, causing considerable damages on plants and hence a loss in terms of yield and crop quality [84,114,115].

Over the past 5 years, a growing number of studies has been published regarding EO antibacterial properties, especially against plant pathogens, depicting a growing interest in biocontrol methods. The number of studies reporting EO as antibacterial agents in a plant pathogen perspective is still limited (Table 2), while most of the studies on antibacterial properties of EO are focusing on a food preservation or health issue perspective.

The response and susceptibility of pathogens to EO or EO major compounds are diverse. It has for instance been demonstrated that the effect of basil EO on different bacteria induced various responses in terms of inhibition [116], against a wide range of pathogens. It has been shown particularly efficient against *Pseudomonas tolaasii*, whereas *Brenneria nigrifluens* was barely affected by the EO. Additionally, *Xanthomonas citri* and *Rhodococcus fascians* were also inhibited but at higher EO concentrations in comparison with *P. tolaasii*. Another study has shown a mitigated success of the *Tanacetum* species EO, being ineffective against *Erwinia amylovora* or *Xanthomonas* sp. [117]. *Origanum onites* has on the contrary proven itself efficient against *Clavibacter michiganensis* and *Xanthomonas* spp. with consistent inhibition zones [118].

Table 2. Examples of EO acting against phytopathogenic bacteria (from 2007 to present).

Essential Oil Distilled from	Target Bacteria and Caused Disease	References
<i>Achillea biebersteinii</i>	<i>Clavibacter michiganensis</i> Ring rot disease	[119]
<i>Achillea millefolium</i>		
<i>Ocimum ciliatum</i>	<i>Rhodococcus fascians</i> Leafy gall disease	[116]
<i>Origanum heracleoticum</i>		[88]
<i>Origanum majorana</i>		
<i>Origanum onites</i>	<i>Clavibacter michiganensis</i> Ring rot disease	[116]
<i>Salmea scandens</i>		[99]
<i>Satureja hortensis</i>		[120]
<i>Satureja spicigera</i>		[119]
<i>Solidago canadensis</i> L.		[82]
<i>Tanacetum aucheranum</i>		[117]
<i>Thymus fallax</i>		[119]

Gram positive bacteria

Table 2. Cont.

Essential Oil Distilled from	Target Bacteria and Caused Disease	References
<i>Achillea biebersteinii</i>	<i>Erwinia</i> spp.	[119]
	<i>Pseudomonas</i> spp. Bacterial canker	
	<i>Xanthomonas</i> spp. Bacterial spots and blights	
<i>Achillea millefolium</i>	<i>Erwinia</i> spp.	[121]
	<i>Pseudomonas</i> spp. Bacterial canker	
	<i>Xanthomonas</i> spp. Bacterial spots and blights	
<i>Citrus aurantium</i> L.	<i>Agrobacterium tumefaciens</i> Crown gall	[121]
	<i>Dickeya solani</i> Black leg and soft rot	
<i>Citrus reticulata</i>	<i>Erwinia amylovora</i> Fire blight	[122]
	<i>Pseudomonas aeruginosa</i> Soft rot	
<i>Cleistocalyx operculatus</i>	<i>Xanthomonas</i> spp. Bacterial spots and blights	[123]
<i>Cynara scolymus</i> (stems)	<i>Erwinia amylovora</i> Fire blight	[124]
	<i>Erwinia carotovora</i> Soft rot	
	<i>Pseudomonas syringae</i> Bacterial canker	
	<i>Xanthomonas vesicatoria</i> Bacterial leaf spot	
<i>Eriocephalus africanus</i> L.	<i>Agrobacterium tumefaciens</i> Crown gall	[125]
	<i>Dickeya solani</i> Black leg and soft rot	
	<i>Erwinia amylovora</i> Fire blight	
	<i>Pseudomonas cichorii</i> Leaf blight and spots	
<i>Juglans regia</i> L. (shells)	<i>Serratia pulmuthica</i>	[124]
	<i>Erwinia amylovora</i> Fire blight	
	<i>Erwinia carotovora</i> Soft rot	
	<i>Pseudomonas syringae</i> Bacterial canker	
<i>Metasequoia glyptostroboides</i>	<i>Xanthomonas vesicatoria</i> Bacterial leaf spot	[89]
	<i>Xanthomonas</i> spp. Bacterial spots and blights	
<i>Ocimum ciliatum</i>	<i>Agrobacterium vitis</i> Crown gall	[116]
	<i>Brenneria nigrifluens</i> Cankers	
	<i>Pantoea stewartii</i> Stewart's wilt and leaf blight	
	<i>Pseudomonas</i> spp. Bacterial canker	
	<i>Ralstonia solanacearum</i> Bacterial wilt	
	<i>Xanthomonas</i> spp. Bacterial spots and blights	
<i>Ocimum basilicum</i>	<i>Pseudomonas aeruginosa</i> Soft rot	[76]
<i>Origanum heracleoticum</i>	<i>Pseudomonas</i> spp. Bacterial canker	[88]
	<i>Xanthomonas</i> sp. Bacterial spots and blights	
<i>Origanum majorana</i>	<i>Pseudomonas</i> spp. Bacterial canker	[116]
	<i>Xanthomonas</i> sp. Bacterial spots and blights	
<i>Origanum onites</i>	<i>Erwinia</i> spp.	[116]
	<i>Pseudomonas</i> spp. Bacterial canker	
	<i>Xanthomonas</i> spp. Bacterial spots and blights	
<i>Origanum vulgare</i>	<i>Erwinia amylovora</i> Fire blight	[124]
	<i>Erwinia carotovora</i> Soft rot	
	<i>Pseudomonas syringae</i> Bacterial canker	
	<i>Xanthomonas vesicatoria</i> Bacterial leaf spot	
	<i>Pseudomonas syringae</i> Bacterial canker	
	<i>Pseudomonas</i> spp. Bacterial canker	[127]

Table 2. Cont.

Essential Oil Distilled from	Target Bacteria and Caused Disease	References
<i>Piper sarmentosum</i>	<i>Xanthomonas oryzae</i> <i>pv. oryzae</i>	Bacterial blight [108]
	<i>Xanthomonas oryzae</i> <i>pv. oryzicola</i>	Bacterial blight
<i>Salmea scandens</i>	<i>Pseudomonas syringae</i>	Bacterial canker
	<i>Erwinia carotovora</i>	Soft rot [99]
	<i>Erwinia spp.</i>	
<i>Salmea scandens</i>	<i>Pseudomonas spp.</i>	Bacterial canker
	<i>Xanthomonas spp.</i>	Bacterial spots and blights [99]
<i>Satureja hortensis</i>	<i>Erwinia carotovora</i>	Soft rot
	<i>Erwinia spp.</i>	
<i>Satureja spicigera</i>	<i>Pseudomonas spp.</i>	Bacterial spots and blights [120]
	<i>Xanthomonas spp.</i>	Bacterial spots and blights
<i>Satureja spicigera</i>	<i>Erwinia spp.</i>	
	<i>Pseudomonas spp.</i>	Bacterial canker [118]
	<i>Xanthomonas spp.</i>	Bacterial spots and blights
<i>Solidago canadensis</i> L.	<i>Pseudomonas spp.</i>	Bacterial canker [82]
	<i>Xanthomonas sp.</i>	Bacterial spots and blights
<i>Syzygium aromaticum</i>	<i>Erwinia amylovora</i>	Fire blight
	<i>Erwinia carotovora</i>	Soft rot [124]
	<i>Pseudomonas syringae</i>	Bacterial canker
	<i>Xanthomonas vesicatoria</i>	Bacterial leaf spot
<i>Tanacetum aucheranum</i>	<i>Agrobacterium tumefaciens</i>	Crown gall
	<i>Erwinia spp.</i>	
	<i>Pseudomonas spp.</i>	Bacterial canker [117]
<i>Tanacetum chiliophyllum</i>	<i>Xanthomonas spp.</i>	Bacterial spots and blights
	<i>Agrobacterium tumefaciens</i>	Crown gall
	<i>Erwinia spp.</i>	
<i>Thymus fallax</i>	<i>Pseudomonas spp.</i>	Bacterial canker [118]
	<i>Xanthomonas spp.</i>	Bacterial spots and blights
	<i>Erwinia spp.</i>	
<i>Thymus vulgaris</i>	<i>Pseudomonas syringae</i>	Bacterial canker [126]
	<i>Pseudomonas spp.</i>	Bacterial canker
<i>Vetiveria zizanioides</i>	<i>Pseudomonas aeruginosa</i>	Soft rot [76]
<i>Zataria multiflora</i>	<i>Xanthomonas campestris</i>	Black rot and leaf spot [127]
11 different plants	<i>Erwinia amylovora</i>	Fire blight [128]
18 egyptian plants	<i>Agrobacterium tumefaciens</i>	Crown gall [129]
	<i>Erwinia carotovora</i>	Soft rot

1.2.3. Herbicidal Properties

Essential oils have been investigated for their suspected effect on seed germination and shoot growth and development (inhibiting either one or both processes [130–132]) and are likely to be used in weeds’ control (Table 3). Several authors previously synthesized EO herbicidal and phytotoxic

properties in reviews [133,134]. These phytotoxic effects have been demonstrated for *Amaranthus retroflexus*, *Chenopodium album* and *Rumex crispus* being completely inhibited in contact with *Origanum acutidens* EO [135], for *Raphanus sativus*, *Lactuca sativa* and *Lepidium sativum* tested with EO of *Thymus vulgaris*, *Verbena officinalis* and *Melissa officinalis* [136] and for *A. retroflexus*, *Cirsium arvense* and *Lactuca serriola* treated with *Achillea gypsicola* and *Achillea biebersteinii* EO [137]. Furthermore, visible damage on a grown weed are reported on *Parthenium hysterophorus* [138] and on little seed canary grass [139] in contact with *Eucalyptus citriodora* EO. However, a few EO have been demonstrated ineffective for the purpose of weed control. *Ocimum basilicum*, *Foeniculum vulgare* and *Pimpinella anisum* EO against *Raphanus sativus*, *L. sativa* and *L. sativum* germination and radicle growth [136] while *Achillea gypsicola* EO was shown ineffective against the germination of *Chenopodium album* and *Rumex crispus* [137].

Table 3. Examples of EO presenting herbicidal properties.

Essential Oil Distilled from	Plant Tested	References
<i>Achillea gypsicola</i>	<i>Amaranthus retroflexus</i>	[137]
	<i>Chenopodium album</i>	
	<i>Cirsium arvense</i>	
	<i>Lactuca serriola</i>	
	<i>Rumex crispus</i>	
<i>Achillea biebersteinii</i>	<i>Amaranthus retroflexus</i>	[137]
	<i>Chenopodium album</i>	
	<i>Cirsium arvense</i>	
	<i>Rumex crispus</i>	
<i>Angelica glauca</i>	<i>Lemna minor</i>	[73]
<i>Citrus x limon</i> L.	<i>Portulaca oleracea</i>	[140]
<i>Citrus aurantiifolia</i>	<i>Avena fatua</i>	[131]
	<i>Echinochloa crus-galli</i>	
	<i>Phalaris minor</i>	
<i>Coriandrum sativum</i> L.	<i>Amaranthus retroflexus</i>	[141]
	<i>Chenopodium album</i>	
	<i>Echinochloa crus-galli</i>	
<i>Eucalyptus</i> spp.	<i>Annual ryegrass</i>	[142]
	<i>Echinochloa crus-galli</i>	[143]
	<i>Lolium multiflorum</i>	
	<i>Nicotiana glauca</i>	[139]
	<i>Phalaris minor</i>	
	<i>Parthenium hysterophorus</i>	[138]
	<i>Portulaca oleracea</i>	[143]
	<i>Sinapis arvensis</i>	[84]
<i>Phalaris canariensis</i>	[36]	
<i>Lavandula</i> spp.	<i>Solanum elaeagnifolium</i>	[144]
	<i>Lolium rigidum</i>	

Table 3. Cont.

Essential Oil Distilled from	Plant Tested	References
12 Mediterranean species	<i>Lactuca sativa</i>	[136]
	<i>Lepidium sativum</i>	
	<i>Raphanus sativus</i>	
<i>Origanum acutidens</i>	<i>Amaranthus retroflexus</i>	[135]
	<i>Rumex crispus</i>	
	<i>Chenopodium album</i>	
<i>Origanum vulgare</i> L.	<i>Hordeum vulgare</i>	[145]
	<i>Lepidium sativum</i>	[146]
	<i>Matricaria chamomilla</i> L.	
	<i>Sinapsis alba</i>	[145]
<i>Peumus boldus</i>	<i>Portulaca oleracea</i>	[140]
<i>Pinus nigra</i>	<i>Phalaris canariensis</i>	[147]
	<i>Trifolium campestre</i>	
	<i>Sinapis arvensis</i>	
<i>Pinus pinea</i>	<i>Sinapis arvensis</i>	[25]
	<i>Raphanus raphanistrum</i>	
	<i>Lolium rigidum</i>	
<i>Plectranthus rugosus</i>	<i>Lemna minor</i>	[73]
<i>Rosmarinus officinalis</i>	<i>Amaranthus retroflexus</i>	[130]
	<i>Matricaria chamomilla</i> L.	[146]
	<i>Phalaris minor</i>	[100]
	<i>Rhaphanus sativus</i>	[130]
	<i>Silybum marianum</i>	[100]
	<i>Trifolium incarnatum</i>	
<i>Syzygium aromaticum</i>	Common lambsquarters	[148]
	Redwood pigweed	
<i>Tagetes erecta</i>	<i>Echinochloa crus-galli</i> L. Beauv.	[149]
<i>Tanacetum aucheranum</i>	<i>Amaranthus retroflexus</i>	[117]
	<i>Chenopodium album</i>	
	<i>Rumex crispus</i>	
<i>Tanacetum chiliophyllum</i>	<i>Amaranthus retroflexus</i>	
	<i>Chenopodium album</i>	
	<i>Rumex crispus</i>	
<i>Tetraclinis articulata</i>	<i>Sinapis arvensis</i>	[89]
	<i>Phalaris canariensis</i>	
25 various plants	<i>Taraxacum officinale</i>	[150]
<i>Valeriana wallichii</i>	<i>Lemna minor</i>	[73]

1.2.4. Essential Oil's Mechanisms of Action

Even if *in vitro* EO biological properties against a wide range of organisms have been well covered, as mentioned previously, their mechanisms of action have scarcely been investigated. In particular, with the limited number of studies on antibacterial properties, the insights towards the understanding of the EO's antibacterial mechanism remains very limited. Yet, a number of notable features are put forth and will be discussed in the present section.

Two recent reviews on secondary metabolites' mechanisms of action, including EO and plant extracts (obtained through organic solvent extraction), pointed out six different mechanisms regarding antifungal properties [151,152]:

- Inhibiting the fungi cell wall formation;
- Disrupting the cell membrane by inhibiting ergosterol synthesis;
- Affecting the fungal mitochondria by inhibiting the mitochondrial electron transport;
- Inhibiting cell division;
- Interfering with either RNA or DNA synthesis and/or inhibiting protein synthesis;
- Inhibiting efflux pumps.

From the current state of knowledge, it appears that the common property of many EO is that they affect membrane permeability or functioning, leading to cell death in fungi [153] or bacteria [154]. For example, coriander EO (*C. Sativum*) has shown an activity against *Candida albicans* by binding itself on membrane ergosterol and this way increasing membrane permeability [155–157]. A prominent activity of terpenoid compounds present in EO has been discussed, highlighting their potential to attack and disrupt cell walls (by inhibiting β -glucans and chitin synthesis, compromising its integrity and causing the cell to lose control over its shape and disrupting homeostasis, eventually leading to cell death [151,158]) and membranes, affecting not only their permeability (and causing cell leakage) but also compromising membrane functions, such as electron transport, protein and enzyme activity or nutrient transport and uptake [84,122,159]. A similar effect has been demonstrated for *Mentha spicata* EO against *A. flavus* [66]. The inhibition of the biofilm formation has also been put forth as a key feature in EO antimicrobial mechanisms, against both bacteria and fungi [152]. In particular, numerous studies reported EO as *C. albicans* biofilm inhibitors [152]. Several EO from *C. sativum* or *Ocimum americanum* demonstrated an inhibitory effect towards *C. albicans* biofilms [152,160,161]. *Citrus* EO were also demonstrated capable of inhibiting bacterial (*P. aeruginosa*) and fungal (*A. fumigatus* and *Scedosporium apiospermum*) biofilm establishment [162]. Bound to this aspect, 'quorum sensing', that is the ability to detect and to respond to cell population density by gene regulation, has been demonstrated to be affected by EO [152]. As an example, *Citrus* EO previously mentioned were shown able to inhibit quorum sensing in both *P. aeruginosa* and *C. albicans*, leading to a membrane permeabilization of both organisms [163].

A study on tea tree EO demonstrated that the EO action against *B. cinerea* resulted in membrane cell permeability modifications and in a loss of cellular organelles' function [90]. Carvacrol, found as a major compound in thyme (45%) and oregano (60 to 74%) EO, is known for its antibacterial activity against a broad range of Gram-positive and Gram-negative bacteria. It has been shown that carvacrol affects Gram-positive bacteria's membranes and modifies their permeability regarding H^+ and K^+ cations. It also alters the outer membrane of Gram-negative bacteria, hence increasing cytoplasmic membrane's permeability to ATP and unleashing lipopolysaccharides [164]. In fact, Gram-negative bacteria are suspected to be less sensitive to EO than Gram-positive ones, as they possess an outer membrane rich in lipopolysaccharides, which indeed restricts the direct contact between the EO and the cytoplasmic membrane, in contrast with Gram-positive bacteria [35,154]. Yet, the number of studies targeting Gram-negative bacteria are predominant (Table 2).

More specifically, on a molecular level, it has been shown that phenolic and terpenoid compounds were more efficient in comparison with esters, alcohols, aldehydes, etc., [152,165,166]. The presence of a phenolic core is suspected to be the reason of the greater efficiency, and that because of a hydroxyl

group resulting in both antifungal and antibacterial activities [167]. The antifungal activity is also likely to be related to the steric hindrance of specific groups: more hydrophobic molecules such as phenolic compounds or aromatic aldehydes are more susceptible to exert a higher antifungal activity [167,168]. The fat-soluble property of EO has been shown essential in their antifungal activity too [169]. Hydrophobicity of EO and their components allows EO to break through lipids of cell membranes and mitochondria, resulting in the previously mentioned increase of bacterial and fungal membrane permeability [159,170,171].

Susceptibility of the pathogen to the EO depends of various factors, such as the composition of the EO in terms of active compounds, concentration of the EO and solubility in the media [35,172]. Exposure time of the pathogen to the oil and the persistency of the EO's effects (depending highly on EO's volatility) in time also are variability factors considering the efficiency of an EO towards a pathogen [27,173,174]. Growth conditions of the pathogen, such as pH, temperature and dioxygen availability in the media are reported as influencing factors regarding EO's efficiency on a specific pathogen. Thymol has shown greater efficiency in anaerobic conditions [35,175], susceptibility of bacteria in contact with different EO has been demonstrated to be higher coupled with a low pH, especially in food [35,170,176,177] while temperature effects on antimicrobial activity are still controversial [35]. Some authors reported an increased activity coupled with a higher temperature [178, 179] whereas others have shown a better efficiency with a decreasing temperature [180–182].

Regarding EO phytotoxic effects, visible symptoms such as growth decrease, severe chlorosis or leaves' burning [150,183], were previously related to several key features, notably [133,183–186]:

- Mitosis inhibition;
- Decrease of the cellular respiration;
- Ion leakage and membrane depolarisation;
- Waxy cuticular layer removal;
- Decrease of the chlorophyll content;
- Oxidative damages through reactive oxygen species' production;
- Microtubule polymerisation.

However, no comprehensive study regarding the detailed herbicidal mechanisms was previously published [183]. The same authors investigated cinnamon and Java citronella EO and some of their respective main chemical components, namely trans-cinnamaldehyde, citronellal and citronellol, regarding their herbicidal effects. They came to the conclusion that all above-mentioned EO or compounds were efficient herbicides against *A. thaliana*, most likely affecting the plant plasma membrane but not resulting in ion leakage. An effect on membrane domains and/or related properties was then put forward [183].

1.3. Market and Regulation

In the recent years, more and more studies about EO have been published, aiming to investigate their biological properties. Unfortunately, from the current perspective, EO are suffering a loss of efficiency when used as such in the field, owing mostly to their volatile nature and degradation susceptibility. In addition, their approval and registration procedure are very costly, because of the inherent cost of toxicity and environmental suitability assessments [9,187,188]. On a worldwide scale, biopesticides including biocontrol agents and EO are evaluated (as part of approval procedure) through the exact same procedure as their synthetic counterparts and similarly for the registration procedure [12,189,190]. A discussion is however currently initiated towards a relief of the process with a streamlined registration procedure for low-risk products (*i.e.*, products that must not exert toxicity towards non target organisms and have a low persistency in soil [12]).

At the present time, biocontrol market (world-widely valued at approximately \$3 billion) accounts for only 5% of the global crop protection sector but this is a relatively fast-growing market segment

expecting to reach more than 7% of the total crop protection market by 2025 (more than \$4.5 billion estimated) with an annual 8.84% growth estimation [191–193].

In comparison to the biocontrol products' market, the global market for EO (natural cosmetics, beauty products, medicines and nutraceuticals) was estimated at about 4.9 billion euros in 2014, growing to 5.5 billion the next year and expected to reach more than 10 billion euros in 2020 [194]. Europe represents the biggest market, with a global market share of 40% in 2014 [194,195]. France is the second provider concerning high value EO to Europe, after the United States, though supplying relatively small [194]. In comparison, the global cosmetics market in the United States, Europe, China and Japan was worth 168 billion euros in 2014 [194].

The number of registered biocontrol agents and in our case more specifically EO-based products are heavily lower in Europe compared to the United States [193]. In the United States, the development of pesticides based on natural products has been facilitated, by exemption from registration for certain oils commonly used in processed foods and beverages [7,196,197]. Owing to this opportunity, some EO-based products have been developed and tested as fungicides, herbicides or insecticides, using thyme, clove and rosemary EO as active ingredients [7,196]. In particular, two American companies have commercialised several EO-based products, including Cinnamite and Valero from Mycotech Corporation (respectively an aphicide/miticide/fungicide and a fungicide) and EcoPCO, EcoTrol Plus, SPoran and Matran from EcoSMART Technologies (respectively insecticide, insecticide/miticide, fungicide and herbicide), among other products, namely Buzz Away or Green Ban [197,198].

In the recent years across Europe, however, an increasing number of EO has been homologated for use in agriculture, especially as biocides. Thus, EO from various plants and origins have been registered for specified uses (in particular biocidal effects), such as *Mentha arvensis* and *Mentha spicata*, *Juniperus mexicana*, *Citrus x sinensis*, *Persicaria odorata*, *Piper nigrum*, *Canarium commune*, *Cinnamomum zeylanicum*, *Boswellia carterii*, *Cymbopogon flexuosus*, *Litsea cubeba*, *Artemisia alba*, *Cistus ladaniferus*, *Copaifera tree*, *Ferula galbaniflua*, *Citrus aurantium* and *Schinus terebinthifolius* [199]. Commercial products are available for use in certain European countries, notably BIOXEDA (clove EO as a fungicide or bactericide on apple and pear trees storage pathogens), BIOX-M (*Mentha spicata* EO as a growth regulator on potato) or LIMOCIDE-OROCIDE-PREV-AM and ESSEN'CIEL (Sweet orange EO against whitefly, potato leafhopper, powdery mildew, blight, tobacco thrips on aromatic and medicinal plants, vegetable, fruit and ornamental crops as well as tobacco and vine). To this significant number of EO can be added extracts from aromatic plants (either main compounds or purified EO obtained by specific extraction technics such as supercritical fluid extraction), such as *Lavandula angustifolia*, *Artemisia alba*, *Citrus bergamia*, *Bulnesia sarmienti*, *Melaleuca leucadendron*, *Cinnamomum camphora*, *Elettaria cardamomum*, *Coriandrum sativum*, *Cupressus sempervirens*, *Eucalyptus globulus* and *Citrus paradisi* (non-exhaustive list [199]).

1.4. Innovative Avenue—Essential Oil Formulation

So as to legitimate and encourage EO application in agriculture as “green pesticides” and especially in the context of agroecology, it is necessary to find suitable options to promote their use, efficiency and persistence of effects in time. In particular, the stability of the EO when they are used in fields and the persistency of their effects in time are often brought forward as limited. In addition, working with EO might represent an expensive option because of the relatively low yield of obtention and a costly approval procedure. A recent study on rosemary EO [130] has put forth encapsulation (starch coating) of the EO as a means to slowly release it in the soil and control the EO diffusion as well as to improve its efficiency as a potential bio-herbicide with a perspective of field use.

From a wider perspective, a product formulation is a homogeneous and stable mixture of active and inert ingredients [200], involving a specific processing of the product to enhance its biological properties as well as their durability and the stability of the product. Because many of them are not suitable for use in their raw state (toxicity, poor solubility, instability, etc., [200,201]), formulations are commonly used for pesticides and the same kind of technic should be applicable to EO so as to obtain

similar benefits. Additionally, coating materials are bio-sourced and biodegradable products [202]. One should note that even though these two technics may represent promising avenues, the choice of a formulation highly depends on the intended use and application method, the target pathogen as well as the potential environmental degradation factors [201,203].

1.4.1. Essential Oils Emulsification

First, an emulsion is a mixture of two immiscible and suspended phases in a liquid state, often in the presence of a surfactant which acts as a stabiliser [200]. Based on this consideration, emulsion may find use for the enhancement of EO's stability.

Emulsions are primarily classified according to their particle diameter and their thermodynamic stability as macroemulsions, nanoemulsions or microemulsions. The nanoemulsion formulations of active substances, including EO, can be used to develop biodegradable coating to enhance both their quality and biological properties [204].

The preparation methods can be distinguished between high-energy (high-pressure or mechanical homogenisation and ultra-sonication) and low energy (divided into isothermal and thermal processes [205]) methods [204]. One should notice that the choice of both surfactant and appropriate formulation are key components in an efficient pathogen management [203].

Specifically, macroemulsions have a wider particle size and are susceptible to break down overtime because of destabilising factors (gravitational separation, flocculation, coalescence, Ostwald ripening [204,205]) and appear turbid because of the particle size which is similar to the light's wavelength. When it comes to nanoemulsions, they are metastable system as well, but the smaller droplet's size confers a better stability regarding gravitational and aggregation phenomena, which would be beneficial regarding EO stability. Regarding microemulsions, they share similar droplets' size with nanoemulsions, but they are thermodynamically stable [200,204,206]. By using nanoemulsions in particular, EO stability issues could be solved and the fine droplet size would also be beneficial by increasing cellular absorption and hence enhancing EO's biological properties [207]. Additionally, both nano- and microemulsions are o/w emulsions, consisting of three main components: oil phase dissolved in organic solvent, water and surfactants and cosurfactants in varying amounts [200]. Eventually, because of their nature and the preparation process, nanoemulsions require smaller amounts of surfactant and are of greater economic interest [200,204,205].

In brief, EO emulsions and in particular nanoemulsions could be of great interest in ensuring either a controlled release of the EO compounds and a better stability of the product or enhanced biological properties [203]. Several studies have already demonstrated higher efficiency [208–212], stability or even enhanced bioavailability [213,214] when EO were prepared in nanoemulsions.

1.4.2. Essential Oils Encapsulation

Encapsulation of the EO is another emerging technic with the potential to enhance EO stability and provide a controlled-release of the product [27,215,216].

There are different processes leading to the obtention of either micro- or nano-capsules. The basic concept of EO encapsulation is summarised as the process of surrounding a particle or molecule of interest with a coating or building a functional barrier between a core and wall material, so as to avoid physical and chemical reactions between the core and the outer molecules [175]. This technic aims in maintaining the properties of the core material (for example EO) such as biological, functional and physicochemical properties and avoid deterioration [27,175,216]. Two processes are mostly used: coacervation and spray-drying. The spray-drying process is a common encapsulation technic used on an industrial scale, which presents the advantages of producing microcapsules in a relatively simple process and that while being quite inexpensive in comparison with other encapsulation technics [175]. This process requires the use of an emulsion. It relies on the atomisation of EO emulsions into a drying chamber, at a relatively high temperature, leading to a very fast water evaporation and therefore a quasi-instantaneous entrapment of the EO in a fast-formed crust [175,217]. The coacervation process

is another widely used encapsulation technic. This technic relies on a phase separation: it involves an electrostatic attraction between biopolymers, which leads to a separation of a liquid phase from the polymer-rich phase, also called coacervate [175]. Coacervation is distinguished into two different processes, either simple or complex [175,218–220].

The encapsulation of EO in polymeric particles has been investigated: in oligomer particles such as cyclodextrins [221,222], in biopolymers [223,224] or in microparticles or microcapsules through complex coacervation [225–227]. Lavandin EO, successfully encapsulated in a biodegradable polymer, displayed a narrow particle size and an appropriate time-release curve favourable for a controlled release of the product [224]. Similarly, *Mentha x piperita* EO in the presence of cyclodextrins was found forming guest-host complexes, hence allowing to obtain a controlled release [38,221].

There is however a consequent downside during the process, as it is required to heat or evaporate during the encapsulation, which is risky when it comes to EO [27]. Encapsulation in liposomes, which are amphiphilic molecules able to ‘self-organise’ in layers in aqueous media, defining several aqueous compartments, is also feasible. These liposomes are commonly used as carriers for other molecules (either hydrophilic, lipophilic or even amphiphilic) in different compartments [228]. The encapsulation in solid lipid nanoparticles (SLN), which can be constituted by lipid or lipid-like molecules, such as waxes or triacylglycerols [27,229,230], is another advanced technology providing the same advantages as previously mentioned [231]. *Artemisia arborescens* EO encapsulated in SLN was demonstrated significantly more stable in comparison with the raw EO [229].

In brief, encapsulation would represent an innovative method to enhance EO stability and efficacy, by solving some of the downsides EO present when used raw. In particular, the issue regarding a reduction of the EO effects when applied in fields may be solved by means of controlled release through the EO encapsulation. The potential of nanotechnologies regarding EO encapsulation has already been reviewed [232]. Moreover, a few studies link above-cited properties with a potential use in agriculture, for either EO [223,224,229,233] or secondary metabolites in a broader sense [234].

2. Conclusions

In the recent years, a large number of studies have been focusing on EO as a source for new biopesticides. A relative effectiveness against pathogens, multiple mechanisms of action and a relatively low toxicity to mammals and human beings have in particular been highlighted [151,235]. However, a small number of them have been homologated and is permitted for use worldwide. This number of EO homologated in agriculture for various biocide usages (herbicide, fungicide and insecticide) as well as being usable as growth regulator, is remaining surprisingly scarce. This could be explained by the different constraints that EO are facing.

Tests including EO properties are in most cases a screening of the properties against one or several pathogens with a narrow screening spectrum [236] and more importantly tests are commonly run *in vitro*, only a few include a glasshouse in planta experiment at least and even scarcer is the number of *in situ* experiments. It is well-known that EO are facing an efficiency drop when used raw in the fields, mostly owing to their volatility. Yet, the fact that the interaction plant-pathogen-EO is not well studied or published leads to a lack of knowledge when it comes to the field, worsening the efficiency drop while not encouraging the use of EO-based products in biocontrol [196].

Because of their stability issues, EO may in addition be affected during either their storage or transport [7]. It should then be kept in mind that although the contact effect of some EO against pathogens is good, a fast volatilisation when used in agriculture could lead to a low persistence of the effect in time, giving more credit to the importance of EO formulation [235]. But once again, field applications’ reports remain scarce, which might be due to the recent emergence of the nano-emulsion technology that only appeared in the middle of the nineties [237] or to a possible lack of efficiency in the current state. Research should be able to provide insights towards the commercialisation of EO-based products at least as efficient as in controlled laboratory conditions, with an improved stability of the compounds under field conditions, yet there is still a lack of knowledge when it comes to the field. On a

societal aspect, it appears necessary to work on essential points so as to encourage EO's use and avoid understanding issues regarding in particular negative and false public perception (e.g., higher cost of the final product for the consumers, health risks due to the use of EO) as well as farmers concerns about the effectiveness of the products [8,190]. As an example, providing a risk assessment of EO's effects on non-target organisms would be beneficial, as well as demonstrating, under field conditions, the efficiency of EO, free or formulated.

Finally, the authorisations regarding biopesticides commercialisation (and synthetic pesticides even more) are granted through a very complex and onerous process [9,238]. These require in particular evidence from a certain number of tests, such as toxicological and environmental studies or efficiency. In many cases, studies on toxicological effects do not exist and are too expensive and time-consuming for local manufacturers for example [198,235]. One of the big constraint in commercialising EO products as biopesticides could then be a consequence of regulatory barriers [196]. Nonetheless, the current trend towards a reduction of synthetic pesticides' use as well as an alleviation of the approval procedure for low-risk substances might enable EO products to be developed and used worldwide.

One should keep in mind that biocontrol is not without its own risks as well [239]. In the present case with EO, the environmental risk is significantly lower, owing to the volatile nature of EO, which leads to a significant reduction in terms of persistency in comparison with synthetic pesticides. Non-target living organisms in the ecosystem may be less impacted, due to a minor residual activity of the EO and EO-based products used as biopesticides [196]. Yet, even though the aim of EO formulation is to provide enhanced stability and efficiency of EO and that coating materials are most the time not toxic for living organisms themselves, nanotechnologies could face undesirable effects, in particular because of the interaction with the highly reactive surface of several types of nanomaterials (especially those containing metallic nanoparticles [202,234]). A careful evaluation regarding their potent toxicity should then be carried out [234].

New emerging techniques such as EO formulation through emulsion or encapsulation might enable EO to appear on a wider scale, as a means to enhance both their biological activity and stability, with considerations however from an economic point of view. In that concern, both technics might represent innovative methods for EO to emerge on the market as viable biocontrol products. A feasible and gentle transition could be the use of EO, preferentially formulated according to the previous considerations, in a traditional pesticide crop management system, in complement with synthetic pesticides, allowing to reduce the amounts of pesticides used towards an integrated pest management system.

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IV. Le microbiote des sols pollués par les ET

1. Importance et diversité des communautés microbiennes du sol

La rhizosphère constitue une mince portion du sol qui se distingue du sol brut par l'influence que la racine exerce sur les organismes qui y sont présents (Bakker et al., 2013; Lee et al., 2019; Wang et al., 2017c). Ces différents compartiments sont un réservoir de diversité microbienne, comprenant notamment les bactéries, les champignons et les archées, interagissant entre eux et avec la racine de multiples façons (Lee et al., 2019; Li et al., 2017; Wang et al., 2017c). En raison de leur proximité avec la racine, le métagénome de ce microbiome est souvent décrit comme le deuxième génome de la plante (Berg et al., 2014; Dagher et al., 2019). Au sein du microbiome, les bactéries ainsi que les champignons constituent des acteurs essentiels au fonctionnement des sols et des écosystèmes, en raison des nombreuses fonctions et des services écosystémiques qu'ils remplissent (Bodelier, 2011; Delgado-Baquerizo et al., 2016; Saccá et al., 2017). Ils sont notamment impliqués dans les cycles biogéochimiques des nutriments, la dégradation de la matière organique, la séquestration du carbone, le maintien de la structure du sol ainsi que la dégradation de polluants organiques ou la détoxification de polluants organiques ou inorganiques (Ding et al., 2017; Saccá et al., 2017). Ils peuvent également contribuer à la croissance des plantes au sein des agroécosystèmes, par la production de composés organiques, la formation d'associations symbiotiques bénéfiques pour la plante hôte ou encore en réduisant l'impact des stress environnementaux, biotiques ou abiotiques, sur le développement des espèces végétales (Bakker et al., 2013; Ding et al., 2017; Saccá et al., 2017). Enfin, en vertu des nombreuses fonctions qu'elles exercent, les communautés microbiennes du sol peuvent servir d'indicateurs de fertilité et de fonctionnement du sol (Ding et al., 2017; Saccá et al., 2017).

Leur abondance au sein du sol rhizosphérique, évaluée par PCR quantitative, a été estimée à 10^8 copies de gènes par gramme de sol pour les bactéries et varie de 10^5 à 10^6 copies de gènes pour les champignons (Lee et al., 2019; Rousk et al., 2010). Ces communautés microbiennes sont principalement représentées par les embranchements bactériens *Proteobacteria*, *Acidobacteria* et *Actinobacteria* (Navarro-Noya et al., 2010; Pacwa-Plóciniczak et al., 2018; Pereira et al., 2015) et par les embranchements fongiques *Ascomycota* et *Basidiomycota* (Bourceret et al., 2016; Dagher et al., 2019; Liang et al., 2020).

2. Les champignons mycorhiziens à arbuscules (CMA)

Au sein des écosystèmes terrestres, la symbiose mycorhizienne (du grec « *mukes* », signifiant champignon et « *rhiza* », racine) formée entre les champignons mycorhiziens à arbuscules (CMA) appartenant au phylum des *Glomeromycota* (Schüßler et al., 2001) et la majorité des végétaux supérieurs, est l'une des associations symbiotiques les plus répandues (Muneer et al., 2019; Smith and Read, 2008). Elle est également l'une des plus anciennes, datant de plus de 400 millions d'années, et ayant probablement permis la colonisation des terres émergées par les végétaux supérieurs (Redecker, 2002). Elle témoigne ainsi de la coévolution entre les CMA et les plantes et concerne à l'heure actuelle plus de 80 % des plantes vasculaires terrestres, dont la majorité des angiospermes et des gymnospermes, à l'exception des Pinacées et des Brassicacées (Bonfante and Genre, 2008; Smith and Read, 2008). Les CMA sont aussi ubiquistes et répartis sur l'ensemble du globe, au sein d'écosystèmes variés (Bonfante and Genre, 2008).

2.1. Cycle de développement des CMA

Le cycle de développement des CMA comprend cinq stades (Figure 8) (Akiyama, 2007) :

Stade 1 : Germination des spores (après sortie de dormance) et émergence d'un mycélium primaire, également appelé pro-mycélium, en direction de la racine.

Stade 2 : Contact entre le mycélium et la racine avec la formation d'un hyphopode.

Stade 3 : Pénétration du CMA au sein de la racine, où il se ramifie. Lorsque le mycélium atteint la partie la plus interne du cortex racinaire, il forme des arbuscules.

Stade 4 : Après différenciation des structures fongiques intra-racinaire, le CMA établit un réseau extra-racinaire à partir duquel seront différenciées de nouvelles spores.

Stade 5 : Une fois à maturité, les spores sont libérées. En germant, elles seront à l'origine d'un nouveau pro-mycélium.

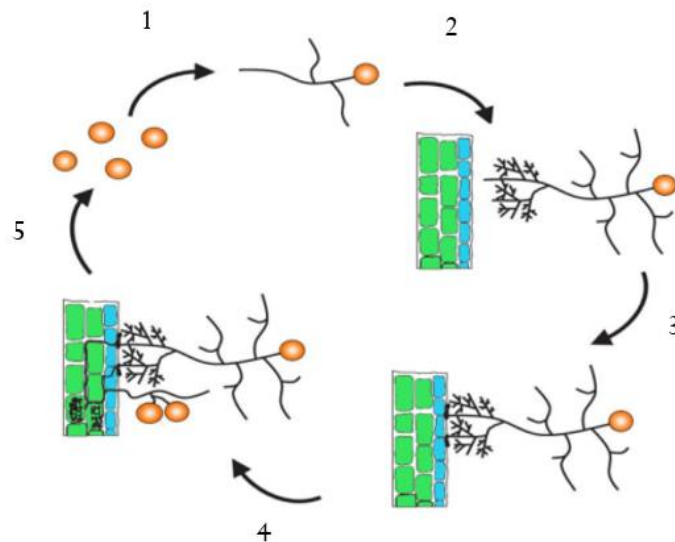


Figure 8: Cycle de développement des CMA (Akiyama, 2007).

2.1.1. Phases a-symbiotique et pré-symbiotique

Les spores de CMA, une fois sorties de dormance, peuvent germer même en l'absence de stimulus exogène. Toutefois, la perception de signaux chimiques diffusibles provenant d'un potentiel partenaire végétal stimule la germination pour favoriser le contact entre les deux partenaires et l'établissement de la symbiose (Smith and Read, 2008). En l'absence de plante hôte après 2 à 4 semaines de croissance, le mycélium issu de la germination de la spore de CMA bloque sa croissance (Smith and Read, 2008). Le CMA ne pouvant pas compléter son cycle de vie est bloqué dans une phase qualifiée « d'asymbiotique » : les ressources cessent d'être mobilisées pour le développement des hyphes, dont le cytoplasme se rétracte, et les spores entrent à nouveau en dormance, dans l'attente de conditions plus favorables (Smith and Read, 2008).

En présence d'un hôte végétal, lorsqu'un signal est perçu par le CMA, le pro-mycélium issu de la germination de la spore va être émis en direction de la racine du partenaire végétal et va se ramifier de manière importante. Cette phase est qualifiée de « pré-symbiotique ». Elle est conditionnée par

l'établissement d'un dialogue moléculaire entre les deux partenaires, préalable à l'établissement de la symbiose (Genre, 2012; Smith and Read, 2008). Avant même le premier contact entre les deux partenaires, le CMA détecte la présence de la plante hôte grâce à l'émission d'exsudats racinaires, sous la forme de strigolactones (Figure 9) (Besserer et al., 2006; Genre, 2012). Ces exsudats sont capables d'activer l'activité métabolique chez le CMA, notamment énergétique, en stimulant l'activité mitochondriale et en favorisant la ramification du pro-mycélium (Besserer et al., 2006). De son côté, l'hôte végétal perçoit également des signaux diffusibles sécrétés par le champignon, modifiant l'expression de ses gènes et son métabolisme et conduisant notamment à la ramification des racines et à l'accumulation de glucides dans les cellules racinaires (Genre, 2012). Il a été établi que des lipochitoooligosaccharides (facteurs Myc) étaient à l'origine de ces signaux et jouaient un rôle important dans la préparation de la racine à la colonisation par le CMA (Maillet et al., 2011). Une fois les premiers signaux diffusibles échangés entre les deux partenaires, une régulation génétique s'établit afin de préparer l'établissement de la symbiose.

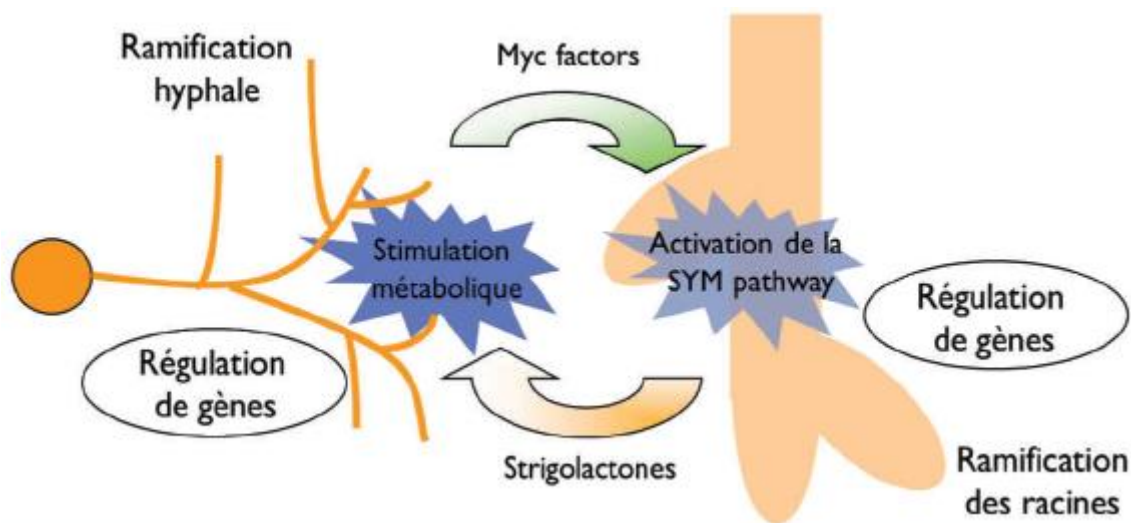


Figure 9: Dialogue moléculaire pré-symbiotique (Genre, 2012). Ce schéma synthétise les principaux signaux moléculaires préalables à la mise en place de la symbiose mycorhizienne. Les strigolactones libérées par la plante hôte activent le métabolisme fongique, stimulent la ramification du mycélium et induisent la régulation de certains gènes chez le CMA. En réponse, la perception par la plante des facteurs « Myc » (lipochitoooligosaccharides) entraîne une induction de la voie de signalisation SYM, à l'origine de l'induction de différents gènes et de la formation de racines latérales.

2.1.2. Phase symbiotique

Au contact de l'épiderme racinaire, l'hyphes différencie une structure appelée « hyphopode ». Cette structure constitue un point d'ancrage pour le champignon sur la racine, mais ne permet pas la pénétration du champignon dans la racine (Genre and Bonfante, 2012). Dans la cellule épidermique végétale située sous l'hyphopode, une réorganisation des organites et en particulier du cytosquelette conduit à la formation d'un appareil de pré-pénétration (Bonfante and Genre, 2008). Ce dernier contrôle la pénétration du CMA au sein de la racine, grâce à la formation d'un pont apoplasmique endocellulaire, permettant le passage du champignon de l'épiderme jusqu'au cortex interne (Bonfante and Genre, 2008; Genre, 2012; Voß et al., 2018).

La phase symbiotique à proprement parler se caractérise par la colonisation des cellules du cortex interne par le mycélium ramifié. Au niveau des cellules corticales, le champignon pénètre sans toutefois traverser la membrane plasmique, formant ainsi les arbuscules, structures hyper-ramifiées, fonctionnelles et caractéristiques de la symbiose mycorhizienne arbusculaire (Voß et al., 2018; Wipf et

al., 2019). Une membrane péri-arbusculaire, dérivée de la membrane plasmique des cellules corticales, sépare l'arbuscule fongique du cytoplasme végétal. C'est au niveau des arbuscules qu'ont lieu les échanges entre les deux partenaires symbiotiques, au travers de la membrane péri-arbusculaire, impliquant notamment des transporteurs spécifiques tels que des transporteurs de phosphate, d'azote, de potassium, des aquaporines ainsi que des transporteurs de sucres (Wipf et al., 2019).

Certains CMA forment également des organes de stockage riches en lipides et en glycolipides, appelés vésicules (Smith and Read, 2008).

2.2. Bénéfices de la symbiose mycorhizienne

2.2.1. Bénéfices pour le CMA

Les CMA sont des biotrophes obligatoires car ils sont incapables de réaliser la photosynthèse et sont par conséquent hétérotrophes pour le carbone (Begum et al., 2019; Finlay, 2008). Ainsi, l'association symbiotique entre les deux partenaires, végétal et fongiques, implique la circulation de composés carbonés issus de la photosynthèse depuis l'hôte végétal vers le champignon et en retour des composés minéraux et de l'eau, du CMA vers la plante (Smith and Read, 2008). Il a notamment été estimé que le transfert de photosynthétats carbonés de la plante hôte vers le CMA pouvait représenter entre 4 et 25 % du carbone fixé par la plante lors de la réalisation de la photosynthèse (Wipf et al., 2019).

2.2.2. Bénéfices pour la plante hôte

Les bénéfices pour l'hôte végétal peuvent être regroupés sous quatre grandes thématiques, synthétisées dans la Figure 10 :

- Amélioration de la nutrition minérale et hydrique
- Amélioration de la structure du sol
- Amélioration de la tolérance aux stress biotiques
- Amélioration de la tolérance aux stress abiotiques

2.2.3. Bénéfices dans le cas du phytomanagement des sols pollués par les ET

L'ensemble des contributions des CMA dans le cadre du phytomanagement des sols pollués par les ET a été détaillé dans la revue 1 précédemment présentée : « Recent advances in microbial aided phytostabilisation of trace element contaminated soils ».

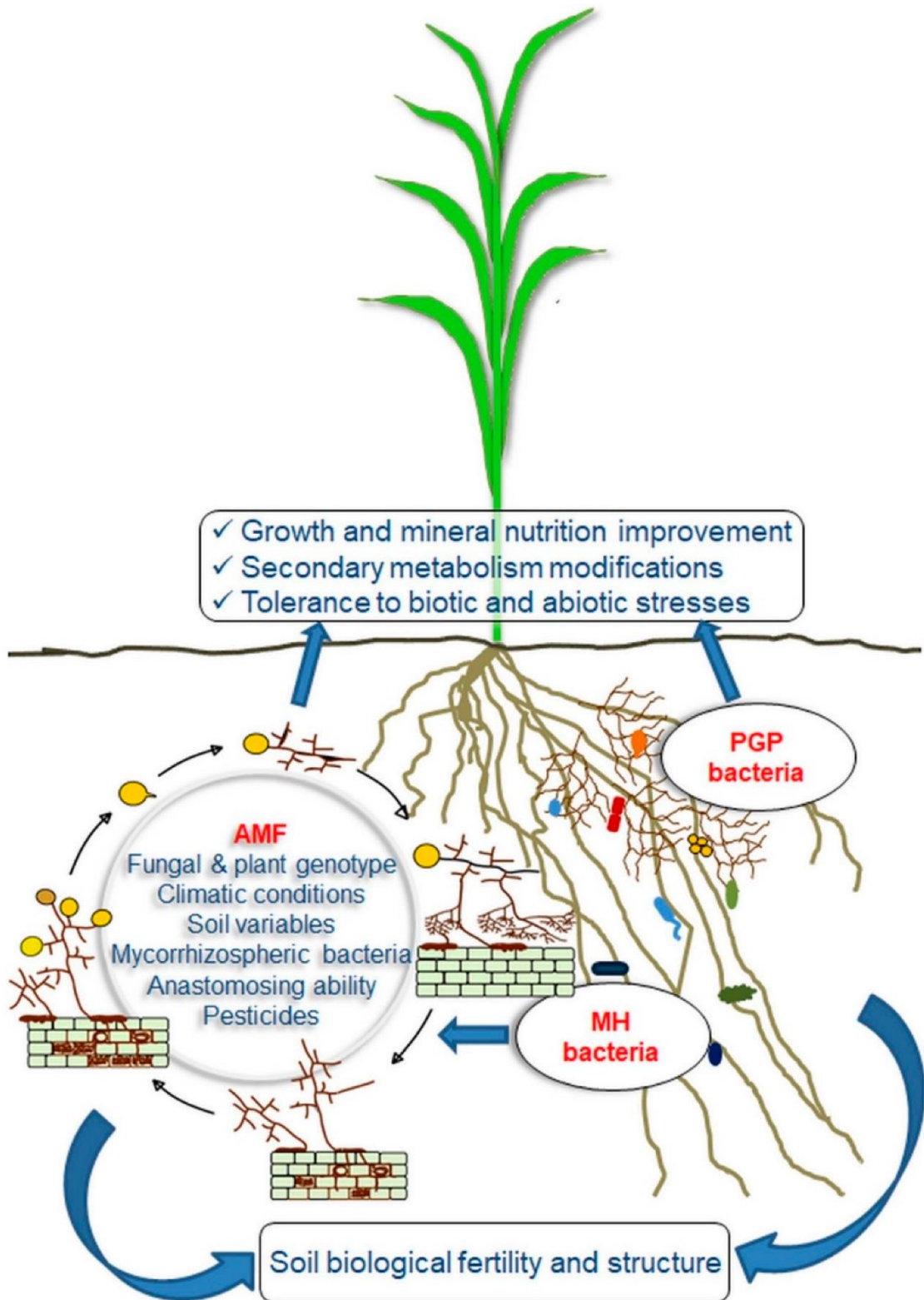


Figure 10: Représentation schématique des bénéfices de la symbiose mycorhizienne et de bactéries bénéfiques du sol sur la fertilité du sol et les performances de la plante (Giovannini et al., 2020).

3. Facteurs responsables de la variation de la structure des communautés microbiennes du sol

L'environnement souterrain est soumis en permanence à des perturbations susceptibles de modifier profondément la structure des communautés microbiennes et leur évolution dans le temps (Delgado-Baquerizo et al., 2016). Si de nombreux travaux ont démontré la résilience et la capacité d'adaptation de ces communautés aux variations de leur environnement (Dagher et al., 2019; Lin et al., 2019; Xue et al., 2018), divers facteurs d'origine écologique (édaphique ou environnementale) ou anthropique sont toutefois susceptibles de faire varier la biomasse microbienne, l'abondance relative des différents phyla et donc la structure de ces communautés, et par conséquent de perturber le fonctionnement des écosystèmes (Ding et al., 2017; Lin et al., 2019; Saccá et al., 2017). Toutefois, il est établi que la réponse des communautés microbiennes aux différents facteurs de variabilité dépend fortement du contexte et varie significativement d'un habitat à l'autre (Bottos et al., 2020; Chen et al., 2016). De plus, plusieurs études suggèrent que les communautés microbiennes sont influencées non pas par un unique facteur mais par leur combinaison (Berg and Smalla, 2009; Chen et al., 2016; Wang et al., 2020).

VI. 3. 1. Facteurs abiotiques

La structure des communautés microbiennes est notamment façonnée par de nombreux facteurs abiotiques. En premier lieu, les paramètres physico-chimiques ainsi que la texture du sol sont largement décrits pour leur influence sur les communautés microbiennes du sol (Bottos et al., 2020). Parmi ceux-ci, le pH est d'importance majeure (Bottos et al., 2020; Wang et al., 2019b). En effet, il est susceptible de modifier la spéciation de certains éléments dans le sol, tels que les nutriments ou les ions métalliques, et ainsi d'en modifier la biodisponibilité (Huang et al., 2017; Neina, 2019). Les variations de pH extrêmes peuvent également imposer un état de stress à certains organismes, dû à des contraintes physiologiques notamment, et favorisant ainsi le développement de certains taxa capables de tolérer ou de s'adapter à ces conditions inhabituelles aux dépens d'autres dont le développement est inhibé (Quatrini and Johnson, 2018; Yun et al., 2016). Malgré la capacité de certaines bactéries à se développer dans des conditions extrêmes de pH (acidophiles et alcalinophiles), la majorité s'épanouit dans une gamme de pH autour de la neutralité et a été démontrée plus sensible aux variations de pH que les champignons (Merino et al., 2019; Rousk et al., 2010; Singh et al., 2009).

La pollution du sol, par des polluants organiques ou inorganiques, conduit à court terme à une diminution de la biomasse, de la diversité et de la richesse microbienne (Bourceret et al., 2016; Ding et al., 2017; Lin et al., 2019). Ces changements affectent la structure des communautés microbiennes ainsi que leur diversité fonctionnelle (Ding et al., 2017). La toxicité des ET envers les populations microbiennes entraîne notamment une diminution des populations sensibles et une augmentation des populations tolérantes et résistantes à cette pollution (Bourceret et al., 2016; Ding et al., 2017; Giller et al., 2009). A titre d'exemple, une concentration importante d'hydrocarbures dans les sols affecte également significativement les communautés bactériennes et fongiques saprotrophes, notamment celles associées aux CMA (Iffis et al., 2016, 2017). Les travaux de Bourceret et al., (2016), conduits en parallèle sur des sols historiquement contaminés par les ET et par des hydrocarbures aromatiques polycycliques, démontrent que la richesse et la diversité des espèces microbiennes augmente au cours du temps. A plus long terme, ces conditions environnementales particulières permettent donc à certaines communautés microbiennes de s'adapter, en favorisant les microorganismes capables de tolérer la présence des polluants, voire d'utiliser les polluants organiques en tant que source de carbone et d'énergie (Bourceret et al., 2016; Peng et al., 2008). La disponibilité des nutriments est aussi

un facteur de variabilité entre les différentes communautés, bactériennes ou fongiques, mais également au sein de ces communautés. En effet, il a été suggéré qu'une faible disponibilité en nutriments pouvait favoriser le développement des champignons au détriment des bactéries (Chen et al., 2016). Certains phyla bactériens, comme les *Betaproteobacteria* ou les *Bacteroidetes*, se développeront en effet lentement et difficilement dans des milieux appauvris en nutriments en raison de leurs besoins nutritifs importants, alors qu'ils auront une cinétique de croissance très rapide sur un milieu riche (Fierer et al., 2007). A l'inverse, les bactéries oligotrophes, telles que le phylum des *Acidobacteria*, présentent une cinétique de croissance relativement lente, leur permettant de s'adapter à des milieux pauvres en nutriments (Fierer et al., 2007).

Parmi les facteurs abiotiques, l'humidité du sol ainsi que la température structurent également fortement les communautés bactériennes notamment (Singh et al., 2009; Wang et al., 2020). Rasche et al., (2011) ont par exemple rapporté dans un sol forestier en milieu tempéré que le phylum des *Actinobacteria* était particulièrement sensible aux variations saisonnières de températures et d'humidité dans les sols, accompagnées de changements dans la disponibilité des ressources en carbone et en azote. Certains phyla bactériens, tels que les *Proteobacteria*, ont aussi montré une forte corrélation avec l'humidité du sol (Zhang et al., 2013).

En complément des facteurs précédemment cités, la structure des communautés microbienne varie également avec la profondeur du sol (Steven et al., 2013; Wang et al., 2018). En effet, des variations en termes d'abondance, de structure et de diversité des communautés bactériennes et fongiques peuvent être observées en fonction de l'horizon de sol (Wang et al., 2018). Notamment, la disponibilité de la matière organique au sein de l'horizon de surface, fortement dépendante de la végétalisation du sol et du type de végétation, favoriserait les phyla bactériens ou fongiques impliqués dans les processus de dégradation et de minéralisation de la matière organique (Su et al., 2020; Wu et al., 2018). A l'inverse, les espèces oligotrophes seraient favorisées en profondeur, où la disponibilité des nutriments est plus limitée (Bourceret et al., 2016).

Si l'effet des conditions climatiques est contrasté et dénote une forte dépendance au contexte du site et souvent un effet moindre par rapport aux paramètres édaphiques, il n'est toutefois pas à négliger (Chen et al., 2016; Wang et al., 2020). L'influence du rythme saisonnier et des variations climatiques qui l'accompagnent sur les communautés fongiques en particulier a par exemple été mis en avant (Davey et al., 2012; Rasche et al., 2011).

VI. 3. 2. Facteurs biotiques

Les facteurs biotiques sont aussi connus pour exercer une pression significative sur la structure ainsi que sur la distribution écologique des communautés microbiennes, par effet direct ou en interaction avec les facteurs abiotiques précédemment mentionnés (Wang et al., 2020). Les plantes notamment peuvent avoir une influence identique voire supérieure à celle exercée par les facteurs abiotiques, aussi bien dans le fonctionnement des écosystèmes que dans la structure des communautés microbiennes présentes dans le sol (Singh et al., 2009). La végétalisation du sol augmente par exemple l'humidité relative du sol, la quantité de biomasse ainsi que la respiration microbienne (Singh et al., 2009), facteurs ayant un rôle dans la modulation des communautés microbiennes. Outre la végétalisation, c'est également l'identité et la diversité des espèces végétales qui jouent un rôle structurant sur les communautés microbiennes, bactériennes ou fongiques (Dagher et al., 2019; Iffis et al., 2017; Wang et al., 2018).

En premier lieu, l'architecture du système racinaire diffère fortement selon l'espèce végétale et est sous l'influence de nombreux paramètres édaphiques, tels que la nature et la texture du sol, son état de compaction, la disponibilité des nutriments et de l'eau (Iannucci et al., 2021; Saleem et al., 2018). Les racines constituant l'interface primaire de contact entre la plante et le sol, de leur architecture dépend donc le volume de sol exploré et les interactions possibles entre la plante, le sol et les microorganismes (Iannucci et al., 2021).

De nombreux travaux ont aussi démontré l'importance des exsudats racinaires, dont la nature et la quantité peut varier, dans la sélection de communautés microbiennes spécifiques par les plantes (Wang et al., 2018). Les microorganismes du sol sont attirés par chimiotactisme par les exsudats racinaires (Bakker et al., 2013). La nature de ces derniers, qui peuvent notamment constituer une source de carbone, induit une compétition au sein des communautés microbiennes présentes (Backer et al., 2018; Chen et al., 2019). Ils peuvent également avoir le rôle de signaux chimiques, impliqués dans le recrutement spécifique de certaines espèces et notamment dans la formation d'associations symbiotiques, telles que la symbiose mycorhizienne ou rhizobiale (Backer et al., 2018; Berg and Smalla, 2009; Chen et al., 2019). La composition de ces exsudats racinaires est très variée, constituée d'acides aminés, phénoliques ou organiques, de polysaccharides de haut poids moléculaire, de vitamines, de glucides simples ou encore d'enzymes (Chen et al., 2019; Montiel-Rozas et al., 2016; Wu et al., 2015). Etant donné que la qualité des exsudats racinaires varie en fonction des espèces végétales, des différences en termes de communautés microbiennes sont à prévoir (Bakker et al., 2013). En comparaison avec le sol brut, le sol rhizosphérique contient un nombre de microorganismes bien plus important, notamment en raison de la libération des exsudats racinaires dans cette portion du sol (Bakker et al., 2013). Au-delà de la biomasse microbienne qui est plus importante, les activités microbiennes sont également plus élevées au niveau de la rhizosphère, en comparaison avec un sol non rhizosphérique (Bakker et al., 2013).

Il est intéressant de noter que la communauté fongique est plus fortement influencée par les facteurs biotiques, ce qui pourrait être attribué à la forte dépendance de celle-ci vis-à-vis de la litière et de la rhizodéposition (Wang et al., 2020).

VI. 3. 3. Facteurs d'origine anthropique

L'action de l'homme contribue aussi grandement à la perturbation de l'équilibre des sols et de leur microbiote. L'usage consacré aux sols, les pratiques culturales telles que la préparation mécanique du sol, ainsi que l'usage intensif d'intrants agricoles, ont des conséquences importantes sur la structure et l'évolution du microbiote (Chen et al., 2016; Girvan et al., 2003; Wang et al., 2018). Les pratiques culturales intensives et le passage d'engins agricoles sont notamment décrits pour leur rôle dans la compaction du sol, la perturbation de ses propriétés physico-chimiques et de sa structure ainsi que dans la perturbation des communautés microbiennes présentes (Chen et al., 2016; Girvan et al., 2003).

Les communautés microbiennes du sol sont également sensibles à l'apport de fertilisants azotés, qui réduisent drastiquement la biomasse ainsi que la respiration microbienne du sol (Leff et al., 2015). Certains groupes fonctionnels, tels que les CMA ou les bactéries nitrifiantes, sont notamment souvent décrits comme étant particulièrement sensibles à une augmentation de la quantité d'azote dans le sol (Leff et al., 2015). Une teneur élevée en phosphate dans les sols est aussi largement décrite comme limitant l'établissement de la symbiose mycorhizienne (Begum et al., 2019; Leff et al., 2015).

V. Les plantes à parfum, aromatiques et médicinales (PPAM) utilisées dans le cadre de cette thèse

Au sein du règne végétal, les PPAM comportent une grande variété d'espèces cultivées ou sauvages. En raison de leurs propriétés intéressantes, les PPAM ont de nombreuses utilisations possibles, dans des secteurs tels que la parfumerie, l'agro-alimentaire, les produits cosmétiques et la pharmaceutique. Le secteur des PPAM se divise ainsi en trois branches complémentaires, en fonction de leurs usages : la filière des plantes à parfum, celle des plantes aromatiques et condimentaires et enfin, la filière des plantes médicinales.

Trois PPAM ont été testées dans le cadre de ce travail :

1. Sauge sclarée

La sauge sclarée, *Salvia sclarea* L., appartient à la famille des Lamiacées. Cette famille est l'une des plus importantes parmi les plantes à fleurs et contient un grand nombre d'espèces aromatiques attrayantes (Sim et al., 2019; Singh and Pandey, 2018). Cette espèce végétale herbacée est originaire du bassin méditerranéen et y est toujours cultivée (Angelova et al., 2016; Tibaldi et al., 2010). Cette plante bisannuelle apprécie les environnements tempérés et possède un caractère xérophytique (Tibaldi et al., 2010). Pouvant atteindre jusqu'à 2 mètres de hauteur, la sauge sclarée présente une tige dressée avec de grandes feuilles opposées (Zutic et al., 2016). La première année, seule une rosette basale proche du sol est visible (Figure 11A.) (Zutic et al., 2016). La tige et l'inflorescence, sommité fleurie composés de fleurs blanches ou violettes (Figure 11B.), n'apparaissent que lors de la deuxième année de culture (Tibaldi et al., 2010). L'ensemble de la plante et en particulier l'inflorescence possède un fort parfum aromatique (Tibaldi et al., 2010).

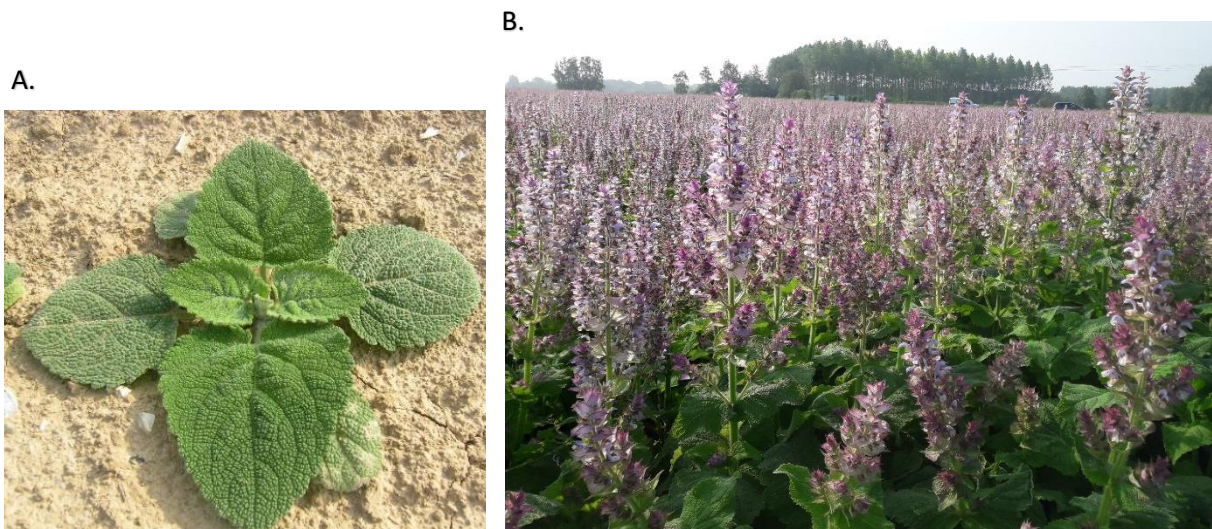


Figure 11: Photographies de la sauge sclarée – rosette basale visible en première année de culture (A.) et sommités fleuries lors de la deuxième année de culture (B.). Photographes : Robin Raveau et Anissa Lounès-Hadj Sahraoui.

La sauge sclarée est avant tout cultivée pour son HE (Grigoriadou et al., 2020; Zutic et al., 2016) et pour le sclaréol, un diterpène produit par les organes floraux de la plante et utilisé en parfumerie pour sa capacité à fixer les arômes (Grigoriadou et al., 2020). L'HE traditionnelle est obtenue par hydrodistillation ou entraînement à la vapeur des sommités fleuries, avec des rendements variant entre 0,01 et 0,4 % (Aćimović et al., 2018; Kuzma et al., 2009). Elle est notamment utilisée en agro-

alimentaire pour parfumer certaines boissons, en parfumerie et cosmétique et est aussi connue pour ses vastes propriétés antiseptiques, anti-inflammatoires et apaisantes (Angelova et al., 2016; Tibaldi et al., 2010).

La sauge sclarée a également été évaluée dans le cadre d'approches de phytoremédiation (Angelova et al., 2016) ou d'essais visant à évaluer la capacité de la sauge à se développer et à accumuler les ET (Chand et al., 2015). Toutefois, les résultats obtenus, s'ils démontrent le potentiel de la sauge sclarée à se développer en conditions polluées par les ET, sont contrastés quant à la capacité d'accumulation ou de stabilisation de la sauge et ne permettent pas de statuer sur son comportement.

2. Angélique

L'angélique (*Angelica archangelica* L.) est une PPAM originaire du nord de l'Europe, appartenant à la famille des *Apiacées* (Chauhan et al., 2016). L'angélique est une plante pérenne de très grande taille, pouvant atteindre jusqu'à 250 cm de hauteur (Chauhan et al., 2016; Joshi, 2016). Les graines possèdent un pouvoir germinatif relativement faible, mais germent dès que les conditions sont favorables, permettant de cultiver l'angélique sur des cycles relativement longs, pouvant atteindre jusqu'à 10 ans (Rautio et al., 2016). De plus, cette espèce végétale est capable de se développer même dans des sols très pauvres (Chauhan et al., 2016). Ses racines sont épaisses et présentent une coloration intérieure blanche voire jaune clair (Bhat et al., 2011). A l'extrémité de la tige, de couleur rougeâtre, l'inflorescence est une ombelle composée, portant 20 à 40 fleurs de petite taille et de couleur blanche ou jaune verdâtre (Figure 12) (Ghedira and Goetz, 2014; Joshi, 2016). La période de floraison s'étend de juillet à août mais peut varier en fonction de la localisation géographique (Pop et al., 2011; Teixidor-Toneu et al., 2020).

A.



B.



Figure 12: Photographies de l'ombelle composée en fleurs (A.) et à la maturité des graines (B.). Photographes : Robin Raveau et Anissa Lounès-Hadj Sahraoui.

Pendant son cycle de développement, l'angélique émet un parfum caractéristique agréable, émanant aussi bien des parties aériennes que souterraines. La plante est principalement cultivée pour sa racine aromatique, valorisée pour les traitements de divers soucis de santé (migraines, bronchites, fatigue chronique, etc), en alimentaire et dans la fabrication de liqueurs (Chalcha and Garry, 1997; Chauhan et al., 2016). Elle est aussi recherchée pour son HE, produite dans l'ensemble des organes de la plante (Roslon et al., 2016). Toutefois, les HE provenant des racines et des graines sont celles affichant le plus de valeur. Il est à noter également que les graines contiennent en proportion davantage d'HE, de l'ordre de 0,6 à 1,5 %, en comparaison des 0,5 à 1 % affichés pour la racine (Sowndhararajan et al., 2017; Zitterl-Eglseer et al., 2015). Les graines peuvent être récoltées à partir de la deuxième année, avec un rendement affiché pouvant varier de 1 à 10 tonnes par hectare, sur des sols agricoles non pollués (Galambosi et al., 2002).

3. Coriandre

Coriandrum sativum L., plus communément connue sous le nom de coriandre, est une plante annuelle herbacée appartenant à la famille des *Apiacées* (Bhuiyan et al., 2009; Freires et al., 2014). Native du bassin méditerranéen, elle est largement cultivée à travers le monde pour la consommation de ses feuilles en tant qu'herbes aromatiques ou pour ses graines, utilisées comme épices (Bhuiyan et al., 2009; Matasyoh et al., 2009; Sharma and Sharma, 2012). De petite taille, atteignant communément 30 à 40 cm de hauteur, elle possède un cycle de développement rapide, fleurissant 45 à 60 jours après semis et à maturité au bout de 65 à 120 jours (Mandal and Mandal, 2015; Sharma and Sharma, 2012). Les feuilles les plus élevées sont fines et délicatement divisées, tandis que les feuilles plus basses sont plus larges, non divisées et à trois lobes (Figure 13A.). Chaque tige se termine en ombelle, portant chacune 10 à 50 fleurs, souvent de couleur blanche (Figure 13B.) (Sharma and Sharma, 2012).

A.



B.



Figure 13: Photographies de la coriandre lors de la croissance des parties aériennes (A.) puis à la floraison (B.). Photographes : Robin Raveau et Anissa Lounès-Hadj Sahraoui.

La coriandre est également connue pour son HE, traditionnellement extraite par hydrodistillation à partir des graines de coriandre, avec des rendements de l'ordre de 0,4 à 0,6 % (Bandoni et al., 1998; Bhuiyan et al., 2009; Sharma and Sharma, 2012). Toutefois, l'HE peut aussi être distillée à partir des parties aériennes de la coriandre, avec des rendements plus faibles variant entre 0,1 et 0,3 %, et

possédant une composition chimique ainsi que des propriétés biologiques distinctes (Bhuiyan et al., 2009; Freires et al., 2014). Historiquement utilisée en tant qu'arôme en agro-alimentaire, l'HE de coriandre graines est aussi utilisée en aromathérapie pour ses propriétés médicinales (analgésique, digestive, antispasmodique et anti-rhumatique) et est reconnue pour ses propriétés antimicrobiennes et antioxydantes (Ghobadi and Ghobadi, 2010; Mandal and Mandal, 2015; Shahwar et al., 2012). La coriandre a également été évaluée, parmi d'autres plantes aromatiques telles que l'aneth, le basilic ou la camomille, pour sa capacité à se développer sur un milieu pollué par des ET (Zheljazkov et al., 2008). Les teneurs en ET retrouvées dans les parties aériennes de la coriandre étaient notamment parmi les plus faibles mesurées au sein des plantes testées, à l'état de traces (9 ppm de Cd, 144 ppm de Pb, 21 ppm de Cu et 80 ppm de Mn). De plus, aucune information n'a été rapportée dans la littérature quant à son utilisation dans la gestion des sites et sols pollués.

Problématique
et
objectifs

Problématique et objectifs

Au travers de cette analyse bibliographique, nous avons montré que les sites pollués, notamment par les ET, sont nombreux à l'échelle nationale et que dans un contexte de forte pression démographique et de raréfaction des sols, la requalification des sites pollués représente un enjeu important pour la santé humaine et pour la préservation de l'environnement. Le choix de la méthode de réhabilitation la mieux appropriée pour leur reconquête représente donc un défi avec des enjeux multiples, à la fois technique, économique, sanitaire, environnemental et social. Dans ce cadre, le phytomanagement, offrant la perspective d'une gestion globale du site, en combinant l'utilisation de plantes pour immobiliser les polluants du sol à la valorisation de la biomasse produite, semble être une option pertinente. Parmi les filières non-alimentaires de valorisation de la biomasse obtenue sur sols pollués, la production de substances biosourcées à haute valeur ajoutée permettrait d'abaisser les coûts globaux de gestion et valoriserait des ressources en sol par ailleurs délaissées. Ainsi, la culture de PPAM, telles que la coriandre, la sauge sclarée et l'angélique pourrait apporter des réponses adéquates en termes de valorisation de produits à hautes valeurs ajoutées telles que les HE et d'immobilisation des ET dans le sol. Toutefois, à notre connaissance, le potentiel de ces PPAM dans le cadre d'une filière de phytomanagement de sols pollués par les ET n'a pas été étudié auparavant.

Par ailleurs, la qualité biologique des sols, qui fait référence à l'abondance, à la diversité et à l'activité des organismes vivants, est généralement affectée dans les sols fortement anthropisés tels que les sols agricoles et de surcroît pollués par les ET (Chen et al., 2016; Ding et al., 2017; Wang et al., 2018). Or, le microbiote du sol qui représente l'une des composantes majeures de la vie des sols, joue un rôle fondamental dans le fonctionnement des sols et notamment dans la dégradation de la matière organique, le recyclage des nutriments (carbone, azote, phosphore...) ou encore la stabilisation des polluants inorganiques (Delgado-Baquerizo et al., 2016; Saccá et al., 2017). Les communautés microbiennes du sol seraient également fortement influencées par la présence d'un couvert végétal (Dagher et al., 2019; Singh et al., 2009). Par conséquent, la revégétalisation du sol par la culture de PPAM pourrait contribuer à la refonctionnalisation de ces sols. Cependant, les effets de la revégétalisation dans le cas de cultures de PPAM ont été très peu étudiés et restent donc méconnus, en particulier dans un contexte de pollution des sols par les ET.

L'objectif général de mon projet de thèse, qui s'inscrit dans une démarche de phytomanagement, consiste donc à évaluer la faisabilité technique et économique de la filière « culture de PPAM/production d'HE » (filière PPAM-HE) ainsi que son impact sur le microbiote rhizosphérique. Il s'appuie sur des expérimentations *in situ* conduites sur deux parcelles agricoles (à l'échelle de l'hectare) polluées ou non par les ET.

Ce travail s'est articulé autour de trois grands axes :

1. Evaluer la faisabilité technico-économique de la filière PPAM-HE en testant trois plantes aromatiques, la coriandre, la sauge sclarée et l'angélique, cultivées sur un sol pollué par les ET.

- ⇒ Quelles sont les caractéristiques pédo-agronomiques des parcelles expérimentales, leur niveau de contamination et leurs caractéristiques biologiques (inventaire phytosociologique et microbiologique) ?
- ⇒ Sur quels critères repose le choix des PPAM à tester ?
- ⇒ Les trois espèces végétales choisies sont-elles capables de se développer sur un sol pollué par les ET ?

- ⇒ La présence d'ET dans le sol affecte-elle la production de biomasse, les rendements et la qualité des HE produites ?
- ⇒ L'introduction d'un amendement biologique à base de CMA influence-t-elle la croissance des PPAM, la production de biomasse, les rendements et la qualité des HE produites ?
- ⇒ Est-ce que la filière PPAM-HE sur sols pollués est viable d'un point de vue économique ?

2. Déterminer les compositions chimiques et les propriétés biologiques des HE distillées à partir des PPAM testées afin d'évaluer le potentiel de la filière PPAM-HE comme nouvelle piste de valorisation de la biomasse produite sur sols pollués.

- ⇒ Quelle est la composition des HE issues des différentes PPAM et/ou organes ?
- ⇒ Est-ce que la pollution des sols et l'inoculation mycorhizienne influencent la qualité des HE (composition chimique, contamination éventuelle) ?
- ⇒ Les HE produites à partir des différentes PPAM testées présentent-elles des propriétés biologiques applicables dans le domaine de la protection des cultures et/ou de la santé humaine ?
- ⇒ Est-ce que l'encapsulation des HE par la β -CD peut améliorer les propriétés biologiques de ces HE ?

3. Etudier, par une approche de métagénomique (Métabarcoding environnemental par séquençage Illumina Miseq), l'impact de la culture de PPAM sur un sol pollué par les ET et de l'inoculation mycorhizienne, sur la structure des communautés microbiennes du sol rhizosphérique et des racines.

- ⇒ Quelles sont les communautés rhizosphériques bactérienne, fongique et mycorhizienne associées aux PPAM et comment évolue leur structure durant le cycle de vie d'une PPAM cultivée sur un sol pollué ?
- ⇒ L'introduction d'un inoculum mycorhizien commercial dans le sol peut-il influencer la structure des communautés microbiennes ?

Dans le cadre du projet PhytEO, une réflexion globale sur la filière de phytomanagement, combinant la phytostabilisation aidée et la valorisation de la biomasse produite sur sols pollués, a été menée. Ainsi, concernant le volet « phytostabilisation aidée », la question de la mobilité et de la biodisponibilité des ET, sous l'influence de la culture de sauge sclarée, de coriandre ou d'angélique, ainsi que les notions de transfert des ET depuis le sol vers la plante ont été abordées et font partie intégrante du projet. Néanmoins, ces aspects ont été traités en partenariat avec l'INERIS (V. Bert) et ne font pas directement partie des résultats des travaux que j'ai réalisés moi-même ([Perlein et al., en cours de rédaction](#)). A ce titre, ils ne figureront pas en tant que résultats dans le cadre de mon manuscrit de thèse. Néanmoins, certains éléments de réponse seront donnés dans la partie synthèse/discussion générale afin d'appréhender la filière PPAM-HE dans sa globalité.

Partie 2 : Matériels et méthodes

Matériels et méthodes

I. Dispositifs expérimentaux *in situ*

1. Présentation des sites expérimentaux

Dans le cadre du projet PhytEO, deux parcelles expérimentales, de 2 ha chacune, sont mobilisées.

1.1. Parcelle historiquement polluée par des ET – Site d'Evin-Malmaison

Une première parcelle (Figure 14), située à Evin Malmaison (Coordonnées GPS : 3°02'25.5", 50°25'55.5") est mobilisée dans le cadre de cette étude. Cette parcelle se trouve dans la zone 3, à la limite de la zone 2 du périmètre du site Metaleurop Nord (Figure 2), défini dans l'arrêté préfectoral du 29 mai 2015 et soumis à un encadrement sanitaire des productions agricoles et présente des teneurs en ET comprises entre 4 et 10 ppm pour le Cd et entre 200 et 500 ppm pour le Pb. Cette parcelle fait partie du site atelier recensé dans le réseau SAFIR (Sites Ateliers Français pour l'Innovation et la Recherche en gestion des sols) et appartient au bassin minier Metaleurop (Figure 1).



Figure 14: Localisation de la parcelle contaminée par des ET – site d'Evin-Malmaison.

1.2. Parcelle non polluée – Site de Rodelinghem

Une seconde parcelle, témoin, non polluée par les ET (Figure 15), située à Rodelinghem (Coordonnées GPS 1°55'46.9", 50°49'55.7") et présentant des concentrations en ET comparables aux bruits de fonds géochimique de sols agricoles (Cd : 0,03 à 1,39 ppm ; Pb : 13,4 à 198,1 ppm ; Zn : 24,2 à 310 ppm - base de données ASPITET de l'INRA), est également mobilisée.






Figure 15: Localisation de la parcelle non contaminée – site de Rodelinghem.

2. Mise en place du dispositif expérimental

2.1. Choix des espèces végétales

Trois espèces de PPAM ont été choisies dans le cadre de cette étude : la coriandre, la sauge et l'angélique. Plusieurs critères ont été pris en compte dans ce choix, à savoir le cycle de végétation et de développement de la plante, son acclimatation à la région d'étude et à son contexte pédo-climatique, les pratiques agricoles associées, le potentiel de l'espèce en termes de phytostabilisation et le potentiel commercial de l'huile essentielle distillée en filière non alimentaire (coût et disponibilité de la semence, prix de vente de l'HE, attractivité du marché, attractivité pour le producteur). Le Tableau 2 regroupe les critères de sélection pour chaque espèce cultivée. En définitive, la sélection comporte des espèces ayant des cycles de développement différents, capables de se développer dans le contexte pédo-climatique de la région des Hauts-de-France avec un nombre d'interventions sur la parcelle limité et possédant un potentiel commercial attractif dans une filière non-alimentaire (sauge sclarée, angélique) ou déjà décrites dans d'autres études en phytostabilisation (sauge sclarée, coriandre).

Tableau 2: Caractéristiques des PPAM de l'étude.

	Angélique <i>Angelica archangelica</i> L. Apiacées (Ombellifères) 	Sauge sclérée <i>Salvia sclarea</i> L. Lamiacées 	Coriandre <i>Coriandrum sativum</i> L. Apiacées (Ombellifères) 
Cycle de végétation	Bisannuelle vivace (Pérenne ≈ 10 ans)	Bisannuelle vivace (de vie courte)	Annuelle
Récolte	Dès la 2^{ème} année	1^{ère} (≈ 10 %), 2^{ème} (100%) et 3^{ème} (70%) années	Année du semis
Partie récoltée	Graines	Sommités fleuries	Plantes fleuries ou graines
Phytoremédiation	Pas d'étude référencée sur le potentiel de l'espèce	✓ (Angelova et al., 2016)	Pas d'étude référencée sur le potentiel de l'espèce

2.2. Délimitation du parcellaire

Les deux parcelles d'étude ont chacune été découpées en 3 surfaces équivalentes afin d'y cultiver l'angélique, la sauge et la coriandre dès la première année (Figure 16 et Figure 17). Pour chaque plante, la surface a été également subdivisée en 3 aires équivalentes. Dans la première, les sols ont été amendés avec un inoculum mycorhizien commercial (AGTIV – cultures spécialisées, Premier Tech agriculture), à base de *Rhizophagus irregularis* ; l'inoculum mycorhizien sous forme de poudre (125 g/ha) est mélangé à la semence (poudrage) lors du semis. La deuxième surface était prévue pour l'ajout d'un amendement minéral (ex: matériaux alcalins, phosphates, oxy(hydroxy)des de fer et de manganèse) à choisir en fonction des résultats obtenus suite au premier essai notamment en termes de mobilité des ET dans le sol et enfin la troisième surface restera non amendée (Témoin - Figure 16 et Figure 17).

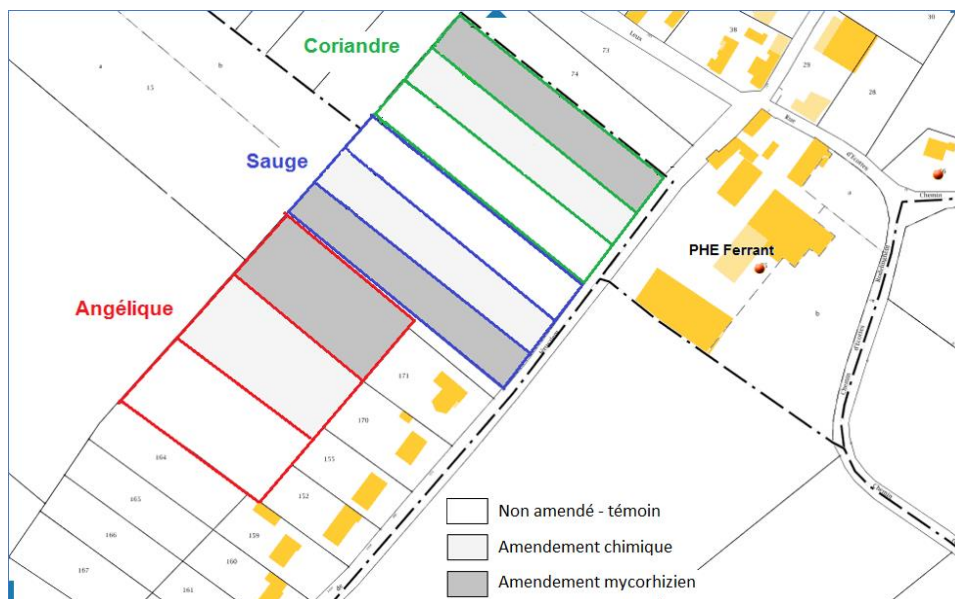


Figure 16: Délimitation du parcellaire de la parcelle non polluée - Site de Rodelinghem.

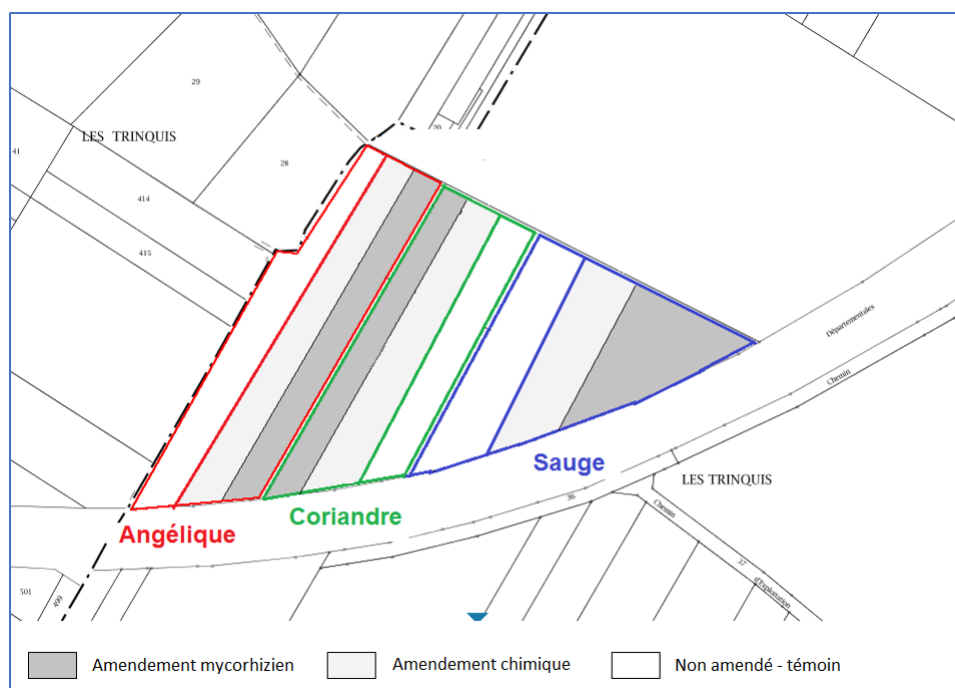


Figure 17: Délimitation du parcellaire de la parcelle polluée - Site d'Evin-Malmaison.

Des bandes non végétalisées sont conservées entre les différentes parcelles amendées ou non (espacées de 2 m) ainsi qu'entre les différentes espèces végétales (espacées d'1 m) comme décrit ci-dessus. Ces bandes non végétalisées permettent de garder un espace entre les différentes modalités afin d'éviter que l'inoculum mycorhizien ne puisse coloniser les racines des plantes de la modalité voisine (non inoculée notamment). Par ailleurs, chaque subdivision de la parcelle pour chaque plante, correspond à une surface de 2400 m² sur le site non contaminé (Rodelinghem – Figure 16) et à une surface de 2200 m² sur le site contaminé (Evin-Malmaison – Figure 17).

2.3. Pouvoir germinatif des semences de PPAM

Le pouvoir germinatif des différentes semences employées a été étudié au laboratoire, dans différentes conditions expérimentales : pour ce faire, des graines conservées à 4 °C ont été placées sur différents supports de culture (papier filtre imbibé d'eau, sol prélevé de la parcelle non polluée, sol prélevé de la parcelle polluée) et la germination des graines a été suivie pendant 15 jours par comptages quotidiens. Ces observations ont été répétées sur trois réplicats indépendants.

3. Caractérisation des sites expérimentaux

3.1. Caractérisation pédo-agronomique et du niveau de contamination par les ET du sol

Des prélèvements de sol ont été réalisés le 6 avril 2017, en coopération avec l'INERIS, d'une part pour caractériser la parcelle d'un point de vue pédo-agronomique et d'autre part pour déterminer les teneurs totales en ET présentes dans les sols ainsi que leur fraction mobile. Les prélèvements ont été réalisés à l'aide d'une tarière manuelle, dans les horizons superficiels du sol (25 premiers centimètres). Chaque point de prélèvement a été également géolocalisé par relevé GPS avec une précision de 50 cm (Appareil Trimble TDC100, antenne R1), le plan de prélèvement est présenté ci-dessous (Figure 18).



Figure 18: Cartographie des points de prélèvement réalisés pour caractériser l'état initial des parcelles - Parcelle non polluée à gauche ; parcelle polluée à droite. Les contours correspondant à la délimitation des parcelles pour l'implantation future des PPAM est indiquée (angélique en bleu – coriandre en noir – sauge en rouge).

3.1.1. Analyse des caractéristiques pédo-agronomiques du sol

L'analyse des caractéristiques pédo-agronomiques (pH, texture du sol, pseudo total des ET, carbone organique total, teneur en matière organique, rapport C/N, capacité d'échange cationique, taux de calcaire, azote total, phosphore échangeable, potassium échangeable, magnésium échangeable, calcium échangeable et sodium échangeable) du sol des parcelles a été sous-traitée à un laboratoire extérieur par l'INERIS.

3.1.2. Analyse des fractions mobiles et totales en ET du sol

La détermination des teneurs en ET totales ainsi que des fractions mobiles a elle été réalisée par l'INERIS dans le cadre du projet PhytEO.

Préparation des échantillons et mesure du pH à l'eau

- Les échantillons de sol sont au préalable pesés (250 g – balance de précision OHAUS, précision 0,01 g), puis placés à l'étuve (40 °C, 48 h) avant de subir un broyage à 2 mm (broyeur RETSCH Fisher Beoblock Scientific) ainsi qu'un tamisage (tamis à maille 2 mm), afin de conserver uniquement le sol de granulométrie inférieure à 2 mm dans des flacons hermétiques de 250 mL.

- Le pH à l'eau est mesuré (NF iso 10390, 2005) à partir de 5 g de sol (balance de précision OHAUS, précision 0,01 g). Dans un flacon de 50 mL, 25 mL d'eau distillée sont ajoutés aux 5 g. Une agitation (mélangeur Heidolph – 1 h) suivie d'une période de repos (2 h) sont observées avant de réaliser la mesure de pH, sous agitation magnétique.

Détermination de la fraction mobile en ET

- Du nitrate d'ammonium (NH_4NO_3 , 1 M) est utilisé pour extraire la fraction mobile en ET. Cet extractant permet de solubiliser la fraction ionique échangeable ainsi que les complexes métalliques solubles (Norme ISO 19730:2008(E)). 10 g de sol sec (balance de précision OHAUS, précision 0.01g) sont placés dans un flacon de 250 mL, au sein duquel est ajouté 25 mL de solution de NH_4NO_3 (1 M), avant agitation (mélangeur Heidolph – 2 h) et filtration (filtre de 45 μm) à l'aide d'un système à air comprimé, dans le but de récupérer le surnageant.
- Ce dernier est analysé par ICP-OES (Inductively Coupled Plasma Optical Spectroscopy, AGILENT 5110), afin de quantifier les ET présents. Si les concentrations mesurées dans les échantillons sont inférieures à la limite de quantification de l'ICP-OES, les concentrations et les éléments concernés sont alors analysés par ICP-MS (Inductively Coupled Plasma Mass Spectrometry), technique analytique possédant une limite de quantification plus basse.

Détermination de la fraction totale en ET

- 0,2 g de sol sec à une granulométrie inférieure à 250 μm sont prélevés dans un matras en téflon, où sont ajoutés : 9 mL d'acide chlorhydrique (HCl), 3 mL d'acide nitrique (HNO_3) et 2 mL d'acide fluoridrique (HF – habilitation nécessaire).
- Le matras est chauffé (micro-onde), afin d'améliorer l'efficacité de l'attaque acide. De l'acide borique est ensuite ajouté pour neutraliser l'HF, une fois le matras froid. Le contenu du matras (minéralisât) est placé dans des tubes d'analyse de 50 mL, complétés par de l'eau distillée milliQ et enfin filtré dans un nouveau tube de même volume, afin d'éliminer les particules de sol éventuellement restées en suspension.
- La fraction totale en ET contenue dans le minéralisât de 50 mL est analysée par ICP-OES (Inductively Coupled Plasma Optical Spectroscopy, AGILENT 5110) selon les protocoles de qualités internes à l'INERIS (acquisition et vérification des résultats d'analyses). Si les concentrations mesurées dans les échantillons sont inférieures à la limite de quantification de l'ICP-OES, les concentrations et les éléments concernés sont alors analysés par ICP-MS (Inductively Coupled Plasma Mass Spectrometry), technique analytique possédant une limite de quantification plus faible.

3.2. Caractérisation biologique

3.2.1. Isolement et quantification de spores de CMA à partir du sol

Des prélèvements d'échantillons de sol ont été réalisés dans les 25 premiers centimètres sur les parcelles témoin et contaminée, avant les semis, afin de rechercher la présence de CMA « autochtones ».

- Méthode de tamisage humide

Afin d'isoler les spores de CMA présentes dans le sol, la méthode de tamisage humide décrite par Gerdemann et Nicolson (1963) a été utilisée. Le sol (50 g) est agité longuement après ajout de 500 ml d'eau de robinet, puis laissé au repos pendant 1min. La solution est ensuite passée à travers une série de tamis, de mailles décroissantes 1 mm, 500, 250, 125, 90 et 45 μm . Les premiers tamis sont utilisés dans le but de retenir les particules les plus larges et les derniers permettent de retenir les spores de CMA. Les filtrats des 4 derniers tamis sont transférés dans un bécher et les spores de CMA sont recherchées à la loupe binoculaire après avoir déposé des petites fractions aliquotes en boîtes de Pétri sur papier filtre. Les spores prélevées à l'aide d'une pince fine, sont conservées dans de l'eau distillée stérile à 4 °C. Cette extraction, identification et quantification des spores est répétée 3 fois avec la même quantité de sol. Les différentes étapes de cette méthode sont présentées sur la Figure 19.

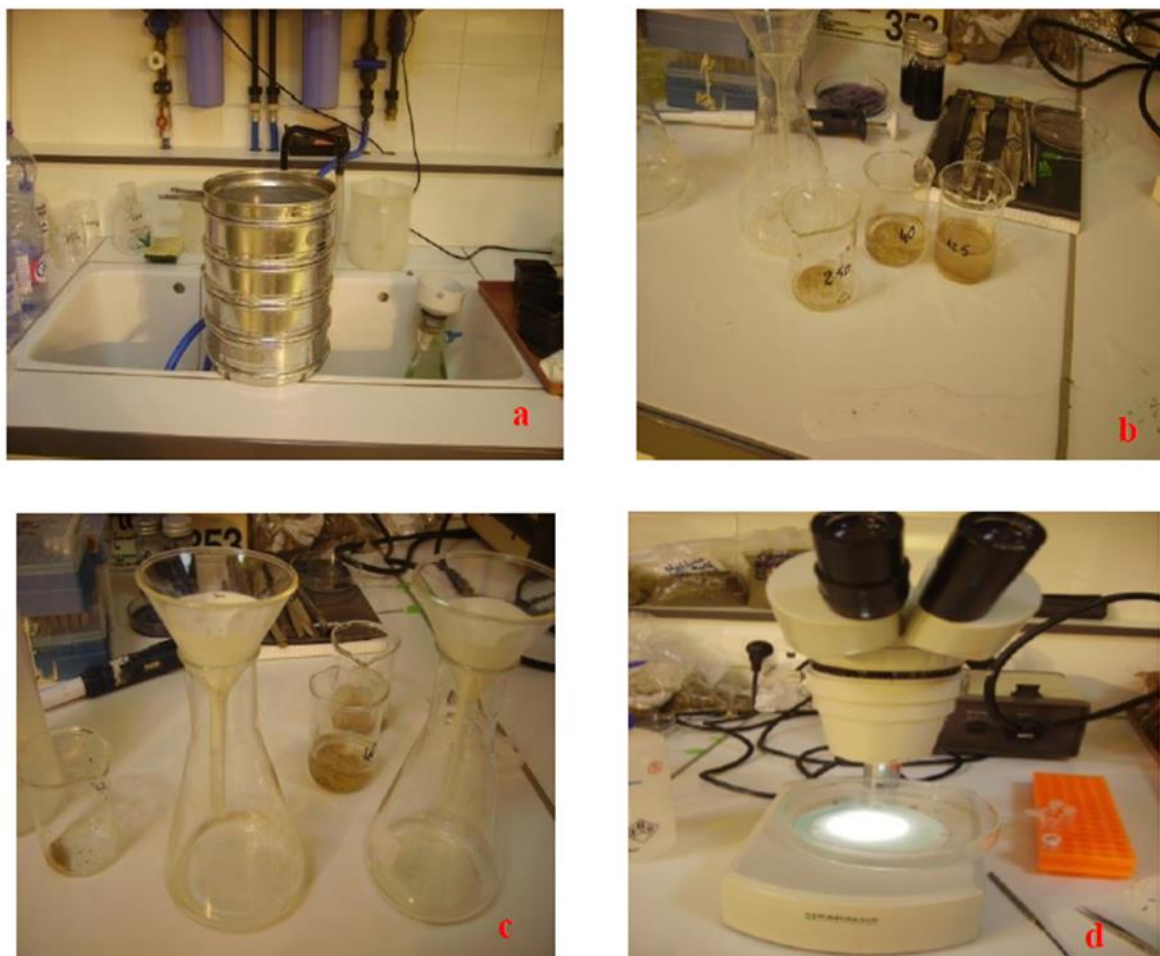


Figure 19: Photographies des différentes étapes de la méthode de tamisage humide du sol ; A. tamisage de la solution aqueuse de sol - B. suspension des filtrats des tamis de maille 250 ; 125 ; 90 et 45 μm dans de l'eau stérile – C. récupération de fractions aliquotes sur papier filtre – D. isolement des spores à la pince fine par observation avec une loupe binoculaire.

3.2.2. Détermination des taux de mycorhization

Afin de mesurer les taux de mycorhization des racines des espèces végétales autochtones ou cultivées sur nos sites expérimentaux, des prélèvements de racines des plantes ont été effectués et colorés selon la technique de Phillips et Hayman (1970) (modifiée). Cette technique se déroule en plusieurs étapes :

- éclaircissement : les racines sont préalablement lavées à l'eau claire et placées dans une solution de KOH 10 % à l'étuve à 70 °C pendant une heure ;
 - acidification : quelques gouttes d'HCl 1 M sont ajoutées (5 min) afin de neutraliser le pH du milieu ;
 - coloration : les racines sont transférées dans une solution aqueuse de bleu de Trypan à 0,05 % (500 mg de bleu de Trypan ; 330 mL acide lactique (C₃H₆O₃) ; 330 mL glycérol (C₃H₈O₃) ; 330 mL eau distillée) et incubées pendant 30 min à l'étuve à 70°C ;
 - stockage : les racines sont conservées à long terme dans une solution de glycérol 50 % à 4 °C.
- A noter qu'un rinçage des racines après chaque étape est indispensable.

Les racines les plus fines sont sélectionnées, coupées en fragments de 1 cm de longueur chacun et placées entre lames et lamelles, à raison de 15 fragments par lame et 3 lames par échantillon, pour une observation sous microscope (x 40). La présence des structures fongiques spécifiques des CMA (arbuscules, vésicules et hyphes fongiques) observées à l'intersection entre l'axe de l'objectif et le fragment racinaire est relevée (Figure 20 - McGonigle *et al.*, 1990). Trois lectures par fragment sont réalisées. Le pourcentage de colonisation total est égal au nombre d'intersections avec les racines mycorhizées × 100 / nombre d'intersections totales. Ce pourcentage de colonisation est exprimé en fonction des pourcentages d'arbuscules et de vésicules distincts.

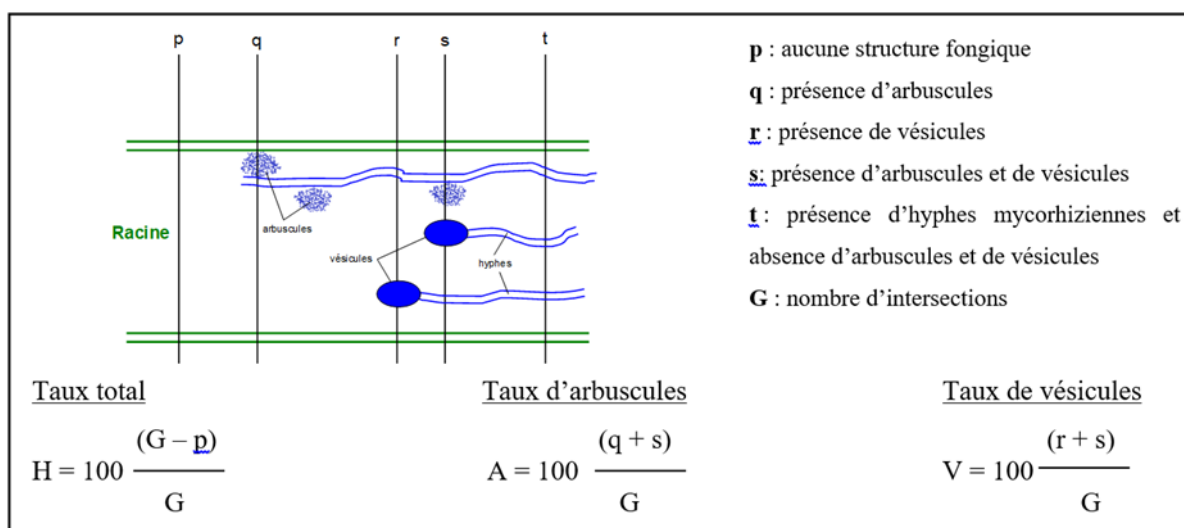


Figure 20: Illustration des possibilités d'intersections entre un axe d'observation microscopique et une racine colonisée par un CMA (McGonigle *et al.*, 1990).

3.2.3. Inventaire floristique des espèces autochtones

L'inventaire floristique de la parcelle expérimentale polluée par les ET a été réalisé au printemps 2019. Le dénombrement et l'identification des espèces végétales présentes ont été réalisés à l'aide de relevés par quadrats d'1 m², au nombre de 13, dont la position a été déterminée aléatoirement sur la parcelle. La position de ces treize quadrats a été géolocalisée. L'identification des plantes a été réalisée selon l'ouvrage de Bonnier *et al.*, (1987).

La diversité α ou diversité au sein d'un habitat ou d'une aire géographique délimitée caractérise la variété et l'abondance des espèces au sein d'une unité d'étude. Un grand nombre d'indices mesurant la diversité peuvent être appliqués (Magurran, 2004 ; Wagner *et al.*, 2018).

Des coefficients d'abondance-dominance et de sociologie de Braun-Blanquet sont attribués à l'ensemble des espèces identifiées dans chaque quadrat. Les relevés sont également traduits en indices de composition : les indices de Menhinick (1964) et de Margalef (1958) qui traduisent à la fois la richesse des espèces et l'abondance des individus d'un groupe biologique étudié ; les indices de diversité de Shannon Weaver (1948) et de Simpson (1949) qui permettent d'identifier si une espèce est dominante ou si toutes les espèces sont présentes avec un effectif similaire.

- Coefficients d'abondance-dominance et de sociabilité de Braun-Blanquet (1951)

Le coefficient d'abondance-dominance de Braun-Blanquet désigne une estimation de l'espace occupé et traduit le recouvrement spatial de l'espèce sur la zone d'étude échantillonnée (Figure 21). Ce coefficient réunit à la fois les notions « d'abondance », nombre d'individus formant la population de l'espèce présente dans le relevé et de « dominance », recouvrement de l'ensemble des individus d'une espèce donnée (Braun-Blanquet, 1951).

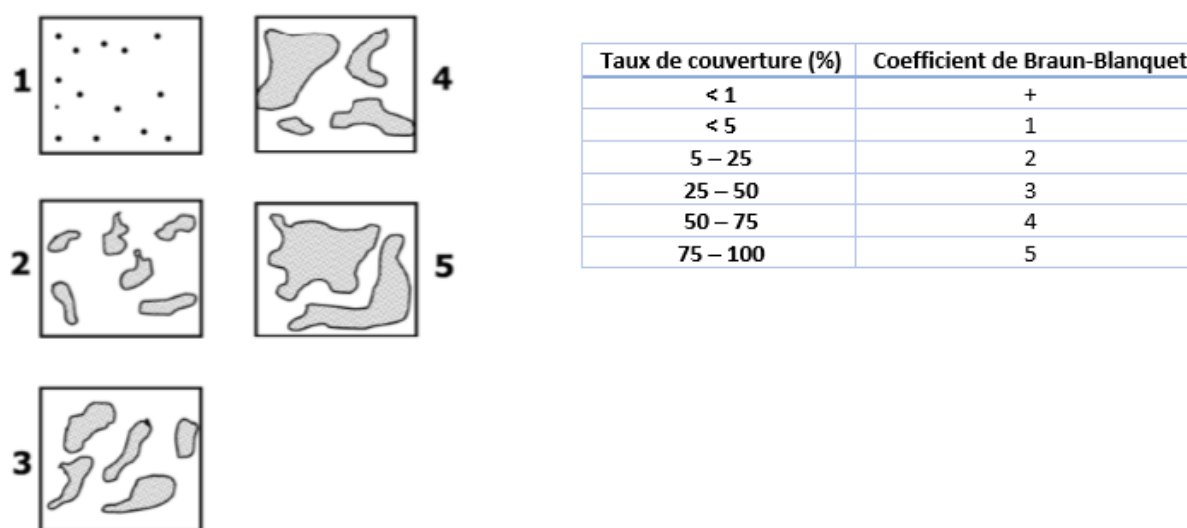


Figure 21: Echelle des coefficients d'abondance-dominance de Braun-Blanquet (1951).

La sociabilité prend en compte le mode d'organisation et de regroupement des espèces au sein de la communauté végétale. Elle traduit un « degré de dispersion spatiale des individus » et exprime l'aptitude des espèces à former des groupements plus ou moins denses au sein d'un espace. Elle est complémentaire du coefficient d'abondance-dominance présenté ci-dessus.

La sociabilité s'apprécie suivant une échelle graduée de 1 à 5 (1 : individus isolés, 2 : groupes, 3 : petites colonies - troupes, 4 : peuplement, 5 : peuplements denses) (Sturbois et Bioret, 2012)

- Indices de diversité α :

- indice de Menhinick, D : tient compte du nombre d'individus et d'espèces (ou taxa) dénombrés dans un échantillon ou milieu donné. Il permet d'exprimer la richesse spécifique d'un milieu et est calculé de la manière suivante :

$$D = \frac{s}{\sqrt{N}}$$

Où s est égal au nombre d'espèces représentées (richesse) et N au nombre d'individus observés (Menhinick, 1964).

- indice de Margalef (1958) : permet de traduire la richesse spécifique d'un milieu (Choudhury et Choudhury, 2013 ; Gamito, 2009). Il s'exprime de la manière suivante :

$$D_{mg} = \frac{(s - 1)}{\ln N}$$

Où s est le nombre d'espèces total et N le nombre d'individus.

- indice de diversité de Shannon-Weaver (1948), H' , également appelé indice de Shannon-Wiener : au départ développé pour les systèmes de communication, cet indice est basé sur la théorie de l'information s'appuyant sur le fait qu'un message peut être transmis sous la forme d'un code binaire (Shannon, 1948 ; Shannon et Weaver, 1964 ; Thukral, 2017).

$$H' = - \sum_{i=1}^S \frac{n_i}{N} \ln\left(\frac{n_i}{N}\right)$$

Où n_i correspond au nombre d'individus au sein d'une espèce i et N au nombre total d'individus observés.

- indice de diversité de Simpson (1949) : représente le complémentaire de l'indice de Simpson original, lorsqu'exprimé sous la forme ci-dessous. Il traduit ainsi la probabilité que deux individus, choisis au hasard dans la population, appartiennent à des espèces différentes. Transformé ainsi, l'indice retranscrit de manière plus intuitive un accroissement de la diversité (Morris *et al.*, 2014).

$$D_S = 1 - \sum_{i=1}^S \frac{n_i(n_i - 1)}{N(N - 1)}$$

Où n_i correspond au nombre d'individus au sein d'une espèce i et N au nombre total d'individus observés.

L'expression de la diversité est souvent le résultat du calcul de différents indices, car ceux-ci, de par leur expression, sont plus ou moins sensibles et plus ou moins affectés par l'échantillonnage (méthode, taille de la population échantillonnée – Thukral, 2017 ; Wagner *et al.*, 2018).

4. Semis et suivi des cultures

4.1. Semis des espèces végétales cultivées

Les densités de semis (300 000 graines / hectare) par espèce végétale ainsi que la période de semis sont basées sur l'expertise de la société Ferrant PHE ainsi que sur des références données par l'ITEIPMAI pour la culture des PPAM. Les différents semis se sont échelonnés comme détaillé dans le Tableau 3 :

Tableau 3: Chronologie des semis des PPAM de l'étude.

ANNEE DE SEMIS	PPAM	DATE DE SEMIS			
		Parcelle non polluée		Parcelle polluée	
2017	Coriandre	10 avril		7 avril	
	Sauge				
	Angélique	10 avril	Re-semis le 15 mai	7 avril	Re-semis le 15 mai
2018	Coriandre	23 avril		20 avril	
	Angélique			20 avril	Re-semis le 19 septembre

4.2. Détermination des taux de levée *in situ*

Le taux de levée associé à chaque espèce végétale a été évalué 1 mois et demi après semis, à 3 positions déterminées aléatoirement dans la parcelle et par condition (modalités inoculées ou non à l'aide de champignons mycorhiziens). Pour chaque position, des comptages ont été réalisés au sein de 3 quadrats d'un mètre carré, selon le dispositif représenté dans la Figure 22 et selon une méthodologie adaptée de Braun-Blanquet (1928) et de la méthode des points quadrats (Daget et Poissonet, 1971). Chaque point de relevé a été géoréférencé à l'aide d'un appareil GPS, avec une précision de 50 cm.

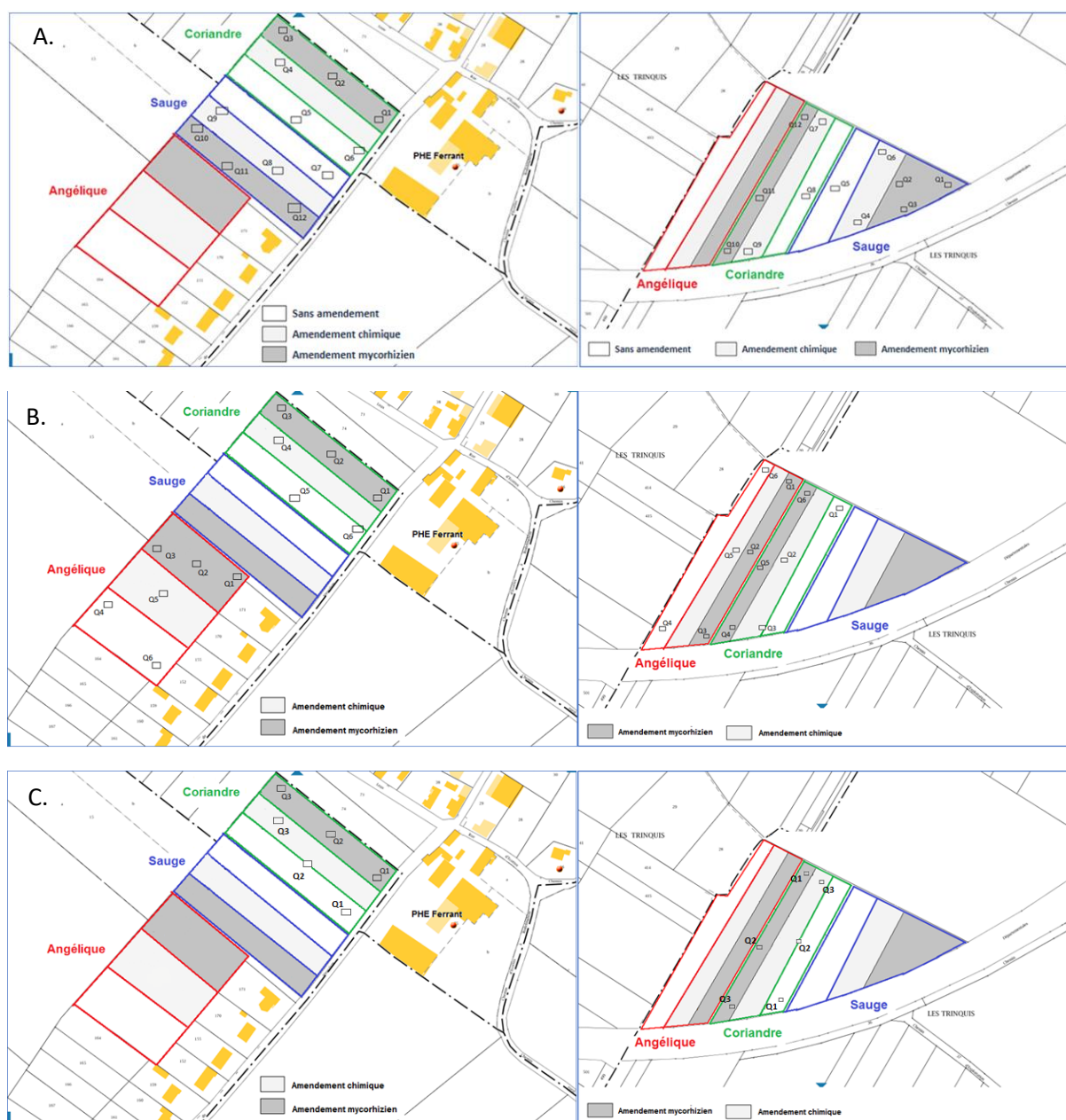


Figure 22: Cartographie des comptages par la méthode des quadrats pour l'année de culture 2017 (A.), 2018 (B.) et 2019 (C.). Site non pollué à gauche – site pollué à droite.

Le développement des plantes est suivi régulièrement dans le but de déceler la présence de maladies et/ou d'espèces adventices sur la parcelle ainsi que de pouvoir définir une date optimale de récolte. Les différentes interventions réalisées sur les parcelles sont également suivies en étroite collaboration

avec Ferrant PHE : le désherbage global de la parcelle (Tableau 4) ainsi que la conservation de bandes non végétalisées, intercalées entre les différentes « sous-parcelles » sont ainsi réalisés chaque année.

Tableau 4: Liste des interventions phytosanitaires réalisées sur les parcelles expérimentales.

PARCELLE	PASSAGE	PLANTE	PRODUIT COMMERCIAL	SUBSTANCE ACTIVE	DICOTYLEDONES	GRAMINEES	
EVIN-MALMAISON	1 ^{er}	Angélique	Challenge 600	Aclonifen	✓	✓	
			Lentagran	Pyridate	✓		
			Prowl 400	Pendimethaline	✓	✓	
		Coriandre	Challenge 600	Aclonifen	✓	✓	
			Lentagran	Pyridate	✓		
			Prowl 400	Pendimethaline	✓	✓	
		Sauge	Lontrel SG	Clopyralid	✓		
	Prowl 400		Pendimethaline	✓	✓		
	Tornado SC		Methamitron	✓	✓		
	2 ^e	Angélique	Round-up	Glyphosate	✓	✓	
			Challenge 600	Aclonifen	✓		
		Coriandre	Prowl 400	Pendimethaline	✓	✓	
			Sauge	Challenge 600	Aclonifen	✓	
				Prowl 400	Pendimethaline	✓	✓
RODELINGHEM	1 ^{er}	Coriandre	Prowl 400	Pendimethaline	✓	✓	
			Linuron	Linuron	✓	✓	
			Centurion (2000 ou 240 EC)	Quizalofop + clethodime		✓	
		Sauge	Prowl 400	Pendimethaline	✓	✓	
			Linuron	Linuron	✓	✓	
			Centurion (2000 ou 240 EC)	Quizalofop + clethodime		✓	
	2 ^e	Coriandre	Prowl 400	Pendimethaline	✓	✓	
			Challenge 600	Aclonifen	✓		
			Asulox	Asulame	✓		
			Centurion (2000 ou 240 EC)	Quizalofop + clethodime		✓	
		Sauge	Asulox	Asulame	✓		
			Challenge 600	Aclonifen	✓		
			Defi	Prosulfocarbe	✓	✓	
			Lentagran	Pyridate	✓		

5. Echantillonnage des plantes et du sol

5.1. Echantillonnage du sol

En coordination avec l'INERIS, des prélèvements de sol (500 g) à l'aide d'une tarière manuelle sont réalisés quelques jours avant la récolte des PPAM. Ces analyses sont réalisées par ICP-MS par l'INERIS. Les différents points de prélèvement sont systématiquement géoréférencés à l'aide d'un appareil GPS, avec une précision de mesure de 50 cm.

Les points de prélèvement correspondant à l'état initial de la parcelle avant toute intervention sur celle-ci ont été projetés sur des fonds de carte (Figure 18). De même pour les points correspondant aux prélèvements avant récolte 2017 (Figure 20B.), 2018 (Figure 20B.) et 2019 (Figure 23C.).

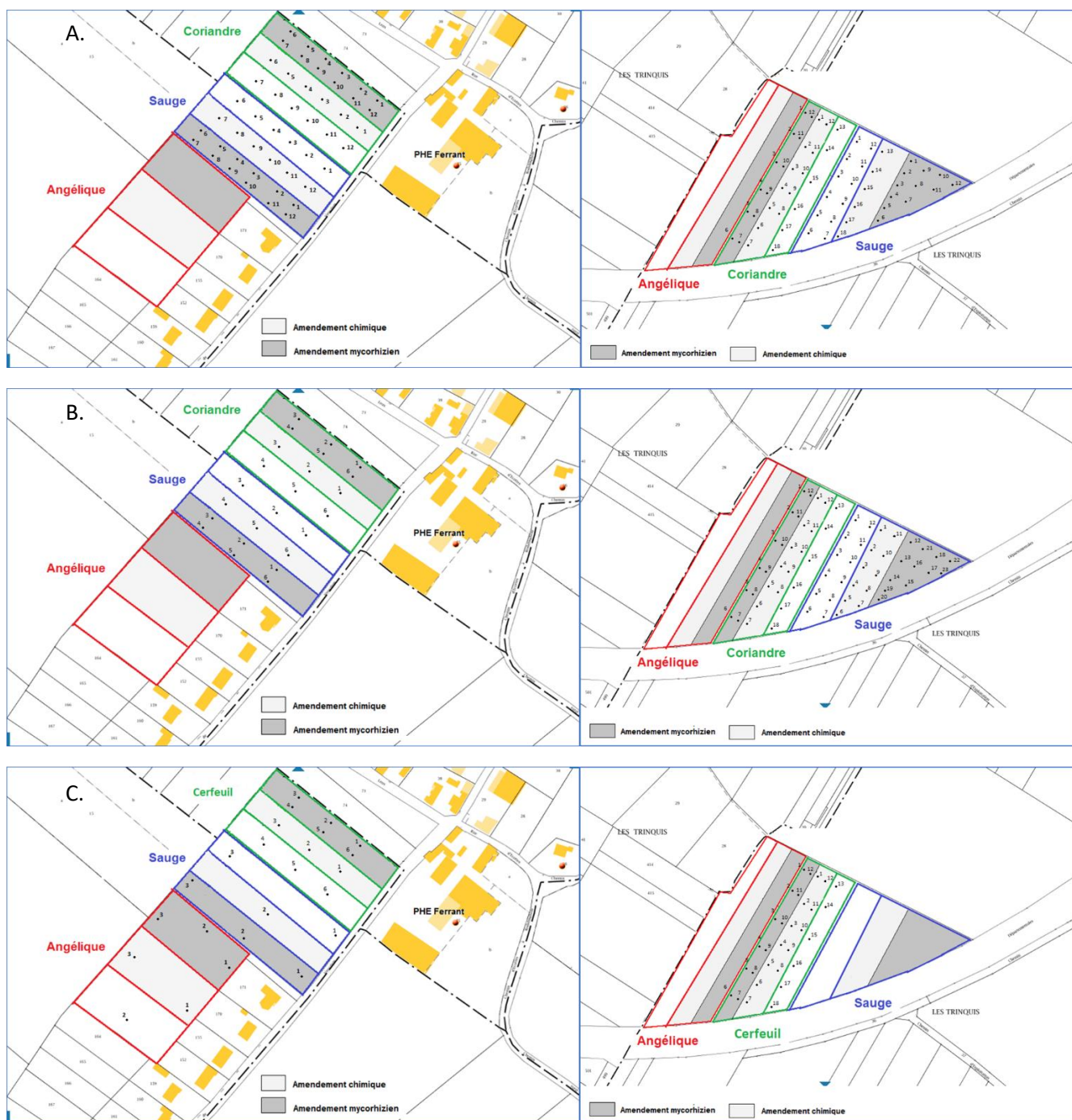


Figure 23: Cartographie des points de prélèvement réalisés avant récolte pour l'année de culture 2017 (A.), 2018 (B.) et 2019 (C.). Parcelle non polluée à gauche ; parcelle polluée à droite. Les contours correspondant à la délimitation des parcelles des PPAM est indiquée (angélique en bleu – coriandre en noir – sauge en rouge).

D'un point de vue technique, l'échantillonnage a été réalisé à intervalles réguliers comme indiqué sur la Figure 24 pour la parcelle non polluée (site de Rodelinghem). En raison de la géométrie de la parcelle, le schéma de prélèvement a été adapté pour la parcelle polluée (site d'Evin-Malmaison - Figure 25).

Matériels et méthodes

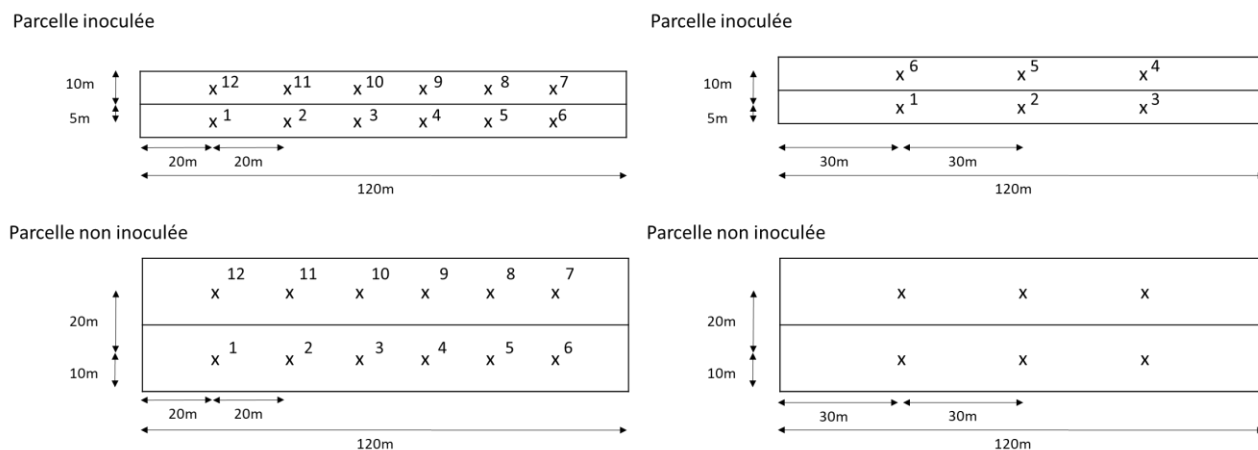


Figure 24: Schéma d'échantillonnage suivi sur la parcelle non polluée (site de Rodelinghem) – pour l'année 2017 à gauche ; pour les années 2018 et 2019 à droite.

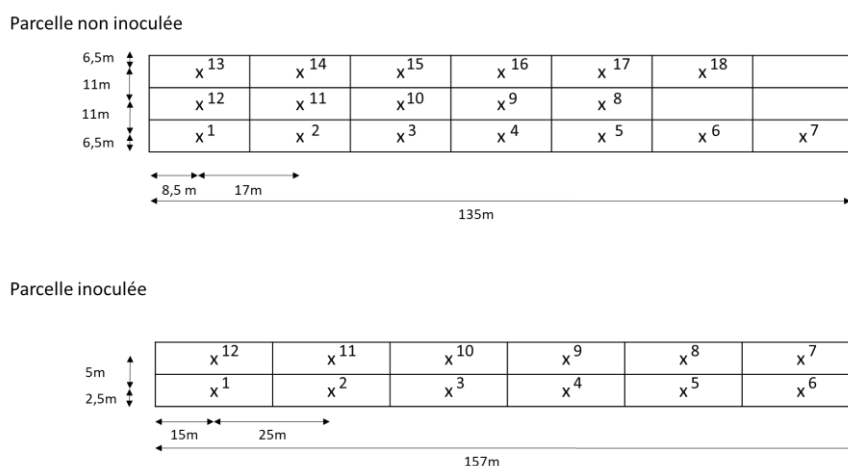


Figure 25: Schéma d'échantillonnage suivi sur la parcelle polluée (site d'Evin-Malmaison) – pour les années 2017, 2018 et 2019.

5.2. Détermination de la hauteur et de la masse sèche de biomasse aérienne

Lors des campagnes d'échantillonnage de sol, dont la méthodologie a été présentée au point précédent, des organes végétaux pour chaque espèce (Tableau 5) ont également été prélevés, à l'endroit même du prélèvement de sol.

Tableau 5: Organes végétaux échantillonnés selon l'année de culture, pour chaque PPAM.

Année de récolte	Plante	Partie récoltée	
		UCEIV	INERIS
2017	Coriandre	Parties aériennes - tiges et feuilles	
	Sauge	Sommités fleuries et plante entière	
2018	Coriandre	Graines	Parties aériennes
	Sauge	Plante entière	Sommités fleuries
2019	Angélique	Parties aériennes - tiges et porte-graines	
	Sauge	Plante entière	Inflorescences

Les échantillons prélevés par l'INERIS donnent lieu à des analyses en ICP-MS, pour évaluer le transfert des ET dans les parties aériennes des PPAM qui sont distillées (voir I.6. Récolte de la phytomasse et distillation des HE) lors des années de récolte 2017, 2018 et 2019.

Par ailleurs, les parties aériennes prélevées sont mesurées puis séchées à l'étuve (72 h à 80 °C), afin d'en déterminer le poids sec.

5.3. Suivi de la contamination et de la mobilité des ET

De manière analogue à la méthodologie présentée au point I.3.1.2., les échantillons de sol sont traités afin de déterminer les fractions mobile et totale en ET.

De plus, comme présenté ci-dessus (I.5.2), des organes végétaux correspondant à la biomasse distillée pour obtenir les huiles essentielles ont été prélevées afin de réaliser le même suivi (fractions mobile et totale). Pour ce faire :

- Chaque échantillon végétal est lavé avant d'être placé à l'étuve (48 h à 40 °C), puis broyé.
- 0,5 g de ce broyat sont pesés puis placés dans un matras, où est ajouté 10 mL d'acide nitrique (HNO₃ – 68 %). Le matras est ensuite chauffé (micro-ondes – 180 °C), puis le minéralisât obtenu est placé dans des tubes d'analyse de 50 mL complétés par de l'eau distillée milliQ et enfin filtré dans un nouveau tube afin d'éliminer les dernières particules végétales.
- La fraction totale en ET contenue dans le minéralisât de 50 mL est analysée par ICP-OES (Inductively Coupled Plasma Optical Spectroscopy, AGILENT 5110) selon les protocoles de qualités internes à l'INERIS (acquisition et vérification des résultats d'analyses). Si les concentrations mesurées dans les échantillons sont inférieures à la limite de quantification de l'ICP-OES, les concentrations et les éléments concernés sont alors analysés par ICP-MS (Inductively Coupled Plasma Mass Spectrometry), technique analytique possédant une limite de quantification plus basse.

6. Récolte de la phytomasse et distillation des HE

La date de récolte est décidée en fonction du stade de développement des plantes et également par rapport à leurs caractéristiques (Tableau 6). La coriandre, qui est une plante herbacée annuelle, peut être récoltée dès la première année et en général dès la neuvième semaine suivant le semis (récolte des parties aériennes en fleurs - croissance très rapide, favorisée par la chaleur). Les graines peuvent aussi être récoltées, à partir de la douzième semaine après semis. La sauge fleurit dès la première année, mais le nombre d'inflorescences attendu est très limité et les rendements de distillation faibles ; l'année de récolte optimale est donc la seconde année de culture, où les meilleurs rendements sont obtenus. Les graines de l'angélique sont également récoltables durant le deuxième été suivant le semis.

Tableau 6: Date de récolte des différentes PPAM selon l'année de culture.

ANNEE DE SEMIS	PPAM	DATE DE RECOLTE	
		Parcelle non contaminée	Parcelle contaminée
2017	Coriandre	12 juillet	19 juillet
	Sauge	29 août	11 septembre
2018	Coriandre	21 août	3 septembre
	Sauge	17 et 19 juillet	16 et 18 juillet
2019	Sauge	23 et 29 juillet	14 et 16 août

Suite à la récolte de chaque espèce, la phytomasse obtenue est acheminée vers le lieu de distillation par la société Ferrant PHE. Un pilote fonctionnel d'extraction des HE est mis à disposition par Ferrant PHE (Figure 26), qui réalise le process de distillation des HE.



Figure 26: Pilote d'extraction des huiles essentielles ; à gauche le réfrigérant et l'essencier pour récupérer les HE, à droite la remorque permettant la distillation de la biomasse.

La matière végétale récoltée est placée dans une enceinte fermée hermétiquement dans laquelle est injectée de la vapeur d'eau. La vapeur d'eau, en passant à travers la matière végétale se charge en composés volatils extraits de la plante et passe ensuite à travers un système de refroidissement, où celle-ci est recondensée. Dans le cas de faibles quantités de phytomasse récoltées ou pour la distillation des graines, de la paille peut être ajoutée afin de réduire la vitesse de circulation de la vapeur d'eau et d'améliorer les rendements de distillation. Après décantation, l'huile essentielle moins dense que l'eau, est recueillie : il s'agit d'un procédé d'entraînement à la vapeur, dont le principe est schématisé sur la Figure 27. La mesure du volume de biomasse est possible grâce à des graduations disposées dans la remorque.

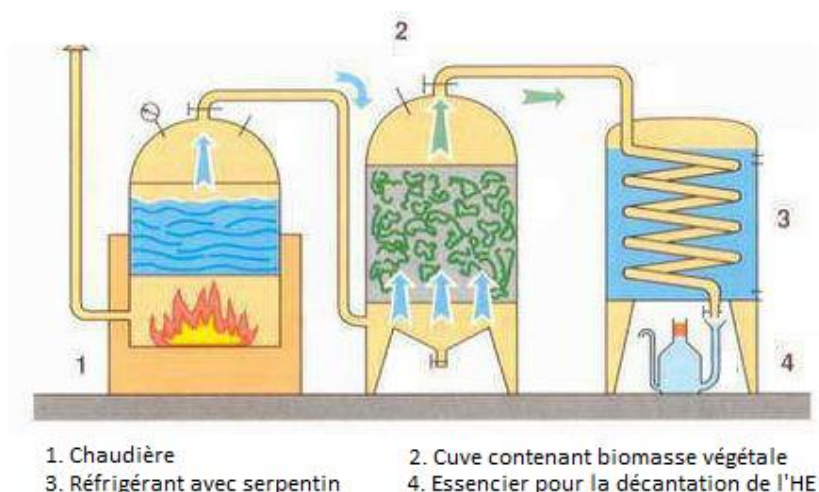


Figure 27: Procédé de distillation des HE par entraînement à la vapeur.

Les résidus de biomasse issus de la distillation ont été échantillonnés par lot et mis à disposition de l'INERIS pour y vérifier les teneurs en ET présentes. Selon les résultats obtenus, la méthode de valorisation des résidus de distillation la plus appropriée sera étudiée (combustion, méthanisation, compostage) dans le cadre du projet PhytEO. La détermination de la teneur en ET dans les HE est également analysée par l'INERIS.

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Figure 28:Chronologie des interventions réalisées sur les sites expérimentaux de Rodelinghem et d'Evin-Malmaison

II. Analyse de la composition chimique des HE et de leurs propriétés biologiques

1. Analyse de la composition chimique des HE

La composition des HE a été déterminée par chromatographie en phase gazeuse, équipée d'une colonne capillaire ZB-5MS (Phenomenex - 5 % Phenyl-arylène / 95% diméthylpolysiloxane - longueur 10 m, diamètre interne 0,10 mm, épaisseur de la phase 0,10 μm) et couplée à un spectromètre de masse (Shimadzu QP 2010 Ultra). Les conditions analytiques ont été définies selon les données bibliographiques. Préalablement diluées dans de l'acétate d'éthyle (ratio 1:200 (v/v)), les HE sont injectées en mode « split » (ratio 1:10) dans un volume de 0,2 μL , à 220 °C. Le système a été maintenu à une vitesse linéaire constante de 60 $\text{cm}\cdot\text{s}^{-1}$ et le débit du gaz vecteur, de l'hélium, est de 0,8 $\text{mL}\cdot\text{min}^{-1}$. Le programme débute à une température de 60 °C et augmente progressivement de 40 °C/min pour atteindre un palier de 280 °C.

Les spectres de masses ont été déterminés en mode « impact électronique » à 70 eV et enregistrés sur une étendue de 35 à 350 (m/z). Les paramètres de températures du spectromètre ont été fixés à 280 °C pour la source d'ions et à 240 °C pour la ligne de transfert.

Les indices de rétention de Kovats ont été déterminés à partir de co-injections d'alcanes. L'identification des composés des HE a été effectuée en comparant les indices de Kovats et les spectres de masse obtenus en sortie de colonne avec ceux des bibliothèques NIST et Wiley 275 (Bandoni *et al.*, 2011 ; Kuźma *et al.*, 2009 ; Pitarokili *et al.*, 2002 ; Ronyai *et al.*, 1999).

2. Etude des propriétés biologiques

2.1. Matériels biologiques

Les HE testées pour leurs propriétés biologiques sont celles qui ont été obtenues par entraînement à la vapeur à partir de la biomasse des différentes PPAM, récoltées sur les deux parcelles d'étude, polluée ou non par les ET.

2.2. Principe de l'encapsulation par la β -CD et détermination du coefficient de rétention des HE par la β -CD

Les cyclodextrines (CD) sont des oligosaccharides cycliques, composés d'un assemblage d'unités glucopyranose (7 pour la β -CD). Les CD se présentent sous une forme tronconique et leur structure tridimensionnelle leur procure des propriétés amphiphiles, dotées d'une surface externe hydrophile permettant la solubilité aqueuse et d'une cavité interne hydrophobe (Abarca *et al.*, 2016; Kfoury *et al.*, 2015). Cette cavité hydrophobe permet l'établissement de complexes d'inclusion avec des hôtes hydrophobes, comme représenté sur la Figure 29, grâce à l'établissement d'interactions de Van der Waals, de liaisons hydrogènes ou d'interactions hydrophobes (Abarca *et al.*, 2016; Fourmentin *et al.*, 2013). Ces forces étant faibles, le phénomène de complexation est réversible et repose sur un état d'équilibre (Abarca *et al.*, 2016; Kfoury *et al.*, 2015).

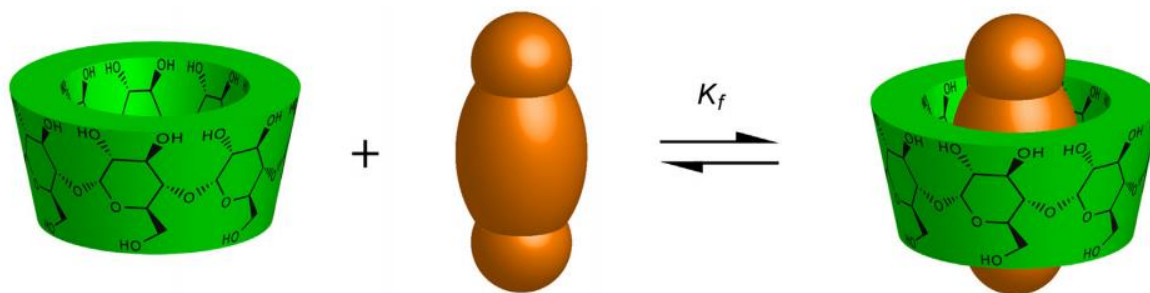


Figure 29: Représentation schématique de la formation d'un complexe d'inclusion entre la β -CD et un hôte hydrophobe (d'après Kfoury et al., 2016).

La capacité de rétention des HE par la β -CD a été déterminée par chromatographie en phase gazeuse en espace de tête, selon la méthode de Kfoury *et al.*, 2015, en comparant la somme des aires des pics chromatographiques pour les HE mises en présence de la β -CD avec les mêmes HE libres dans le milieu.

Les HE ont été ajoutées soit à un volume de 10 mL d'eau ultra pure, soit à une solution aqueuse de β -CD (10 mM), placés dans des fioles de 22 mL en verre, ensuite scellées à l'aide de septa en silicone et d'aluminium et thermostatées à 25 ± 0.1 °C. Une fois l'équilibre entre les phases aqueuse et gazeuse atteint (30 min), 1 mL de la phase vapeur présente dans la partie supérieure de la fiole, au-dessus de la solution aqueuse d'HE, est prélevée à l'aide d'une seringue étanche au gaz et injecté directement en tête de colonne via une ligne de transfert (250 °C). De l'azote est utilisé comme gaz vecteur.

Toutes les mesures ont été réalisées à l'aide d'un échantillonneur « Headspace » automatique Agilent couplé à un appareil Perkin Elmer Autosystem XL, équipé d'un détecteur FID et d'une colonne chromatographique DB624.

Les conditions de température sont les suivantes :

- Température initiale de 50 °C, maintenue 2 min
- Augmentation progressive jusqu'à 190 °C, à un taux constant de 5 °C/min
- Durée totale d'analyse de 32 min

Le coefficient de rétention (r) des HE par les CD a été déterminé à 25 °C, à l'aide de l'expression suivante (1) :

$$r (\%) = \left(1 - \frac{\sum A_{CD}}{\sum A_0}\right) \times 100 \quad (1)$$

Où $\sum A_0$ and $\sum A_{CD}$ correspondent à la somme des aires des pics chromatographiques de chacun des composés des HE en présence (A_{CD}) ou absence (A_0) de CD. Toutes les mesures ont été réalisées en triplicats pour chaque HE, libre ou en présence de CD.

2.3. Activités antifongiques

L'activité antifongique des différentes HE a été testée contre deux champignons phytopathogènes, *Zymoseptoria tritici* (par contact direct, en milieu liquide) et *Fusarium culmorum* (par contact direct et par volatilité sur milieu solide, en boîte de Pétri).

2.3.1. Préparation des milieux de culture

Deux milieux nutritifs sont utilisés pour la réalisation des tests antifongiques :

- Du Glucose-Peptone, pour les expériences en milieu liquide avec *Zymoseptoria tritici*, est préparé dans les proportions suivantes :
 - 14,3 g de Glucose D
 - 7,1 g de Peptone bactériologique
 - Eau distillée : Q.s.p. 1 L de milieu
- Du PDA (Potato Dextrose Agar) préparé à une concentration de 39 g.L⁻¹ et ensuite stérilisé à l'autoclave (20 min à 121 °C), est utilisé comme support de culture pour le champignon *Fusarium culmorum*.

2.3.2. Activité antifongique par contact direct

2.3.2.1. Contre *Zymoseptoria tritici*

Les expériences en milieu liquide ont pour but d'étudier l'effet antifongique des HE sur les spores des champignons : celles-ci sont effectuées dans des microplaques 96 puits operculées à l'aide d'un film étanche.

- Incorporation des HE au sein de la microplaque

Les HE testées sont incorporées directement dans les puits contenant du Glucose-Peptone, en présence ou absence de β -CD (10 mM). Chaque ligne de 12 puits représentant une concentration de produit comporte 4 puits témoins du caractère coloré de l'HE, sans spore de champignon, et 8 puits qui constituent les répétitions avec le champignon. Un témoin positif correspondant à un fongicide de référence (Aviator XPRO, à base de Bixafen 75 g.L⁻¹ et Prothioconazole 150 g.L⁻¹ – homologué sur grandes cultures contre septorioses et fusarioses) a également été réalisé, avec la même gamme de concentrations que les HE. Le plan de plaque utilisé est présenté sur la Figure 30 suivante :

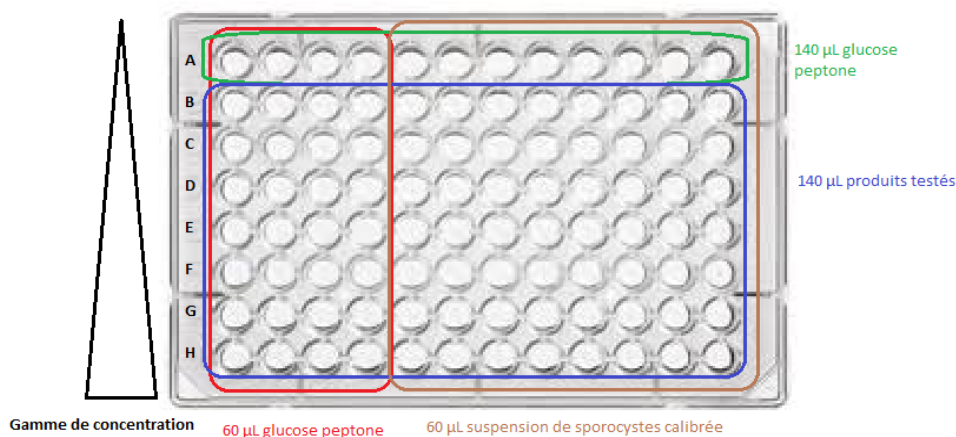


Figure 30: Plan de microplaque utilisé lors des tests antifongiques des HE vis-à-vis de *Z. tritici*. Chaque ligne correspond à une concentration en produit, la ligne A étant à 0 mg.L⁻¹ (témoin de croissance du champignon) et la ligne H étant la plus forte concentration utilisée.

- Suspension de spores

La mise en suspension du champignon est réalisée avec un inoculum âgé de 4 jours pour la souche de *Z. tritici*. La suspension de spores est calibrée par comptage sur cellule de Malassez pour contenir 2×10^5 spores/mL selon un protocole du FRAC (Fungicide Resistance Action Comitee - 2006). La même quantité de spores (60 μ L par puit) est ensuite ajoutée à chacun des huit puits pour chaque ligne de la microplaque.

- Période d'incubation et lecture des résultats

Les microplaques sont mises à l'incubation en chambre de culture à 20 °C, à l'obscurité et sous agitation mécanique (110 RPM) pendant 6 jours.

Après ce délai, qui correspond à un temps optimal de développement pour le champignon, les microplaques sont analysées à l'aide d'un spectromètre. Les valeurs de Densité Optique (DO) sont lues à 620 nm sans agitation par le spectromètre et analysées manuellement.

Pour chaque concentration de produit, les DO nettes sont calculées selon la formule suivante :

$$\text{DO nette} = \text{DO moyenne avec champignon} - \text{DO moyenne témoin sans spores}$$

Les valeurs de CI_{50} sont obtenues grâce à une régression non linéaire à l'aide du logiciel XLStat, avec la fonction suivante (Sahmer *et al.*, 2015) :

$$f(x) = \text{bottom} + \frac{\text{top} - \text{bottom}}{1 + (x \times 10^{-\log IC_{50}})}$$

Où « bottom » correspond à la valeur vers laquelle la courbe converge pour des valeurs élevées de concentration en HE (inhibition complète pour un modèle optimal) ; « top » correspond à la valeur pour une absence d'inhibition et « $\log IC_{50}$ » au logarithme de base 10 de la CI_{50} .

Les valeurs de DO nettes sont utilisées comme valeurs dépendantes et les logarithmes des concentrations en produit comme valeurs explicatives. Le modèle donne une valeur de R^2 associée à valeur logarithmique de la CI_{50} (nommée pr3), ensuite exprimée pour obtenir la CI_{50} de chaque produit selon la formule suivante : $CI_{50} = 10^{\text{pr3}}$. Pour chaque modalité, trois répétitions indépendantes sont réalisées.

2.3.2.2. Contre *Fusarium culmorum*

L'activité antifongique des HE par contact direct a été évaluée selon une méthodologie adaptée de Abdollahi *et al.*, (2011) et Znini *et al.*, (2013). Les HE sont introduites dans le milieu de culture (PDA) refroidi à 50 °C avant d'être coulées (avant prise en masse du milieu) dans des boîtes de Pétri de 9 cm de diamètre sous un poste de sécurité microbiologique. Une gamme de concentrations en HE est réalisée (entre 0,005 et 1 %) dans un volume final de 25 mL de PDA par boîte. Du Diméthylsulfoxyde (DMSO) est utilisé afin d'améliorer la solubilisation des HE dans la solution aqueuse. Des boîtes de Pétri contenant juste la solution avec du DMSO à 1 % sont préparées servant comme témoin. Un témoin positif correspondant à un fongicide de référence (Aviator XPRO, à base de Bixafen et Prothioconazole – homologué sur grandes cultures contre septorioses et fusarioses) a également été réalisé (concentrations variant de 5×10^{-5} à 0,5 %).

Une fois le milieu refroidi, les boîtes sontensemencées avec un inoculum de 9 mm de diamètre, prélevé à l'aide d'un emporte-pièce stérile, à partir de la périphérie d'un tapis mycélien provenant d'une culture du champignon âgée de 7 jours. Les inocula sont déposés avec le mycélium en contact direct avec la gélose, au centre des boîtes. L'activité antifongique par contact direct est réalisée en absence et en présence des β -CD, à une concentration finale de 10 mM dans le milieu de culture. Le diamètre de la croissance radiale du mycélium fongique est mesuré après 7 jours d'incubation à 20 °C. Chaque essai est répété trois fois.

Le pourcentage d'inhibition de la croissance mycélienne est exprimé comme suit :

$$\text{Pourcentage d'inhibition} = \frac{X_0 - X_i}{X_0} \times 100 \quad (2)$$

Où X_0 correspond au diamètre moyen de la croissance mycélienne du témoin ; X_i au diamètre moyen de la croissance mycélienne en présence des HE. La concentration nécessaire pour inhiber la croissance du mycélium fongique de 50 % (CI_{50}) par rapport au témoin, est obtenue par interpolation graphique complétement par une méthode statistique (Sahmer *et al.*, 2015).

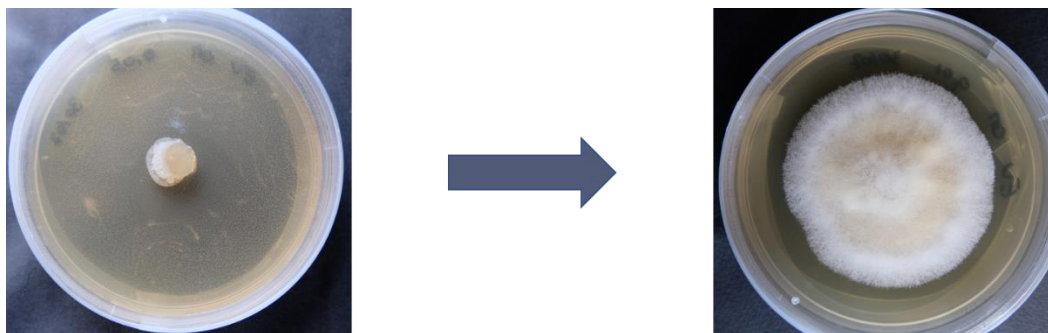


Figure 31 : Activité antifongique par contact direct contre *Fusarium culmorum* (présentée ici pour l'HE de coriandre feuilles). Photographie Robin Raveau.

2.3.3. Activité antifongique par volatilité

L'activité antifongique des HE par volatilité a été évaluée selon une méthode adaptée d'Abarca *et al.*, (2016) et de Chutia *et al.*, (2009), en absence et en présence de β -CD (10 mM). Une gamme de concentrations en HE (entre 0,005 et 1 %) est préparée dans une solution aqueuse avec du DMSO à 1 % ou dans une solution de β -CD (10 mM). Des boîtes de Pétri contenant juste la solution avec du DMSO à 1 % ou bien des β -CD seules sont préparées et servent de témoin. Un témoin positif correspondant à un fongicide de référence (Aviator XPRO, à base de Bixafen et Prothioconazole – homologué sur grandes cultures contre septorioses et fusarioses) a également été réalisé (concentrations variant de 5×10^{-5} à 0,5 %). 10 mL de PDA sont introduits (avant prise en masse du milieu) dans une boîte de Pétri de 5,5 cm. Cette petite boîte est déposée au centre d'une boîte de Pétri de 9 cm de diamètre, contenant elle 10 mL d'une solution aqueuse des HE (Figure 32).

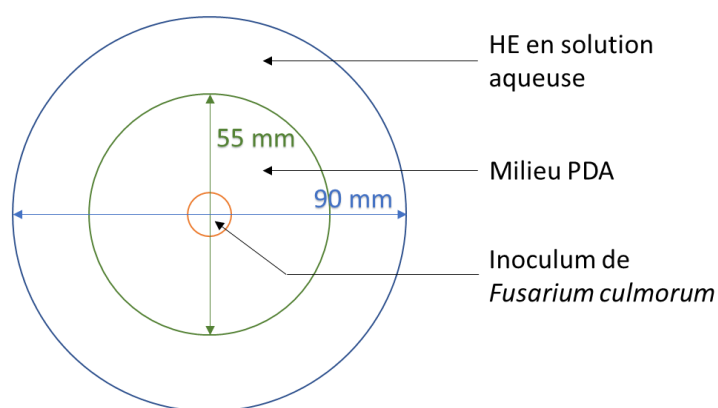


Figure 32 : Représentation schématique du dispositif utilisé pour les tests antifongiques par volatilité.

Un inoculum de 9 mm de diamètre, prélevé à l'aide d'un emporte-pièce stérile à partir de la périphérie d'un tapis mycélien provenant d'une culture du champignon âgée de 7 jours, est déposé au centre de la boîte de 5.5 cm de diamètre, avec le mycélium en contact direct avec la gélose. Le diamètre de la croissance radiale du mycélium fongique est mesuré après 5 jours d'incubation à 20 °C. Chaque essai est répété trois fois.

Le pourcentage d'inhibition de la croissance mycélienne est exprimé suivant l'équation (2).

La concentration nécessaire pour inhiber la croissance du mycélium fongique de 50 % (CI_{50}) par rapport au témoin, est obtenue par interpolation graphique complétement par une méthode statistique (Sahmer *et al.*, 2015).

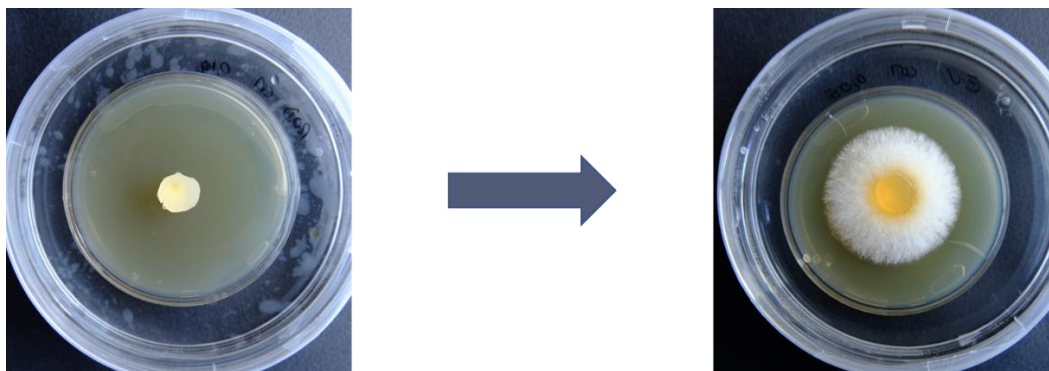


Figure 33 : Activité antifongique par volatilité contre *F. culmorum* (présentée ici pour l'HE de coriandre feuilles).
Photographe Robin Raveau.

2.4. Activités anti-germinatives et herbicides

Les propriétés anti-germinatives et herbicides sont évaluées vis-à-vis de deux espèces végétales : le ray-grass (monocotylédone) ainsi que la laitue (dicotylédone), communément utilisées comme espèces bioindicatrices pour les tests de phytotoxicité (Park *et al.*, 2016) et recommandées dans les directives ISO (1995), de l'OECD (2003) et de l'US EPA (1996).

2.4.1. Inhibition de la germination des graines

L'activité antigerminative des HE par contact direct, a été évaluée selon une méthodologie adaptée de Judd *et al.*, (2015), Lyu *et al.*, (2018) et Paul *et al.*, (2001). Les HE sont introduites dans un milieu gélosé Agar (1 %), non complétement, refroidi à 50 °C avant d'être coulé (avant prise en masse du milieu) dans des boîtes de Pétri carrée de 12 cm × 12 cm, placées sous un poste de sécurité microbiologique. Les essais *in vitro* ont été réalisés en présence et absence de β -CD (10 mM) dans le milieu gélosé. Une gamme de concentration en HE (0,0005 à 0,5 %) est évaluée, dans un volume final de 60 mL par boîte. Du DMSO est utilisé afin d'améliorer la solubilisation des HE dans le milieu. Deux témoins sans HE sont également réalisés pour chaque espèce, l'un contenant simplement la β -CD en solution aqueuse, dans les mêmes proportions que les modalités testées et l'autre contenant uniquement du DMSO 1 % en solution aqueuse, ainsi qu'un témoin positif correspondant à un herbicide systémique non sélectif (Glyphosate, sel d'isopropylamine - 360 g.L⁻¹), testé sur la même gamme de concentrations que les HE.

Une fois le milieu refroidi, les graines de laitue (12 par boîte –Figure 31A.) ou de ray-grass (10 par boîte – Figure 34B.) sont déposées à la surface de la gélose, régulièrement espacées.

Les boîtes de pétri sont ensuite placées en position verticale dans des armoires de culture en conditions contrôlées en incubation pendant 8 jours, avec une photopériode de 16 h, une température de 20 °C à la lumière et 12 °C à l'obscurité, un taux d'humidité de 70 % et une intensité lumineuse de 4 LS (Figure 35).

A l'aide d'un scanner « Winrhizo », des photos sont prises des boîtes après 8 jours d'incubation. Le nombre de graines germées par modalité est ainsi dénombré et donne lieu à une évaluation de valeur de CI_{50} , correspondant à une inhibition de la germination de 50 % par rapport au témoin et déterminée par interpolation graphique et par analyse statistique (Sahmer *et al.*, 2015).

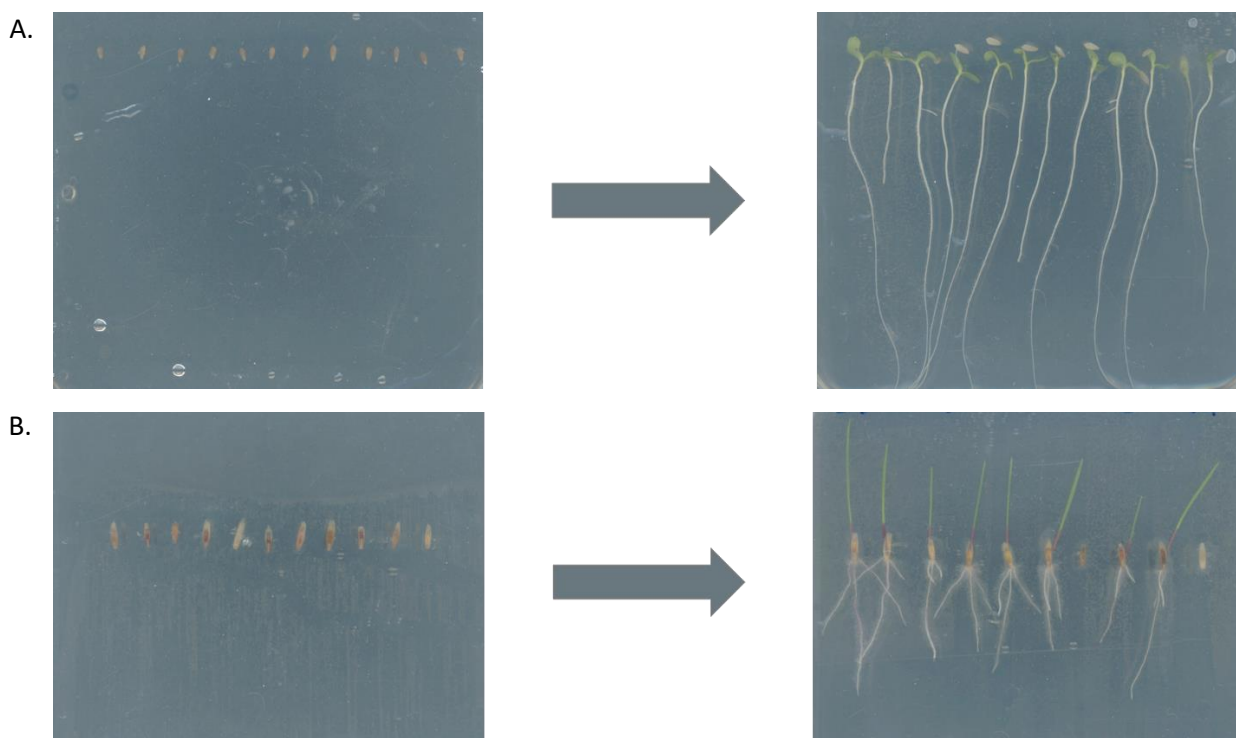


Figure 34 : Activité herbicide par contact direct contre la laitue (A.) et le ray-grass (B.). Photographe Robin Raveau.

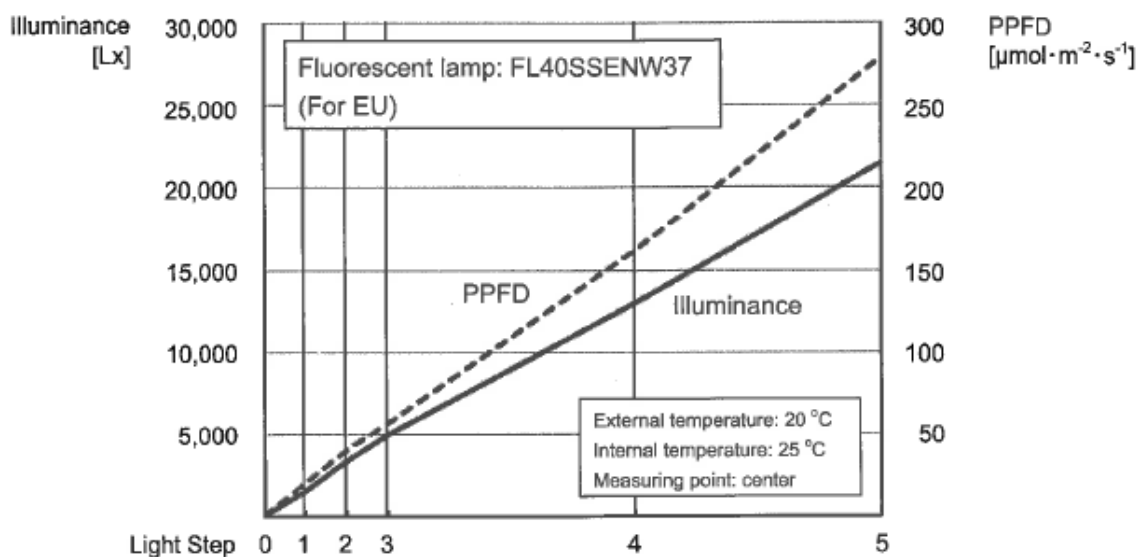


Figure 35: Correspondance entre la phase lumineuse et l'éclairage (en Lux).

2.4.2. Activité herbicide – inhibition de l'élongation racinaire

L'activité inhibitrice des HE vis-à-vis de l'élongation racinaire des deux espèces végétales a été évaluée de manière analogue au protocole décrit dans le paragraphe précédent.

A l'aide d'un scanner « Winrhizo », des photos sont prises des boîtes après 8 jours d'incubation. Les photos sont analysées à l'aide du logiciel ImageJ afin de déterminer l'élongation de la racicule, lorsqu'elle est présente. Une valeur de CI_{50} , correspondant à une inhibition de la croissance racinaire de 50 % par rapport au témoin est déterminée par interpolation graphique et par analyse statistique (Sahmer *et al.*, 2015).

2.5. Activité antioxydante

Le test de piégeage du radical 2,2-diphényl-1-picrylhydrazyl (DPPH*) a été utilisé pour déterminer l'activité antioxydante des différentes HE selon une méthodologie adaptée de Kfoury et al. (2019). Les composés présentant des propriétés antioxydantes réduisent le radical stable DPPH* en diphényl-picrylhydrazine. Cette réduction du DPPH* s'accompagne d'un changement de couleur quantifiable par spectrométrie UV/Vis. Pour ce faire, une gamme de concentrations croissantes d'HE (jusqu'à 4×10^{-3} %) a été préparée dans de l'éthanol pour chacune des HE testées. 1 mL de chaque solution d'HE éthanolique a ensuite été mélangé à 2 mL de solution de DPPH* éthanolique (0,1 mM). Tous les mélanges ont été agités dans l'obscurité pendant 1 h à 20 ± 1 °C. L'absorbance a par la suite été mesurée à l'aide d'un spectromètre UV/Vis (Perkin Elmer Lambda 2S) à la longueur d'onde maximum du DPPH*, soit 517 nm. L'activité antioxydante, par piégeage des radicaux libres, a été évaluée à l'aide de l'équation suivante :

$$\text{Activité antioxydante (AA) \%} = \left(1 - \frac{A_S}{A_0}\right) \times 100$$

Où AS et A0 représentent respectivement l'absorbance de l'échantillon et du blanc.

Les résultats obtenus pour chaque HE ont été rapportés en valeurs de CE50, correspondant à la concentration d'antioxydant qui provoque une diminution de 50 % de l'absorbance du DPPH*, par interpolation graphique. En outre, les valeurs de CE50 des HE ont été comparées à celles obtenues pour l'acide 6-hydroxy-2,5,7,8-tétraméthylchroman-2-carboxylique (Trolox®), un analogue hydrosoluble de la vitamine E, avec des résultats exprimés en Capacité Antioxydante en Equivalent Trolox® (TEAC).

2.6. Activité anti-inflammatoire

Une lignée de cellules épithéliales bronchiques humaines (BEAS-2B) a été cultivée dans des microplaques 96 puits de type CellBIND® (Corning - ThermoFisher Scientific, France). L'ensemencement a été réalisé dans 100 µL de milieu LHC-9, de manière à obtenir une densité de 40 000 cellules par puits le jour de l'évaluation (soit initialement 20 000 et 10 000 cellules pour 200 µL de milieu, pour une évaluation après 24 et 48 h d'exposition respectivement). Les plaques sont incubées à 37 °C sous atmosphère humide contenant 5 % de CO₂.

La cytotoxicité des HE a d'abord été évaluée sur la lignée cellulaire BEAS-2B à différentes concentrations, allant de 9×10^{-5} à $1,8 \times 10^{-3}$ %. Les cellules BEAS-2B sont incubées pour 24 ou 48 h, exposées ou non aux différentes HE et aux différentes concentrations testées. Après cette durée d'exposition, la cytotoxicité a été évaluée en mesurant l'activité extracellulaire de la lactate déshydrogénase (LDH) dans les surnageants (Cytotoxicity Detection Kit LDH, Roche Diagnostics), et l'activité de la déshydrogénase mitochondriale (MDH) (Cell Proliferation Reagent WST-1, Roche Diagnostics), comme décrit précédemment (Dergham et al., 2015 ; Kfoury et al., 2016). Des cellules non exposées à l'HE et exposées au Triton X-100 (2 %) ont été respectivement utilisées comme témoins négatifs et positifs.

Le potentiel anti-inflammatoire des HE a ensuite été évalué grâce à une méthode adaptée de Dergham et al. (2015). Les cellules BEAS-2B ont été préalablement ensemencées dans des microplaques CellBIND® de 96 puits, afin d'obtenir 40000 cellules/puits à la fin du temps d'exposition. Les cellules ont ensuite été exposées à des PM_{2,5}, c'est-à-dire à des particules d'un diamètre aérodynamique inférieur à 2,5 µm (15 µg/cm²), afin d'induire une réponse inflammatoire et cultivées pendant 48 h, en présence d'HE aux deux concentrations testées ($1,8 \times 10^{-4}$ et 9×10^{-4} %). Des cellules non exposées à l'HE et non exposées à la fois à l'HE et aux PM_{2,5} ont été utilisées respectivement comme témoins

positifs et négatifs. Après 48 heures d'exposition, une fraction aliquote des surnageants de cultures sans cellule a été collectée et congelée (-80 °C) pour la détermination des concentrations en cytokines et pour l'analyse de la teneur en protéines totales. Les concentrations en cytokines ont été déterminées par une analyse Elisa (Kits ELISA Quantikine, R&D Systems Europe, Ltd.) tandis que la teneur totale en protéines des surnageants de cultures cellulaires a été quantifiée à l'aide du kit Acide BiCinchoninique (Sigma-Aldrich, France). Deux interleukines, à savoir IL-6 et IL-8, ont été sélectionnées comme cytokines d'intérêt pour l'évaluation du potentiel anti-inflammatoire des HE testées, étant donné leur implication connue comme médiateurs pro-inflammatoires dans les cellules exposées aux particules atmosphériques (Dergham et al., 2015 ; Kfoury et al., 2016).

III. Caractérisation des communautés microbiennes rhizosphériques

1. Extraction de l'ADN génomique

1.1. Extractions réalisées à partir du sol

L'ADN génomique des microorganismes présents dans le sol a été extrait à partir de 250 mg de sol humide, préalablement stocké à -20 °C immédiatement après la réalisation des prélèvements sur le terrain (état initial des parcelles en 2017 – sol avant récolte 2017 – sol avant récolte 2018). Pour ce faire, un kit Nucleospin Soil® (Macherey-Nagel, Allemagne) a été employé, suivant les recommandations fournies par le fabricant :

- extraction : l'ADNg du sol est extrait du sol à l'aide d'une solution de lyse SL₁, éventuellement complétée d'un « Enhancer SX » pour en améliorer le rendement d'extraction. Cette étape est réalisée à l'aide de tubes à billes Nucleospin®, placés dans un broyeur à billes (2 cycles de 15 s à 5000 RPM) ;
- purification : une étape de purification par précipitations des contaminants (notamment protéines) est réalisée, par ajout d'un tampon de lyse SL₃ et centrifugation (1 min – 11 000 g) après une période d'incubation (5 min à 4 °C) ;
- filtration et liaison de l'ADN : le surnageant clair est centrifugé sur une nouvelle colonne Nucleospin® (1 min – 11 000 g) afin d'éliminer des inhibiteurs. L'ADN est ensuite lié à une seconde colonne Nucleospin® par ajout d'un tampon SB puis centrifugation (2 × 1 min – 11 000 g) ;
- lavages successifs : les substances humiques présentes dans le sol, ainsi que d'autres substances inhibitrices de PCR sont éliminées par des lavages successifs avec le tampon de liaison SB dans un premier temps (30 s – 11 000 g) puis avec des solutions de lavage SW₁ (30 s – 11 000 g) et SW₂ (30 s – 11 000 g).
- élution de l'ADN : après séchage de la membrane de la colonne Nucleospin® à laquelle l'ADN a été lié (centrifugation 2 min à sec – 11 000 g), l'ADN est élué à l'aide de 50 µL d'un tampon d'élution SE (5 mM Tris-HCl, pH 8.5).

La qualité des ADNg extraits du sol a été vérifiée par dépôt sur gel d'agarose (1 %) colorés au Gel Red.

La quantité d'ADNg présente dans les échantillons et sa qualité ont également été déterminées à l'aide d'un spectrophotomètre UV-Visible (SpectraMax iD3, Molecular Devices LLC, Sunnyvale, Etats-Unis) : 5 µL d'ADNg extraits à l'aide du kit Nucleospin Soil® sont dilués dans 65 µL d'eau ultra pure stérile et placés dans une Uvette Eppendorf. Une Uvette contenant uniquement 70 µL d'eau ultra pure stérile est utilisée pour réaliser l'étalonnage. Trois lectures sont réalisées, à 230, 260 et 280 nm afin d'obtenir les rapports d'absorbance A260/A280 (rapport de pureté par rapport aux protéines) et A260/A230

(rapport de pureté par rapport aux polysaccharides) et la quantification de l'ADNg présent dans l'échantillon.

1.2. Extractions réalisées à partir des racines des plantes cultivées

L'ADN génomique des microorganismes présents dans les racines de la sauge cultivée, prélevées *in situ*, lavées puis congelées à -20 °C, a été extrait en suivant la méthode d'Abu-Romman (2011) et d'Aleksic *et al.*, (2012). Un broyage préalable au mortier/pilon des racines dans de l'azote liquide est réalisé afin de réduire celles-ci en poudre.

- extraction : l'ADNg est extrait des racines réduites en poudre (500 mg), à l'aide d'un tampon d'extraction Cetyltriméthylammonium Bromide (CTAB – 1,4 M NaCl, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 2 % CTAB), auquel ont été ajoutés du Polyvinylpyrrolidone (PVP 1 %), du β -Mercaptoéthanol (5 %) et du charbon activé (0,5 % p/v). Une période d'incubation (30 min à 55 °C) est observée avant centrifugation (10 min – 16 000 g) ;
- purification : une étape de purification par précipitations des contaminants (notamment protéines) est réalisée, par deux extractions successives à l'aide d'un mélange chloroforme-alcool isoamylique (24:1) ;
- précipitation de l'ADN : la précipitation de l'ADNg est réalisée par ajout d'un volume d'isopropanol (100 %) puis incubation (1 h à 25 °C). Une étape de centrifugation est ensuite réalisée (10 min – 700 g) ;
- lavages successifs : le culot présent après centrifugation est rincé 3 fois successivement par ajout d'éthanol glacial (70 %). Une étape de centrifugation (10 min – 900 g) est réalisée après élimination de l'éthanol pour favoriser le séchage du culot d'ADNg ;
- élution de l'ADN : après séchage à température ambiante du culot d'ADNg (1 h – 20 °C), le culot d'ADNg est remis en suspension à l'aide de 50 μ L d'un tampon d'élution TE (10 mM Tris-HCl, pH 8.0 ; 1,0 mM EDTA, pH 8.0).

La qualité des ADNg extraits du sol a été vérifiée par dépôt sur gel d'agarose (1 %) colorés au Gel Red.

La quantité d'ADNg présente dans les échantillons et sa qualité ont également été déterminées à l'aide d'un spectrophotomètre UV-Visible : 5 μ L d'ADNg extraits à l'aide du protocole détaillé ci-dessus sont dilués dans 65 μ L d'eau ultra pure stérile et placés dans une Uvette Eppendorf. Une Uvette contenant uniquement 70 μ L d'eau ultra pure stérile est utilisée pour réaliser l'étalonnage. Trois lectures sont réalisées, à 230, 260 et 280 nm afin d'obtenir les rapports d'absorbance A260/A280 (rapport de pureté par rapport aux protéines) et A260/A230 (rapport de pureté par rapport aux polysaccharides) et la quantification de l'ADNg présent dans l'échantillon.

2. Amplification de l'ADN génomique par PCR

2.1. Amplification de l'ADNr 16S bactérien

Pour étudier les communautés bactériennes présentes dans le sol et les racines de sauge cultivée, une amplification par PCR d'une région de l'ADNr 16S bactérien a été réalisée à l'aide d'un couple d'amorces 341F / 805R ciblant les régions variantes V3 et V4 bactériennes (Figure 36). La taille attendue des amplicons obtenus par PCR est d'environ 400 pb (Herlemann *et al.*, 2011).

Ces amorces ont été complétées à leur extrémité 5' d'une séquence de 20 nucléotides spécifique, « adapter », permettant la fixation des fragments d'ADN simple brin sur un support solide pour le

séquençage. L'ajout d'un « Barcode » lors du séquençage, permettra également d'identifier les échantillons (Figure 37).

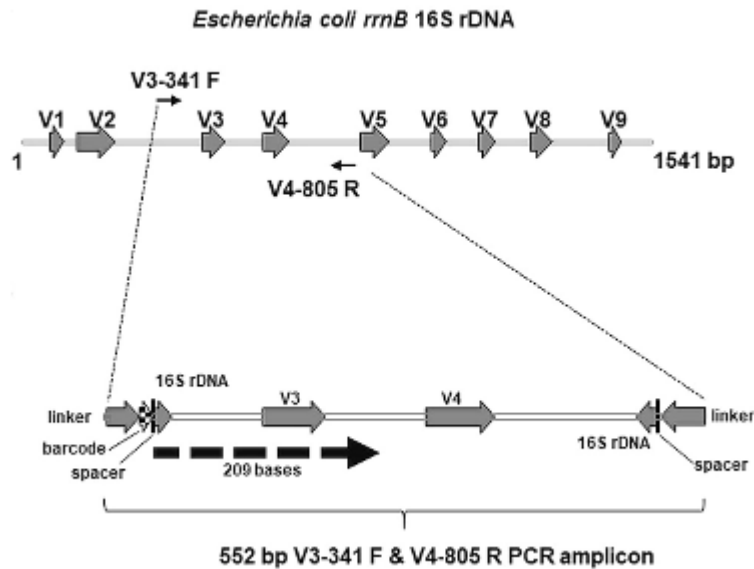


Figure 36: Représentation de l'ADNr 16S bactérien. V3-341-F indique la position de la séquence complémentaire pour l'amorce sens (340-356) et V4-805-R la position pour l'amorce anti-sens (784-804) – source Murugesan et al., 2018.

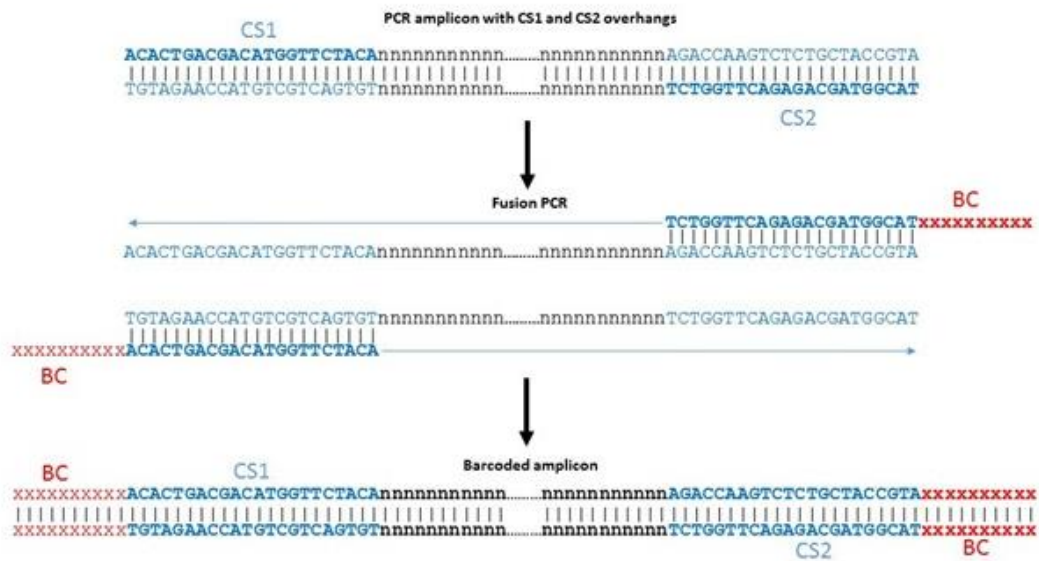


Figure 37: Principe de l'utilisation d'adaptateurs et de « barcode » pour le séquençage (<https://www.seqme.eu>).

La séquence du couple d'amorces, avec les adaptateurs CS1 et CS2 indiqués en gras est comme suit :

CS1_341-F (5' - **ACA CTG ACG ACA TGG TTC TAC ACC** TAC GGG NGG CWG CAG - 3')

CS2_805-R (5' - **TAC GGT AGC AGA GAC TTG GTC TCT** GAC TACC AGG GTA TCT AAT C - 3')

Deux réactions de PCR sont réalisées au sein d'un thermocycleur (Agilent Surecycler 8800), dans un volume de 25 µL par réaction. Le mélange réactionnel final contient 0,2 mM de dNTP, 0,4 µM de

chaque amorce (sens et anti-sens), 1 µL de DMSO, de la Séralbumine bovine (100 µg/mL), 1 ng d'ADNg, 0,5 U de Q5® High-Fidelity Polymérase (New England Biolabs France, Evry) et son tampon réactionnel.

Les conditions réactionnelles ont été adaptées d'après les préconisations du fournisseur et sont comme suit (Tableau 7) :

Tableau 7: Conditions réactionnelles de l'amplification par PCR avec les amorces 341F et 805R.

	Etape	Température	Durée
35 cycles	Dénaturation initiale	95 °C	3 minutes
	Dénaturation	95 °C	30 secondes
	Hybridation	56 °C	30 secondes
	Elongation	72 °C	50 secondes
	Extension finale	72 °C	5 minutes

2.2. Amplification de l'ADNr fongique

Pour étudier les communautés fongiques présentes dans le sol et les racines de sauge cultivée, une amplification par PCR d'une région de l'ADNr fongique a été réalisée à l'aide d'un couple d'amorces ITS₃ KYO₂-F / ITS₄ KYO₃-R ciblant l'internal transcribed spacer 2 (ITS₂ - Figure 38), situé entre l'ARNr 5.8S et l'ARNr 28S. La taille attendue des amplicons obtenus par PCR est de 327 pb.

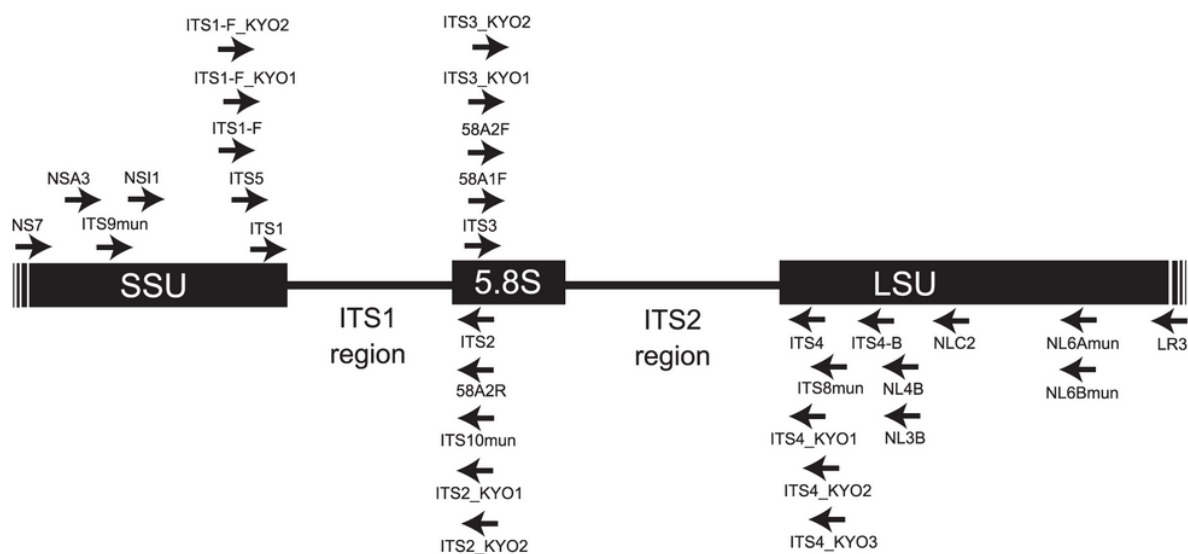


Figure 38: Cartographie des régions ITS1 et ITS2 et des gènes avoisinants codant pour l'ADNr – la position des amorces sens (flèche pointant à droite) et anti-sens (flèches pointant à gauche) est indiquée – source Toju et al., 2012.

Ces amorces ont été complétées à leur extrémité 5' d'une séquence de 20 nucléotides spécifique, « adapter », permettant la fixation des fragments d'ADN simple brin sur un support solide pour le séquençage. L'ajout d'un « Barcode » lors du séquençage, permettra également d'identifier les échantillons (Figure 37).

La séquence du couple d'amorces, avec les adaptateurs CS1 et CS2 indiqués en gras est comme suit :

CS1_ITS3-KYO2-F (5' - **ACA CTG ACG ACA TGG TTC TAC** AGA TGA AGA ACG YAG YRA A - 3')

CS2_ITS4-KYO3-R (5' - **TAC GGT AGC AGA GAC TTG GTC TCT** BTT VCC KCT TCA CTC G - 3')

Deux réactions de PCR sont réalisées au sein d'un thermocycleur (Agilent Surecycler 8800), dans un volume de 25 µL par réaction. Le mélange réactionnel final contient 0,2 mM de dNTP, 0,4 µM de chaque amorce (sens et anti-sens), 1 µL de DMSO, de la Séralbumine bovine (100 µg/mL), 1 ng d'ADNg, 0,5 U de Q5® High-Fidelity Polymérase (New England Biolabs), son tampon réactionnel ainsi que de l'« enhanceur Q5 », fourni avec la polymérase pour aider à la fixation des amorces.

Les conditions réactionnelles ont été adaptées d'après Toju *et al.*, (2012) et sont comme suit (Tableau 8) :

Tableau 8: Conditions réactionnelles de l'amplification par PCR avec les amorces CS1_ITS3-KYO2F et CS2_ITS4-KYO3R.

	Etape	Température	Durée
	Dénaturation initiale	95 °C	10 minutes
35 cycles	Dénaturation	94 °C	20 secondes
	Hybridation	47 °C	30 secondes
	Elongation	72 °C	20 secondes
	Extension finale	72 °C	7 minutes

2.3. Amplification de l'ADNr 18S des champignons mycorhiziens à arbuscules

Pour étudier la diversité des groupes de champignons mycorhiziens à arbuscules présents dans le sol et les racines de sauge cultivée, une amplification par PCR nichée d'une région de l'ADNr 18S fongique a été réalisée, à l'aide de deux couples d'amorces utilisés successivement : AML₁F / AML₂R puis CS1_nu-SSU-0595-5-F / CS2_nu-SSU-0948-3-R (Stefani *et al.*, en préparation). La taille attendue des amplicons obtenus pour la première étape de la PCR nichée est de 800 pb et de 400 pb pour la deuxième étape, comme présenté sur la Figure 39 :

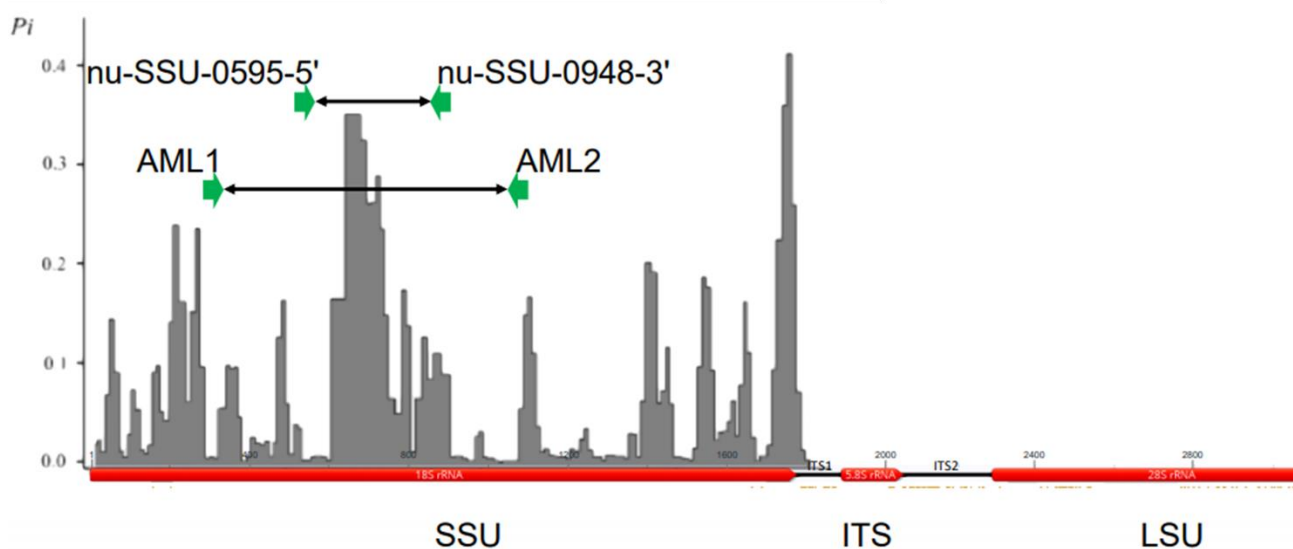


Figure 39 : Cartographie de la région amplifiée par PCR nichée (ADNr 18S - source Stefani *et al.*, 2020).

Ces dernières ont été complétées à leur extrémité 5' d'une séquence de 20 nucléotides spécifique, « adapter », permettant la fixation des fragments d'ADN simple brin sur un support solide pour le séquençage. L'ajout d'un « Barcode » lors du séquençage, permettra également d'identifier les échantillons (Figure 37).

La séquence des deux couples d'amorces utilisés pour les deux phases successives, avec les adaptateurs CS1 et CS2 indiqués en gras est comme suit :

AML₁-F (5' - ATC AAC TTT CGA TGG TAG GAT AGA - 3')

AML₂-R (5' - GAA CCC AAA CAC TTT GGT TTC C - 3')

CS1_nu-SSU-0595-5-F (5' - **ACA CTG ACG ACA TGG TTC TAC ACG** GTA ATT CCA GCT CCA ATA G - 3')

CS2_nu-SSU-0948-3-R (5' - **TAC GGT AGC AGA GAC TTG GTC** TTT GAT TAA TGA AAA CAT CCT TGG C - 3')

Deux réactions de PCR sont réalisées au sein d'un thermocycleur (Agilent Surecycler 8800), dans un volume de 25 µL par réaction. Le mélange réactionnel final contient 0,2 mM de dNTP, 0,4 µM de chaque amorce (sens et anti-sens), 1 µL de DMSO, de la Séralbumine bovine (100 µg/mL), 1 ng d'ADNg, 0,5 U de Q5[®] High-Fidelity Polymérase (New England Biolabs) et son tampon réactionnel.

Les conditions réactionnelles ont été adaptées d'après Lee *et al.*, (2008) et sont comme suit (Tableau 9) :

Tableau 9: Conditions réactionnelles de l'amplification par PCR nichée.

	Etape	Température	Durée	
Phase 1 Amorces AML1F / AML2R	Dénaturation initiale	94 °C	3 minutes	
	35 cycles	Dénaturation	94 °C	1 minute
		Hybridation	45 °C	1 minute
		Elongation	72 °C	1 minute
	Extension finale	72 °C	5 minutes	
Phase 2 Amorces CS1_nu-SSU- 0595-5-F / CS2_nu-SSU- 0948-3-R	Dénaturation initiale	94 °C	3 minutes	
	35 cycles	Dénaturation	94 °C	1 minute
		Hybridation	58 °C	1 minute
		Elongation	72 °C	1 minute
	Extension finale	72 °C	5 minutes	

3. Séquençage à haut débit – Illumina sequencing

Le séquençage des produits d'amplification PCR (ADNr 16S bactérien, ITS2 fongique et ADNr 18S des champignons mycorhiziens à arbuscules) pour l'analyse de la structure et de la diversité des communautés microbiennes a été réalisé par la technologie Illumina NGS (Illumina Next Generation Sequencing), au moyen d'un appareil MiSeq, par la plateforme de séquençage du Centre d'Innovation Génome Québec de l'Université de McGill (Montréal, QC, Canada).

Cette technique est basée sur une amplification en ponts et repose sur l'incorporation de terminateurs réversibles, marqués par des fluorophores, permettant l'identification des bases nucléotidiques et de la séquence d'ADN. Le processus est comme suit :

Les amplicons préalablement obtenus par PCR, auxquels ont été intégré un adaptateur, sont dénaturés afin d'obtenir des simples brins puis sont fixés aléatoirement sur un support solide « flowcell », grâce à l'adaptateur. L'extrémité libre comporte le second adaptateur et peut donc s'hybrider avec le

support, formant un pont. Une fois ancrés, les produits de PCR sont amplifiés grâce à une polymérase et ce cycle est répété, afin de former localement des groupes de séquences d'ADN identiques (clusters). Les brins anti-sens générés sont clivés après amplification pour ne garder que les brins sens.

Une fois ces clusters formés, l'amorce de séquençage vient s'hybrider sur l'extrémité 3' libre des brins d'ADN. Des dNTP - correspondants aux quatre types de nucléotides (A/T/C/G) - sont additionnés d'une étiquette fluorescente (fluorophore), caractéristique à chacun. Lorsque le nucléotide est incorporé à la séquence par la polymérase, un signal fluorescent est émis, permettant d'identifier la base, puis le fluorophore subit un clivage enzymatique afin de permettre l'intégration du nucléotide suivant et la suite de la lecture, nucléotide par nucléotide, jusqu'à obtenir la totalité de la séquence d'ADN. Les fluorophores possèdent sinon un groupement bloquant qui tant qu'il n'est pas clivé, empêche la formation de la liaison nucléotidique.

Le principal avantage de ce type de technique est qu'il se base non pas sur la lecture d'un seul fragment d'ADN pour le séquençage, mais d'une multitude de séquences identiques groupées localement et lues simultanément. Les séquences ainsi obtenues sont ensuite alignées et comparées avec une référence, afin d'identifier des différences. L'ensemble des étapes du séquençage Illumina peut être trouvé sur la Figure 40 suivante.

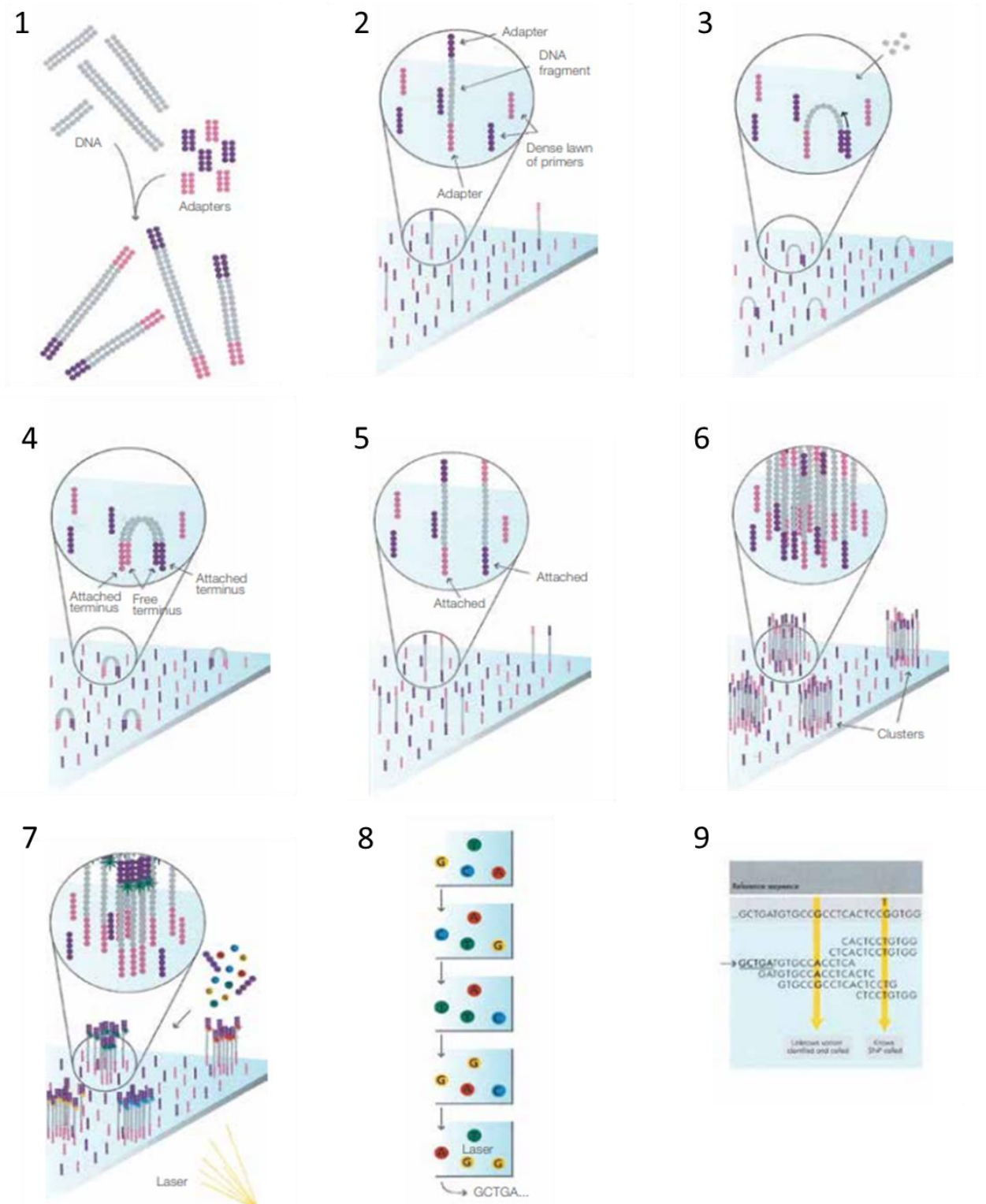


Figure 40 : Principe de la technologie de séquençage Illumina (<http://www.illumina.com>). 1, amplification de l'ADN par PCR et fixation des adaptateurs. 2, liaison des amplicons au support grâce aux adaptateurs. 3, formation de ponts par hybridation de l'extrémité 3' libre au support grâce au 2^{ème} adaptateur et amplification en pont. 4, l'amplification permet la synthèse de fragments double-brins. 5, libération de l'extrémité 3' après dénaturation de l'ADN double-brin pour obtenir des fragments simple-brins toujours ancrés au support. 6, nouveaux cycles d'amplification successifs, permettant la formation de clusters. 7, fixation de l'amorce de séquençage à l'extrémité 3' libre des fragments simple-brins et amorçage du séquençage avec la fixation et la détection de la première base. 8, répétition des cycles de lecture, base par base, jusqu'à obtention de la séquence complète. 9, alignement des séquences obtenues et comparaison à une référence.

4. Analyses bioinformatiques

Un pipeline bioinformatique, DADA2 (v. 1.12), a été utilisé afin de traiter l'ensemble des données issues du séquençage et de regrouper les séquences avec une identité de 100 % (Variants de Séquences d'Amplicons – ASV). Ce pipeline a été exécuté sous R 3.6.1 (R core Team, 2019), en suivant la procédure décrite (<https://benjjneb.github.io/dada2/tutorial.html>) par l'auteur du pipeline (Callahan et al., 2016). Ce pipeline se décompose en plusieurs grandes étapes:

- Vérification des profils de qualité : cette première étape permet de visualiser la qualité des séquences en sortie de séquençage, grâce à un Q score attribué à chaque nucléotide. L'analyse des profils de qualité permet de déterminer les paramètres pour l'étape suivante.
- Filtrage et troncage des séquences : l'utilisation d'une fonction « filterAndTrim » permet de filtrer les séquences en fonctions de paramètres définis.
- Apprentissage des taux d'erreur : cette étape spécifique au pipeline DADA2 consiste à estimer le taux d'erreur de séquençage selon un modèle d'erreur. Le but est de pouvoir différencier les séquences erronées des séquences mutantes.
- Déréplication : au sein de cette étape, toutes les séquences vont être regroupées ensemble en séquences uniques, auxquelles vont être attribuées une abondance.
- Inférence des échantillons : modèle d'inférence propre à DADA2 qui va permettre d'attribuer les ASV.
- Fusion des séquences par paires : cette étape va fusionner les deux brins complémentaires afin d'accroître la fiabilité de la méthode.
- Obtention des tableaux d'ASV.
- Suppression des chimères : cette étape consiste à éliminer toutes les séquences non biologiques éventuellement obtenues.
- Assignation taxonomique : grâce à l'utilisation de bases de données taxonomiques connues et à l'aide de la fonction « assignTaxonomy », l'assignation taxonomique est réalisée (attribution naïve Bayésienne). Cette assignation taxonomique a été réalisée à l'aide de la base de données Silva v132 pour les bactéries, et de la base de données Unite pour les Fungi et CMA.

En sortie de pipeline, les séquences ont donc été filtrées et triées selon les profils de qualité, un taux d'erreur modèle est calculé selon un algorithme puis les séquences sont dérépliquées avant d'attribuer les ASV. Le pipeline bioinformatique a été exécuté séparément sur les différents jeux de données, issus du séquençage d'échantillons provenant des racines ou du sol rhizosphérique, et résultant d'amplifications par PCR avec des amorces différentes, d'où la nécessité pour chacun de modéliser un taux d'erreur spécifique. Ainsi, les jeux de données « Bactéries – sol », « Bactéries – racines », « Fungi – sol », « Fungi – racines » et « CMA – sol », « CMA – racines » ont été traités séparément.

IV. Analyses statistiques

Pour l'ensemble des résultats obtenus, un test de Shapiro-Wilk ainsi qu'un test de Bartlett sont utilisés afin de vérifier la normalité des échantillons ainsi que l'homoscédasticité des données. Ces résultats conditionnent l'utilisation de tests paramétrique (Analyse de la Variance – Test ANOVA) ou non paramétrique (Mann-Whitney et Kruskal-Wallis). Ces tests permettent notamment d'étudier la dispersion entre les variables et d'étudier la significativité des différences observées. Ceux-ci sont réalisés à l'aide du logiciel R 3.6.1 (R core Team, 2019) ou du logiciel XLSTAT, Version 2018.2.50198

(Addinsoft, France), avec un risque d'erreur fixé de 5%, et combinés à un test de Tukey, afin d'obtenir des groupes statistiques, selon les différences observées. Les tests statistiques sont basés sur 3 ou 5 réplicats indépendants.

Dans le cas de données initialement exprimées en pourcentage, celles-ci ont été transformées en coordonnées angulaires (arcsinus de la racine carrée) afin de pouvoir leur appliquer un test statistique.

Dans le cadre des tests de propriétés biologiques, les valeurs de CI_{50} et CE_{50} ont été déterminées à l'aide d'une analyse en régression non linéaire à partir de trois réplicats et sont exprimées en tant que valeur moyenne complétée d'un écart type. La comparaison de ces valeurs a été réalisée à l'aide d'une ANOVA à deux facteurs (HE issue des différentes conditions expérimentales / présence ou absence de la β -CD) complété par un test *post-hoc* de Tukey-HSD afin d'établir les groupes statistiques. Une analyse en composantes principales (ACP) a également été utilisée pour évaluer les corrélations potentielles entre les compositions des différentes HE testées ainsi que d'éventuelles différences en termes de composition entre les différences conditions expérimentales.

Dans le cadre de l'étude des communautés microbiennes, l'ensemble des tests statistiques a été réalisé à l'aide du logiciel R 3.6.1 (R core Team, 2019). L'analyse de raréfaction a été conduite à l'aide de la fonction « rarecurve » du package *Vegan*. Une analyse multivariée des variances par permutations (PERMANOVA), basée sur une matrice de dissimilitude de Bray-Curtis, a été employée afin d'évaluer l'influence des conditions expérimentales (inoculation, temps de prélèvement) sur les communautés bactériennes ou fongiques. Des diagrammes de Venn ont été tracés à l'aide du package *Vegan* sous R. Les heatmaps de corrélation (basée sur une matrice de corrélation de Pearson) et d'abondance relatives ont été réalisées à l'aide de la fonction « heatmap2 » à partir du package *gplots*. Les indices de richesse (Chao1) et de diversité (Simpson, Shannon et Inv. Simpson) ont été estimés à l'aide du package *Phyloseq*.

Partie 3

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Résultats et discussions

Publication 1

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In situ cultivation of aromatic plant species for the phytomanagement of an aged-trace element contaminated soil: plant biomass improvement options and techno-economic assessment of the essential oil production channel

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Présentation de la Publication 1

Dans un contexte de forte pression démographique et de raréfaction des sols, la requalification de sites pollués, impropres aux activités agricoles, représente des enjeux économiques, environnementaux et sociaux. Parmi les méthodes émergentes de requalification des sols pollués, les phytotechnologies et la phytostabilisation en particulier, pourraient constituer une solution adaptée aux sols pollués par les ET. Jugées *a priori* plus en adéquation avec les enjeux du développement durable que les techniques conventionnelles physico-chimiques de traitement et/ou de gestion des sols pollués, elles bénéficient d'une bonne acceptabilité sociétale, sont applicables à de larges surfaces de sols pollués et sont relativement peu onéreuses à mettre en place (Burges et al., 2018; Gong et al., 2018). Néanmoins, deux points critiques conditionnent leur succès.

En premier lieu, le développement des plantes peut être entravé par les conditions pédo-agronomiques du site et la phytotoxicité élevée des polluants (Burges et al., 2018; Mench et al., 2010). C'est pourquoi, avant le lancement de tout projet de phytomanagement, une caractérisation du site est indispensable. Celle-ci permet de connaître précisément la nature et la concentration des polluants, l'étendue de la pollution ainsi que les caractéristiques pédo-agronomiques du sol. A cet égard, l'ajout d'amendements biologiques tels que les CMA, capables d'établir une relation symbiotique avec 80 % des végétaux supérieurs (Smith and Read, 2008) et décrits pour leur capacité à stimuler la croissance des plantes, à améliorer leur tolérance au stress généré par la présence des polluants dans le sol (Ferrol et al., 2016; Garg et al., 2017) et à contribuer à l'immobilisation des ET au niveau de la rhizosphère (Begum et al., 2019; Garg et al., 2017), pourrait aider à améliorer l'efficacité de la phytotechnologie choisie.

En second lieu, le problème de la rentabilité socio-économique de la méthode est souvent pointé comme étant relativement limité, lorsque la biomasse végétale produite n'est pas valorisée (Bert et al., 2017; Kumpiene et al., 2019). A ce titre, le développement dans le cadre d'une approche de phytomanagement, d'une filière rentable s'appuyant sur des produits biosourcés issus de la biomasse végétale produite sur ces sols pollués, pourrait permettre d'abaisser les coûts de gestion et de faciliter la requalification de ces sites en répondant aux différentes attentes. Parmi les filières éco-innovantes de valorisation de la biomasse, la culture de plantes aromatiques et la distillation d'HE, produits biosourcés à forte valeur ajoutée, est proposée comme une option novatrice et prometteuse.

Malgré le caractère pérenne de l'angélique et un marché porteur pour la commercialisation de son HE, cette espèce de PPAM n'a pu être évaluée dans le cadre de ce projet. En effet, un faible pouvoir germinatif de la semence et des conditions climatiques exceptionnelles (sécheresse estivale et températures moyennes supérieures aux normales saisonnières – Archives climatiques Infoclimat, 2018 et 2019) ont conduit à des très faibles taux de levée *in situ*. L'angélique s'est par la suite bien développée suite à un semis en septembre 2018, mais du fait de sa floraison en deuxième année, la récolte des graines n'a pu avoir lieu que fin juin 2020

Dans le cadre de cette première publication, nous nous sommes donc attachés à étudier la viabilité technico-économique de la filière associant la culture de deux PPAM, la coriandre et la sauge sclarée, à la production d'HE à activités biologiques. Cette étude a été menée sur deux sites expérimentaux, pollués (Evin-Malmaison) ou non (Rodelinghem) par les ET. La coriandre, espèce annuelle, a été semée deux années successives afin de comparer la culture et la production d'HE à partir des parties aériennes (année 1) ou des graines (année 2). La sauge sclarée présentant elle un cycle de développement

bisannuel, a été suivie sur l'ensemble de son cycle de vie (3 ans) et les inflorescences ont été récoltées pour la distillation des HE. Dans ce contexte, la pertinence ou non de l'ajout d'un inoculum mycorhizien commercial (AGTIV®, Premier Tech) a également été évaluée en termes de croissance des PPAM, de production de biomasse et de rendements en HE.

Dans un premier temps, les deux sites expérimentaux ont été caractérisés d'un point de vue pédo-agronomique (en collaboration avec Valérie Bert, INERIS) et biologique. Les résultats indiquent des teneurs en ET de 0,4 ppm de Cd, 22 ppm de Pb et 48 ppm de Zn sur la parcelle non polluée, tandis que la parcelle localisée dans le périmètre du site Metaleurop présentait les teneurs en ET suivantes : 7 ppm de Cd, 394 ppm de Pb et 443 ppm de Zn. La disponibilité des nutriments ainsi que les indicateurs de fertilité des sols (teneur en matière organique, carbone organique total, teneur en azote) ont également été déterminés, indiquant des valeurs plus élevées sur le site pollué par les ET. Avant semis, 143 et 157 spores/100 g de sol ont par ailleurs été isolées et dénombrées, sur les parcelles polluée et non polluée respectivement. L'étude de la colonisation racinaire par les CMA des espèces végétales qui se développent spontanément sur les deux sites a également révélé la présence de structures mycorhiziennes (notamment arbuscules, vésicules), avec des taux de mycorhization d'environ 4 et 7 % sur les parcelles polluée et non polluée respectivement. Un inventaire phytosociologique a enfin permis d'identifier 24 espèces végétales, appartenant à 14 familles botaniques différentes, sur la parcelle polluée par les ET. La biodiversité végétale, évaluée au travers de différents estimateurs, indique une richesse en espèces de 1,09 et 3,72 (indices de Menhinick et Margelef resp.) et une diversité de 2,15 et de 0,85 (indices de Shannon & Weaver et Simpson resp.).

Nous nous sommes ensuite attachés à évaluer la levée et la croissance des PPAM sur les sites expérimentaux. Nos résultats montrent que les deux espèces végétales testées, la coriandre et la sauge sclarée, parviennent à se développer sans difficulté sur le sol historiquement pollué par les ET. Pour la coriandre, des taux de levée variant entre 50 et 84 % ont été déterminés *in situ*, sans différence significative entre les deux parcelles expérimentales polluée et non polluée, quelle que soit l'année de culture et la modalité inoculée ou non. Pour la sauge sclarée, les taux de levée étaient significativement supérieurs sur la parcelle polluée, atteignant en moyenne 68 %, alors qu'ils étaient à hauteur de 45 % sur la parcelle non polluée par les ET, quelle que soit la condition inoculée ou non par le CMA.

En termes de croissance, la coriandre atteignait à la récolte des parties aériennes (année 1) une hauteur de 66 et 55 cm en moyenne, sur les parcelles non polluée et polluée respectivement, et des poids secs respectifs de 10 et 4 g/plante. Les valeurs affichées étaient notamment similaires entre conditions inoculées ou non. A la récolte des graines (année 2), les plants de coriandre atteignaient une hauteur de 77 cm en moyenne, quelle que soit la modalité, tandis que les poids secs étaient de 11 et 7 g/plante, sur les parcelles non polluée et polluée respectivement et quelles que soient les modalités, inoculées ou non. Concernant la sauge sclarée, sa hauteur moyenne atteignait 39 et 32 cm la première année, s'accroissant à 160 et 145 cm lors de la deuxième année de culture, puis diminuant à 108 et 104 cm lors de la troisième année, sur les parcelles non polluée et polluée, respectivement. Ces valeurs étaient similaires entre les conditions inoculées ou non, quelle que soit l'année de culture. Par ailleurs, une différence en termes de poids sec et de hauteurs de la sauge sclarée, entre les parcelles polluées ou non, a seulement été mise en évidence en année 2.

Une mycorhization spontanée des espèces végétales a été mise en évidence dans nos conditions expérimentales, pour les deux espèces cultivées. Les taux de mycorhization obtenus pour la coriandre

et la sauge sclarée étaient respectivement compris entre 3 et 8 % et entre 4 et 30 % pour la condition non inoculée. Par ailleurs, l'introduction d'un amendement mycorhizien commercial dans le sol a permis une augmentation des taux de mycorhization comprise entre 50 et 75 % par rapport à la modalité non inoculée, pour la coriandre ou la sauge sclarée. De plus, il est à noter que pour la sauge sclarée, les taux de mycorhization ont augmenté significativement au cours du temps, de 4 % en moyenne la première année, quelle que soit la modalité, à 40 % la troisième année.

Les rendements de distillation d'HE obtenus pour la coriandre étaient en moyenne de 10 et 6 kg/ha pour les HE issues des parties aériennes et des graines respectivement, sans différences significatives entre les modalités inoculées ou non par le CMA et les parcelles expérimentales polluée ou non par les ET. Concernant la sauge, les meilleurs rendements en HE ont été obtenus en deuxième année de culture, compris entre 13 et 34 kg d'HE /ha, avec des quantités en HE similaires entre les parcelles polluées ou non par les ET.

Par ailleurs, la qualité des HE obtenues a été vérifiée en recherchant la présence éventuelle d'ET et de résidus de pesticides dans les HE. Nos résultats démontrent que les HE ne contiennent que des teneurs à l'état de traces ou inférieures aux limites de quantifications, que ce soit pour les résidus de pesticides ou les ET.

Enfin, l'étude de faisabilité technico-économique repose sur les coûts de production et de distillation (300 € pour un chargement de 14 m³ de biomasse) des HE et leur valeur marchande. Elle a révélé que les coûts de productions annuels s'élevaient à 706, 772 et 386 €/ha pour la coriandre (parties aériennes et graines) et la sauge sclarée, respectivement. En tenant compte de ces coûts de productions, les marges brutes dégagées pour la coriandre étaient respectivement de 150 €/ha et - 400 €/ha pour les parties aériennes et les graines et de 1600 €/ha en moyenne pour la sauge sclarée, s'élevant jusqu'à 4485 €/ha sur la parcelle polluée par les ET.

Dans le cadre de cette étude, je me suis chargé de l'ensemble de la caractérisation biologique ainsi que du suivi des cultures, de l'évaluation des paramètres de croissance et de la colonisation racinaire par les CMA. J'ai également réalisé l'ensemble des analyses statistiques. La mise en place du dispositif expérimental ainsi que les prélèvements de sol et de plantes ont été réalisés en collaboration avec l'INERIS. L'analyse des ET dans les HE est aussi le fruit du travail réalisé par A. Perleïn (INERIS). Les étapes de semis, récolte et distillation ont été réalisés avec l'aide de P. Ferrant (FERRANT PHE). Les bases de l'analyse technico-économique ont été dressées en collaboration avec P. Ferrant ainsi que F. Dutertre (Chambre d'agriculture du Nord-Pas-de-Calais). J'ai également rédigé cette publication avec l'aide des co-auteurs.

In situ cultivation of aromatic plant species for the phytomanagement of an aged-trace element polluted soil: plant biomass improvement options and techno-economic assessment of the essential oil production channel

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Abstract

Due to the presence of trace element (TE) in agricultural soils, wide areas are unproper for food production. Considering the potential remediation options, the use of aromatic plants producing a high quantity of biomass and developing high-added value sectors such as essential oil (EO) production could be valuable regarding a reduction in global costs of phytomanagement approaches and towards the restoration of an economic activity. The purpose of this study was hence to evaluate *in situ* the suitability of two aromatic crops, clary sage and coriander, for the phytomanagement of TE-polluted soils, taking into account plants' growth, development and biomass production, essential oil (EO) content and quality as well as a techno-economic feasibility analysis of the channel. Experiments have been carried out *in situ* on two agricultural plots of 1.5 ha, a TE-polluted one (Pb: 394 ppm - Zn: 443 ppm - Cd: 7,2 ppm) and an unpolluted one. Our findings have shown the ability of coriander and sage to grow similarly on both unpolluted and TE-polluted soil, to produce significant amounts of biomass and to result in satisfactory amounts and qualities of EO. Notably, the pesticide residue and TE analyses demonstrated that the EO only contained trace amounts of the contaminants, below or close to the limit of quantification of the method used and similar to marketed products. Mycorrhizal inoculation has also shown promising results by increasing the colonization rates of both aromatic plants, but did not result in higher biomass or EO amounts. Our study brings new evidence towards the potential of clary sage to be used for the phytomanagement of TE-polluted areas, given its perennial vegetation cover, tolerance to TE and obtained EO yields.

Keywords: Essential oil, Trace Elements, *Salvia sclarea*, *Coriandrum sativum*, Arbuscular mycorrhizal fungi

1. Introduction

Soil pollution is worldwide a major environmental and health concern. A recent study has estimated the number of potentially polluted sites in Europe to more than 2 Million (van Liedekerke *et al.*, 2014; Mench *et al.*, 2018). Trace Elements (TE), mainly originating from anthropogenic sources (including wastewater discharge, agricultural inputs and practices, refining and mining activities, atmospheric fallout originating from smelting activity and fossil fuels' combustion as well as human-induced soil erosion), are the most frequently listed soil pollutants in Europe (37 % - Antoniadis *et al.*, 2017; Gong *et al.*, 2018; Mench *et al.*, 2018; Panagos *et al.*, 2013). Owing mostly to their non-biodegradability, TE are a major threat for ecosystems, as they are susceptible to affect both soil and water (surface and groundwater) quality and accumulate into living organisms, causing ecological disturbances (Ali *et al.*, 2019; Antoniadis *et al.*, 2017).

One of the main issues with TE is the pollution of agricultural soils. Trace elements are likely to affect not only the plant growth and productivity, but also human and animal health when accumulated in edible food crops which will further be consumed either for food or feeding purposes and lead to a contamination of the food chain (Ali *et al.*, 2019; Gong *et al.*, 2018). Notably, health risks in the case of an exposure to excessive TE concentrations include various disorders such as kidney dysfunction, bones fragility or neurological disorders and reduced cognitive ability (Järup, 2003).

Conventional physicochemical soil remediation techniques usually result in elevated costs and may cause a degradation of the ecosystem as well as a potential risk of displacement of the pollution during transportation of the excavated soil for further *ex situ* processing or storage (Kumpiene *et al.*, 2019). Additionally, these methods may not be applicable on large areas as it is often the case for agricultural fields. Therefore, one of the existing options to environmentally and economically manage TE-polluted agricultural soils, developed during the last decades, is the use of phytotechnologies (Barbosa and Fernando, 2018; Burges *et al.*, 2018). In particular, phytostabilisation, which relies on the use of plants and their associated microorganism's ability to reduce TE mobility in the soil and availability for the living organisms, is brought forward as a promising tool. In addition to the reduction of TE bioavailability, (re)establishing a vegetation cover aims at reducing leaching and erosion phenomena and potentially restoring soil structure and function (Barbosa and Fernando, 2018; Dongmei and Changqun, 2008; Kidd *et al.*, 2015). This technology might be aided through the use of adequate either biological, organic or mineral amendments, aiming at enhancing the TE immobilization or facilitating both plant establishment and growth (Barbosa and Fernando, 2018; Kidd *et al.*, 2015). Among microbial amendments, arbuscular mycorrhizal fungi (AMF) could be particularly relevant for aided phytostabilisation. These soil-borne fungi are obligate biotrophs, able to establish symbiotic relationships with nearly 80 % of the vascular plant species (Ferrol *et al.*, 2016; Smith and Read, 2008). These symbiotic fungi are widely studied for their benefits in cropping systems, not only for the improved plant growth through enhanced water and nutrient uptake, but also for their contribution to an increased tolerance towards biotic and abiotic environmental stresses (Begum *et al.*, 2019; Smith and Read, 2008). AMF benefits could be translated in aided phytostabilisation process, in particular, for their contribution in improving plant biomass production (Smith and Read, 2008). Additionally, their potential to alleviate TE stress in plants and TE transfer and accumulation in aerial parts and to enhance TE immobilization were previously highlighted (Ferrol *et al.*, 2016; Gong *et al.*, 2018). Notably, the contribution of glomalin (glomalin-related soil protein) produced by AMF, has been evidenced regarding soil aggregation and TE stabilisation (Ferrol *et al.*, 2016; Wang *et al.*, 2019). Nonetheless, aided phytostabilisation faces several obstacles and among them, the fact that if the vegetation cover is not valued, the economic profitability remains scarce, which may be prohibitive (Burges *et al.*, 2018; Vigil *et al.*, 2015). Biomass valorisation thus appears as a key feature.

Hence, the combination of a phytotechnology aiming at reducing the risk bound to the presence of TE with a sustainable and profitable land and biomass use, defined as phytomanagement, offers the asset of generating an economic profit, along with environmental and health benefits, while mitigating the TE-risk (Burges *et al.*, 2018; Cundy *et al.*, 2016; Mench *et al.*, 2018). The selection of appropriate plant species is another critical aspect towards a successful phytomanagement (Burges *et al.*, 2018; Yang *et al.*, 2016). In particular, plants have to be able to grow in TE-polluted conditions and to develop a vegetation cover in a relatively short period of time, while producing high amounts of biomass towards a potential valorisation of the vegetation cover (Evangelou *et al.*, 2015; Yang *et al.*, 2016). On this last point, non-food valorisation, such as bioenergy crops or renewable materials, including high-added value products, could be particularly relevant regarding phytomanagement.

In that matter, aromatic crops used for essential oils' (EO) production on TE polluted agricultural soils, may be a suitable alternative. First, EO are high added-value products, widely used as aromatic agents in various non-food industries, such as perfumery, cosmetics and medicine, as well as potential crop protection products, which could provide economic benefits with the produced biomass (Lydakis-Simantiris *et al.*, 2015; Raveau *et al.*, 2020; Zheljazkov *et al.*, 2008). Additionally, as in the current state agricultural TE-polluted soils are unsuitable for consistent and economically viable food production, this channel would not compete with feeding agriculture. Moreover, the cultivation of aromatic crops for phytostabilisation purposes has often been highlighted as a feasible option on TE-polluted sites, since TE potentially accumulated in the distilled biomass were not present or in really low amounts in the EO (Angelova *et al.*, 2016; Zheljazkov *et al.*, 2008). Finally, the global market EO and on a broader level for plant products, is steadily rising and expected to reach more than \$10 billion in 2020 and \$15.6 billion in 2026 (Allied Market Research, 2020; CBI, 2020). Therefore, cultivation of aromatic plant species, based on decision criteria such as pedo-climatic site conditions, TE plant tolerance and life cycle, as well as their potential regarding phytostabilisation and valorisation perspectives, could be a profitable and economically viable option. In particular, the effects of TE-pollution on the growth and EO production and quality of commercially important aromatic crops such as coriander (*Coriandrum sativum* L.) or clary sage (*Salvia sclarea* L.) have received little attention and insufficient information regarding their potential for TE immobilisation and their possible use for phytostabilisation are available. Coriander is an annual plant, belonging to the *Apiaceae* family, grown for both its aerial parts and seeds, while clary sage, belonging to the *Lamiaceae* family, is a biennial or short-lived perennial plant, cultivated for its inflorescences (Angelova *et al.*, 2016; Grigoriadou *et al.*, 2019; Mandal and Mandal, 2015). Both plant species are native from the Mediterranean area and their respective EO are widely used in pharmaceutical products as well as in cosmetics and in traditional medicine (Angelova *et al.*, 2016; Mandal and Mandal, 2015; Saharkhiz *et al.*, 2009). Additionally, they were previously demonstrated tolerant to TE and capable of growing in TE-polluted conditions (Angelova *et al.*, 2016; Chand *et al.*, 2015; Zheljazkov *et al.*, 2008).

Therefore, the aim of this study was to evaluate the techno-economic feasibility of the EO production channel, distilled from aromatic crops grown on TE-polluted fields. It addresses three main questions: (1) Are aromatic crops, in particular coriander and clary sage, able to grow in TE-polluted soils in the north of France and to produce significant amounts of biomass, sufficient to be distilled in EO with satisfactory yields? (2) Is the quality of the produced EO faithful, regarding the potential presence of both TE and chemical residues in the final product, intended for non-food valorisation? (3) Can the addition of a biological amendment based on AMF improve biomass and EO production yields in TE-polluted *in situ* conditions?

2. Material and methods

2.1. Experimental site

Experiments have been carried out *in situ* on two agricultural plots of more than one hectare in the north of France. The unpolluted site is located in Rodelinghem (1.4 ha - 50°49'55.7" N, 1°55'46.9" E). The TE-polluted site, in Evin-Malmaison (1.3 ha - 50°25'55.5" N, 3°02'25.5" E), is a former agricultural field, located approximately 600 m away from a former lead smelter, Metaleurop Nord. This smelter, located in the historical mining area, has been in activity for more than a century until 2003, and has generated significant amounts of TE through atmospheric emissions and TE-enriched dusts (Labidi *et al.*, 2015; Sterckeman *et al.*, 2002). These emissions have led to a topsoil (0-30cm) pollution of the surrounding area on more than 700 ha of agricultural soils.

2.2. *In situ* experimental design

Both aromatic plants, clary sage (*Salvia sclarea* L.) and coriander (*Coriandrum sativum* L.), were cultivated on the two unpolluted and polluted experimental sites. The plots were divided into equivalent areas for each plant species and devoted to their cultivation. Additionally, the available surface for each plant was subdivided in two plots. The first plot was amended (I) with a commercial mycorrhizal inoculant while the other one was not amended and was considered as the non-inoculated control on each experimental site (NI – Figure S1).

So as to evaluate the phytomanagement channel from an agricultural perspective, sowing, cultivation practices and harvest were realised with farm scale equipment. Soil preparation was achieved through conventional tillage (10 cm-depth ploughing) and the sowing steps were carried out in April (2017), for the two experimental sites and the two plant species (300 000 seeds per hectare, 3-4 cm deep). Additionally, coriander was sown again in April (2018) for a second year of cultivation.

2.3. Floristic inventory of the native species

The floristic inventory was based on observations realised before cultivation practices, in April, on the TE-polluted site, naturally colonized by plant species. The position of 13 quadrats (1 m² each) was randomly determined on the experimental site and geo-referenced. All plant species naturally growing were sampled (three individuals per species), identified using the manuals from Bonnier and de Layens, 1986 and Lambinon *et al.*, 2004 and compared with regional data (Centre Botanique National de Bailleul, url: <http://www.cbnbl.org/centrederdigit.htm>).

A cover-abundance value was allocated to each identified species according to Braun-Blanquet cover-abundance scale (1951). Several biodiversity indices were also calculated to estimate plant diversity: First, Menhinick and Margalef indexes that indicate species richness (Margalef, 1958; Menhinick, 1964). Then, Shannon index that represents the spatial distribution as well as the abundance of identified plant species, and may additionally reveal rare species (Shannon, 1948; Shannon and Weaver, 1964). Eventually, Simpson index that measures the probability that two randomly selected individuals from a sample belong to the same species (heterogeneity - Simpson, 1949).

2.4. Isolation of autochthonous AMF spores and root mycorrhizal colonization

To assess the presence of AMF in soils on both experimental sites and hence guide the choice towards the use a mycorrhizal inoculum or not, 12 soil samples have been collected before any cultivation practice, from randomly chosen positions and where plants were naturally growing. AMF spores were extracted from the soil samples by wet sieving (Oehl *et al.*, 2004) and counted under microscopic

observation (Nikon Eclipse E600, x100 magnification). The AMF spores' content (number of spores per 10 gram of soil) was assessed in triplicates.

To evaluate AMF root colonization, fresh roots of the plants naturally growing on the experimental sites were sampled and cleared in a KOH 10 % (v/v) solution for 1h at 70 °C and then stained with trypan blue (0.05 %) in a water, glycerol and lactic acid mixture (1:1:1, v/v/v) for 1 h at 70 °C (Phillips and Hayman, 1970). The stained root fragments were stored at 6 °C in a water/glycerol (1:1, v/v) solution until they were observed. Roots' fragments were examined with microscopic observations (Nikon Eclipse E600, x100 magnification) to detect the presence of specific mycorrhizal structures (arbuscules, vesicles and intraradical hyphae) and to determine mycorrhizal rates. Root colonization was evaluated using the grid line intersect method (McGonigle et al., 1990). Root fragments from three replicates (45 fragments in total per condition) were observed and 3 intersections per root fragment were examined.

2.5. Plant species and amendment

Seeds of *C. sativum* and *S. sclarea* were purchased from the French research institute ITEIPMAI, providing certified seeds. A commercial AMF inoculant (AGTIV® Specialty Crops) containing the AMF *Rhizophagus irregularis* (12000 viable spores per gram of product) kindly provided by PremierTech was used. The powdery inoculant was introduced during sowing, directly mixed with seeds and applied at a rate of 125 g/ha.

2.6. Determination of emergence rates of sage and coriander on unpolluted and polluted sites

Six weeks after sowing, the emergence of the seedlings has been determined *in situ* for each plant and for each condition (plants inoculated with the AMF or not). For each condition, three randomly chosen positions were determined and for each location, the number of seedlings was visually determined in a one square meter quadrat, replicated 3 times (adapted from Gunaratne *et al.*, 2010 and Yan *et al.*, 2007). Each quadrat was also geo-referenced.

2.7. Weed control

Based on ITEIPMAI recommendations, only two herbicide applications have been carried out with a three-week interval to regulate the spontaneous growth of gramineous and dicotyledonous species (Table S1). In particular, the presence of *Matricaria recutita* L. was necessary to avoid as it is likely to contaminate and alter EO composition and quality. No fungicidal or insecticidal treatments have been carried out. The chemicals used were applied in half doses. Additionally, during the second and third years of sage's cultivation, the number of crop protection products and the number of applications were reduced (respectively to 3 and 1 – Table S1).

2.8. Root and aerial plant part samplings

Sampling locations were equally spaced and determined to best match the plot's shape and each one was geo-referenced. The roots and whole aerial parts of the 3 closest growing plants (sage or coriander) were collected to characterize the biomass development. For each sampled plant (three replicates per sampling point), the height was measured and they were then placed in an oven (72 h at 80 °C), to determine their dry weights. Root samples were prepared for further evaluation of the root mycorrhizal colonization in cultivated plants, using the same methodology as previously described (Section 2.4.).

2.9. Biomass harvesting

Plants have been harvested with farm equipment, based on recommendations given by a French institute (ITEIPMAI) providing technical and crop management advice regarding aromatic plants' cultivation. Aerial parts of coriander (annual life cycle) were harvested with a mow-picker on the first year, after 13 weeks of cultivation. Seeds of coriander were harvested using a combine on the second year, after 19 weeks of cultivation.

The cultivation of clary sage (short-lived perennial life cycle) was carried out on three successive years. During the first year of sage's cultivation, aerial parts (mostly large leaves) were harvested, using a mower and a mow-picker. Flowery parts of sage were then harvested on the second and third years of cultivation using successively a mower and a pick-up loader wagon.

2.10. Sage and coriander EO distillation

The distillation of the harvested plant biomass was performed on the harvest day. The harvested biomass was loaded in a distillation tank (14 m³) and water steam heated by a wood-fired boiler (saturated water steam, 0.3 bar) was injected. The formed vapours were recondensed in a cooling-unit and the distillate was collected in a receiver/separator. The process was conducted during a three-hour cycle, until no more EO was recovered. The EO yield expressed as a percentage represents the distillation yield and was calculated according to the following equation (1):

$$(1) \text{ EO yield (\%)} = \frac{\text{Raw plant biomass (m}^3\text{)}}{\text{Essential oil amount (m}^3\text{)}} \times 100$$

The agronomic EO yield is expressed as the quantity of EO produced per hectare, and was calculated using equation (2):

$$(2) \text{ EO yield (kg/ha)} = \frac{\text{Essential oil amount (kg)}}{\text{Plot surface (ha)}}$$

2.11. Essential oil's quality assessment

2.11.1. Trace elements assessment in distilled EO

Essential oil obtained from each plant and experimental condition were aliquoted (5 mL) and analysed through ICP-MS (Agilent 7500). Briefly, sample digestion was carried out in a microwave oven at 180 °C for 20 min, using an aqueous solution of nitric acid (3:10 – 10 mL). In addition, samples from one market product of clary sage and two of coriander EO (aerial parts and seeds) were analysed to compare their quality in terms of TE content. All TE analyses were led in triplicates.

2.11.2. Pesticide residues analysis

Multi-residue analysis targeting about 600 active molecules, were performed through either GC-MS/MS (method MOC3/05(S1), version 0) or LC-Q-TOF (method MOC3408(S1), version 2) screenings, by Phytocontrol Analytics – France.

2.12. Techno-economic feasibility analysis

To estimate the viability of the EO production channel on both unpolluted and TE-polluted soils, a techno-economic analysis has been carried out. The production costs for EO production, involving the cultivation of aerial parts of coriander, seeds of coriander or sage inflorescences have been evaluated on each crop and for each experimental condition (polluted or unpolluted site, I or NI conditions) separately and were expressed per hectare. They include the operating costs (material amortisation and maintenance, fuel, labour cost, etc) and the supply expenses (seeds, fertilizers, AMF inoculant and

crop protection products). They were estimated according to two reference handbooks: the Field Crop Protection Guide (updated each year by the Chamber of Agriculture of each French region) which provides data about crop protection products and their costs and a second guide providing technical information about agricultural equipment, regarding material and operating costs. They included soil preparation, sowing, inputs, harvest and potential post-harvest costs. These were compared to other phytoremediation or phytomanagement methods and also to the production costs of common field crops (wheat, winter barley and rapeseed).

In addition, a gross profit is estimated from the obtained EO yields for the 3 conditions (sage and coriander aerial parts or seeds), using the following equation:

$$(3) \text{ Gross Proceeds} = \text{EO yield} \times \text{EO selling price (per kg)}$$

One should note that the EO selling price is the price paid by companies before marketing EO as a final product. The distillation process was considered subcontracted in the present evaluation.

3. Statistical analysis

Statistical analysis has been done using the R software, version 3.5 and XLSTAT 2018.1.1. Data were first subject to Shapiro-Wilk (normality), Bartlett (homoscedasticity) and Fligner Killeen (variances' homogeneity) tests, before further analyses. According to the results of the previously mentioned tests, parametric (pairwise t test and ANOVA) or non-parametric (Kruskal-Wallis) statistical tests were chosen, complemented with a post-hoc Tukey HSD (Honestly significant difference) test. Data expressed in percentages (in particular emergence and colonization rates) were converted to arcsine values before using statistical tests.

4. Results and discussion

4.1. Physico-chemical and biological characterisations of the experimental site soils

This study was carried out on two agricultural plots, an unpolluted plot and an aged TE-polluted one. The soil physico-chemical parameters and initial TE content of the experimental plots have been evaluated before cultivation practices and are summarized in Table 1. Briefly, on both experimental sites, the soil is a silt loam, with a neutral/slightly alkaline water pH and a relatively low limestone content (less than 1 %). On the unpolluted site, the concentrations of TE are similar to the regional background levels for agricultural topsoils (Sterckeman *et al.*, 2007). On the TE-polluted site, the soil is mainly polluted with Cd ($7 \pm 0.4 \text{ mg.Kg}^{-1}$), Pb ($394 \pm 16 \text{ mg.Kg}^{-1}$) and Zn ($443 \pm 12 \text{ mg.Kg}^{-1}$), with concentrations respectively 17, 11 and 6 times higher than those in regional background levels (Sterckeman *et al.*, 2007). Regarding the physico-chemical parameters, total organic carbon and total nitrogen content, C/N ratio, organic matter content and CEC were higher on the TE-polluted site (Table 1). Before tillage and sowing, about 143 ± 68 and 157 ± 47 AMF spores/100 g of soil were respectively isolated from the aged TE-polluted and unpolluted soils, displaying no significant difference between the two conditions. Microscopic observations of stained roots of the plant species initially present on both experimental sites, such as *Urtica dioica* L., *Veronica arvensis* L., *Senecio vulgaris* L. or *Mercurialis annua* L., revealed the presence of AMF structures such as arbuscules, vesicles and intraradical hyphae. Total root mycorrhizal rates were relatively low, ranging from 3.8 to 6.5 %. In fact, AMF spores' densities usually vary from 300 to 1400 spores/100 g in unpolluted or TE-polluted soils (Oehl *et al.*, 2004; Yang *et al.*, 2015; Zak *et al.*, 1982). Besides, our data have shown similar results between unpolluted and TE-polluted sites. Hence, the low spores' density and root colonization by AMF observed in the polluted site cannot be attributed to the soil pollution. However, the past cultivation

practices on the two agricultural plots, involving both soil preparation and use of phytopharmaceutical products (esp. for potato or sugar beet cultivation), were likely to affect soil microorganisms' survival (Büneman *et al.*, 2006; Tilston *et al.*, 2010). From that perspective, the addition of a mycorrhizal inoculum seemed appropriate, to assess its effect on cultivated plants' growth and biomass production. Besides, from an agronomic perspective, coriander and sage are relatively low demanding crops (Sharma and Sharma, 2012; Tibaldi *et al.*, 2010). In that regard, MgO was considered higher than average in comparison with reference thresholds (Denoroy *et al.*, 2004), while K₂O was considered average on both plots. Regarding P₂O₅ content, its amount is equal to the reference threshold for low demanding crops on the polluted site, while particularly high on the unpolluted one (Comifer, 2019; Denoroy *et al.*, 2004). There is in fact a trend towards a better chemical fertility of the soils on the polluted site, according to the previously mentioned parameters.

Table 1: Main soil physico-chemical, biological properties (number of AMF spores and native species' mycorrhizal rates) and pseudo-total and NH_4NO_3 extractable (expressed in % of pseudo total) concentrations of 8 TE (As, Cd, Cr, Cu, Ni, Pb, Sb and Zn) in the soil of the experimental sites – initial state before sowing. Values are means \pm SD.

Parameters	Unpolluted site	Polluted site
pH-H₂O	7.1 \pm 0.52	7.86 \pm 0.13
As	9 \pm 0.6 (0.08 % \pm 0.02)	16 \pm 2 (0.06 % \pm 0.02)
Cd	0.4 \pm 0.03 (2.28 % \pm 1.4)	7 \pm 0.4 (1.63 % \pm 0.51)
Cr	50 \pm 3 (0.02 % \pm 0.006)	59 \pm 3 (0.02 % \pm 0.02)
Pseudo-total (mg.Kg⁻¹)		
Cu	16 \pm 0.3 (0.41 % \pm 0.22)	27 \pm 3 (1.07 % \pm 0.41)
Ni	16 \pm 1 (0.95 % \pm 2.18)	25 \pm 1.8 (0.19 % \pm 0.04)
Pb	22 \pm 2 (0.07 % \pm 0.06)	394 \pm 16 (0.02 % \pm 0.006)
Sb	0.7 \pm 0.05 (0.96 % \pm 0)	5 \pm 0.3 (0.97 % \pm 0.28)
Zn	48 \pm 2 (1.27 % \pm 0.73)	443 \pm 12 (0.21 % \pm 0.07)
Clay (%)	14 \pm 1	26 \pm 2
Silt (%)	70 \pm 4	53 \pm 10
Sand (%)	17 \pm 3	18 \pm 3
Total organic carbon (g.Kg⁻¹)	10.2 \pm 1.4	17 \pm 2
Organic matter (g.Kg⁻¹)	18 \pm 2	29 \pm 4
C/N	8.6 \pm 0.8	10.5 \pm 0.7
CEC Metson (me.Kg⁻¹)	99 \pm 3	183 \pm 50
CaCO₃ (%)	0.73 \pm 0.34	0.48 \pm 0.13
N (g.Kg⁻¹)	1.18 \pm 0.05	1.62 \pm 0.24
Available P Joret-Hebert (g.Kg⁻¹) - P₂O₅	0.65 \pm 0.26	0.17 \pm 0.02
Available K (g.Kg⁻¹) - K₂O	0.21 \pm 0.04	0.26 \pm 0
Available Mg (g.Kg⁻¹) - MgO	0.18 \pm 0.07	0.21 \pm 0.02
Available Ca (g.Kg⁻¹) - CaO	3.5 \pm 1.3	6 \pm 0.5
Available Na (g.Kg⁻¹) - Na₂O	0.02 \pm 0	0.03 \pm 0
Number of AMF spores (per 100g of soil)	157 \pm 47	143 \pm 68
Mycorrhizal rates (%)	6.5 \pm 5.6	3.8 \pm 3.6

4.2. Floristic inventory of the native species on the polluted site

The main objectives of this floristic inventory were to identify species naturally growing on TE-polluted soils. In addition, it should provide useful information regarding the cautious selection of the cultivated plant species and the associated cultivation practices, notably the use of herbicidal compounds: if the wild growing plant species belonged to the same family as the cultivated ones, this would entail an additional constraint, especially regarding weed control. Moreover, in addition to their potential effect on plants' growth, the presence of certain weeds and in particular aromatic species, such as *M. recutita*, are also binding considering harvest, as they are likely to contaminate and alter EO's quality. Before the beginning of the experiment, despite the TE-pollution and its potential phytotoxicity, 24 plant species belonging to 14 different families were able to grow under these conditions (Table 2). *Asteraceae* and *Poaceae* are the two most abundant plant families, with 5 members each. *Sonchus asper* is the most abundant and dominant plant species on the site, whereas *Phragmites australis*, *U. dioica*, *Geranium pusillum* and *Rumex crispus* were sparsely visible on the experimental site. Vegetation naturally covers 70 to 100 % of the soil. Amongst the inventoried plant species, most of the identified *Asteraceae*, namely *Erigeron canadensis*, *M. recutita* and *S. vulgaris* were previously identified in TE-polluted soils, as well as *Erysimum* sp. (*Brassicaceae*), *Lolium perenne* and *Festuca* sp. (*Poaceae*), *U. dioica* L. (*Urticaceae*) and *Rumex* sp. (*Polygonaceae* – Dazy *et al.*, 2008; Hajiboland and Manafi, 2007; Jeannin *et al.*, 2020; Masarovičová *et al.*, 2010). Interestingly, amidst these plant species, *M. recutita* was already demonstrated as a Cd-accumulator species (Masarovičová *et al.*, 2010).

Species richness was about 1.09 and 3.72, according respectively to Menhinick and Margalef indexes. In addition, biodiversity indexes were about 2.15 and 0.85 for Shannon & Weaver and Simpson indexes respectively (Table 3). Diversity indexes have in previous studies been demonstrated negatively correlated with increasing TE-amounts (Hernández and Pastor, 2008; Koptsik *et al.*, 2003; Vangronsveld *et al.*, 1996). Despite this, our results have shown that the polluted site is higher in plant biodiversity in comparison with other unpolluted agricultural fields or polluted soils described in the literature (Chateil *et al.*, 2013; Dazy *et al.*, 2008; Fédoroff *et al.*, 2005; Hernández and Pastor, 2008; Koptsik *et al.*, 2003; Vangronsveld *et al.*, 1996; Zhou *et al.*, 2018). Nonetheless, one should note that beside TE-content, diversity indexes were demonstrated highly related to soil properties, in particular to the nutrient contents such as K, Mg or N (Hernández and Pastor, 2008; Koptsik *et al.*, 2003). In our case, they were relatively high for low-demanding crops.

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Table 2: Inventoried plant species' families, life cycle and cover-abundance study of the experimental TE-polluted site

		Quadrat Number	1	2	3	4	5	6	7	8	9	10	11	12	13
Family	Species	Soil cover Life cycle	80%	95%	95%	90%	85%	80%	100%	95%	90%	100%	70%	75%	85%
			Cover-abundance value												
Asteraceae	<i>Erigeron canadensis</i> L.	Annual	+	+	.	.	+	.	+	.	1.1	.	2.2	1.1	1.1
	<i>Lactuca virosa</i> L.	Biennial	2.2	.	+	.	2.2
	<i>Matricaria recutita</i> L.	Annual	1.1
	<i>Senecio vulgaris</i> L.	Annual	2.2	.	2.2	.	.	2.2	2.2	.	.	1.1	1.1	.	+
	<i>Sonchus asper</i> L. Hill	Annual	2.2	5.5	2.2	4.4	4.3	2.2	3.3	5.5	2.2	2.2	3.3	3.4	2.2
Brassicaceae	<i>Erysimum cheiranthoides</i> L.	Annual	.	.	+	.	.	.	1.1	+	4.4	.	.	1.1	.
	<i>Sinapis arvensis</i> L.	Annual	+
Convolvulaceae	<i>Convolvulus arvensis</i> L.	Perennial	1.1	.	4.3	1.1	1.1	1.1	1.1	+	1.1	2.2	.	.	+
Equisetaceae	<i>Equisetum arvense</i> L.	Perennial	.	.	2.3
Euphorbiaceae	<i>Euphorbia helioscopia</i> L.	Annual
	<i>Mercurialis annua</i> L.	Annual	1.1	1.1	.	1.1	+	+	1.1	+	.	1.1	+	+	.
Fumariaceae	<i>Fumaria officinalis</i> L.	Annual	2.2	.	.	.
Geraniaceae	<i>Geranium pusillum</i> L.	Annual
Papaveraceae	<i>Papaver rhoeas</i> L.	Annual	+
Plantaginaceae	<i>Veronica arvensis</i> L.	Annual	1.1	.	+	.	.	1.1	+	.	1.1
	<i>Alopecurus myosuroides</i> Huds.	Annual	3.2	1.1	1.1	2.3	3.3	3.4	3.3	2.2	.	4.4	+	+	.
	<i>Bromus sterilis</i> L. Nevski	Annual	+
Poaceae	<i>Festuca pratensis</i> Huds.	Perennial	+
	<i>Lolium perenne</i> L.	Perennial	+	2.2	3.2
	<i>Phragmites australis</i> Cav.	Perennial
Polygonaceae	<i>Rumex crispus</i> L.	Perennial
Rubiaceae	<i>Galium aparine</i> L.	Annual	.	+	.	.	+
Sapindaceae	<i>Acer</i> sp.	Perennial	.	.	.	+	.	+	+	+
Urticaceae	<i>Urtica dioica</i> L.	Perennial

Table 3: Plant biodiversity indexes measured on the TE-polluted site

Plant biodiversity indexes	Value
Menhinick	1.09
Margalef	3.72
Shannon and Weaver	2.15
Simpson	0.85

4.3. *In situ* emergence rates

For first and second years of coriander's cultivation, emergence rates were ranging from 49 to 84 % (Table 4). No significant differences have been observed between the polluted and unpolluted sites, and between I and NI conditions, for both cultivation years. Previous studies have highlighted adverse effects of Zn on coriander seedling's persistence (Marichali *et al.*, 2014) and of Cd and Cu on both germination rates and seedling's emergence and survival of coriander (Faizan *et al.*, 2012). However, in our experimental conditions, seedling's emergence was not adversely affected by soil pollution. For sage, emergence rates were significantly higher on the polluted site in comparison with the unpolluted one. They were about 71 and 65 % on the polluted plot in NI and I conditions respectively, while on the unpolluted one they were around 45 % whatever the condition (Table 4). TE-pollution does not seem to alter seedlings' emergence; the difference observed between polluted and unpolluted sites may be due to the differences in terms of soil physicochemical properties.

Table 4: *In situ* emergence rates assessed for both cultivated plants - Significant differences ($\alpha = 0.05$) between conditions are indicated by different letters ($n=12$). NI: non-inoculated; I: inoculated.

	Plant Plot	Coriander – aerial parts	Coriander – seeds	Sage
Unpolluted	NI	72 ± 26 a	58 ± 31 ab'	45 ± 6 B
	I	68 ± 15 a	49 ± 28 b'	45 ± 10 AB
Polluted	NI	78 ± 11 a	84 ± 15 a'	71 ± 9 A
	I	77 ± 10 a	72 ± 15 ab'	65 ± 8 A

4.4. Coriander and sage growth

It should first be remembered that the study of coriander was carried out on two successive years. The first year's aim was to distil the whole coriander aerial parts and the second one to distil the seeds.

At the harvest of coriander's aerial parts, the plants' height reached an average of 66 cm on the unpolluted site and 55 cm on the polluted one (Figure 1A). Coriander's dry weight were respectively an average 10 g/plant on the unpolluted site and 3.5 g/plant on the polluted one. Additionally, coriander's dry weight and height were similar in I and NI conditions, on both polluted and unpolluted sites.

At the harvest of coriander's seeds, the height of coriander's plants reached respectively 77 and 80 cm on the NI and I conditions on the unpolluted site, while reaching 81 and 70 cm respectively for NI and I conditions on the polluted site (Figure 1B). Furthermore, the recorded coriander dry weights were about 11 and 7 g/plant when cultivated on unpolluted and polluted sites respectively. A significant difference was observed for coriander grown on both unpolluted and polluted sites for the I condition, which displays a lower dry weight on the polluted one (50 % reduction). Moreover, a significant difference has been observed between NI and I conditions in terms of plant height (respectively 81 and 70 cm) on the polluted site. For both coriander cultivation years, similar behaviour in terms of

plant's growth (dry weight and height) have been obtained on the unpolluted plot. These results are in accordance with previous studies (Ghobadi and Ghobadi, 2010; Inan *et al.*, 2014).

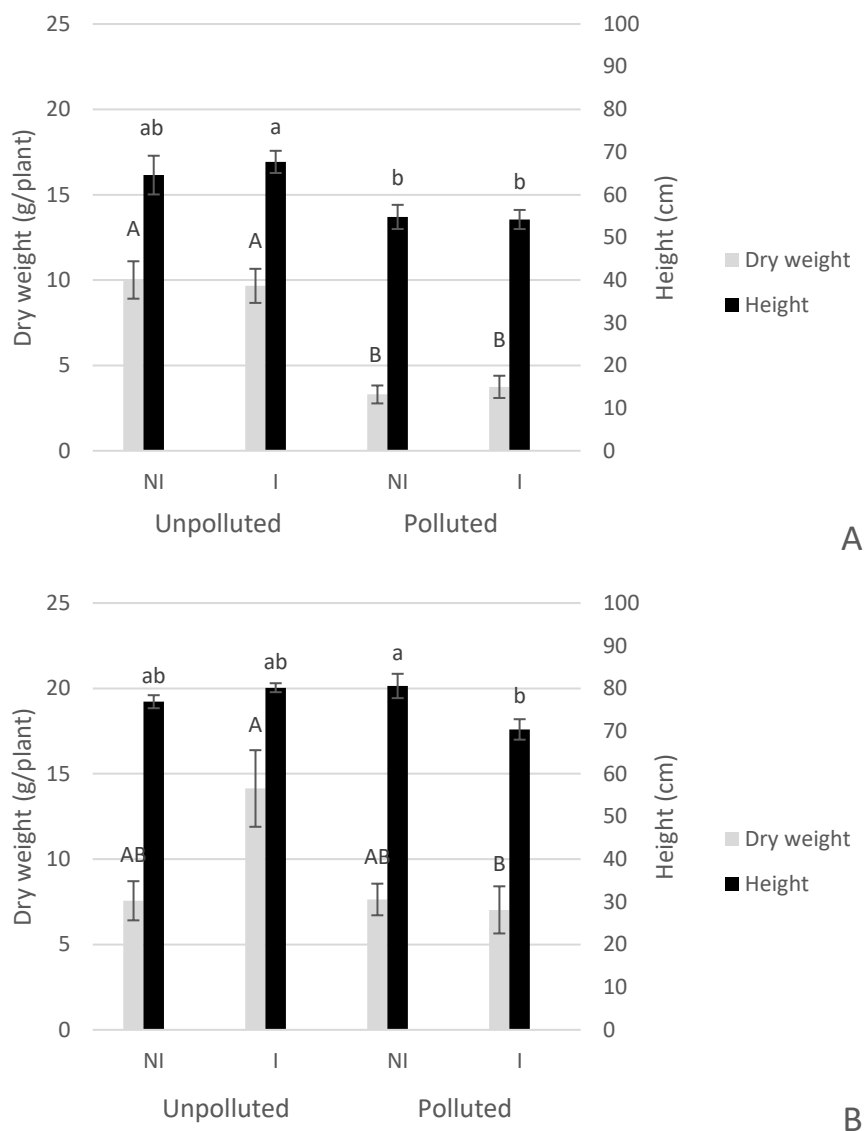


Figure 1: Dry weight and height of sampled aerial parts (A) or seeds (B) of coriander - Significant differences between conditions are indicated by different letters ($\alpha = 0.05$). Values are means \pm SE ($n=12$). NI: non-inoculated; I: inoculated.

In contrast with coriander, clary sage is rather displaying a short-lived perennial life cycle and is cultivated for at least two successive years (Tibaldi *et al.*, 2010). It has hence been monitored over time for three cultivation years. Regarding plant height, clary sage attained an average of 38.5 and 32.4 cm at first year's harvest, reaching 160 and 145 cm (respective 76 and 78 % increases) at second year's harvest and decreasing of about 10 and 29 % in third year, respectively on unpolluted and polluted sites (Figure 2). Regarding dry weights, sage was about 48.9 and 51.9 g/plant at first harvest. At second year's harvest it increased to 127 g/plant on the unpolluted site, regardless of the condition, while remaining similar to first year on the polluted one. During the third year of cultivation, data ranged from 152.5 to 207 g/plant on the unpolluted site, while ranging from 160 to 181 g/plant on the polluted one (60 % increase). Overall, no significant differences were observed between I and NI conditions, for both plant height and dry weight, whatever the cultivation year. Besides, a significant difference between polluted and unpolluted sites has only been measured for the NI condition in year 2.

Furthermore, due to the inflorescences' large size in year 2, measurements have been carried out specifically on the inflorescences as well (Figure 3). Inflorescences' heights ranged from 69 to 80 cm, while their dry weights ranged from 71 to 95 g/plant. The number of stems carrying inflorescence varied from 5 to 6. Whatever the condition (polluted or unpolluted sites, I or NI), no significant differences in sage growth parameters (inflorescences' height and dry weight or number of stems carrying inflorescences) were observed (Figure 3).

As expected, due to the short-lived perennial life cycle, sage mostly developed large leaves on the ground level during the first year and displayed a really limited number of flowers. During the second year, a high number of tall inflorescences have been observed, whereas on the third year, there were still numerous flowers, but the inflorescences were smaller and sage grew larger leaves, increasing the total plant's weight, which is similar to what was previously reported for sage cultivated on successive years (Carruba *et al.*, 2006). Previous studies have demonstrated that sage was tolerant to TE and able to grow in TE-polluted conditions without being significantly affected (Angelova *et al.*, 2016; Chand *et al.*, 2015; Grigoriadou *et al.*, 2019). It should however be noted that a lower plant development has been observed on the polluted site, especially during year 2. Due to its short-lived perennial life cycle, sage is likely to produce high amounts of biomass during the second year of cultivation (Carruba *et al.*, 2002; Zutic *et al.*, 2016). Moreover, sage's plant density was previously highlighted as a key component towards a successful sage's cultivation (Tibaldi *et al.*, 2010). Because of the higher emergence rates measured on the polluted site (Table 4), resulting in a higher plant density per square meter, sage's development may have been hindered, which would result in lower plant weight.

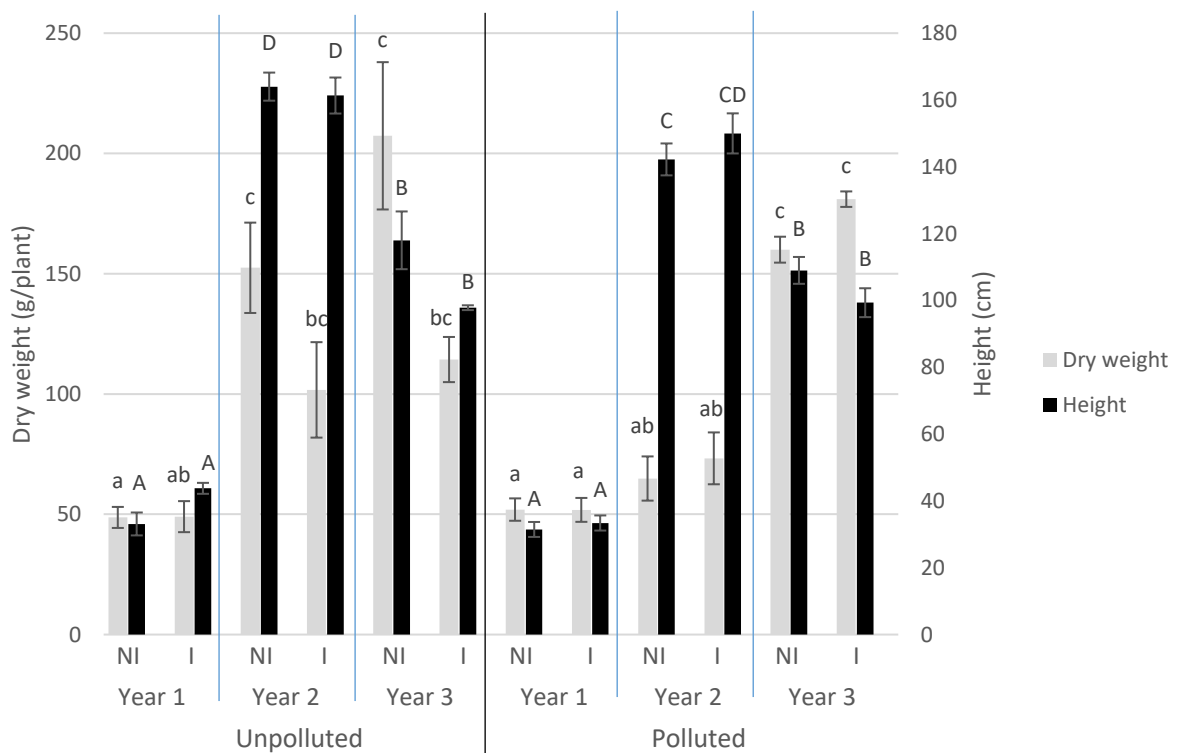


Figure 2: Dry weight and height of sampled sage (whole plant) in first, second and third year of cultivation - Significant differences between conditions are indicated by different letters ($\alpha = 0.05$). Values are means \pm SE ($n=6$). NI: non-inoculated; I: inoculated.

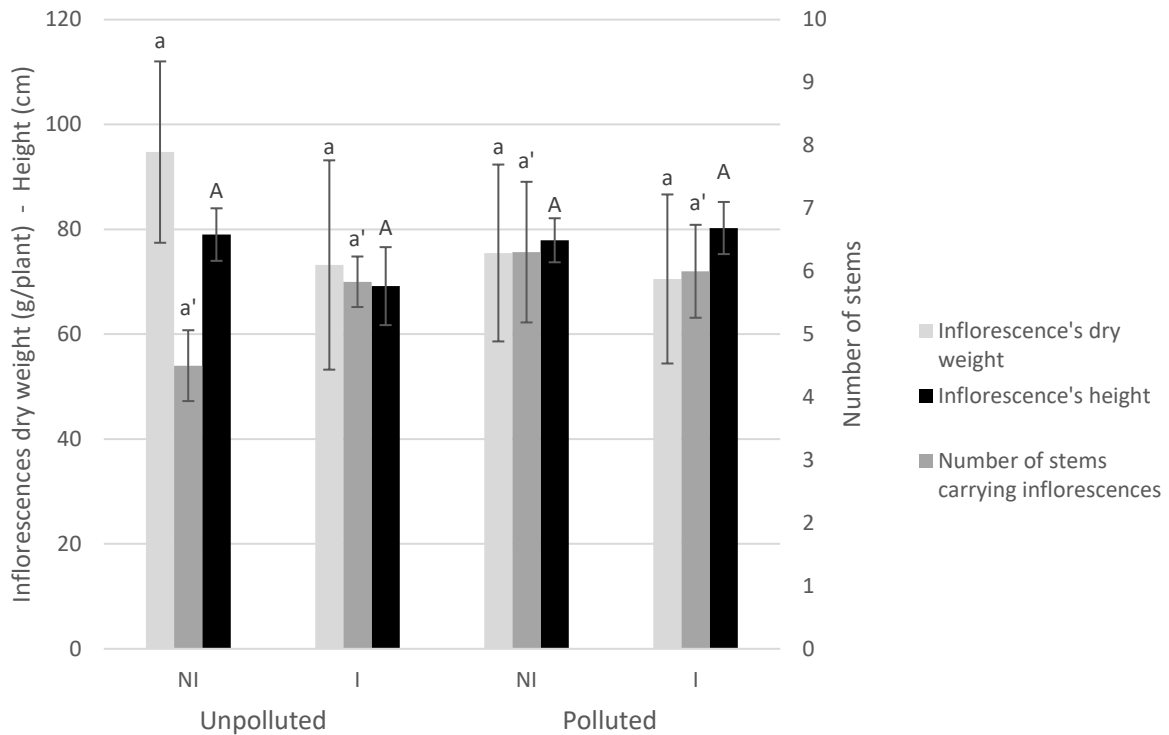


Figure 3: Number of sage stems carrying inflorescences and dry weight and height of inflorescences of sampled sage during year 2 of cultivation - Significant differences between conditions are indicated by different letters ($\alpha = 0.05$). Values are means \pm SE (n=6). NI: non-inoculated; I: inoculated.

4.5. AMF mycorrhizal colonization of coriander and sage

At the harvest of coriander's aerial parts, while no differences were observed between NI conditions on both sites (Figure 4A), a significant improvement in total mycorrhizal colonization rates has been observed on the I condition, in comparison with the NI condition on both sites, with respective increases of 65 and 73 % on the unpolluted and polluted sites. Arbuscular and vesicular mycorrhizal rates were respectively about 4 and 21.5 %.

At the harvest of coriander's seeds, significantly higher mycorrhizal rates have been displayed on the unpolluted site for the I condition (13 %), in comparison with the NI one (3 %), while no significant differences between NI and I conditions were observed on the polluted site (Figure 4B). It is noteworthy that total, arbuscular and vesicular mycorrhizal rates displayed a similar trend.

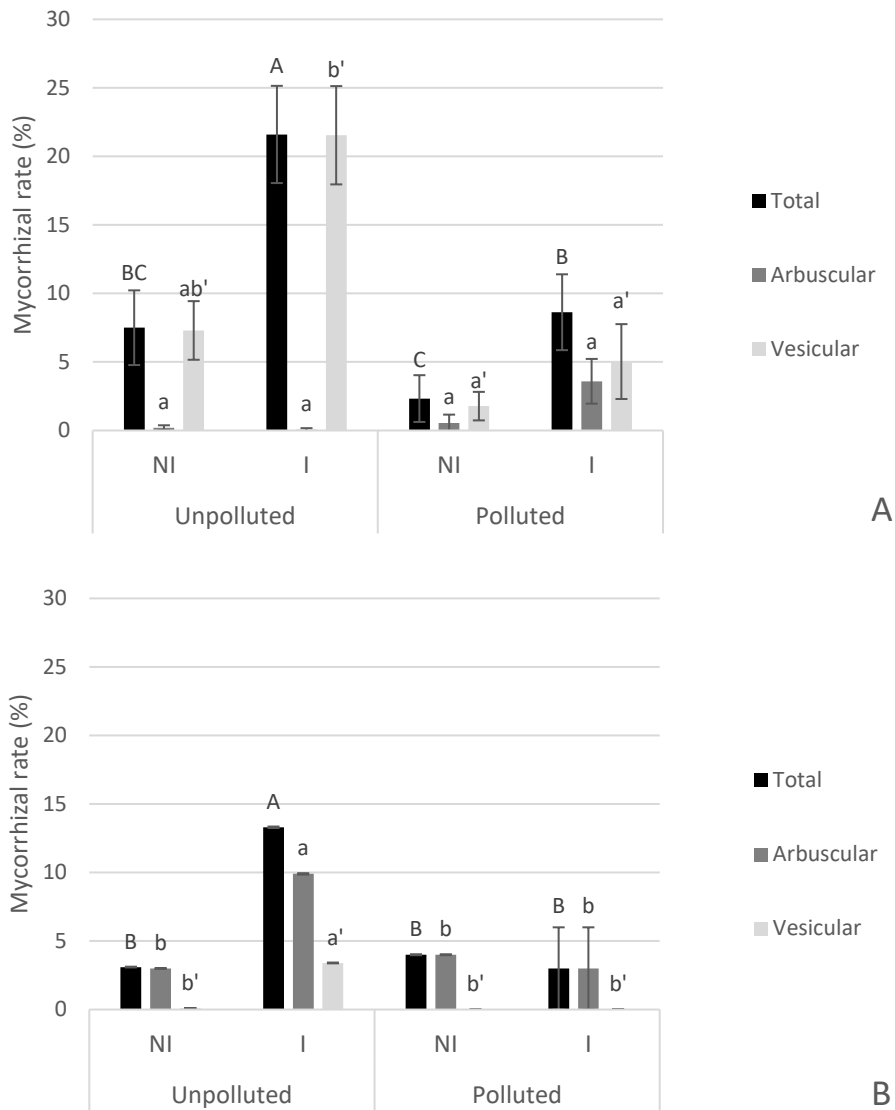


Figure 4: Mycorrhizal rates of coriander (A) aerial parts and (B) seeds - Significant differences between conditions are indicated by different letters ($\alpha = 0.05$). Values are means \pm SE (n=12). NI: non-inoculated; I: inoculated.

Our results on sage have shown that total mycorrhizal rates were significantly increased overtime and at highest during the third year of cultivation, on both experimental sites, raising from 4 to 35 % and from 3 to 34 %, respectively on the unpolluted and polluted sites (Figure 5). No significant differences were observed between unpolluted and polluted sites, excepted in year 2 where the total mycorrhizal rates were 50 % lower on the polluted site. Regarding inoculation's effects, significantly higher mycorrhizal rates have been obtained during year 2 and year 3 for the I condition in comparison with the NI one, especially on the polluted site, with respective 59 and 34 % increases. Arbuscular mycorrhizal rates followed the same trends as the total mycorrhizal rates, for year 2 and 3, whereas no significant differences were observed for vesicular rates, whatever the year and the condition (polluted or unpolluted sites, I or NI conditions).

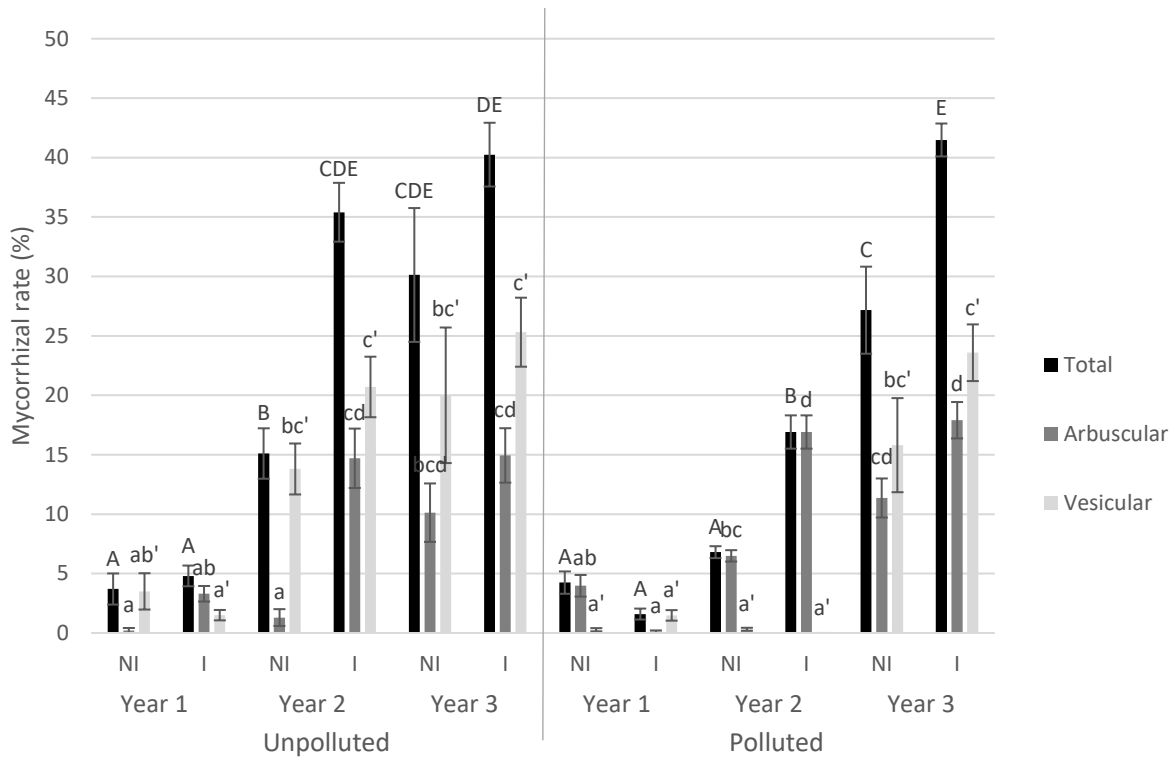


Figure 5: Sage mycorrhizal rates – time monitoring of the successive cultivation years - Significant differences between conditions are indicated by different letters ($\alpha = 0.05$). Values are means \pm SE (n=6). NI: non-inoculated; I: inoculated.

As a whole, our results have shown that spontaneous root mycorrhizal colonization was observed on NI plots, whatever the plant species, which is due to the presence of autochthonous AMF spores in both experimental soils, as previously reported (Table 1). Interestingly, despite this spontaneous mycorrhization which remains relatively low, the introduction of the exogenous mycorrhizal inoculum during sowing led to a significant improvement of mycorrhizal rates of coriander and sage roots, during second and third years of cultivation. However, despite the presence of arbuscules, seat of nutrient exchange between both symbiosis partners (Ambrosino *et al.*, 2020; Smith and Read, 2008), no beneficial effect on biomass production was observed whatever the cultivated plant and the experimental site. Nonetheless, due to the presence of root mycorrhization even in NI conditions, their relative contribution towards plant's growth in I condition may be difficult to identify. Additionally, the physicochemical characteristics of the soil seemed sufficient for low-demanding crops and have been complemented with fertilizers (in particular 120 units of nitrogen) one month after sowing, which is likely to ensure a good nutrient availability for the plants and does not put the crops in a situation where they depend on the establishment of the mycorrhizal symbiosis. One should note that in this work, the mycorrhizal symbiosis was established in both polluted and unpolluted conditions for both cultivated plants, sage and coriander, depicting the fact that at least some AMF species are able to tolerate the presence of TE in soils. This may even result in an alleviated TE stress for cultivated plants they are associated with. In fact, previous studies have demonstrated that *Glomus* or *Acaulospora* species were found in Zn and Pb polluted soils (Zarei *et al.*, 2008), or that glomalin was secreted in Cu and Zn polluted soil, revealing the presence of AMF species (Cornejo *et al.*, 2008). In the latter case, the authors have highlighted a high correlation between the amounts of glomalin in soil and the ability of plants to grow in the area, depicting a contribution of the AMF to the plants' growth and/or tolerance to TE (Cornejo *et al.*, 2008).

4.6. Sage and coriander EO distillation yields

Regarding aerial parts of coriander's harvest, the distillation yields were lower on the polluted site, for both I and NI conditions, in comparison with the unpolluted site with about 50 % of reduction. These distillation yields are 15 to 77 % lower in comparison with those commonly obtained for the distillation of aerial parts of coriander (0.1 to 0.3 % - Bhuiyan *et al.*, 2009; Nurzyńska-Wierdak, 2013; Shahwar *et al.*, 2012). The previously displayed results were yet obtained using laboratory scale Clevenger-type apparatus, whereas this study's results were obtained with a large-scale distillation unit, which may result in significant differences. For the harvest of coriander seeds, the distillation yields were higher than those obtained for aerial parts of coriander (Table 5), which is in agreement with previously published results, since coriander seeds were demonstrated to possess a higher EO content than the aerial parts (Bhuiyan *et al.*, 2009; Sharma and Sharma, 2012).

Regarding sage during its second year of cultivation, expected to be the best harvest year (Carruba *et al.*, 2006; Tibaldi *et al.*, 2010), distillation yields were at highest and ranged from 0.019 up to 0.035 % on the polluted NI condition (Table 6). Similar distillation yields were also obtained between year 1 and year 3, regardless of the condition, which were significantly lower than those obtained in year 2.

Regarding EO yields, aerial parts of coriander displayed no significant difference in terms of EO's amounts obtained between the polluted and unpolluted sites and between the I or NI conditions (Table 5). EO's yields were higher than 10 kg per hectare on both experimental sites, which is satisfactory in comparison with the literature (Smallfield *et al.*, 1994). Interestingly, an improvement lever towards an increase of the distillation yields could be the addition of straw in the distillation process, as sometimes done by the distiller when biomass amounts are really low, to slow down the water steam diffusion through the biomass (Smallfield *et al.*, 1994).

For the harvest of coriander's seeds, the obtained EO's yields were in average up to 6 kg per hectare on both experimental sites. Equivalent yields have been obtained between the two experimental sites, but a higher EO yield has been obtained on the I condition (7.5 kg/ha) in comparison with the NI one (4.4 kg/ha) for the unpolluted site. This same trend has not been identified on the polluted site, where the EO yields were equivalent between I and NI conditions. The EO yields per hectare are significantly lower than those expected for this crop when cultivated on unpolluted soils (up to 18 kg/ha – NIIR Board, 2003; Priyadarshi and Borse, 2014). Two factors may explain these lower yields: on one hand, a late harvest was carried out, while on the other, coriander seeds are commonly crushed to enhance the distillation yields (Geed *et al.*, 2014; NIIR Board, 2003; Saim *et al.*, 2008), a step that was not done in this work. However, it is interesting to note that the obtained EO yields are higher by about 30 % than those obtained on TE-polluted soils and reported by Zheljzakov *et al.* (2008).

Regarding sage, high EO amounts have been obtained particularly in year 2, with yields up to 34 kg/ha. The EO amounts obtained on the polluted site were higher with respective 60 and 16 % increases on the NI and I conditions. On the unpolluted site, there is a higher yield obtained for the I condition (20.3 kg/ha) in comparison with the NI one (12.9 kg/ha), but the trend is opposite on the polluted site (respectively 33.7 and 24.2 kg/ha for NI and I conditions). It is furthermore well known that the second year of cultivation is the optimal harvest year for sage's EO production (Carruba *et al.*, 2002; Zutic *et al.*, 2016). In comparison with reference values, the obtained EO yields, in particular on the polluted site, are on the high range (expected yields range from 10 to 30 kg/ha – CRIEPPAM website; Yaseen *et al.*, 2015). For the first and third years of sage cultivation, obtained yields were lower and ranged from 0.5 to 2.7 kg/ha. However, contrary to first year where low yields were expected due to the very small number of inflorescences, yields on the third year of cultivation are expected to reach up to 50 or 60 % of those obtained during the second year (Carruba *et al.*, 2006). This trend was not observed in the

present case since obtained yields were barely higher than those obtained during the first year of cultivation. This could be explained by a late harvest period due to climatic conditions, implying that many inflorescences had started to wither, hence reverberating on the yields.

Table 5: EO distillation from aerial parts and seeds of coriander. NI: non-inoculated; I: inoculated.

Condition	Plant	Coriander aerial parts		Coriander seeds	
		Distillation yield (%)	EO yield (kg/ha)	Distillation yield (%)	EO yield (kg/ha)
Unpolluted	NI	0.075	10.2	0.183	4.4
	I	0.085	10.6	0.206	7.5
Polluted	NI	0.047	11.1	0.133	6.3
	I	0.040	9.6	0.166	6.2

Table 6: EO distillation from sage harvested during 3 years of cultivation. NI: non-inoculated; I: inoculated; NC: not calculated.

Condition		Year 1		Year 2		Year 3	
		Distillation yield (%)	EO yield (kg/ha)	Distillation yield (%)	EO yield (kg/ha)	Distillation yield (%)	EO yield (kg/ha)
Unpolluted	NI	0.005	0.93	0.021	12.9	0.0012	0.48
	I	0.007	0.53	0.019	20.3	0.0059	2.7
Polluted	NI	NC	0.17	0.035	33.7	0.0059	1.4
	I	NC	0.15	0.020	24.2	0.0058	2.3

4.7. Essential oil's quality assessment

4.7.1. Trace elements assessment in distilled EO

The assessment of TE amounts in EO has been carried out on both EO from coriander as well as on the EO of sage obtained during the second year of cultivation (optimal harvest year for sage). The results from the analyses have shown that Cd was below the limit of quantification (LoQ) in any of the distilled EO (Table 7). Similarly, trace amounts of Pb and Zn have been detected, below the limit of quantification in most of the EO samples, exception made for the EO from aerial parts of coriander. Previous studies have also shown that TE were detected in the distilled EO of coriander (As, Cr, Cu, Mn, Pb and Zn) and within the same range (Zheljazkov and Jekov, 1996), or sage (Cd, Pb and Zn), depicting higher Pb and Zn amounts in the EO than those highlighted in this work (Angelova *et al.*, 2016). Interestingly, coriander seeds were previously demonstrated to accumulate lesser amounts of TE than other edible parts of the plant (Kisku *et al.*, 2011), which is in accordance with our results with lower amounts of Pb and Zn detected in coriander seeds' EO. Furthermore, it should be mentioned that even though TE were found in EO, their presence was quantified in trace amounts, equal to or barely higher than the LoQ, depicting a very limited transfer of the different TE in the EO thanks to the distillation process. It should also be highlighted that scarce are the differences obtained between the EO originating from the polluted and the unpolluted conditions. Finally, one should note that there is currently no regulatory limit set regarding TE content in EO for non-food purposes in France or more broadly in Europe. Interestingly however, all the EO distilled in this study display TE amounts equal to or below the ones found in the analysed marketed products (Table 7), which is a key factor towards the feasibility of the method.

Table 7: Concentrations (mg/kg of EO) of Cd, Pb and Zn in EO obtained from aerial parts and seeds of coriander and from inflorescences (year 2) of sage. Market: results obtained with the EO originating from a market product; D: detected but below the limit of quantification of the method.

	Coriander aerial parts			Coriander seeds			Sage inflorescences		
	Unpolluted	Polluted	Market	Unpolluted	Polluted	Market	Unpolluted	Polluted	Market
Cd	D < 0.01	D < 0.01	D < 0.01	D < 0.005	D < 0.005	D < 0.01	D < 0.005	D < 0.005	D < 0.01
Pb	0,041	0,042	0,63 ± 0,04	D < 0.01	D < 0.01	D < 0.01	D < 0.01	D < 0.01	0,01
Zn	0,078	0,052	5,05 ± 0,14	0,063	D < 0.05	0,11 ± 0,06	D < 0.05	0,086	0,11 ± 0,01

4.7.2. Pesticide residues analysis

The assessment has been carried out on both EO from aerial parts and seeds of coriander as well as on the EO of sage obtained during the second year of cultivation. The results from the pesticide multi-residues analysis indicate that out of the 600 investigated active compounds, only 14 were detected in the distilled EO from coriander and sage (Table 8). Additionally, the identified residues are present in small amounts or below the LoQ for the majority. It should be mentioned that even though these analyses were conducted, the EO have in practice for non-food valorisation no maximum residue limits set, by European legislation or worldwide, with the only exception being citrus derivatives in the US, framed by the Environmental Protection Agency (EPA – Quarré, 2018; Tascone *et al.*, 2013). Amongst the 14 detected pesticide residues, the majority was detected in the EO distilled from the coriander's aerial parts. Previous studies have already reported the presence of pesticide residues in aerial parts and leaves of coriander (Ahmed *et al.*, 2019; Dogheim *et al.*, 2004). However, there seem to be an effect of the distilled plant part, as the number and amounts of detected pesticide residues were significantly lower in the EO from seeds of coriander, despite the same chemical interventions on the experimental sites. The case of Malathion accumulation in coriander and its transfer in EO was previously discussed, evidencing that lower amounts were accumulated in seeds compared to the leaves, but that there was a concentration effect between Malathion's amounts in seeds and in the EO (Abd El-Rahman *et al.*, 2005). On another note, sage's EO displayed a really good quality regarding the presence of residues, as Dimethenamid was the only detected one and below limit of quantification. Aclonifen and Pendimethalin were the two active compounds detected and quantified with the highest concentrations (respectively up to 1.1 and 1.7 mg/kg of EO), while Hexachlorobenzene, Pendimethalin and Triallate were identified in the wider number of samples. Pendimethalin, aclonifen and linuron, that were detected and quantified in the highest amounts in both coriander EO, were used in the crop management system. DDT, pentachloroanisol and heptachlor, listed as Persistent Organic Pollutants (POPs – Stockholm Convention, 2001), are likely to persist for an extended period of time in soils and to bio-accumulate in living organisms (Jing *et al.*, 2018; Lee *et al.*, 2018; Zacharia, 2019). Their potential past use may hence be reflected in their presence in the cultivated plants and in the EO. Yet, these amounts were barely higher than the LoQ for both DDT and heptachlor and below LoQ for pentachloroanisol, meaning that they were detected but in trace amounts. Finally, the rest of the detected molecules is often found in commercial products homologated for use on beetroot and potato, which were previous crops on the experimental sites, or on rapeseed, which is commonly cultivated in the neighbouring plots. To sum up, the detected residues may be the result of an old soil pollution, of the carried out chemical interventions or of incidental contamination.

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Table 8: Residual amounts of pesticides in distilled EO of coriander and sage. The quantification limit (LoQ) is 0.01 mg/kg. nd: not detected; D < 0.01: detected compound but not quantified given the LoQ.

Essential oil from:		Coriander				Sage	
Cultivation year		1		2		2	
Site		Unpolluted	Polluted	Unpolluted	Polluted	Unpolluted	Polluted
Active substance	Activity spectrum						
Aclonifen	Herbicide	nd	1.1 ± 0.3	nd	nd	nd	nd
Chlorpyrifos	Insecticide	nd	nd	nd	D < 0.01	nd	nd
Clomazone	Herbicide	0.079 ± 0.040	0.028 ± 0.014	nd	nd	nd	nd
DDT	Insecticide	nd	0.010 ± 0.005	nd	nd	nd	nd
Dimethenamid	Herbicide	0.030 ± 0.015	0.076 ± 0.038	nd	nd	D < 0.01	nd
Ethofumesate	Herbicide	nd	0.034 ± 0.017	nd	nd	nd	nd
Hexachlorobenzene	Fungicide	0.016 ± 0.008	0.012 ± 0.006	0.012 ± 0.006	nd	nd	nd
Heptachlor	Insecticide	nd	0.051 ± 0.026	nd	nd	nd	nd
Linuron	Herbicide	0.073 ± 0.037	0.020 ± 0.010	nd	nd	nd	nd
Metolachlor	Herbicide	nd	0.019 ± 0.010	nd	nd	nd	nd
Pendimethalin	Herbicide	0.52 ± 0.18	1.7 ± 0.5	0.12 ± 0.058	nd	nd	nd
Pentachloroanisol	Pentachlorophenol (general biocide) degradation product	nd	D < 0.01	nd	nd	nd	nd
Prosulfocarb	Herbicide	nd	0.21 ± 0.08	nd	nd	nd	nd
Triallate	Herbicide	0.094 ± 0.047	0.027 ± 0.014	0.052 ± 0.026	nd	nd	nd

4.8. Techno-economic feasibility

The purpose of the study was finally to evaluate the techno-economic feasibility of EO production on polluted soils. First, it should be noted that physicochemical methods for soil remediation often result in elevated costs and are not applicable to large areas (Borges *et al.*, 2018; Kumpiene *et al.*, 2019). In fact, in our case a topsoil pollution (0-30 cm) on more than 1 hectare of land would result in approximately 4000 m³ of polluted soil to remediate. Recently, an average estimated cost of about 180 €/m³ for the physico-chemical remediation of TE-polluted soil has been brought forward (Zhang, 2019), which in our case would translate into more than 700 000 euros for the site remediation, which seems prohibitive.

In this study, the economic evaluation is presented for each aromatic crop and for each experimental condition (I or NI) separately. Production costs for the biomass on the NI condition, regardless of the experimental site (polluted or not) and after deduction of the inoculum's cost representing an amount of 170 €/ha, are as follows: 706 €/ha for aerial parts of coriander (one-year cultivation – Table 9), 772 €/ha for coriander seeds (one-year cultivation – Table S3) and 1158.5 €/ha for sage (three-year cultivation cycle – Table S4). There was no significant difference between the production costs in TE-polluted or unpolluted conditions, as the followed procedure and the material used were equivalent. In addition, the cost for subcontracting EO distillation is about 300 €.

Table 9: Estimation of the production costs for the cultivation of coriander's aerial parts (one-year cost estimation)

	Description	Per-unit cost	Unit	Amount	Operation cost	Unit
Step 1: soil preparation						
Tractor + plough	1 intervention: 5 shears plough - 130-hp tractor	59.8	€/ha	1	59.8	€/ha
Tractor + rotary harrow	1 intervention: Vibrashank (4m) - 110-hp tractor	37.9	€/ha	1	37.9	€/ha
Total					97.7	€/ha
Step 2: sowing						
Tractor + beet seeder	8 rows seeder - 80-hp tractor	67.8	€/ha	1	67.8	€/ha
Seeds	300 000 seeds/ha <=> 3-4 kg/ha	40	€/kg	4	160	€/ha
Total					227.8	€/ha
Step 3: inputs						
Tractor + fertilizer distributor	2 interventions	14,5	€/ha	2	29	€/ha
Fertilizer 1	Sulfate of ammonia	200	€/t	0.3	60	€/ha
Fertilizer 2	Sulfate of ammonia	200	€/t	0.3	60	€/ha
Tractor + sprayer	2 interventions / year	15	€/ha	2	30	€/ha
Crop protection products	2 treatments / year				104.9	€/ha
Other inputs	Mycorrhizal inoculant	1.36	€/g	125	170	€/ha
Total					453.9	€/ha
Step 4: harvest						
Tractor + mower	70-hp tractor + 5-disc rotary mower (2m)	37	€/ha	1	37	€/ha
Tractor + pick-up loader wagon	120-hp tractor + pick-up loader wagon	60	€/ha	1	60	€/ha
Total					97	€/ha
Total production cost - coriander aerial parts					876.4	€/ha

Aerial parts of coriander

An average of 10 kg/ha of coriander EO was produced on experimental sites, with no significant differences between experimental conditions (I or NI, polluted or unpolluted conditions). Using equation (3) described in Section 2.10., taking into account a recorded EO price of about 110 €/kg (ultranl Market report, 2019), the resulting gross proceed is about 1100 €/ha. The production costs evaluated in this work, 706 €/ha, should be deduced for the NI condition, as well as the distillation cost (300 €), resulting in a margin of about 100 and 200 € for the unpolluted and TE-polluted sites respectively. In that regard, there were no significant differences between polluted and unpolluted conditions.

Coriander seeds

An average of 6 kg/ha of coriander EO was produced on experimental sites, whatever the condition. Using equation (3), taking into account a recorded EO price of about 62 €/kg (ultranl Market report, 2019), the resulting gross proceed is about 372 €/ha. With the simple deduction of the production costs of about 770 €/ha for the NI condition for both polluted and unpolluted sites, the resulting margin appears negative. In that regard, in the current state, the production of coriander's seeds on both unpolluted and polluted soils, does not seem to be economically profitable with our experimental conditions. By combining these results with the previously discussed EO yields, it appears necessary to enhance both distillation (*e.g.* by grinding seeds) and per hectare yields, so as to significantly raise the benefits coming from the cultivation of coriander's seeds. In comparison, the aerial parts of coriander for EO production on both unpolluted and TE-polluted soils seem to be a moderately satisfactory candidate, since the obtained margins are weakly positive for the NI condition, on both polluted and unpolluted soils.

Clary sage

For a 3-year cultivation cycle, an average of 14.3, 23.5, 35.3 and 26.7 kg/ha of sage EO were produced for NI and I conditions on unpolluted and polluted sites respectively. The sage EO selling price is about 127€/kg, with minor fluctuations (France Agrimer data, 2016). Using equation (3), it would represent a gross proceed of respectively 1816; 2985; 4485 and 3391 €/ha. To these amounts should be deduced the production costs as well as the distillation subcontracting, *i.e.* a total amount of 1458 €/ha for the NI condition. This results in margins of about 358 and 3024 € for the NI condition on the unpolluted and polluted sites respectively. Due to the higher amounts of EO obtained, the margin is significantly higher on the polluted site.

Regarding the contribution of the inoculation, for both coriander EO, considering equivalent yields between I and NI conditions and the inoculum's cost, the addition of the inoculum does not seem beneficial with our experimental conditions, resulting in negative margins in both cases. In the case of sage, EO yields were higher in the inoculated condition on the unpolluted site, while the trend was opposite on the polluted one. In addition, despite the higher EO amounts obtained for the inoculated condition, the yields as well as the margin remain lower in comparison with the polluted site. Overall, considering inoculum's cost and limited contribution regarding EO yields, it does not appear advantageous in our experimental conditions, whatever the plant.

Another phytoremediation channel previously described on a TE-polluted area, investigated the potential of TE-hyperaccumulator species *Pteris vittata* and *Sedum alfredii* Hance to extract TE from the soil, in an intercropping system involving cash crops (such as sugar cane), and highlighted

operational cost of more than 10 000 € a year due to very costly inputs (Wan *et al.*, 2016). Comparatively, any of the cultivated aromatic plant in this work displayed significantly lower costs. Additionally, a study from Włóka *et al.*, (2019), using *Phalaris arundinacea* L. and *Brassica napus* L. for the remediation of a PAH-polluted site (PAH biodegradation), has brought forward annual costs of about 699.2 and 707.2 € for the cultivation and harvest of *P. arundinacea* L. and *B. napus* L. respectively, highlighting lower costs than any of the coriander cultivation years, but significantly higher than those obtained for sage cultivation in this work.

In the following section, the case of clary sage only will be discussed, as it appears as the only potentially viable option, regarding the obtained margins. In addition, since inoculation did not appear as beneficial, it will not be further investigated. Clary sage production costs have been compared to the production costs of wheat, winter barley and rapeseed (mean of three successive years, including operational and material costs), which are respectively 720; 669 and 761 €/ha (Casdar Project, 2015). The estimated cost of 1158.5 €/ha for a three-year cycle of sage cultivation (NI condition), resulting in a mean annual cost of 386.2 €/ha, is in average 46 % lower than those reported for wheat, barley and rapeseed. In addition, wheat yields commonly reported are about 7.3 t/ha, with a selling price of 195 €/t. With a similar method as previously described, wheat gross proceeds over 3 years should be around 4271 €/ha, with a deduction of 2160 €/ha corresponding to the production costs, for a resulting margin totalling 2111 €/ha. This amount is 80 % higher in comparison with sage grown on unpolluted soils, while 30 % lower in comparison with sage grown on polluted soils (NI condition). In addition, it should be noted that the results concerning sage have been obtained with particularly low EO yields for the third year of cultivation. With the assumption that yields in the third year are expected to reach 50 % of those obtained in the second year, which would represent in average an additional 10 kg of EO per hectare, the crop margin would increase of about 1200 €/ha, thus being considerably valuable. According to our results, sage cultivation on either unpolluted or TE-polluted soils appears to be a profitable option, but could be enhanced through increased yields, especially during the third cultivation year.

5. Conclusions - perspectives

The purpose of this study was to evaluate *in situ* the suitability of two aromatic crops for the phytomanagement of TE-polluted soils, from several perspectives: plants' growth, development and biomass production, EO content and quality as well as techno-economic feasibility. In particular, their cultivation on TE-polluted fields, improper for food production, could represent a profitable land use for marginal lands. Based on the obtained results, the following conclusions should be highlighted.

In our experimental conditions, both coriander and sage depicted a tolerance to the TE-content of soils. In fact, both aromatic plants grew normally, without displaying any morphological symptom resulting from the TE-pollution. Emergence and growth as well as biomass production were not compromised by TE-pollution, rather influenced by soil characteristics and weeds' presence. Their potential regarding TE-stabilisation has been evaluated in another related paper. Besides, the addition of the mycorrhizal commercial inoculant displayed an increase of the mycorrhizal rates for both coriander and sage in both polluted and unpolluted conditions, which could be particularly relevant in either nutrient shortage or stressful environmental conditions, such as drought or increasing temperatures, that should be considered especially in a context of global warming.

According to our results, sage in particular appears as a strong candidate for EO oil production on TE-polluted soils, displaying a higher margin in comparison with coriander or with other commonly cultivated plant species on agricultural lands, such as wheat, barley or rapeseed. Thanks to its short-perennial life cycle and its relatively low nutrient requirements, it may also reflect in lower

management costs, highly valuable in a phytomanagement approach. One should however keep in mind that TE bioavailable amounts, accumulation in aromatic plants and translocation to the aboveground tissues are highly dependent from the combination of the soil physicochemical properties, the site climatic conditions, the present TE and the chosen plant species. From a global perspective, several improvement levers could be relevant, regarding a costs' reduction for the production of biomass on both unpolluted and polluted soils: increasing the number of cultivated hectares and the diversity of aromatic plants cultivated and considering crop rotations are some considerations in that regard. It is furthermore relevant to consider a collaboration with a local distiller, as it was the case in the current study, to benefit from a nearby valorisation channel, hence reducing the transportation costs. However, one should avoid a highly competitive market, which may compromise the start and the sustainability of the channel. On another note, the TE and pesticide residue analyses provided key information, notably highlighting a good quality of the EO with trace amounts below or close to the limit of quantification of the method used and similar to marketed products. An improvement of the cultivation practices and in particular of the weed control methods (reduction of the number or the quantity of products) appears nonetheless as a suitable option to enhance the EO quality.

Besides, a further investigation of the channel's social acceptability, namely biomass production on TE-polluted soils and EO distillation from this biomass, which is an important factor to take into account, will be carried out in a successive part of the project. Regarding biomass distillation, the composition of the EO as well as their biological properties will be evaluated separately.

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Supplemental data

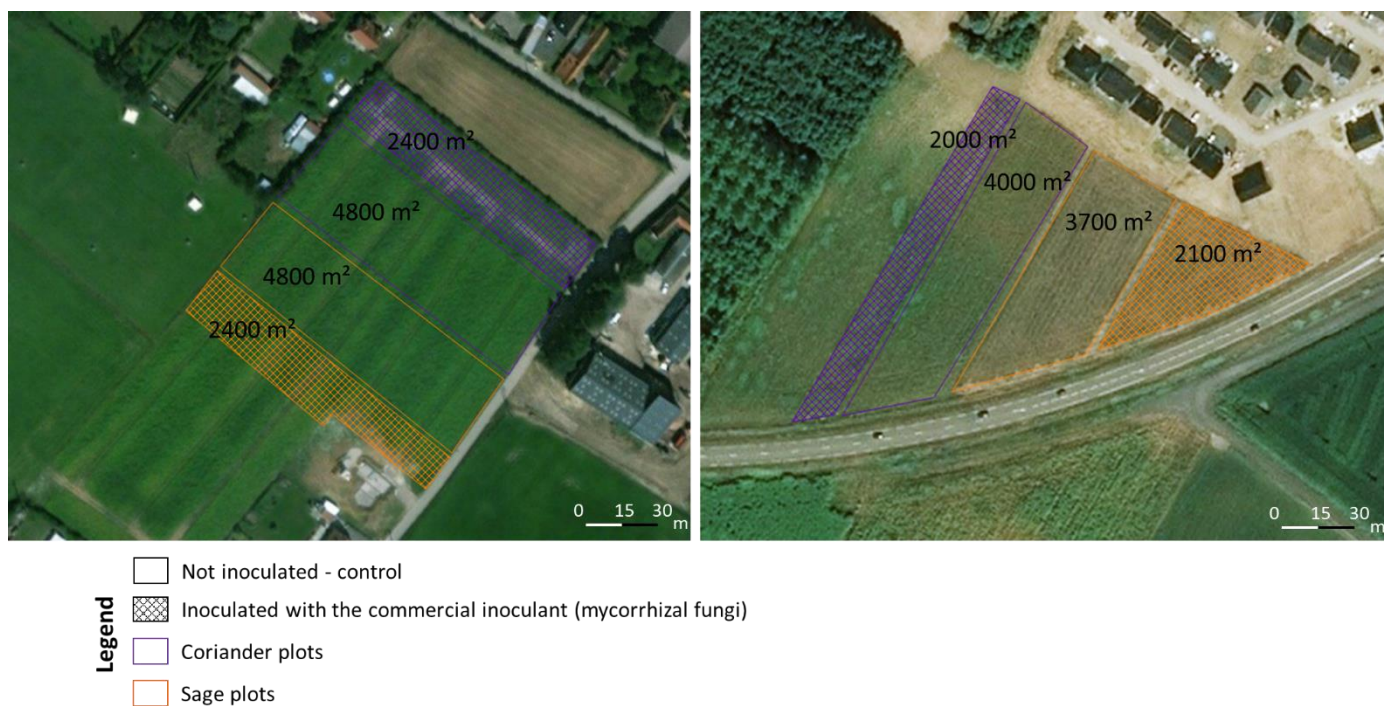


Figure S1: in situ experimental design on unpolluted (left side) and TE-contaminated (right side) sites. Plots' contours have been obtained from georeferencing data (50cm accuracy – scale 1:1500).

Table S1: commercial products and contained active substances used for the chemical interventions on experimental sites. Products applied on: "C": coriander and "S": sage.

Commercial product	Target weeds		Active substance	Unpolluted site		Contaminated site	
	Dicotyledons	Grasses		Application 1	Application 2	Application 1	Application 2
Prowl 400	✓	✓	Pendimethalin	C S	C	C S	C S
Linuron	✓	✓	Linuron	C S			
Centurion 240 EC		✓	Clethodim	C S	C		
Challenge 600	✓		Aclonifen		C S	C	C S
Asulox	✓		Asulam		C S		
Defi	✓	✓	Prosulfocarb		S		
Lentagran	✓		Pyridate		S	C	
Lontrel SG	✓		Clopyralid			S	
Tornado SC	✓	✓	Methamitron			S	

Table S2: estimation of the production costs for the cultivation of coriander's seeds (one-year cost estimation)

	Description	Per-unit cost	Unit	Amount	Operation cost	Unit
Step 1: soil preparation						
Tractor + plough	1 intervention: 5 shears plough - 130-hp tractor	59.8	€/ha	1	59.8	€/ha
Tractor + rotary harrow	1 intervention: Vibrashank (4m) - 110-hp tractor	37.9	€/ha	1	37.9	€/ha
	Total				97.7	€/ha
Step 2: sowing						
Tractor + beet seeder	8 rows seeder - 80-hp tractor	67.8	€/ha	1	67.8	€/ha
Seeds	300 000 seeds/ha <=> 3-4 kg/ha	40	€/kg	4	160	€/ha
	Total				227.8	€/ha
Step 3: inputs						
Tractor + fertilizer distributor	2 interventions	14.5	€/ha	2	29	€/ha
Fertilizer 1	Sulfate of ammonia	200	€/t	0.3	60	€/ha
Fertilizer 2	Sulfate of ammonia	200	€/t	0.3	60	€/ha
Tractor + sprayer	2 interventions / year	15	€/ha	2	30	€/ha
Crop protection products	2 treatments / year				104.9	€/ha
Other inputs	Mycorrhizal inoculant	1.36	€/g	125	170	€/ha
	Total				453.9	€/ha
Step 4: harvest						
Combine	170 or 190-hp combine with 5 agitators	114	€/ha	1	114	€/ha
Tractor + wagon	130-hp tractor (1ha/h) + 16-tonne wagon (2 axles)	49	€/h	1	49	€/ha
	Total				163	€/ha
Total production cost - coriander seeds					942.4	€/ha

Table S3: estimation of the production costs for the cultivation of sage (3-year cycle cost estimation)

	Description	Per-unit cost	Unit	Amount	Operation cost	Unit
Step 1: soil preparation						
Tractor + plough	1 intervention: 5 shears plough - 130-hp tractor	59.8	€/ha	1	59.8	€/ha
Tractor + rotary harrow	1 intervention: Vibrashank (4m) - 110-hp tractor	37.9	€/ha	1	37.9	€/ha
	Total				97.7	€/ha
Step 2: sowing						
Tractor + beet seeder	8 rows seeder - 80-hp tractor	67.8	€/ha	1	67.8	€/ha
Seeds	300 000 seeds/ha <=> 3-4 kg/ha	25	€/kg	4	100	€/ha
	Total				167.8	€/ha
Step 3: inputs						
Tractor + fertilizer distributor	2 interventions per year, during 3 successive years	14.5	€/ha	6	87	€/ha
Fertilizer 1	Sulfate of ammonia	200	€/t	0.45	90	€/ha
Fertilizer 2	Sulfate of ammonia	200	€/t	0.45	90	€/ha
Tractor + sprayer	4 interventions in 3 years	15	€/ha	4	60	€/ha
Crop protection products	4 treatments in 3 years				215	€/ha
Other inputs	Mycorrhizal inoculant	1.36	€/g	125	170	€/ha
	Total				712	€/ha
Step 4: harvest						
Tractor + mower	70-hp tractor + 5-disc rotary mower (2m)	37	€/ha	3	111	€/ha
Tractor + pick-up loader wagon	120-hp tractor + pick-up loader wagon	60	€/ha	3	180	€/ha
	Total				291	€/ha
Step 5: post-harvest						
Tractor	Plot upkeep	60	€/ha	1	60	€/ha
	Total				60	€/ha
Total production cost - sage cultivated for 3 years					1328.5	€/ha

Publication 2

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Biological activities of clary sage and coriander essential oils: *in vitro* screening towards their potential use as crop protection and healthcare products

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Présentation de la Publication 2

D'après les résultats obtenus dans la publication précédente, les HE de sauge sclarée et de coriandre produites sur les parcelles polluée (Evin-Malmaison) et non polluée (Rodelinghem) présentent une bonne qualité au regard de l'analyse des ET et des résidus de pesticides. Grâce à leur richesse naturelle en principes actifs, les HE, composés biosourcés à haute valeur ajoutée, possèdent des propriétés biologiques variées, avec un potentiel d'application dans divers domaines, notamment à vocation non-alimentaire, tels que la protection des cultures (Pavela and Benelli, 2016; Raveau et al., 2020a) ou la santé humaine (Elshafie and Camele, 2017; Kostić et al., 2017). Leur valorisation dans de tels secteurs pourrait permettre d'accroître le développement de la filière PPAM, en augmentant l'offre (création et diversification des domaines d'applications) et en stimulant la demande.

A cet égard, différents travaux ont notamment démontré l'efficacité de certaines HE à inhiber le développement de champignons phytopathogènes (De Clerck et al., 2020; Lagrouh et al., 2017; Muchembled et al., 2017), la germination des graines ou la croissance des plantes (Amri et al., 2013; Blázquez, 2014; El-Alam et al., 2020). Grâce à leurs diverses propriétés biocides, ces composés pourraient donc constituer une solution plus durable en agriculture pour le contrôle des agents phytopathogènes ou des adventices et notamment, dans le cadre du plan Ecophyto II+, permettraient de réduire l'utilisation de produits phytosanitaires chimiques qui présentent des effets nocifs pour la santé de l'Homme et de l'environnement (Raveau et al., 2020a; Rienth et al., 2019). Par ailleurs, certaines HE possèdent des propriétés anti-oxydantes (Duarte et al., 2016; Hsouna et al., 2013; Do Nascimento et al., 2020) ou anti-inflammatoires (Kfoury et al., 2016b; Kostić et al., 2017) et pourraient trouver une application dans le domaine pharmaceutique.

En outre, il est connu que la composition des HE est fortement influencée par les facteurs environnementaux susceptibles de modifier le développement des plantes aromatiques (Zutic et al., 2016). Ainsi, la localisation géographique, les conditions climatiques ainsi que l'ensoleillement et les caractéristiques du sol peuvent affecter la composition des HE (Aćimović et al., 2018; Kumar et al., 2013). La présence de polluants dans le sol peut également modifier la quantité et la qualité des HE produites par la plante (Biswas et al., 2011; Prasad et al., 2014). De même, l'introduction d'un inoculum mycorhizien dans le sol, comme cela a été réalisé dans le cadre de cette étude (Publication 1, Raveau et al., 2020, à soumettre), est aussi susceptible de modifier la composition et la teneur en HE dans la plante hôte (Golubkina et al., 2020; Khaosaad et al., 2006). D'autre part, il est à signaler que les HE, de par la nature aromatique de leurs composés, sont très volatiles et la persistance de leurs effets biologiques dans le temps est souvent décrite comme étant limitée (Kfoury et al., 2019; Singh and Pandey, 2018). Par ailleurs, l'encapsulation des HE par la β -cyclodextrine (β -CD), un oligosaccharide cyclique, a été décrite pour sa capacité à améliorer la stabilité et à renforcer les propriétés biologiques de diverses molécules (Ciobanu et al., 2013; Kfoury et al., 2019). C'est pourquoi, les activités biologiques des HE encapsulées ou non par la β -CD ont été comparées. Ce volet a été étudié en collaboration avec Sophie Fourmentin (équipe CS-UCEIV-ULCO).

Ainsi, en complément de l'étude précédente portant sur la viabilité technico-économique de la filière PPAM-HE, cette publication vise dans un premier temps à caractériser la composition des HE issues de la sauge sclarée (inflorescences) et de la coriandre (graines et parties aériennes), cultivées en présence ou non d'un inoculum mycorhizien sur un sol pollué ou non par les ET. Dans un second temps, les propriétés biologiques des HE ont été évaluées par des méthodes *in vitro* en vue d'une application

potentielle dans deux domaines non alimentaires, la protection des cultures (activités antifongique, anti-germinative et herbicide) et la santé humaine (activités antioxydante et anti-inflammatoire).

L'activité antifongique des différentes HE a été évaluée vis-à-vis de deux champignons phytopathogènes, *Fusarium culmorum* et *Zyloseptoria tritici*, responsables respectivement de la fusariose et de la septoriose chez les céréales (Hellin et al., 2018; Mejri et al., 2018). De plus, *F. culmorum* produit également des métabolites secondaires, les mycotoxines, toxiques pour l'Homme (Ji et al., 2019). Les effets anti-germinatifs et herbicides des HE ont été évalués sur deux espèces végétales fréquemment utilisées dans les tests normés de phytotoxicité, la laitue (*Lactuca sativa* L.) et le ray-grass (*Lolium perenne* L.) (Park et al., 2016). Les effets anti-inflammatoires des HE ont été évalués dans le cadre d'une réponse inflammatoire induite par l'exposition d'une lignée cellulaire bronchiale humaine à des particules atmosphériques. Par ailleurs, l'effet antioxydant des HE a été évalué selon leur capacité à piéger les radicaux libres (activité anti-radicalaire), d'après un test de piégeage du radical libre DPPH^{*} (Brand-Williams et al., 1995). Ces deux derniers aspects ont été abordés en collaboration avec Anthony Verdin (équipe TCEA-UCEIV-ULCO) et Sophie Fourmentin (équipe CS-UCEIV-ULCO), respectivement.

Nos résultats relatifs à la composition chimique des HE, établie au moyen d'une analyse chromatographique par GC-MS, indiquent que l'HE distillée des inflorescences de sauge sclérée est composée majoritairement de monoterpènes (jusqu'à 85 %) dont les principaux sont le linalol (10-17 %) et l'acétate de linalyle (51-63 %), ainsi que de sesquiterpènes, tels que le germacrène-D (7-17 %). L'HE issue des graines de coriandre présente une très forte proportion de linalol (75-83 %), accompagnée d' α -pinène et de γ -terpinène variant respectivement de 3 à 5 % et de 6 à 9 %, tandis que l'HE obtenue à partir des parties aériennes de coriandre présente une majorité de 2-décénal (38-51 %), du linalol, du décanal ainsi que du 2-dodécénal. Ces compositions des HE sont conformes aux compositions décrites dans la littérature (Aćimović et al., 2018; Bhuiyan et al., 2009; Satyal and Setzer, 2020). Elles sont similaires quelles que soient les conditions expérimentales testées (parcelle polluée ou non et inoculée ou non à l'aide d'un amendement mycorhizien).

Au niveau de leurs propriétés biologiques, les HE testées présentent des propriétés fongistatiques, caractérisées par des valeurs de CI_{50} comprises entre 0,20 et 2,83 g.L⁻¹ et entre 0,001 et 0,08 g.L⁻¹ contre *F. culmorum* et *Z. tritici* respectivement. Dans ces mêmes conditions, le fongicide Aviator[®] testé comme témoin positif a affiché des CI_{50} respectives de 1,2.10⁻⁴ et 0,001 g.L⁻¹. Dans le cadre de l'analyse des effets anti-germinatifs, des valeurs de CI_{50} comprises entre 0,05 et 44,0 g.L⁻¹ et entre 0,15 et 145 g.L⁻¹ ont été déterminées, contre la laitue et le ray-grass respectivement. Pour la propriété herbicide, les HE ont affiché des valeurs de CI_{50} variant entre 0,011 et 1,17 g.L⁻¹ et entre 0,044 et 0,77 g.L⁻¹ contre la laitue et le ray-grass respectivement. Le glyphosate, évalué comme témoin positif, a affiché des CI_{50} respectives de 1,0.10⁻⁴ et 1,6.10⁻³. Les valeurs de CI_{50} étaient par ailleurs similaires pour les HE, quelles que soient les conditions expérimentales (parcelle polluée ou non par les ET et inoculée ou non à l'aide d'un amendement mycorhizien). A noter également qu'en présence de la β -CD, les valeurs de CI_{50} étaient dans la majorité des cas inférieures à celles mesurées pour les HE libres (non encapsulées).

Par ailleurs, les trois HE testées ont présenté dans nos conditions, des propriétés antioxydantes, avec des valeurs de CE_{50} comprises entre 0,009 et 0,038 g.L⁻¹. Ces effets sont toutefois inférieurs à ceux d'un témoin positif, le Trolox[®], quelle que soit l'HE et les conditions expérimentales testées. Un effet anti-inflammatoire a également été démontré, pour l'HE issue des parties aériennes de coriandre

uniquement, réduisant la réponse inflammatoire d'une lignée cellulaire bronchiale humaine, exposée à des particules atmosphériques.

Les HE de sauge sclarée et de coriandre graine ont conduit à l'effet inverse, affichant un effet pro-inflammatoire, se traduisant par une réponse inflammatoire supérieure au témoin positif. Les valeurs obtenues étaient toutefois similaires quelles que soient les conditions expérimentales testées (parcelle polluée ou non et inoculée ou non à l'aide d'un amendement mycorhizien). De plus, les réponses inflammatoires en présence des HE et de la β -CD étaient globalement supérieures à celles mesurées pour les HE libres.

Dans l'ensemble, quelle que soit la propriété biologique testée, l'HE issue des parties aériennes de coriandre a présenté des effets plus importants que les HE distillées à partir des sommités fleuries de sauge sclarée et des graines de coriandre. De plus, bien que la β -CD complexe efficacement les différentes HE (taux de rétention variant entre 63 et 81 %), les effets biologiques des HE n'ont pas été améliorés en sa présence.

Pour cette étude, je me suis chargé de l'ensemble des tests biologiques relatifs aux propriétés antifongiques, anti-germinatives et herbicides. L'analyse des compositions chimiques des HE par GC-MS a été réalisée avec l'aide de F. Laruelle (UCEIV). La distillation à grande échelle des HE, préalable à cette étude, a été réalisée par la société FERRANT PHE (P. Ferrant). Les analyses de rétention des HE par la β -CD ainsi que les propriétés antioxydantes ont été réalisées dans le cadre d'une collaboration avec S. Fourmentin (équipe CS-UCEIV-ULCO) et les propriétés anti-inflammatoires ont été évaluées en collaboration avec A. Verdin (équipe TCEA-UCEIV-ULCO). Je me suis également chargé de l'ensemble des analyses statistiques. L'exploitation des données ainsi que la rédaction du manuscrit sont le fruit d'un travail collectif avec les co-auteurs.

Biological activities of clary sage and coriander essential oils: in vitro screening towards their potential use as crop protection and healthcare products

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Abstract

The phytomanagement of trace elements (TE)-polluted soils combines their ecological remediation and the production of non-food crops for the bioeconomy. In this context, aromatic plants, producing high amounts of biomass and whose end-purpose is the distillation of high added-value essential oils (EO), are emerging as promising candidates as part of phytomanagement approaches. Indeed, EO are nowadays arousing great interest, owing to their various application fields, including crop protection and healthcare products. In this work, two aromatic plants – clary sage (*Salvia sclarea* L.) and coriander (*Coriandrum sativum* L.) – were cultivated on a TE-polluted soil with the addition of a mycorrhizal inoculant, evaluated for its contribution regarding plant establishment, growth and biomass production. Besides, to overcome EO stability issues and low persistence of effects, the encapsulation of EO in β -cyclodextrin (β -CD) could represent a promising avenue. Thus, the purpose of this study was to assess *in vitro* the potential of EO distilled from clary sage inflorescences and coriander seeds and aerial parts, in view of applications as crop protection or healthcare products. Notably, the evaluation of EO biological properties (antifungal, anti-germinative and herbicidal, antioxidant and anti-inflammatory) in the presence and in the absence of β -CD, with considerations regarding the potential influence of the TE-pollution and of the mycorrhizal inoculation on the EO chemical compositions, were the key focuses. Firstly, clary sage EO was characterized by high proportions of linalyl acetate (51-63 %) and linalool (10-17 %), coriander seeds EO by a high proportion of linalool (75-83 %) and lesser relative amounts of γ -terpinene (6-9 %) and α -pinene (3-5 %) and coriander aerial parts EO by 2-decenal (38-51 %) and linalool (22-39 %). EO chemical compositions were unaffected by both soil pollution and mycorrhizal inoculation. Besides all tested EO were able to inhibit the growth of two phytopathogenic fungi, *Zymoseptoria tritici* and *Fusarium culmorum*, displaying IC₅₀ ranging from 0.20 to 2.83 g.L⁻¹ and displayed anti-germinative and herbicidal effects. They also exerted promising antioxidant and anti-inflammatory effects. Of the three tested EO, the one from aerial parts of coriander displayed the most significant biological effects, whatever the tested activity. However, EO encapsulation in β -CD has not shown a significant improvement of EO biological properties in our experimental conditions. Our findings suggest that marginal lands polluted with TE could be used for the production of EO displaying faithful chemical compositions and valuable biological activities, with a non-food perspective.

Keywords: Essential oils, aromatic plants, antifungal, antioxidant, anti-inflammatory, anti-germinative, herbicidal, phytomanagement

1. Introduction

Soil pollution by trace elements (TE) is attracting global concern due to their toxicity, persistency and ability to negatively affect soil quality, plant growth and food quality, as well as animal and human health. For these reasons, reclamation of TE-polluted soils should be considered as a primary objective worldwide (Burgess et al., 2018; Lydakis-Simantiris et al., 2016). In that respect, phytotechnologies have emerged during the last decades as promising tools to remediate TE-polluted areas. Based on the use of plants and their associated microorganisms, phytotechnologies are considered as environmental-friendly, cost-effective and benefit from a high public acceptance (Burgess et al., 2018; Cundy et al., 2016; Oustriere et al., 2016). One of the main strategies, referred to as phytostabilisation, resides in the immobilisation of the pollutants in the plant rhizosphere to reduce their bioavailability and alleviate wind and water erosion phenomena (Garaiyurrebaso et al., 2017; Radziemska et al., 2018). However, increased concentrations of TE may impair plant growth and weaken the success of the method (Burgess et al., 2018; Radziemska et al., 2018). Phytostabilisation can hence be supported through the addition of organic or inorganic amendments, aiming at further reducing TE bioavailability, while improving soil quality and plant's growth (Martínez-Martínez et al., 2019). Among the available amendments, microbial inoculants and in particular arbuscular mycorrhizal fungi (AMF), able to develop symbiotic associations with most of the terrestrial plants, have long been described for their contribution towards an improved plants' fitness in agroecosystems (Njeru et al., 2015; Smith and Read, 2008). Furthermore, their contribution to TE immobilization in the rhizosphere may represent a valuable addition to the technology (Antoniadis et al., 2017; Gong et al., 2018). However, one of the major obstacles that aided phytostabilisation is facing resides in the lack of economic profit when the vegetation cover is not valued. To overcome this issue, the concept of phytomanagement combines risk mitigation through aided phytostabilisation with a sustainable and profitable valorisation method for the produced biomass (Burgess et al., 2018; Evangelou et al., 2015).

In that view, the cultivation of aromatic plants for the reclamation of TE-polluted lands appears as a promising approach. Intended for cultivation on marginal lands, they would not compete with feeding agriculture and as non-food crops, aromatic plants minimize the risk bound to the food-chain contamination (Pandey et al., 2019). In addition, the production of essential oils (EO), being high added-value biosourced products, could help producing an economic profit.

In the plant kingdom, the *Lamiaceae* family is one of the biggest flowering plants' family, comprising a wide number of valuable aromatic species (Sim et al., 2019; Singh and Pandey, 2018). It has notably been demonstrated that some of them were displaying a tolerance to TE and hence could be valuable in phytomanagement approaches (Pandey et al., 2019; Sá et al., 2015). Clary sage (*Salvia sclarea* L.) is a biennial aromatic plant cultivated worldwide for its high-value EO, widely known for its use in perfumery and cosmetics but also for its applications in medicine (Aćimović et al., 2018; Blaskó et al., 2017). Coriander (*Coriandrum sativum* L.) is another annual herbaceous aromatic plant, belonging to the *Apiaceae* (Bhuiyan et al., 2009; Freires et al., 2014). Several studies have depicted an interest of both clary sage and coriander EO, given their biological effects, reporting in particular antifungal properties against the phytopathogenic fungi *Phoma* spp., *Alternaria alternata* or yeasts such as *Candida* spp. (Aćimović et al., 2018; Freires et al., 2014; Karpiński, 2020), anti-inflammatory potential (Kostić et al., 2017) and antioxidant activity (Duarte et al., 2016; Kačániová et al., 2020). EO composition is strongly influenced by environmental factors, including geographical location, sunlight, climatic conditions (Aćimović et al., 2018; Kumar et al., 2013) and soil characteristics, including the presence of pollutants. It was in particular suggested that the presence of TE in soil resulted in modified EO yield, composition and quality (Biswas et al., 2011; Jezler et al., 2015; Prasad et al., 2014). Likewise, the establishment of the mycorrhizal symbiosis has been reported as a variation factor for the EO composition and amount (Golubkina et al., 2020; Khaosaad et al., 2006).

Historically used in the traditional medicine, EO are nowadays arousing great interest, owing to their various application fields (Camele et al., 2019; Michel et al., 2020; Zaïri et al., 2019). Among fungal

phytopathogens, *Fusarium culmorum* and *Zymoseptoria tritici* are of major importance, respectively responsible for *Septoria tritici* blotch and *Fusarium* head blight on cereals, whose damage on host plant are considerable and may cause yield losses up to 50 % (Hellin et al., 2018; Mejri et al., 2018). *Fusarium* spp. are also known to produce a wide range of mycotoxins, secondary metabolites that may be highly toxic to human and animal health (Hellin et al., 2018; Ji et al., 2019). The control of both *F. culmorum* and *Z. tritici* is mostly achieved through the use of triazole fungicides (Hellin et al., 2018). However, resistance levels to triazoles have significantly increased since their marketing authorization and hence compromise their reliability (Hellin et al., 2018; Mejri et al., 2018). Moreover, from a wider perspective, the excessive and inappropriate use of pesticides is controversial, because of their noxious impact on both environmental and human health (Lamichhane et al., 2016; Raveau et al., 2020).

In recent years, EO have been reported as potential alternatives to commercial pesticides in crop protection, given their promising biological properties against plant pathogens, pests and weeds (Camele et al., 2019; Raveau et al., 2020). As part of an integrated pest management system, the use of natural products including EO, considered as biocontrol products and regarded as less harmful to both environmental and human health, in comparison with commercial pesticides, is greatly encouraged (Barzman et al., 2015; Pavela and Benelli, 2016), especially in the current context, where regulatory restrictions lead to the withdrawal of commercial products (Gossen and McDonald, 2019; Lamichhane et al., 2016).

On the other hand, EO could also be beneficial for human health and find applications as healthcare products, owing to their antioxidant and anti-inflammatory capacities (Do Nascimento et al., 2020; Elshafie and Camele, 2017). In particular, one of the main concerns in healthcare problems resides in adverse effects of airborne particulate matter (PM) on human health. The resulting diseases occur from the production of reactive oxygen species (ROS) and/or free radicals that induce lipid, DNA, RNA and proteins' damages in organisms (Dergham et al., 2015; Ndong Ba et al., 2019). In response to infection or injuries, the inflammatory process also implies the production of ROS as well as inflammatory mediators such as cytokines, which may increase damages caused to surrounding tissues by lipid oxidation of cell membranes and organelles (Kostić et al., 2017; Schieber and Chandel, 2014).

Currently, synthetic antioxidants and anti-inflammatory compounds are suspected carcinogen and responsible for health disorders (Kfoury et al., 2015; Kostić et al., 2017). Classified as "Generally Recognized As Safe (GRAS)" by the Food and Drug Administration, eco-friendly and well-accepted by consumers (Abarca et al., 2016; Duarte et al., 2016; Kostić et al., 2017), EO are getting more and more attractive and could be particularly helpful as alternatives to synthetic agents.

Nevertheless, EO are very susceptible to degradation and possess low aqueous solubility and bioavailability, which could be challenging for biological applications. Owing to their volatile nature, their stability as well as the persistency of their effects over time are often brought forward as limited and appear prohibitive for both field and healthcare applications. To overcome this issue, the use of a β -cyclodextrin (β -CD), a cyclic oligosaccharide allowing the encapsulation of hydrophobic guests in aqueous solutions, is a promising avenue (Dima et al., 2014; Fourmentin et al., 2013; Kfoury et al., 2015).

Thus, the aim of this study was first to investigate the potential influence of the soil pollution by TE and of the mycorrhizal inoculation on the chemical composition of the EO distilled from clary sage and from both aerial parts and seeds of coriander and on their biological properties in the presence or in the absence of β -CD. The antifungal, anti-germinative and herbicidal potential of the EO were respectively evaluated against two major phytopathogenic fungi, namely *F. culmorum* and *Z. tritici*, and two plant species commonly used for chemicals' herbicidal assessments, namely *Lactuca sativa* L. and *Lolium perenne* L, so as to investigate their potential use as crop protection products. Their anti-inflammatory and antioxidant properties were also assessed, in view of using the EO as healthcare products.

2. Materials and Methods

2.1. Essential Oils

Essential oils tested in the current study were obtained through the steam distillation of two aromatic species, namely coriander (*Coriandrum sativum* L.) and clary sage (*Salvia sclarea* L.), cultivated *in situ* on two experimental plots: a TE-polluted (50°25'55.5" N, 3°02'25.5" E) one, displaying elevated amounts of TE (Pb: 394 ppm - Zn: 443 ppm - Cd: 7,2 ppm) and an unpolluted one (50°49'55.7" N, 1°55'46.9" E). Experimental plots were inoculated (I) or not (NI) with a commercial inoculant (AGTIV® Specialty Crops, Premier Tech, QC, Canada), containing the AMF *Rhizophagus irregularis*, kindly provided by Premier Tech. The distillation of the harvested plant biomass was performed during a three-hour cycle, until no more EO was recovered, on a steam-distillation unit (14 m³ tank – saturated water steam, 0.3 bar). Coriander's distillation was performed using either the harvested aerial parts or the harvested seeds, whereas sage's distillation was performed using harvested inflorescences at full blossoming during the second year of cultivation. EO were stored at 4 °C in tightly closed brown glass vials until their analysis.

2.2. Determination of the EO chemical composition

EO were analysed by electron ionization gas chromatography–mass spectrometry (Shimadzu QP 2010 Ultra). The system was operated using helium as a carrier gas at a constant linear velocity (60 cm.s⁻¹). Essential oil's samples were first diluted in ethyl acetate (ratio 1:200 (v/v)) and 0.2 µL of the resulting solution was injected in a split mode (split ratio 1:10) in a ZB-5MS (Phenomenex – 5 %-phenyl-arylene / 95 % dimethylpolysiloxane – 10 m length, 0.10 mm inner diameter, 0,10 µm phase thickness) capillary column. The column temperature was programmed to linearly increase at a constant rate of 40 °C.min⁻¹, from 60 °C to 280 °C, respectively held for 2 and 1 min.

Mass spectra were recorded with an ionisation energy of 70 eV and an interface temperature of 280 °C, on a mass range of 35.0 to 350 (m/z). Kovats indexes were calculated from the retention times after co-injection with n-alkanes and the identification of individual EO compounds was carried out based on the comparison of their obtained mass spectra and kovats indexes with those listed in the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) and Wiley 275 computer libraries, as well as those found in the literature (Aćimović et al., 2018; Bhuiyan et al., 2009; De Figueiredo et al., 2004; Kumar et al., 2013; Matasyoh et al., 2009; Zutic et al., 2016). Relative percentages of oil constituents were measured from the GC peak areas.

2.3. Biological activities of EO

2.3.1. Determination of the retention capacity of EO by β-CD

The capacity of the β-CD to encapsulate EO (retention capacity) was quantified through a static headspace-gas chromatography (SH-GC) analysis, performed according to El-Alam et al., (2020). Briefly, EO were added to 10 mL of either water or 10 mM aqueous β-CD solutions in 22 mL headspace glass vials. The vials were equilibrated at 25 ± 0.1 °C before analysis. Then, 1 mL of vapour present in the upper part of the vial at equilibrium was withdrawn by using a gas-tight syringe and injected directly into the chromatographic column. A total runtime of 32 minutes was required per sample (initial temperature of 50 °C for 2 min, increased to 190 °C at a constant rate of 5 °C.min⁻¹). All analyses and measurements were carried out using an Agilent headspace autosampler and a Perkin Elmer Autosystem XL, equipped with a FID detector. A DB624 (6 % cyanopropylphenyl/94 % Dimethylpolysiloxane, bonded and crosslinked phase – 30 m length, 0.53 mm inner diameter and 3 µm phase thickness) chromatographic column was also used. The measurement of the retention capacity was expressed as follows:

$$(1) \ r (\%) = \left(1 - \frac{\sum A_{CD}}{\sum A_0} \right) \times 100$$

where $\sum A_0$ and $\sum A_{CD}$ stand for the sum of each EO component's peak area in the absence and the presence of CD, respectively. Measurements were led in triplicates for each EO, in the presence or absence of the β -CD.

2.3.2. Antifungal activity

2.3.2.1. Phytopathogenic fungal strains

Antifungal activities of the different EO were evaluated against two major phytopathogenic fungi: *Fusarium culmorum* and *Zymoseptoria tritici*, by using *in vitro* assays.

F. culmorum strain was maintained on PDA (Potato-Dextrose-Agar) medium. The hemibiotrophic fungus *Z. tritici* (strain R2596) was conserved at -80 °C in cryopreservation tubes. Five days prior the assays, the fungus was cultivated on a PDA medium in order to produce spores (Siah et al., 2010).

2.3.2.2. Determination of *in vitro* antifungal activity against *F. culmorum*

Essential oils' antifungal activities against *F. culmorum* were evaluated by using direct contact and micro-atmosphere *in vitro* assays.

The antifungal activity by direct contact was evaluated according to the methods described in El-Alam et al., (2020) and Znini et al., (2013), with slight modifications. A PDA medium (39 g.L⁻¹) was first prepared, complemented with 1 % (v/v) DMSO, in which EO were mixed at 50 °C, so as to obtain a final scale of 5 EO concentrations, ranging from 0.005 to 1.0 % in the medium. Discs of *F. culmorum* (0.9 cm) were then cut out from the periphery of a 7-day-old fungal colony and placed at the centre of a 9 cm Petri dish containing the PDA medium complemented with EO. The assay was carried out in the absence and the presence of β -CD in the medium, at 10 mM. After a seven-day incubation (20 ± 1 °C), the mycelium radial growth was measured.

The inhibition rate was calculated following equation (2):

$$(2) \text{ Inhibition rate (\%)} = \frac{X_0 - X_i}{X_0} \times 100$$

where X_0 and X_i stand for the average diameter of the fungal colony in control and in treatment, respectively.

A similar assessment was carried out for the micro-atmosphere assay, in the presence and in the absence of β -CD (10 mM). A concentration of EO ranging from 0.005 to 1.0 % was prepared in an aqueous solution of 1 % (v/v) DMSO. Discs of *F. culmorum* were cut and placed at the centre of a 5.5 cm Petri dish containing PDA medium, stuck into a 9 cm-wide one. The aqueous solution of EO was then placed into the 9 cm-wide Petri dish and the whole was sealed using parafilm. An incubation period of 5 days was observed (20 ± 1 °C), and subsequently, the radius of mycelium growth was measured. The inhibition rate was calculated using equation (2).

For both assays, aqueous solutions of DMSO 1 % (v/v) or β -CD (10 mM), as well as a marketed fungicide, Aviator XPro (prothioconazole – 150 g.L⁻¹ and bixafen – 75 g.L⁻¹), were respectively tested as negative and positive controls. The positive control was evaluated with concentrations ranging from 5×10^{-5} to 0.5 %. Analyses were led in triplicates for each condition.

2.3.2.3. Determination of *in vitro* antifungal activity against *Z. tritici*

Essential oils' antifungal activities against *Z. tritici* were evaluated by using *in vitro* microplate assays, adapted from Fungicide Resistance Committee methods (FRAC, 2006), with a range of eight concentrations (from 0 to 0.8 % of EO in the medium) for each and all EO.

These natural products were compared to a homologated and marketed product: Aviator XPro (prothioconazole – 150 g.L⁻¹ and bixafen – 75 g.L⁻¹) within the same range of concentrations.

Spores of *Z. trici* were collected and placed in a glucose-peptone suspension. The microplates were then inoculated with 60 µL of the calibrated pathogen suspension (2×10^5 spores/mL). The microplates were then incubated in darkness and under agitation at 110 rpm (20 ± 1 °C), with an incubation time determined accordingly to the pathogen's optimal growth time (6 days). The evaluation of its growth was carried out using a spectrometer (620 nm). For each EO concentration, eight replicate wells were used. Additionally, each assay has been carried out in triplicates to compare the products. Controls include four non-inoculated wells per EO concentration. Additionally, EO's effects were assessed in the presence and in the absence of β-CD in the culture medium (10 mM).

2.3.2.4. Determination of *in vitro* antifungal properties of EO

The half-maximal inhibitory concentration (IC₅₀) of EO (expressed in g.L⁻¹) required to obtain a fungal pathogens' growth inhibition of 50 % was calculated for all *in vitro* assays. A graphical interpolation complemented with a statistical analysis based on a nonlinear regression were used to calculate the IC₅₀ value (Sahmer et al., 2015).

The IC₅₀ of each of the tested EO was also classified as fungicidal or fungistatic considering its effects. The fungistatic or fungicidal nature of EO was tested by observing revival of growth of the inhibited mycelial disc following its transfer on EO-free PDA medium: no mycelial return to growth defined fungicidal effect, whereas fungistatic effect was characterized by a fungal regrowth capacity on the EO-free medium.

2.3.3. Anti-germinative and herbicidal activities

Inhibitory effects on seedlings' emergence and growth of the different EO, were assessed against two plant species, *Lolium perenne* L. (monocotyledon) and *Lactuca sativa* L. (dicotyledon), commonly used for chemicals' herbicidal assessments (Park et al., 2016) and listed in the OECD guidelines (2003). An *in vitro* method was adapted from Judd et al., (2015); Lyu et al., (2018) and Paul et al., (2001). EO aqueous solutions (DMSO 1 % (v/v)) were prepared, in the presence or in the absence of β-CD (10 mM) and mixed in an agar non-complemented medium (50 °C), then poured into square Petri dishes (120*120 mm). EO concentrations used ranged from 5×10^{-4} up to 0.5 %. Seeds' samples were then placed on the solidified agar medium, in sealed Petri dishes and incubated for 8 days on a day/night cycle, with a 16-hour photoperiod (20 ± 1 °C) and an obscurity period of 8 hours (16 ± 1 °C). Glyphosate (isopropylamine salt – 360 g.L⁻¹) was used as a positive control within the same concentrations' range as the tested EO, while aqueous solutions of DMSO 1% (v/v) or β-CD (10 mM) were tested as negative ones.

After the incubation period, germination rates were evaluated by counting germinated seeds while root elongation was assessed through an imaging software (ImageJ), by measuring root length (Paul et al., 2001; Yazdanbakhsh and Fisahn, 2010). The analyses were led in triplicates. Graphical interpolation complemented with a statistical analysis (Sahmer et al., 2015) were used to calculate the IC₅₀ values, regarding both germination and root elongation parameters.

2.3.4. Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH*) scavenging assay was used to determine the antioxidant activity of the different EO, according to Kfoury et al., (2019), followed with some minor modifications. An EO concentration scale (up to 4×10^{-3} %) was prepared in ethanol for each and all of the tested EO. 1 mL of each ethanolic EO solution was then mixed with 2 mL of ethanolic DPPH* solution (0.1 mM). All mixtures were shaken for 1 h in the dark at 20 ± 1 °C. The absorbance was measured using a UV/Vis spectrometer (Perkin Elmer Lambda 2S) at 517 nm.

The radical scavenging activity was evaluated using the following equation:

$$(3) \text{ Antioxidant Activity (AA) \%} = \left(1 - \frac{A_s}{A_0}\right) \times 100$$

where A_s and A_0 respectively stand for the absorbance of the sample and of the blank.

Obtained results for each EO were expressed as EC_{50} values, defined as the concentration of antioxidant that causes a 50 %-decrease in the DPPH* absorbance, by graphical interpolation. Additionally, EO EC_{50} values were compared to those obtained for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), a water-soluble analog of vitamin E, with results expressed as TEAC (Trolox® equivalent antioxidant capacity).

2.3.5. Anti-inflammatory activity

2.3.5.1. Cell lines and culture conditions

Human bronchial epithelial (BEAS-2B) cell line was cultured in CellBIND® surface plastic flasks (Corning - ThermoFisher Scientific, France) in LHC-9 medium and incubated at 37 °C in a humidified atmosphere containing 5 % of CO₂.

2.3.5.2. EO cytotoxicity assessment

The EO cytotoxicity was firstly assessed at different EO concentrations (*i.e.*, 9×10^{-5} to 1.8×10^{-3} %) using the BEAS-2B cell line. BEAS-2B cells, exposed or not to the different EO, were seeded in 96-well CellBIND® microplates at a density of 20000 or 10000 cells/200 µL medium. After 24 or 48 h of exposure, cytotoxicity was evaluated by measuring the extracellular lactate dehydrogenase activity in supernatants (Cytotoxicity Detection Kit LDH, Roche Diagnostics), and mitochondrial dehydrogenase (MDH) activity (Cell Proliferation Reagent WST-1, Roche Diagnostics), as previously described (Dergham et al., 2015; Kfoury et al., 2016a). Cells not exposed to EO and exposed to Triton X-100 (2 %) were respectively used as negative and positive controls.

2.3.5.3. Cell exposure for the study of EO anti-inflammatory potential

The anti-inflammatory potential of EO was assessed through a method adapted from Dergham et al., (2015). BEAS-2B cells were previously seeded in 96-well CellBIND® microplates, in order to obtain 40000 cells/well at the end of the exposure time. Cells were then exposed to PM_{2.5}, *i.e.* particulate matter with an aerodynamic diameter inferior to 2.5 µm (15 µg/cm²) to induce an inflammatory response and cultured for 48 h, in the presence of EO at the two tested concentrations (1.8×10^{-4} and 9×10^{-4} %), either free or encapsulated in β-CD (0.1 mM). Cells unexposed to EO and unexposed to both EO and PM_{2.5} were respectively used as positive and negative controls. After 48 h of exposure, aliquots of cell-free culture supernatants were collected and frozen (-80 °C) for the determination of cytokines' concentrations and total protein content analysis. Cytokines' concentrations were determined by a sandwich-based Elisa analysis (Quantikine ELISA Kits, R&D Systems Europe, Ltd.) and the total protein contents in cell-free culture supernatants were quantified using the BiCinchoninic Acid kit (Sigma-Aldrich, France). Two interleukins, namely IL-6 and IL-8, were selected as cytokines of interest for the assessment of the anti-inflammatory potential of the tested EO, given their known implication as pro-inflammatory mediators in cells exposed to atmospheric particles (Dergham et al., 2015; Kfoury et al., 2016a).

2.4. Statistical analyses

Statistical analyses were performed using XLSTAT 2018.1.1 (Adinsoft, Paris, France) software and R 3.6.1 (R Core Team, 2019). Prior any statistical analysis, Shapiro-Wilk and Bartlett tests were performed

on the data to verify respectively the normality and the homoscedasticity. When necessary, non-normal data were “square-root” or “log10” transformed.

For all biological properties evaluated, IC₅₀ or EC₅₀ values resulted from non-linear regression analyses from triplicate assays and were expressed as mean values and standard deviation (mean ± SD). The comparison of IC₅₀ or EC₅₀ values was carried out using two-way analysis of variance (ANOVA), complemented with a *post-hoc* Tukey-HSD (Honestly Significant Difference) test. Interleukins' concentrations resulting from the different experimental conditions were also subjected to two-way ANOVA and Tukey-HSD tests.

Moreover, a Principal Component statistical Analysis (PCA) has been carried out to identify the possibly existing correlations between the different EO and to investigate the separation between EO samples according to their main chemical constituents.

3. Results

3.1. Determination of the EO chemical composition

The EO chemical composition revealed that EO from seeds of coriander and sage were containing mostly terpenes, whereas EO from the aerial parts of coriander was mostly containing aldehydes and some terpene compounds. 22 compounds were identified in sage EO, while 11 and 15 compounds were respectively identified in EO of coriander from aerial parts and seeds. Linalool was the only compound found in all distilled EO and was particularly abundant in coriander seeds' EO (ranging from 75 to 83 % - Table 1). (Z)-2-decenal and linalool were the two compounds presenting the greatest proportions (respectively ranging from 37.9 to 51.4 % and from 21.8 to 39.2 %) in coriander aerial parts' EO, whereas linalyl acetate and germacrene D were the two main compounds in sage EO (respectively ranging from 50.6 to 63 % and from 6.7 to 17.1 %).

Our results have shown that EO from aerial parts of coriander originating from the different conditions were strongly correlated (correlation coefficients between 0.914 and 0.995, $\alpha = 0.05$ – data not shown), whatever the condition (polluted or unpolluted sites, I or NI conditions). High correlation coefficients from the PCA analysis correspond to highly similar chemical composition between EO and conversely. Similarly, EO from seeds of coriander (correlation coefficients equal to 0.999, $\alpha = 0.05$ – data not shown) and from sage (correlation coefficients between 0.972 and 0.999, $\alpha = 0.05$ – data not shown) were strongly correlated. As such, no significant differences were observed between EO from a same plant part under the different experimental conditions. Besides, sage EO were not correlated to any EO from coriander, while EO from aerial parts and seeds of coriander were slightly correlated together (correlation coefficients ranging from 0.344 to 0.695, $\alpha = 0.05$).

These conclusions are also visible on the correlation circles obtained with the PCA analysis (Figure 1A and 1B). The first two principal components, F1 and F2, represented 83.7 % of the initial variability of the data. In order to avoid misinterpretation, an additional projection on F1 and F3 (67.3 % of the initial variability of the data) was executed.

Table 1: chemical composition of the EO distilled from aerial parts or seeds of coriander and from sage inflorescences. “-“: undetected compound. Data are relative percentages of EO compounds.

Distilled plant Biomass origin	Aerial parts of coriander				Seeds of coriander				Sage inflorescences			
	Unpolluted		Polluted		Unpolluted		Polluted		Unpolluted		Polluted	
	NI	I	NI	I	NI	I	NI	I	NI	I	NI	I
(Z)-2-decenal	48	51.4	47.1	37.9	-	-	-	-	-	-	-	-
Linalool	29.3	21.8	32.1	39.2	75	76.8	83.2	79.3	10.0	10.4	16.5	14.8
Decanal	6.6	7.9	7.2	8.0	-	-	-	-	-	-	-	-
2-dodecenal	4.6	5.8	3.0	3.7	-	-	-	-	-	-	-	-
2-tridecenal	3.6	4.0	2.0	2.4	-	-	-	-	-	-	-	-
γ -terpinene	1.6	1.9	1.4	1.8	8.9	8.6	6.6	8.4	-	-	-	-
β -farnesene	1.5	1.4	0.3	0.0	0.8	-	-	-	-	0.1	0.1	-
Camphor	1.3	1.0	1.4	1.7	3.7	3.5	3.6	3.7	-	-	-	-
2-undecenal	1.2	1.7	-	1.2	-	-	-	-	-	-	-	-
α -pinene	0.9	1.3	1.0	1.3	5.4	4.5	3.3	2.7	-	-	-	-
Sesquiphellandrene	0.7	-	-	-	-	-	-	-	-	-	-	-
Dodecanal	0.5	0.6	0.8	0.8	-	-	-	-	-	-	-	-
p-cymene	0.3	0.3	0.2	1.1	1.6	1.6	1.3	1.2	-	-	-	-
Linalyl acetate	-	-	-	-	-	-	-	-	53	50.6	63.0	62.6
Germacrene D	-	-	-	-	-	-	-	-	14.8	17.1	6.7	7.3
α -copaene	-	-	-	-	-	-	-	-	3.6	4.5	1.9	2.0
β -caryophyllene	-	-	-	-	-	-	-	-	3.2	3.9	2.6	2.0
Geranyl acetate (trans)	-	-	-	-	1.6	3.0	0.3	1.8	2.5	2.1	1.5	2.5
β -myrcene	-	-	-	-	-	-	-	-	2.1	1.5	0.9	1.2
α -terpineol	-	-	-	-	-	-	-	-	1.9	1.4	1.8	2.4
Germacrene B	-	-	-	-	-	-	-	-	1.7	2.0	0.9	0.9
Geranyl acetate (cis)	-	-	-	-	-	-	-	-	1.3	0.9	0.7	1.0
δ -cadinene	-	-	-	-	-	-	-	-	1.2	1.6	0.4	0.6
β -copaene	-	-	-	-	-	-	-	-	1.1	1.4	0.7	0.5
Limonene	-	-	-	-	1.7	1.6	1.3	1.7	0.8	-	0.5	0.1
Ocimene	-	-	-	-	-	-	-	-	0.7	0.6	0.4	0.7
Sclareol oxide	-	-	-	-	-	-	-	-	0.4	0.6	0.3	0.4
Humulene	-	-	-	-	-	-	-	-	0.4	0.4	0.1	0.2
Sclareol	-	-	-	-	-	-	-	-	0.3	0.3	0.4	0.3
Caryophyllene oxide	-	-	-	-	0.2	-	-	-	0.3	0.4	0.2	0.2
α -cubebene	-	-	-	-	-	-	-	-	0.2	-	0.1	0.1
β -phellandrene	-	-	-	-	-	-	-	-	0.2	0.1	0.1	0.1
4-carene	-	-	-	-	0.3	-	-	0.8	0.2	0.1	0.1	0.2
Camphene	-	-	-	-	0.6	0.5	0.4	0.3	-	-	-	-
Undecanal	-	-	3.5	0.9	-	-	-	-	-	-	-	-
(Z)-5-tridecene	-	0.9	-	-	-	-	-	-	-	-	-	-

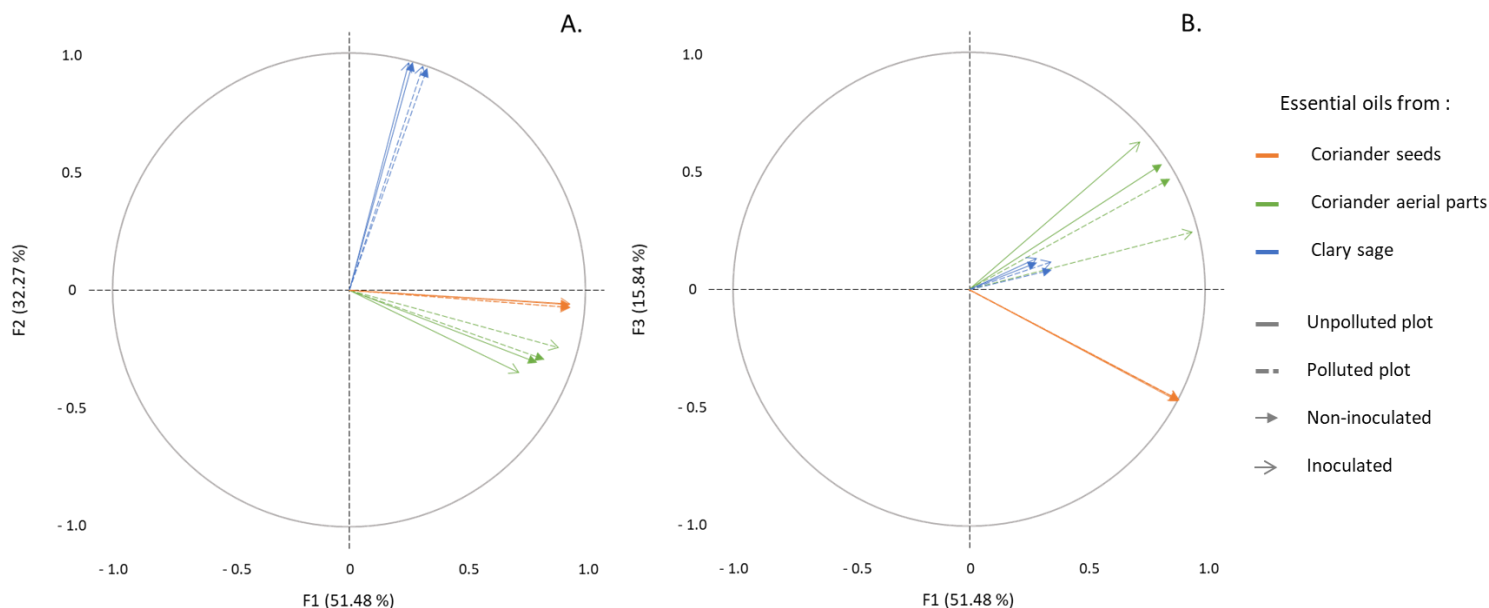


Figure 1: Correlation circles from the PCA analyses based on chemical composition of EO from aerial parts or seeds of coriander and from clary sage, with projection on F1 and F2 axes (a.) and on F1 and F3 axes (b.).

3.2. Determination of the retention capacity of EO by β -CD

All the EO evaluated in this study were able to be encapsulated in β -CD. EO retention percentages by β -CD are listed in Table 2.

Table 2: EO's percentages of retention in the presence of β -CD. Values are expressed as means \pm SD (n=3). Significant differences ($\alpha = 0.05$) between conditions are indicated by different letters (two-way ANOVA).

	Distilled plant part	Aerial parts of coriander	Seeds of coriander	Sage inflorescences
Unpolluted plot	NI	74 \pm 1.4 a	73 \pm 1.0 a	63 \pm 7.1 c
	I	72 \pm 1.4 a	74 \pm 2.1 a	63 \pm 5.7 c
Polluted plot	NI	75 \pm 2.8 a	76 \pm 2.1 a	81 \pm 3.5 b
	I	74 \pm 0.7 a	74 \pm 0.7 a	73 \pm 2.1 a

The obtained EO retention percentages by β -CD were ranging from 63 to 81 %. There were no significant differences in terms of EO retention between all the EO distilled from aerial parts of coriander, ranging from 72 to 75 %, or from seeds of coriander, varying from 73 to 76 %. However, significant differences have been observed between sage's EO, especially between the EO distilled from the biomass obtained on the NI unpolluted (63 % retention) and polluted conditions (81 % retention). Additionally, there is no difference between the I and NI conditions, whatever the EO.

3.3. EO antifungal activity

3.3.1. In vitro antifungal activity against *F. culmorum*

Our results have shown that the EO from coriander and sage presented antifungal properties against the phytopathogenic fungus, *F. culmorum*, by both direct contact and micro-atmosphere bioassays. It was characterized as fungistatic, since fungal regrowth was observed when the disks containing the fungus were transferred on an EO free medium. IC₅₀ obtained for the direct contact assay ranged from 0.46 to 2.63 g.L⁻¹, with no significant difference observed between the EO from a same plant, either aerial parts of coriander, seeds of coriander or sage, but cultivated under the different experimental

conditions (I or NI condition, polluted or unpolluted site – Table 3). Additionally, EO distilled from either aerial parts or seeds of coriander demonstrated a higher efficiency (displaying lower IC₅₀) than those from sage, in particular for the EO distilled from the unpolluted site, with IC₅₀ ranging from 0.46 to 0.61 g.L⁻¹ and from 2.08 to 2.63 g.L⁻¹, respectively for coriander (pooled data from aerial parts and seeds) and sage. On another note, no significant improvement was observed in the presence of β-CD, displaying either similar or higher IC₅₀ in comparison with the β-CD-free condition. In comparison with the positive control, all the obtained IC₅₀ were significantly higher, up to 10⁴ times.

Regarding the micro-atmosphere assay, obtained IC₅₀ ranged from 0.20 to 2.83 g.L⁻¹ (Table 3). As for the direct contact assay, no significant difference was observed between the EO from a same plant, cultivated under the different experimental conditions (I or NI condition, polluted or unpolluted site). In the same way, EO distilled from either aerial parts or seeds of coriander demonstrated a significantly higher efficiency than those from sage, with IC₅₀ ranging from 0.20 to 0.80 g.L⁻¹ and from 1.99 to 2.83 g.L⁻¹, respectively for coriander (pooled data from aerial parts and seeds) and sage. In the presence of β-CD no significant improvement was observed, in comparison with the β-CD-free condition. As the positive control is intended for use as a contact fungicide, the determination of an IC₅₀ regarding volatility effects was not possible.

Table 3: EO's IC₅₀ values (g.L⁻¹) resulting from the antifungal in vitro direct contact and micro-atmosphere bioassays against *F. culmorum*. Values are means ± SD.

Bioassay	Distilled plant part	Aerial parts of coriander		Seeds of coriander		Sage inflorescences		Positive control			
		Without β-CD	With β-CD	Without β-CD	With β-CD	Without β-CD	With β-CD				
Direct Contact assay	Unpolluted	NI	0.46 ± 0.30 b	1.67 ± 0.64 c	0.46 ± 0.14 b	2.18 ± 0.14 c	2.08 ± 0.41 c	8.39 ± 2.41 d	0.00012 ± 0.00074 a		
		I	0.61 ± 0.25 b	2.16 ± 2.02 c	0.42 ± 0.03 b	2.89 ± 1.62 c	2.63 ± 1.08 c	11.2 ± 7.80 d			
	Polluted	NI	0.53 ± 0.37-b	1.63 ± 0.55 c	0.53 ± 0.13 b	2.26 ± 0.61 c	1.47 ± 0.21-bc	5.24 ± 1.28-cd			
		I	0.76 ± 0.28-b	1.57 ± 0.23 c	0.80 ± 0.31 b	2.04 ± 0.05 c	1.54 ± 0.22-bc	3.91 ± 0.84-cd			
	Micro-atmosphere assay	Unpolluted	NI	0.59 ± 0.09 a'	1.98 ± 0.43 b'	0.80 ± 0.11 a'	2.52 ± 0.17 b'	2.63 ± 0.10 b'		11.8 ± 2.17 c'	NC
			I	0.20 ± 0.03 a'	0.47 ± 0.07 a'	0.42 ± 0.23 a'	2.63 ± 0.48 b'	2.83 ± 0.64 b'		14.5 ± 3.27 c'	
Polluted		NI	0.31 ± 0.07-a'	1.01 ± 0.62 ab'	0.40 ± 0.34 a'	2.39 ± 0.07 b'	1.99 ± 0.54-b'	8.29 ± 3.23-c'			
		I	0.78 ± 0.12-a'	2.20 ± 0.13 b'	0.45 ± 0.22 a'	2.44 ± 0.15 b'	2.05 ± 0.57-b'	7.38 ± 1.53-c'			

3.3.2. In vitro antifungal activity against *Z. tritici*

Our results from the microplate assay against *Z. tritici* have shown that the EO from coriander aerial parts or seeds and sage presented fungistatic properties against the phytopathogenic fungus. IC_{50} obtained for this assay ranged from 0.001 to 0.08 $g.L^{-1}$, with no significant difference observed whatever the EO and the plant part it is distilled from (aerial parts or seeds of coriander, sage inflorescences) and the experimental cultivation conditions (I or NI conditions on polluted or unpolluted sites – Table 4). No significant improvement was observed in the presence of β -CD, displaying either similar or higher IC_{50} in comparison with the β -CD-free condition. In comparison with the positive control, the IC_{50} obtained for both coriander EO (aerials parts or seeds) and sage were similar in our experimental conditions.

Table 4: EO's IC_{50} values ($g.L^{-1}$) resulting from the antifungal in vitro microplate bioassay against *Z. tritici*. Values are means \pm SD.

Bioassay	Distilled plant part	Aerial parts of coriander		Seeds of coriander		Sage inflorescences		Positive control	
		Without β -CD	With β -CD	Without β -CD	With β -CD	Without β -CD	With β -CD		
<i>Z. tritici</i>	Unpolluted	NI	0.001 \pm	0.09 \pm	0.03 \pm	0.24 \pm	0.08 \pm	0.25 \pm	0.001 \pm 0.001 a
			0.001 a	0.05 b	0.02 ab	0.18 bc	0.11 ab	0.12 bc	
		I	0.001 \pm	0.08 \pm	0.02 \pm	0.41 \pm	0.04 \pm	0.19 \pm	
			0.001 a	0.11 b	0.01 ab	0.52 c	0.06 ab	0.22 bc	
	Polluted	NI	0.005 \pm	0.08 \pm	0.01 \pm	0.09 \pm	0.03 \pm	0.21 \pm	
			0.006 a	0.10 b	0.01 ab	0.11 b	0.03 ab	0.20 bc	
		I	0.04 \pm	0.15 \pm	0.03 \pm	0.12 \pm	0.01 \pm	0.12 \pm	
			0.02 ab	0.16 b	0.04 ab	0.12 b	0.02 ab	0.07 b	

3.4. EO anti-germinative and herbicidal activities

3.4.1. Seedlings' emergence inhibition bioassay

Our results have shown that all tested EO exerted a significant anti-germinative effect on both lettuce and rye-grass. The obtained IC₅₀ ranged from 0.05 to 44.0 g.L⁻¹ and from 0.15 to 145 g.L⁻¹ for EO tested on lettuce and rye-grass respectively (Table 5). EO from aerial parts and seeds of coriander have demonstrated a higher efficiency on both lettuce and rye-grass than those from sage. Additionally, no difference was found between the EO distilled from a same coriander part, but under the different experimental conditions (I or NI, polluted or unpolluted soil).

In the presence of β-CD, no significant improvement has been demonstrated in our experimental conditions, with even significantly higher effects in the absence of β-CD for the EO of coriander's aerial parts and of sage on both tested plants. Due to the retention of EO by β-CD, it sometimes resulted in a very limited efficiency at the tested concentrations. Thus, IC₅₀ have not been calculated (NC) for several conditions, as it would have resulted in particularly high and inaccurate values. On another note, in comparison with the positive control, only the EO from aerial parts of coriander are in the same range of efficiency on lettuce, whereas all the tested EO are at least as efficient as the control on rye-grass.

Table 5: EO's IC₅₀ (g.L⁻¹) resulting from the seedlings' emergence inhibition bioassay against lettuce and rye-grass. Values are means ± SD.

Bioassay	Distilled plant part Conditions		Aerial parts of coriander		Seeds of coriander		Sage inflorescences		Positive control		
			Without β-CD	With β-CD	Without β-CD	With β-CD	Without β-CD	With β-CD			
Lettuce	Unpolluted plot	NI	0.05 ± 0.01 a	603 ± 138 e	0.56 ± 0.12 b	1.87 ± 0.17 bc	6.22 ± 2.72 c	NC	0.014 ± 0.006 a		
		I	0.13 ± 0.79 a	NC	0.56 ± 0.24 b	1.81 ± 0.06 bc	44.0 ± 21.4 d	4315 ± 743 f			
	Polluted plot	NI	0.08 ± 0.01-a	NC	0.73 ± 0.24 b	1.86 ± 0.02 bc	4.21 ± 1.08 c	8617 ± 741-f			
		I	0.13 ± 0.76 a	604 ± 107 e	0.76 ± 0.21 b	1.92 ± 0.07 bc	36.3 ± 26.0 d	4320 ± 743-f			
	Rye-grass	Unpolluted plot	NI	0.18 ± 0.08 a'	500 ± 317 e'	0.60 ± 0.06 b'	1.73 ± 0.26 bc'	145 ± 139 d'		3093 ± 975 f'	36.5 ± 15.1 cd'
			I	0.15 ± 0.03 a'	868 ± 568 e'	0.81 ± 0.22 b'	1.87 ± 0.23 bc'	102 ± 72 d'		847 ± 532 e'	
Polluted plot		NI	0.19 ± 0.08-a'	95.1 ± 158 d'	0.77 ± 0.17 b'	1.93 ± 0.15 bc'	2.6 ± 0.39 c'	1273 ± 555 f'			
		I	0.16 ± 0.05 a'	782 ± 679 e'	0.74 ± 0.13 b'	1.80 ± 0.26 bc'	9.9 ± 8.3 c'	13.1 ± 11.1 c'			

3.4.2. Seedlings' growth inhibition bioassay

IC₅₀ obtained regarding radicle growth inhibition varied from 0.011 to 1.17 g.L⁻¹ and from 0.044 to 0.77 g.L⁻¹, respectively for lettuce and rye-grass bioassays (Table 6). On both lettuce and rye-grass, EO from coriander aerial parts displayed the highest efficiency, in comparison with those from coriander seeds and from sage. In addition, EO from coriander seeds displayed higher efficiency than those from sage on lettuce. On another note, no difference was observed between the EO distilled from a same plant, but under the different experimental conditions (I or NI, polluted or unpolluted soil), against both lettuce and rye-grass. Additionally, in the presence of β-CD, no significant improvement has been observed in our experimental conditions, with even significantly higher effects in the absence of β-CD in most cases. In comparison with the positive control, the tested EO displayed IC₅₀ more than 100 times higher in the case of both lettuce and rye-grass assays.

Table 6: EO's IC₅₀ (g.L⁻¹) resulting from the growth inhibition bioassay against lettuce and rye-grass. Values are means ± SD.

Bioassay	Distilled plant part		Aerial parts of coriander		Seeds of coriander		Sage inflorescences		Positive control		
	Conditions		Without β-CD	With β-CD	Without β-CD	With β-CD	Without β-CD	With β-CD			
Lettuce	Unpolluted plot	NI	0.017 ± 0.001 b	0.31 ± 0.07 c	0.22 ± 0.06 c	2.09 ± 0.14 e	1.16 ± 0.45 d	3.96 ± 2.89 e	0.0001 ± 0.0001 a		
		I	0.011 ± 0.005 b	0.10 ± 0.06 c	0.28 ± 0.05 c	2.44 ± 0.52 e	1.17 ± 0.56 d	1.77 ± 0.15 e			
	Polluted plot	NI	0.028 ± 0.010 b	0.19 ± 0.08 c	0.29 ± 0.07 c	1.39 ± 0.24 de	0.54 ± 0.09 d	2.49 ± 0.50 e			
		I	0.031 ± 0.010 b	0.15 ± 0.21 bc	0.35 ± 0.02 c	1.90 ± 0.36 e	0.82 ± 0.45 d	2.38 ± 0.54 e			
	Rye-grass	Unpolluted plot	NI	0.050 ± 0.014 b'	0.14 ± 0.02 c'	0.25 ± 0.07 c'	1.93 ± 0.33 e'	0.77 ± 0.77 cd'		0.84 ± 0.27 d'	0.0016 ± 0.0006 a'
			I	0.066 ± 0.014 b'	0.19 ± 0.02 c'	0.26 ± 0.04 c'	2.45 ± 0.56 e'	0.66 ± 0.14 d'		3.09 ± 2.2 e'	
Polluted plot		NI	0.053 ± 0.010 b'	0.94 ± 0.80 cd'	0.25 ± 0.04 c'	1.41 ± 0.24 de'	0.50 ± 0.04 d'	2.52 ± 1.52 e'			
		I	0.044 ± 0.019 b'	0.84 ± 0.40 cd'	0.27 ± 0.01 c'	1.71 ± 0.59 de'	0.34 ± 0.15 cd'	58.1 ± 74.2 f'			

3.5. EO antioxidant activity

Antioxidant activity of the different tested EO was evaluated through a DPPH• scavenging assay, displaying EC₅₀ values ranging from 0.009 to 0.038 g.L⁻¹ (Figure 2). These results have indicated that coriander aerial parts' EO showed the highest free radical scavenging ability, in comparison with sage and coriander seeds EO, given the lower EC₅₀ values that were obtained. Their efficiency was also compared to a well-known antioxidant standard, namely Trolox®, and expressed in terms of TEAC, inversely proportionate to the EC₅₀ values obtained (results not shown). EO from coriander aerial parts displayed TEAC higher than 1 μmol Trolox®.g⁻¹ of EO, while comparatively those from coriander seeds and sage have shown TEAC ranging from 0.4 to 0.9 μmol Trolox®.g⁻¹ of EO. The antioxidant capacity increases in the following order: coriander seeds EO < sage EO < coriander aerial parts EO < Trolox®. Additionally, significant differences were obtained between the different experimental conditions (NI or I, unpolluted or polluted site) for each evaluated EO (coriander seeds or aerial parts and sage).

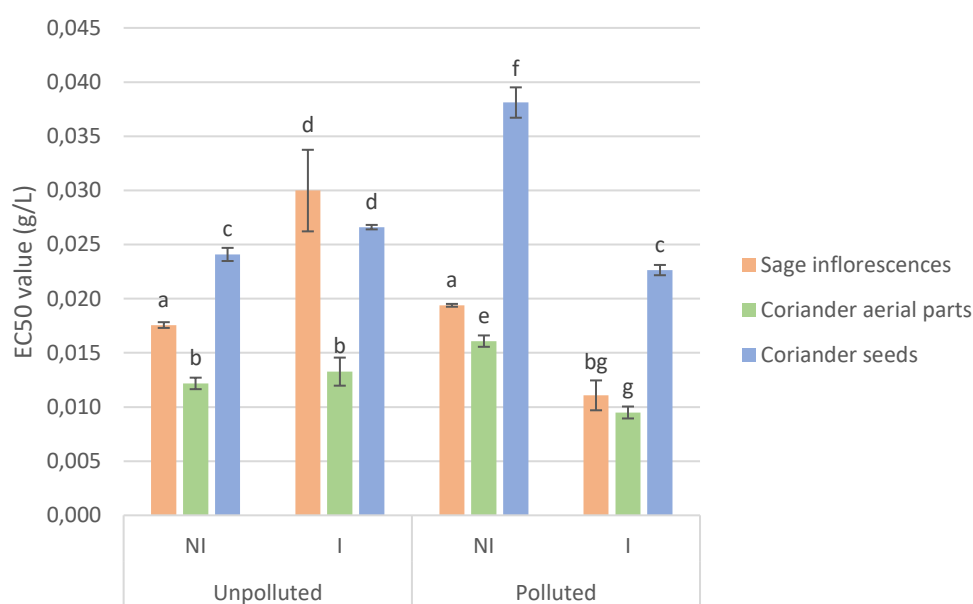


Figure 2: EO's EC₅₀ values (g.L⁻¹) resulting from the DPPH• scavenging assay. Values are means ± SD (n=3). Significant differences between experimental conditions, using a two-way ANOVA comparison ($\alpha = 0.05$), are indicated by different lowercase letters.

3.6. Anti-inflammatory activity

Regarding the cytotoxicity assessment (supplementary material – figure S1), two EO concentrations of 1.8×10^{-4} and 9×10^{-4} % were chosen for the evaluation of the EO anti-inflammatory effects.

After 48 h of exposure to PM_{2.5} (15 μg.cm⁻²), with or without addition of free or β-CD-encapsulated EO, the concentrations of IL-6 (Figure 3a.) and IL-8 (Figure 3b.) were measured and compared to their respective controls. The obtained results for the EO of coriander aerial parts exhibited a reduction of the inflammation, as depicted by the significant decrease observed in both IL-6 and IL-8 levels in comparison with the positive control and that regardless of the condition (Figure 3a. and 3b.). Conversely, in the case of EO from sage and coriander seeds, IL-6 and IL-8 levels were significantly higher, up to 4 times, in comparison with the positive control.

In the case of coriander aerial parts EO, in the presence of β-CD, levels of both IL-6 and IL-8 were not modified, indicating that EO encapsulation did not have any effect on the cells' inflammatory status. Regarding EO of sage and coriander seeds however, in the presence of β-CD, higher levels IL-6 in particular, were measured in most of the experimental conditions, in comparison with both the positive control and the β-CD free condition. Besides, no clear trend in terms of antioxidant capacity

was observed for EO distilled from a same plant part but under the different experimental conditions, regarding both IL-6 and IL-8 levels.

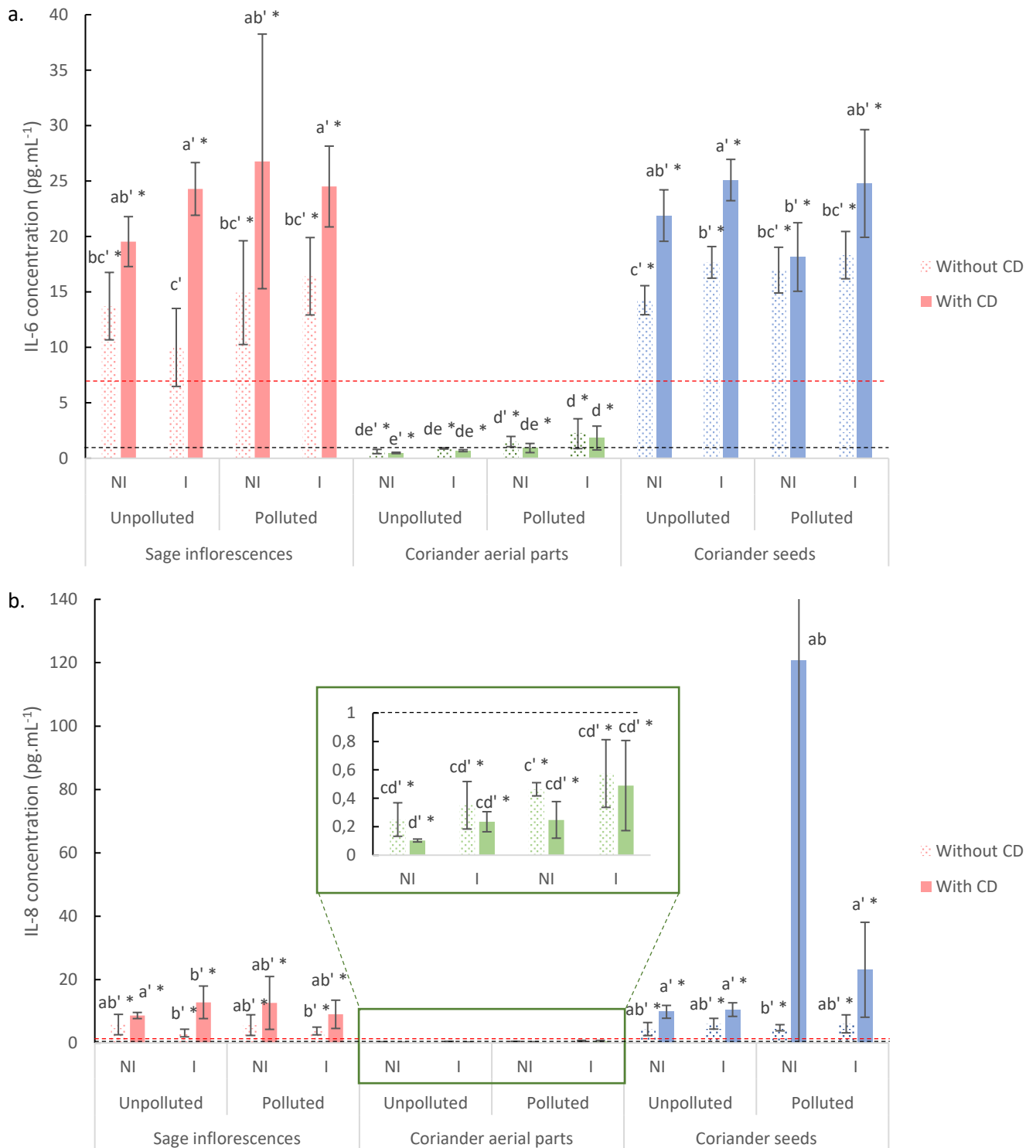


Figure 3: Cytokine levels in BEAS-2B cells exposed for 48 h to PM_{2.5} in the presence of free or β -CD encapsulated EO: a. IL-6 concentrations in BEAS-2B cells, b. IL-8 concentrations in BEAS-2B cells. The negative and positive control values are represented by the black and red dotted lines respectively. Data are presented as mean \pm standard deviation ($n = 3$). CD: Cyclodextrins; BEAS-2B: human bronchial epithelial cells; IL-6: interleukin 6; IL-8: interleukin 8; PM_{2.5}: particulate matter with an aerodynamic diameter inferior to 2.5 μ m. Significant differences using a two-way ANOVA comparison ($\alpha = 0.05$), between results obtained in free β -CD condition and in the presence of β -CD, are indicated by different lowercase letters, differences between experimental conditions and the negative control are displayed with an apostrophe ('), while significant difference with the positive control are displayed with an asterisk (* - $p < 0.05$).

4. Discussion

This study was carried out in the framework of a phytomanagement approach, aiming at cultivating high economic value aromatic plants on TE-polluted soils, to initiate the reclaiming of such areas. In this work, the influence of the soil pollution by TE and mycorrhizal inoculation on the EO chemical compositions were first assessed. The potential of EO coming from clary sage and coriander (aerial parts and seeds) regarding their antifungal, anti-germinative, herbicidal, antioxidant and anti-inflammatory activities were also investigated *in vitro*, in the presence or in the absence of β -CD.

4.1. Chemical compositions of EO distilled from clary sage inflorescences and coriander aerial parts and seeds

Although the relative abundances may slightly vary, all previous reports on clary sage EO seem to agree on the EO composition, highlighting the monoterpenes linalyl acetate and linalool as major compounds (Aćimović et al., 2018; Kumar et al., 2013; Zutic et al., 2016) and corroborating our results. The EO is mostly constituted of oxygenated monoterpenes, up to 85 % of the EO composition, followed by sesquiterpenes. Besides, the relatively high proportion of Germacrene-D obtained in our experimental conditions matches with one of the described chemotypes and is similar to what was previously reported (Aćimović et al., 2018; Sharopov and Setzer, 2012). Similarly, our data regarding the composition of EO from coriander aerial parts and coriander seeds are in line with previous reports, depicting the prevalence of alcohols and aldehydes in the EO from aerial parts (Bhuiyan et al., 2009; Freires et al., 2014; Matasyoh et al., 2009; Satyal and Setzer, 2020), while the EO from coriander seeds is mostly constituted of monoterpenes (Bhuiyan et al., 2009; Satyal and Setzer, 2020). In fact, our results demonstrated a high proportion of linalool (up to 83 %) in the EO from coriander seeds and lesser fractions of α -pinene (from 3 to 5 %) and γ -terpinene (from 6 to 9 %). As previously reported, the EO from coriander aerial parts was significantly different from the one distilled from the seeds, largely composed of 2-decenal (up to 50 %), linalool, decanal and 2-dodecenal.

4.2. Effect of soil pollution by TE and mycorrhizal inoculation on EO composition

In our experimental conditions, the soil pollution by TE did not significantly alter EO composition. However, among environmental conditions, the presence of TE in soil was previously demonstrated as susceptible to affect EO yield, composition and quality (Biswas et al., 2011; Jezler et al., 2015; Prasad et al., 2014). Notably, it was suggested in the case of *Mentha arvensis* L., *Mentha piperita* L. and *Vetiveria zizanioides* Linn. Nash, that the stress induced by the presence of TE resulted in a modification of the secondary metabolism, following an inactivation of enzymes involved in the biosynthesis pathways. This could lead to substantial reductions of the EO amount and yield (Amirmoradi et al., 2012; Jezler et al., 2015; Prasad et al., 2014). Conversely, a significant increase of secondary metabolites amounts was also demonstrated under TE stresses, depicting an elicitation of specific biosynthesis pathways in response to TE exposure (Asgari Lajayer et al., 2017; Gautam and Agrawal, 2017; Sá et al., 2015). It should be noted that the response greatly varies from one aromatic plant species to another and depends on the TE involved (Biswas et al., 2011; Prasad et al., 2014). In this instance, aromatic plants from the *Lamiaceae* family and the genus *Salvia* in particular, were depicted as consistently growing even in TE-polluted conditions and displaying unaffected EO composition and amount (Angelova et al., 2016; Biswas et al., 2011), corroborating our results.

In this work, mycorrhizal inoculation did not result in significant changes in the EO composition either, even though the colonization of plant roots by AMF has previously been reported as significantly modifying both the EO quantity and quality (Golubkina et al., 2020; Khaosaad et al., 2006). In the presence of the mycorrhizal symbiosis, the metabolism of the host plant has in fact been found modified (Ferrol et al., 2016; Harrison, 1999; Hause and Fester, 2005). However, it rather pointed towards an augmentation of the EO content, by affecting the qualitative and quantitative patterns of

secondary metabolites synthesis (Kapoor et al., 2002). It should be noted that in field experiments, inoculation using a commercial inoculum might result in a very limited root colonization by AMF species (Janoušková et al., 2017). In that respect, it could have led to non-significant changes in terms of EO composition.

4.3. EO biological activities towards a potential application in crop protection

EO from both coriander and clary sage were previously investigated for their antifungal activity against a wide spectrum of fungal pathogens, but scarce are the reports targeting plant pathogens in particular. Until now, positive results have been reported regarding the antifungal effects of EO from coriander seeds and from *Lamiaceae* species on the development of *Fusarium* spp. (Sumalan et al., 2019). Our data regarding antifungal activity, against both *F. culmorum* and *Z. tritici* have shown that all the tested EO were able to inhibit fungal growth. Notably, the observed antifungal effect was defined as fungistatic rather than fungicidal, depicted by the revival of hyphae and mycelial growth after transfer on a medium exempted from EO. This feature could be valuable in preventive applications as a means to control pre- and postharvest fungal diseases. The efficiency of the tested EO increased in the following order: Clary sage EO < Coriander seeds EO ≤ Coriander aerial parts EO. Although the concentrations of the EO were up to 1000 times higher than those of the commercial fungicides, the tested EO still displayed consistent antifungal activity. Besides, EO are known to exert lower harmful effects on non-target organisms and on environment and human health. Notably, they are known to possess a low persistence in soils, owing to their volatility (Isman and Machial, 2006; Regnault-Roger et al., 2012; Seixas et al., 2018), while the occurrence of resistance phenomena bound to the use of EO has not been reported so far. It should also be noted that in the case of *Z. tritici*, all the tested EO were in the same range of efficiency as the positive control consisting in a marketed fungicidal product.

Beside antifungal properties, the tested EO revealed a significant anti-germinative effect and herbicidal activity on both lettuce and rye-grass. Our results have shown a promising activity of the EO, especially the one distilled from aerial parts of coriander, which displayed lower IC₅₀ values than those from coriander seeds and clary sage, whatever the bioassay and the target plant. In comparison with glyphosate, which is a systemic herbicide and was evaluated as a positive control in this work, the tested EO have displayed a consistent herbicidal activity. In previous studies, reported glyphosate IC₅₀ varied from 15.3 mg.L⁻¹ (Ghanizadeh et al., 2015) to 23 and 46.2 mg.L⁻¹ (Martin and Ronco, 2006) regarding the inhibition of ryegrass growth, while the results reported on lettuce ranged from 8.9 mg.L⁻¹ (Ronco et al., 2008) to 20 mg.L⁻¹ (Kuang et al., 2017), which are comparable to those obtained in this work. In addition, the EO from aerial parts of coriander, reported as the most efficient in terms of *in vitro* herbicidal activity, exerted effects similar to those of glyphosate and even higher on ryegrass. Since glyphosate is not homologated as an anti-germinative product, the use of EO to fulfil this purpose could be promising.

4.4. EO potential use as healthcare products

Regarding antioxidant activity, all the tested EO revealed a clear antioxidant potential but displayed different ranges of efficiency, with IC₅₀ ranging from 0.009 to 0.038 g.L⁻¹. Our results have indicated that the EO from aerial parts of coriander possessed the highest free radical scavenging capacity using the DPPH[•] scavenging assay. These results are in line with results found in the literature, depicting IC₅₀ values ranging from 0.001 to 1.5 g.L⁻¹, depending on the tested EO, including *Allium cepa*, *Citrus aurantium*, *Myrtus communis* and *Eucalyptus oleosa* EO (Berka-Zougali et al., 2012; Hsouna et al., 2013; Marzoug et al., 2011; Ye et al., 2013). So as to compare the antioxidant capacity of the EO to that of a well-known antioxidant standard, Trolox[®], the obtained results were also expressed in terms of TEAC. TEAC values ranged from 0.4 up to 1.62 μmol Trolox[®].g⁻¹ of EO, for coriander aerial parts and seeds EO respectively, indicating that EO from coriander aerial parts exerted an antioxidant capacity slightly higher than the two other EO, while sage and coriander seeds EO have shown a limited antioxidant

capacity. This result is in agreement with previous findings on coriander aerial parts EO, demonstrating an antioxidant potential by displaying an IC_{50} value lower than that of the reference synthetic antioxidant compound, but which was only slightly lower (Duarte et al., 2016). These findings suggest that the EO from coriander aerial parts in particular could be of interest to prevent lipid oxidation and ROS generation into the human body, preventing oxidation damages (Duarte et al., 2016; Miguel, 2010). It has been hinted that natural antioxidant, such as EO, can avoid ROS formation by acting as physical barriers, preventing the access to biological sites and binding metallic ions (Miguel, 2010). Moreover, they were also shown capable of scavenging and inactivating ROS (Miguel, 2010).

In addition to their ability to scavenge free radicals and prevent ROS formation, the EO were also tested for their anti-inflammatory properties. As previously shown, BEAS-2B cells exposed to $PM_{2.5}$ exhibited an inflammatory response, when compared to control cells (Dergham et al., 2015; Kfoury et al., 2016a). The response of the BEAS-2B cells to the addition of the EO varied, since coriander aerial parts' EO drastically decreased the levels of secreted IL-6 and IL-8, exhibiting a strong anti-inflammatory effect. Conversely, the addition of clary sage and coriander seeds' EO led to the opposite effect, with concentrations of interleukins up to 4 times higher than the control. Hence, only the EO from aerial parts of coriander exerted an anti-inflammatory effect, protecting the cells against the stress induced by $PM_{2.5}$ intoxication.

Altogether, our results suggest that the presence of TE in soil did not alter the EO biological effects, whatever the assessed property. Whether they were evaluated for applications in crop protection or healthcare fields, EO originating from the biomass cultivated on the polluted site displayed similar efficiencies as the one distilled from the unpolluted one. This finding goes along with what has been reported regarding the chemical compositions, which were similar between unpolluted and polluted conditions. Likewise, the mycorrhizal inoculation did not lead to significant changes in the EO effects, whatever the biological property. This result is also consistent regarding the similar EO compositions displayed between inoculated and non-inoculated conditions.

4.5. Insights on the relationships between EO composition and their biological effects

It was previously suggested that mono- and sesquiterpenes present in the EO were responsible for the EO antimicrobial, but also herbicidal, antioxidant and anti-inflammatory activities (Choi et al., 2015; Duarte et al., 2016; Freires et al., 2014; Matasyoh et al., 2009). As an example, linalool is often brought forward as the main explaining factor of the EO activity (Duarte et al., 2016). However, in this work, linalool is present in all the tested EO in various proportions, which alone are not sufficient to explain the different ranges of efficiency that have been assessed regarding the biological activities. It has in fact repeatedly been demonstrated that EO's activities were the result of a synergism between the EO constituents, since the evaluation of the latter isolated often lead to decreased performances (Bakkali et al., 2008; Freires et al., 2014). In that regard, studying the mechanisms of action of the EO and of some of their compounds, alone or in combination, could help towards the understanding of the interconnection between the EO composition and the related biological activity. As an example, several key features were brought forward in the case of herbicidal assessments, however no comprehensive study has so far been carried out on the detailed herbicidal mechanisms (Lins et al., 2019), which could be a valuable addition to the field.

4.6. Essential oil encapsulation in β -CD

On another note, EO stability as well as the persistency of their effects over time are often brought forward as limited. To overcome this issue, encapsulation in β -CD could protect the EO from external factors favouring degradation, prevent EO volatilization and allow a controlled-release of the EO (Abarca et al., 2016; Kfoury et al., 2019a, 2015). β -CD encapsulation may hence be of great interest to address EO downsides and to legitimate their use as natural alternatives in the agriculture or healthcare sectors (Campos et al., 2018; del Toro-Sánchez et al., 2010; Kfoury et al., 2016b). In our experimental conditions, all the tested EO have shown a relatively high capacity to be efficiently

complexed with β -CD, with retention percentages ranging from 63 to 80 %. β -CD among other CD, was in fact previously demonstrated to possess a higher retention capacity (Ciobanu et al., 2013; Kfoury et al., 2015). Interestingly, the measured retention capacities, whatever the considered EO, were in the same range as what was previously described for β -CD but with other EO, such as *Citrus reticulata*, *Cymbopogon nardus*, *Origanum majorana*, *Rosmarinus officinalis* or *Satureja montana* (Kfoury et al., 2015). These results suggest that β -CD could efficiently form inclusion complexes with the component of EO and hence reduce their volatility.

However, in our experimental conditions, EO encapsulation in β -CD did not provide a significant enhancement in terms of EO efficiency in any of the tested biological activity, although it was previously demonstrated (Alipour et al., 2019; Kfoury et al., 2019a). In some specific cases, notably in the antifungal and herbicidal assays, it even resulted in a lower efficiency of the EO, owing to their complexation with β -CD and consequently their reduced volatility and availability.

From an agricultural perspective, encapsulation could nonetheless significantly raise the persistence of the EO's effects, given their efficient retention by β -CD, allowing a controlled-release (Ciobanu et al., 2013). It could particularly be valuable towards a lengthening of the fungistatic effects in time. Similarly, regarding the healthcare application, the encapsulation of EO could provide an efficient tool to enhance the EO solubilization and diffusion throughout the organism and facilitate its accession to the target (Kfoury et al., 2016a).

5. Conclusions

Altogether, the above findings have shown that the three tested EO, from clary sage (inflorescences) and coriander (aerial parts and seeds), displayed faithful chemical compositions, despite the soil pollution by TE and the presence of a mycorrhizal commercial inoculum. EO from clary sage inflorescences was dominated by linalyl acetate (51-63 %), with lesser proportions of linalool (10-17 %) and germacrene D (7-17 %). Regarding coriander, seeds EO was mostly constituted of linalool (75-83 %), γ -terpinene (6-9 %) and α -pinene (3-5 %), while the one distilled from the aerial parts was characterized by 2-decenal (38-51 %), linalool (22-39 %), decanal (7-8 %) and 2-dodecenal (3-6 %).

They were also demonstrated to possess valuable antifungal, anti-germinative, herbicidal, antioxidant and anti-inflammatory activities. Notably, these suggest that the EO distilled from aerial parts of coriander possess a higher efficiency, whatever the tested biological activity. It should be noted that even though the richness in mono- and sesquiterpenes is often brought forward as responsible for the EO biological activities, it was in our experimental conditions not sufficient to explain the different ranges of efficiency that have been assessed regarding the biological activities, suggesting that it's important to consider the presence of minor compounds as well. Hence, a further assessment of the EO mechanisms of actions could provide valuable information, especially regarding the herbicidal effect which has not been thoroughly investigated. Afterwards, if such *in vitro* assays allow a first screening of the EO biological activities and may indicate their potential towards applications in crop protection or as healthcare products, further *in planta* or *in vivo* assays should be carried out to validate EO-based biopesticides and healthcare products' efficiency. In addition, these EO could be tested alone or in association, to investigate potential synergistic effects, as well as in combination with conventional marketed products, so as to reduce the amounts used. Besides, in our experimental conditions, even though the encapsulation of the tested EO in β -CD did not significantly improve the EO efficiency, β -CD efficiently formed inclusion complexes with the EO and reduced their volatility. Further assessments should be conducted to confirm the efficiency of the controlled release of EO in glasshouse of field conditions.

From a wider perspective, our study supports the idea that aromatic plant cultivation on TE-polluted areas and EO distillation from the produced biomass can be a means to reclaim these marginal sites. In particular, EO are high added value products and as depicted in this work could be valued for their

biological properties, provided that the stability and persistence issues are addressed. In addition, their market globally and steadily increases, offering economic opportunities.

Authors contributions

Conceptualization-methodology-validation, R.R., J.F., A.V., F.L., S.F., L.M., P.F., A.L.-H.S.; writing—original draft preparation, R.R.; writing—review and editing, R.R., J.F., A.V., F.L., S.F., L.M., A.L.-H.S.; supervision A.L.-H.S. All authors have read and agreed to the published version of the manuscript.

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Supplemental Data

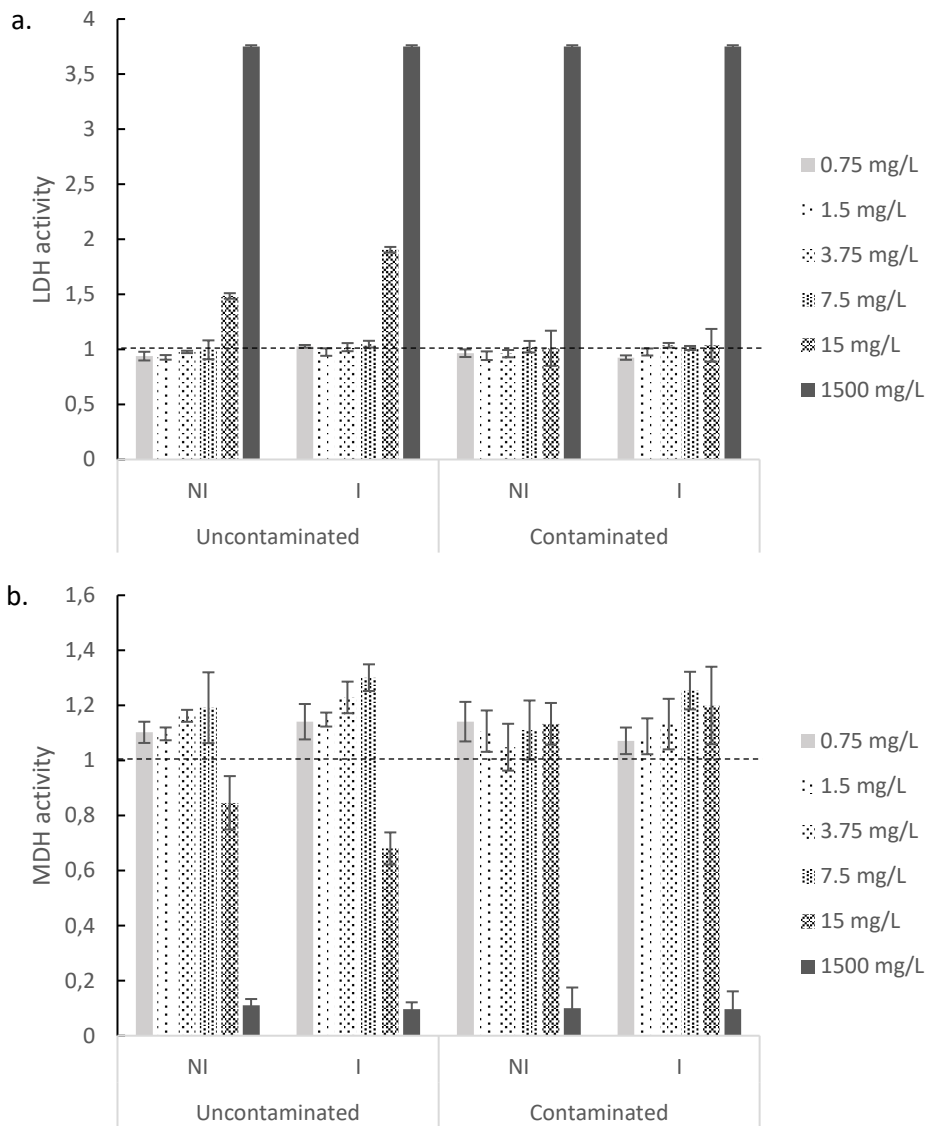


Figure S1: Lactate dehydrogenase (LDH – a.) and mitochondrial dehydrogenase (MDH – b.) activities in cell-free culture supernatants of BEAS-2B cells, after 48 h of exposure to coriander aerial parts EO at different concentrations. Cells exposed to the culture medium only were used as a negative control (dotted line). Results are depicted as mean \pm SD of 3 replicates for each exposure condition.

Publication 3

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The aromatic plant clary sage shaped bacterial communities in the roots and in the trace element-contaminated soil more than mycorrhizal inoculation – a two-year monitoring field trial

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Présentation de la Publication 3

Outre leurs effets nocifs sur la santé humaine, les ET peuvent affecter le développement, la survie et la productivité des espèces végétales mais aussi la vie microbienne du sol (Antoniadis et al., 2017; Ding et al., 2017; Gong et al., 2018). Or le microbiote tellurique joue un rôle fondamental dans le fonctionnement du sol (Chen et al., 2020; Lin et al., 2019). Les communautés bactériennes sont, par exemple, impliquées dans les cycles géochimiques du carbone et de l'azote, la nutrition des végétaux, la régulation des agents phytopathogènes ou même l'immobilisation des polluants inorganiques dans le sol (Lin et al., 2019; Saccá et al., 2017). Elles peuvent également servir d'indicateurs de fertilité et de qualité du sol (Delgado-Baquerizo et al., 2016; Saccá et al., 2017). Ainsi, la modification de la structure des communautés microbiennes telluriques peut affecter durablement la fonctionnalité du sol et par conséquent les services écosystémiques rendus par celui-ci (Ding et al., 2017; Saccá et al., 2017).

De nombreux travaux ont par ailleurs montré que la revégétalisation des sols pouvait s'accompagner d'une refunctionalisation, en contribuant à la séquestration du carbone, à l'enrichissement du sol en matière organique et en carbone organique en particulier et enfin à l'activité microbienne du sol (Burgess et al., 2018; Buta et al., 2019; Durand et al., 2018). En fonction de l'espèce végétale choisie et de la nature de ses exsudats racinaires, la structuration des communautés microbiennes peut être modulée (Dagher et al., 2019; Wang et al., 2018). Il a par ailleurs été suggéré que l'inoculation des plantes par un amendement mycorhizien pouvait également induire des changements structurels au niveau des communautés bactériennes (Akyol et al., 2019; Monokrousos et al., 2019). En effet, les CMA peuvent agir indirectement en modifiant l'exsudation racinaire de leurs plantes hôtes et/ou directement en produisant des exsudats au niveau des hyphes extraracinaires, utilisés comme source de carbone et d'énergie par les bactéries (Gui et al., 2017; Toljander et al., 2006). Ainsi, la nature des exsudats excrétés participe au recrutement d'espèces bactériennes spécifiques, modifiant par conséquent la composition de la communauté bactérienne rhizosphérique (Gui et al., 2017; Toljander et al., 2006).

Ainsi, dans le contexte d'une démarche de phytomanagement d'un sol pollué par les ET, nous nous proposons dans le cadre de cette publication de caractériser et de suivre les communautés bactériennes présentes dans le sol rhizosphérique de la sauge sclarée ainsi que dans ses racines via une approche de métagénomique par séquençage grâce à la technologie Illumina Miseq et ce pendant les deux années de culture de la sauge sclarée et à trois temps de prélèvement (état initial de la parcelle avant végétalisation – année 1 avant récolte – année 2 avant récolte). Les communautés bactériennes ont été étudiées spécifiquement à l'aide d'amorces ciblant la région V3-V4 de l'ARNr 16S procaryote (Herlemann et al., 2011). L'impact de l'inoculation mycorhizienne sur la structure et l'évolution des communautés bactériennes du sol rhizosphérique et des racines a aussi été évalué.

Nos résultats démontrent qu'avant revégétalisation de la parcelle, les phyla bactériens *Actinobacteria* (45 %), *Proteobacteria* (32 %), *Gemmatimonadetes* (12 %) étaient majoritaires. Plus spécifiquement, les genres bactériens *Pseudarthrobacter*, *Sphingomonas*, *Nocardioides* et *Gaiella* étaient les plus représentés. Les indices de richesse (estimateur Chao1) et de diversité (Shannon et Inv. Simpson) calculés sont de 1772 ; 6,7 et 335 respectivement. Il est intéressant de signaler que les phyla *Actinobacteria*, *Proteobacteria*, *Firmicutes* et *Bacteroidetes* ont précédemment été décrits pour leur tolérance aux ET (Chen et al., 2018; Cui et al., 2018; Hemmat-Jou et al., 2018).

Nos résultats suggèrent que les communautés bactériennes du sol rhizosphérique et des racines de la sauge sclarée évoluent distinctement. En effet, les indices de richesse et de diversité dans le sol décroissent au cours du temps et sont supérieurs à ceux observés au niveau des racines. Au niveau des racines, au contraire, les valeurs des indices augmentent entre l'année 1 et l'année 2.

La revégétalisation du sol par la culture de sauge sclarée module de façon significative la structure des communautés bactériennes du sol rhizosphérique et des racines. En effet, les résultats d'une analyse PERMANOVA couplée à une analyse en coordonnées principales ont démontré une variabilité importante et significative au cours du temps ($P < 0,001$) et en particulier entre l'année 1 et l'année 2 de culture. Une chute des indices de richesse et de diversité et une diminution du nombre d'ASV bactériens au niveau du sol rhizosphérique ont ainsi été observées, conjointement avec des modifications en termes d'abondances relatives, au niveau du phylum ou du genre. Les proportions des phyla *Gemmatimonadetes* et *Proteobacteria* connaissent en effet une diminution entre l'année 1 et l'année 2, tandis que le phylum *Firmicutes* suit la tendance inverse, avec une surreprésentation en année 2. Le phylum *Actinobacteria* est lui largement majoritaire quel que soit le temps de prélèvement et la modalité (inoculée ou non par le CMA) et connaît même une progression de 30 % entre l'année 1 et l'année 2. Ces résultats se retrouvent également au niveau des genres bactériens et en particulier dans les racines. En effet, les genres *Clostridium*, *Lelliottia* et *Pseudomonas*, appartenant respectivement aux phyla *Firmicutes* et *Proteobacteria*, étaient particulièrement représentés en année 1, mais leurs abondances relatives chutent significativement en année 2. À l'inverse, les genres *Pseudarthrobacter*, *Nocardioides* et *Streptomyces*, appartenant tous au phylum *Actinobacteria*, étaient faiblement représentés en année 1 et deviennent prédominants en année 2.

L'apport d'un amendement mycorhizien n'a en revanche pas eu d'effet significatif sur la structure des communautés bactériennes, que ce soit au niveau du sol rhizosphérique ou des racines, comme en témoigne l'analyse PERMANOVA ($P > 0,05$). Cette information se traduit au niveau du diagramme de Venn par un nombre élevé d'ASV partagé entre les conditions inoculées ou non. Toutefois, il est à signaler que la chute en termes de richesse et de diversité observée entre l'année 1 et l'année 2 notamment, était significativement moins marquée pour la condition inoculée.

Enfin, les résultats de l'analyse des corrélations entre la biomasse de la sauge sclarée ou les taux de mycorhization et les ASV majoritaires indiquent une forte corrélation entre ces deux paramètres et les genres bactériens *Pseudarthrobacter* et *Streptomyces* notamment, quel que soit le biotope (sol rhizosphérique ou racines). Les résultats de l'assignation fonctionnelle suggèrent finalement une augmentation au cours du temps de la proportion des genres bactériens contenant des espèces décrites comme promotrices de la croissance des plantes (PGPR), tels que *Streptomyces*, *Pseudarthrobacter*, *Pseudomonas* ou *Bacillus* (Durand et al., 2018; Ferreira et al., 2019; Foulon et al., 2016a; Vurukonda et al., 2018).

Pour cette étude, les prélèvements et le conditionnement des échantillons ont été réalisés par mes soins, ainsi que l'ensemble des étapes d'extraction et d'amplification de l'ADN par PCR (optimisation, amplification de l'ADN avec les conditions de séquençage et quantification des amplicons issues de PCR). Le séquençage a été réalisé par Génôme Québec. Le traitement bioinformatique a été réalisé sous la supervision de M. Hijri, lors d'un séjour de formation à l'Institut de Recherche en Biologie Végétale de Montréal. L'ensemble des analyses statistiques suivant le traitement informatique ont été réalisés

par mes soins. L'exploitation des données est issue d'un travail collectif, ayant donné lieu à la rédaction de cette publication.



The Aromatic Plant Clary Sage Shaped Bacterial Communities in the Roots and in the Trace Element-Contaminated Soil More Than Mycorrhizal Inoculation – A Two-Year Monitoring Field Trial

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To cope with soil contamination by trace elements (TE), phytomanagement has attracted much attention as being an eco-friendly and cost-effective green approach. In this context, aromatic plants could represent a good option not only to immobilize TE, but also to use their biomass to extract essential oils, resulting in high added-value products suitable for non-food valorization. However, the influence of aromatic plants cultivation on the bacterial community structure and functioning in the rhizosphere microbiota remains unknown. Thus, the present study aims at determining in TE-aged contaminated soil (Pb – 394 ppm, Zn – 443 ppm, and Cd – 7ppm, respectively, 11, 6, and 17 times higher than the ordinary amounts in regional agricultural soils) the effects of perennial clary sage (*Salvia sclarea* L.) cultivation, during two successive years of growth and inoculated with arbuscular mycorrhizal fungi, on rhizosphere bacterial diversity and community structure. Illumina MiSeq amplicon sequencing targeting bacterial 16S rRNA gene was used to assess bacterial diversity and community structure changes. Bioinformatic analysis of sequencing datasets resulted in 4691 and 2728 bacterial Amplicon Sequence Variants (ASVs) in soil and root biotopes, respectively. Our findings have shown that the cultivation of clary sage displayed a significant year-to-year effect, on both bacterial richness and community structures. We found that the abundance of plant-growth promoting rhizobacteria significantly increased in roots during the second growing season. However, we didn't observe any significant effect of mycorrhizal inoculation neither on bacterial diversity nor on community structure. Our study brings new evidence in TE-contaminated areas of the effect of a vegetation cover with clary sage cultivation on the microbial soil functioning.

Keywords: phytomanagement, trace elements-contaminated soils, microbiota, *Salvia sclarea*, aromatic plant, essential oils

INTRODUCTION

Inorganic and organic soil contamination resulting mainly from anthropogenic practices, is a worldwide concern particularly in urban areas. Contamination with trace elements (TE), especially originating from industrial activities such as smelting and fossil fuel combustion, agriculture and mining activities are among the most frequent inorganic soil contaminants in Europe. They represent more than 30% of the total soil contamination and are found in nearly 2 million hectares of contaminated land (Panagos et al., 2013; Mench et al., 2018). A large number of the contaminated sites remains marginal, due to very-costly remediation methods, large areas to remediate or unprofitable long-term use of the site (Van Liedekerke et al., 2014; Mench et al., 2018). Beside their noxious effects on human health, TE are also likely to affect soil biology and fertility as well as plant development and productivity, representing a major constraint for contaminated agricultural lands (Antoniadis et al., 2017; Gong et al., 2018). It has previously been demonstrated that TE reduce microbial biomass, hence affecting functional diversity and microbial community structure resulting in an impairment of ecosystem services (Ding et al., 2017). Indeed, soil organisms contribute to a wide range of ecosystem services that are essential to the sustainable functioning of natural and managed ecosystems (Delgado-Baquerizo et al., 2016; Saccá et al., 2017). In particular, the soil microbiota of terrestrial plants is of crucial importance, regarding soil fertility, as many microorganisms play a role in biogeochemical cycles, and regarding their contribution to plant nutrition and health (Ding et al., 2017; Misra et al., 2019; Chen et al., 2020). Among them, soil Bacteria are well known for regulating pests and diseases, sequestering or degrading contaminants, cycling nutrients and mineralizing organic matter as well as being potential biological indicators for soil quality and fertility (Saccá et al., 2017).

Among the potential remediation options, the phytomanagement of contaminated soils, relying on the use of plant species to reduce the risk due to the presence of the contaminant combined with a sustainable and profitable land and biomass use, appears as a promising method to rehabilitate and increase the market value of contaminated sites (Cundy et al., 2016; Mench et al., 2018). It encourages the use of remediation phytotechnologies as part of an integrated site management, providing economic, aesthetic, environmental and social benefits (Burgess et al., 2018). Due to the non-biodegradability of TE, the use of phytostabilization, involving the natural ability of plants and their associated microorganisms to immobilize inorganic contaminants such as TE in their rhizosphere, could be particularly relevant. The success of phytomanagement in agricultural contaminated soils will hence depend on the capacity of cultivated plants to grow in contaminated conditions and to develop large amounts of biomass for valorization perspectives (Evangelou et al., 2015). Currently, the research on the valorization of biomass produced on TE-contaminated soil mainly focuses on high biomass plants intended for bioenergy production, such as *Miscanthus*, maize (*Zea mays* L.), poplar (*Populus* spp.) or willow (*Salix* spp.) (Evangelou et al., 2015;

Chalot et al., 2020; Zgorelec et al., 2020). The cultivation of aromatic plants, such as clary sage (*Salvia sclarea* L.), belonging to family Lamiaceae, could nonetheless represent a profitable and innovative alternative for the reconversion of agricultural contaminated soils. In particular, due to its biennial or short-lived perennial life-cycle, clary sage is able to quickly develop a sustainable vegetative cover over a 2- or 3-year period, and to produce large amounts of biomass (Tibaldi et al., 2010; Lydakis-Simantiris et al., 2016; Grigoriadou et al., 2020). Moreover, the cultivation of aromatic crops such as clary sage, for phytostabilization purposes on TE-contaminated sites, has previously been brought forward as a possible alternative, owing to the good quality of the produced EO regarding a potential TE-contamination (Zheljazkov et al., 2008; Angelova et al., 2016). Furthermore, clary sage was previously demonstrated tolerant to TE (Chand et al., 2015; Angelova et al., 2016), although its behavior regarding TE accumulation remains unclear (Angelova et al., 2016). Such biomass can be harvested and exploited for essential oils' (EO) production, therefore providing high-added value marketable products. In fact, EO from clary sage are valuable in food industry as well as in perfumery and cosmetics but are also known for their antimicrobial properties (Yaseen et al., 2015; Angelova et al., 2016; Zutic et al., 2016). Besides, based on recent forecasts, the global plant products and EO market is expected to consistently increase to reach more than \$10 billion in 2020 and \$15.6 billion in 2026 (Allied Market Research, 2020; CBI, 2020). Based on these criteria, EO production was hence the intended valorization channel for the biomass originating from the TE-contaminated site in the present work.

However, the development of the cultivated plant species can be severely affected by the high levels of bioavailable TE present in the contaminated site (Burgess et al., 2018). Hence, plant growth and phytostabilization can benefit from the addition of amendments, therefore referred to as aided phytostabilization. These either mineral, organic or microbial amendments aim at enhancing plant's health and growth or at improving TE immobilization by precipitation, complexation or root adsorption, hence reducing their mobility and bioavailability for the plant and the living organisms (Antoniadis et al., 2017; Burgess et al., 2018; Gong et al., 2018). In particular, microbial inoculants such as arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi have previously been used in phytomanagement trials (Dagher et al., 2020b). Indeed, AMF are known to be able to establish mycorrhizal symbiosis with most of the terrestrial plants, resulting in an improved plant growth through enhanced mineral nutrient and water acquisition as well as in a mitigation of environmental stresses (Smith and Read, 2008; Ferrol et al., 2016), valuable in both plant establishment and biomass production. In addition, AMF may be beneficial regarding TE tolerance, since they were previously described as contributing to TE immobilization and reduced uptake (Gong et al., 2018; Kumpiene et al., 2019).

The cultivation of plant species is a key and determining factor, as well as the introduction of exogenous microbial species, which are susceptible to modify the structure of the bacterial communities and their diversity in soil (Wang X. et al., 2018;

Dagher et al., 2019). It is believed that the distinctive microbiome around aromatic plants is highly specific and dependant on the plant species and on the related nature and abundance of secondary metabolites, present in the root exudates (Köberl et al., 2013; Adamovic et al., 2015; Misra et al., 2019). Apart from the effects of plant's driven changes on microbial communities, it has been previously reported that mycorrhizal inoculation can also influence the microbial community structure (Lioussanne et al., 2010; Akyol et al., 2019; Monokrousos et al., 2019; Dagher et al., 2020a). Soil bacteria may obtain carbon from AMF hyphae exudates or use the hyphae themselves as substrate (Qin et al., 2016; Gui et al., 2017), creating a niche for the growth of specific bacteria (Toljander et al., 2006; Gui et al., 2017). Furthermore, some specific bacterial taxa have been reported to be highly associated with AMF as they develop and form biofilms on the surface of the fungal hyphae or colonize AMF mycelia (Iffis et al., 2014; Qin et al., 2016). In these regards, AMF inoculation is likely to result in a shift of the bacterial communities in soil and in root of the cultivated plant.

Although the plant identity can strongly shape the bacterial communities' structure, the combined effects of perennial clary sage inoculated with AMF under TE-contaminated conditions on bacterial diversity and communities' structure, remain unexplored. Thus, the present work aims at (i) characterizing the bacterial richness and communities' structure in an aged-TE agricultural contaminated soil, (ii) determining changes of both the rhizosphere soil and root's communities over two growing seasons, and (iii) evaluating the impact of the AMF inoculation on the bacterial richness and communities' structure overtime.

MATERIALS AND METHODS

Experimental Design

Experimental Site

This study was conducted *in situ* on an aged TE-contaminated site (50°25'55.5" N, 3°02'25.5" E; altitude 23 m) in the north of France. A warm temperate humid climate (Csb – Köppen-Geiger classification) with cool summers characterizes the climate. The mean annual temperature is 12°C, with mean monthly temperatures ranging from 3.6°C in January to 20.3°C in July. Annual precipitation is 742.5 mm, almost equally distributed over the year (Infoclimat, 2020). The site is a former agricultural field of about 1.3 ha, located approximately 600 m away from a former lead smelter, Metaleurop Nord, whose activity during more than a century led to a topsoil (0–30 cm) contamination by TE, resulting mostly from atmospheric deposition (Sterckeman et al., 2002; Labidi et al., 2015). In particular, high amounts of Cd, Pb and Zn (7, 394, and 443 ppm, respectively) were recorded, with concentrations approximately 17-, 11-, and 6-fold higher, respectively, than those reported in regional background levels for agricultural soils (Sterckeman et al., 2007). Briefly, the soil is a silt loam, with a slightly alkaline (7.86) water pH. The main characteristics have been evaluated from 19 soil

samples collected before sowing and are as follows: soil organic matter, $29.0 \pm 4.0 \text{ g.kg}^{-1}$; total N, $1.62 \pm 0.24 \text{ g.kg}^{-1}$; C/N ratio, 10.5; available (water extractable) P (Joret-Herbert), $0.17 \pm 0.02 \text{ g.kg}^{-1}$; available K, $0.26 \pm 0 \text{ g.kg}^{-1}$; available Mg, $0.21 \pm 0.02 \text{ g.kg}^{-1}$; available Ca, $6.0 \pm 0.5 \text{ g.kg}^{-1}$; available Na, $0.03 \pm 0 \text{ g.kg}^{-1}$.

Clary sage (*S. sclarea L.*), a biennial or short-lived perennial aromatic plant species, has been sown in late April 2017 (300 000 seeds per hectare) and cultivated for 2 years in the contaminated site. Seeds were purchased from the French research institute ITEIPMAI, providing certified seeds.

The experimental design included two conditions [non-inoculated (NI) and inoculated with the mycorrhizal inoculum (I)] and a time monitoring (initial state of the plot, unplanted – state at first sage harvest, year 1 – state at second sage harvest, year 2), with five replicates each. A commercial mycorrhizal inoculum (AGTIV® Specialty Crops, Premier Tech, QC, Canada) containing the AMF *Rhizophagus irregularis* isolate DAOM 197198 in a powder formulation (12000 viable spores per gram of product, 125 g/ha) was kindly provided by Premier Tech. It has been introduced during sowing, mixed with sage seeds.

Soil and Sage Root Sampling

In April 2017, five soil samples have been randomly collected from the surface horizon (0–30 cm) with a manual auger on the experimental site, to assess the site's initial characteristics. Two other sampling campaigns have been done in August 2017, to assess the state at first sage harvest (after 21 weeks of cultivation) and in July 2018 (after 66 weeks of cultivation), to assess the state at the second sage harvest, totalizing 10 soil samples each. The study was conducted in a completely randomized experimental design and all the sampling points have been geo-referenced. The three closest growing sage plants for each sampling point were collected with their root systems. All samples were collected over a half-day period and transported within 2 h. After sampling, sage roots were vigorously shaken to remove slightly attached soil. Rhizospheric soil was then collected by means of a careful clean-up, using sterile brush and spatula tip (Sun et al., 2018; Yi et al., 2019). Sage roots were finally rinsed three more times with sterilized water to get rid of the potentially remaining soil particles. Soil and sage roots were frozen and stored at -20°C for further use.

Plant Growth

As previously described, sage above-ground parts have been sampled after 21 and 66 weeks of cultivation, to characterize biomass development. For each condition, fifteen plants were used as biological replicates. They were placed in an oven (72 h – 80°C), to determine their dry weights.

Arbuscular Mycorrhizal Root Colonization

Fresh roots were stained using Trypan blue (0.5 g.L^{-1}), according to the method described by Mustafa et al. (2016) and root colonization was estimated by microscopic observation (Nikon Eclipse E600, $\times 100$ magnification) using the grid line intersect method (McGonigle et al., 1990). In that regard, root fragments of 1 cm from three replicates per sampling point

(45 fragments in total) were observed and 3 intersections per root fragment were examined. In total, more than 600 observations per condition were analyzed to assess total mycorrhizal colonization.

DNA Extraction

From Soil

Genomic DNA was extracted in triplicates directly from 250 mg of soil using Nucleospin Soil® kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. The quality of the extracted DNA was verified using 1% (w/v) agarose gels. Quantification of extracted DNA was carried out on a SpectraMax® iD3 spectrophotometer (Molecular Devices LLC, Sunnyvale, CA, United States). The concentration of all samples was determined and DNA extracts were diluted to 25 ng·μL⁻¹ for further analyses. The extracted DNA was stored at -20°C until use.

From Sage Roots

The fine roots were kept in Eppendorf tubes and frozen at -20°C. Genomic DNA from *S. sclarea* roots was extracted in triplicate using a method adapted from Abu-Romman (2011) and Aleksić et al. (2012). Briefly, sage roots were beforehand frozen in liquid nitrogen (-196°C) in a sterilized mortar and ground into fine powder. 500 mg of the ground roots were then subject to Cetyltrimethylammonium bromide (CTAB - 1.4 M NaCl, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 2% CTAB), Polyvinylpyrrolidone (PVP 1% w/v), β-Mercaptoethanol (5% v/v) and activated charcoal (0.5% w/v) extraction (30 min - 55°C). After this incubation period, a centrifugation step was carried out (10 min - 16000 g) and the lysate extraction was then performed using two successive steps with chlorophorm:isoamylalcohol (24:1). DNA precipitation then occurred in the presence of isopropanol (1 h incubation - 25°C), followed by another centrifugation (10 min - 700 g). The DNA pellet was next washed three times in a row by addition of ice-cold ethanol (70%) and centrifuged (10 min - 900 g), before air drying at room temperature (approximately 1 h 30 - 20°C). Finally, DNA pellet was dissolved in 50 μL of TE buffer (10 mM Tris-HCl, pH 8.0; 1.0 mM EDTA, pH 8.0). The quality of the extracted DNA was verified using 1% (w/v) agarose gels. DNA extracts' quantification was carried out on a SpectraMax® iD3 device (Molecular Devices LLC, Sunnyvale, CA, United States). The concentration of all samples was determined and DNA extracts were diluted to 25 ng·μL⁻¹ for further analyses. The extracted DNA was stored at -20°C until use.

Polymerase Chain Reaction and Sequencing

16S rRNA Bacteria Gene

The V3-V4 hypervariable regions of the 16S rRNA gene were amplified from genomic DNA using a PCR thermal cycler (Agilent Surecycler 8800) with CS1 and CS2 barcoded degenerated primers as follows: forward primer: **CS1_341_F** **ACACTGACGACATGGTTCTACACCTACGGGNGGCWGCA**

G, reverse primer: **CS2_805_R** **TACGGTAGCAGAGACTTG** **GTCTCTGACTACCAGGGTATCTAATC**, with an expected length of about 400 bp (Herlemann et al., 2011). CS1 and CS2 tags are highlighted in bold.

Triplicate reactions per DNA sample were performed. The reaction mixtures (25 μL) contained 5 μL of Q5 (5×) reaction buffer and 0.25 μL (2 U μL⁻¹) of Q5® High-Fidelity DNA Polymerase (New England Biolabs France, Évry, France), 0.8 μL of each primer (0.4 μM), 1 μL of dNTPs (0.2 mM), 1 μL of DMSO, 1 μL of Bovine Serum Albumin (BSA - 100 μg/mL) and 1 ng of DNA template. The PCR conditions were as follows: initial denaturation at 95°C for 3 min followed by thirty-five cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 50 s, and a final elongation step at 72°C for 5 min.

Illumina MiSeq Sequencing

The triplicates for each PCR product ($n = 25$ for soil; $n = 20$ for roots) were pooled together and sent for sequencing at the Genome Quebec Innovation Centre (Montreal, QC, Canada), using Illumina MiSeq paired-end 2 bp × 300 bp.

Data Analysis – Bioinformatic Processing

Bioinformatic processing and statistical analyses were performed using R 3.6.1 (R Core Team, 2019). A sequencing pipeline, namely DADA2 (v. 1.12), was used to process the data and to infer amplicon sequence variants (ASV), following the described procedure¹. Briefly, DADA2 is an open-source program implemented in R package (Callahan et al., 2016), allowing to trim and filter forward and reverse reads separately, according to quality thresholds (removal of primers and low-quality nucleotides). Error rates were estimated using the error model algorithm of DADA2 for each sequencing dataset separately and reads were then deduplicated and ASVs were inferred. These ASVs were merged at the end of the pipeline, after a denoising step carried out on the forward and reverse reads independently and before a final step of chimera removal. One should note that the bioinformatic analysis was run separately for each dataset, namely “bacteria-root” and “bacteria-soil,” as these were separately sequenced and therefore a specific error model for each dataset was calculated.

Taxonomic assignment was performed through the DADA2 pipeline, using the Ribosomal Database Project (RDP) naïve Bayesian classifier (Wang et al., 2007). The Silva v132 database formatted for DADA2 (Callahan, 2018) was used to assign bacterial taxa from Kingdom to genus (minimum bootstrap 80). Rarefaction curves were visualized with the “rarecurve” function in the Vegan R package.

Nucleotide Sequence Accession Numbers

The 16S rRNA gene sequences of the whole dataset have been deposited in NCBI Sequence Read Archive (SRA) database and can be found under accession number PRJNA648983.

¹<https://benjjneb.github.io/dada2/tutorial.html>

Statistical Analysis

Shapiro and Bartlett tests were used prior any statistical analysis, so as to verify the normality and the homoscedasticity of the data, respectively. If both these conditions were verified, ANOVA analysis was carried out. Otherwise, Kruskal–Wallis non-parametric test (“kruskal.test” function in R) was used. All statistical tests were considered significant with $\alpha = 0.05$. Permutational multivariate analyses of variance (PERMANOVA), based on Bray–Curtis dissimilarity was run with 1000 permutations using “adonis” function and the numbers of shared ASVs between the experimental conditions were highlighted using Venn diagrams, with the help of Vegan package in R. Correlation (based on Pearson’s correlation matrix) and abundance heatmaps were plotted using “heatmap2” function from gplots package. Richness (Chao1) and diversity (Shannon and Inverse Simpson) indexes were calculated using Phyloseq package. The Chao richness estimator is a non-parametric method estimating the number of species in a community by considering rare species (singletons and doubletons – Kim et al., 2017). Both Shannon and Inv. Simpson indexes are diversity measurements. The biological diversity is widely explained as follows: “Biological diversity means the variability among living organisms from the ecological complexes of which organisms are part, and it is defined as species richness and relative species abundance in space and time” (Kim et al., 2017). Alpha diversity is assessed locally, within a specific ecosystem or area (Thukral, 2017). In that regard, both indexes are measurements of alpha diversity, based on relative abundances, but Shannon index gives a greater weight to species richness, while Simpson index gives greater prominence to species evenness (Shannon, 1948; Simpson, 1949; Kim et al., 2017).

RESULTS

Descriptive Results of the Bioinformatic Outcomes

A total of 1 794 423 and 1 332 221 bacterial raw MiSeq reads were obtained for soil and root biotopes, respectively, from 25 soil samples and 20 root samples. After quality filtering and chimera removal, 21 770 and 25 415 bacterial sequences were retained for further analyses on community composition in soil and root biotopes, respectively. Bioinformatic analysis details are shown in **Supplementary Table 1**.

Rarefaction curves for each sample tend to reach saturation at approximately 10,000 reads (**Supplementary Figure 1**). As such, the rarefaction curves suggest that the depth of sequencing efforts was adequate and that most of the bacterial diversity either associated with the roots or in the rhizosphere of clary sage has been recorded. In addition, rarefaction curves reach the plateau at the same magnitude for both root and rhizospheric soil samples.

Sage Growth and Mycorrhizal Root Colonization

In non-inoculated (NI) treatment, mycorrhizal colonization rates did not change between year 1 and year 2. They ranged between 4 and 5% (**Table 1**). However, in inoculated (I) treatment,

TABLE 1 | Sage dry weight and root total mycorrhizal colonization in response to NI and I conditions.

	Year 1		Year 2	
	NI	I	NI	I
Total mycorrhizal rate (%)	4 ± 4 ^a	1 ± 1 ^a	5 ± 2 ^a	16 ± 5 ^b
Dry weight (g.plant ⁻¹)	30 ± 9 ^{a'}	34 ± 10 ^{a'}	68 ± 16 ^{b'}	72 ± 31 ^{b'}

Values are means ± SD (n = 5). Different lowercase letters indicate significant differences between the four tested experimental conditions and are displayed without and with apostrophe for total mycorrhizal rates and dry weight, respectively, as the statistical significance has been evaluated separately (ANOVA – $\alpha = 0.05$). NI, non-inoculated; I, inoculated.

a significant increase in the mycorrhizal colonization rates was observed during the second growing season, reaching 16% ($P < 0.001$). In addition, during this second season, the mycorrhizal inoculation has shown a significant increase of root colonization (69%) compared to non-inoculated plants. Regarding shoot dry weights, they increased by 50% between year 1 and year 2. They varied from 30 to 68 g.plant⁻¹ and from 34 to 72 g.plant⁻¹ [$F_{(3)} = 7.031$; $P < 0.001$], for non-inoculated and inoculated plants, respectively. No significant differences were observed for plant’s dry weights between NI and I conditions, whatever the year [$F_{(3)} = 0.476$; $P = 0.701$].

Bacterial α -Diversity in Root and Soil Biotopes

The bacterial α -diversity was calculated separately for each experimental condition, *i.e.*, culture duration (initial state, year 1 and year 2) and mycorrhizal inoculation (NI or I), and accordingly for root and soil biotopes. According to the Chao1 index, the bacterial species richness in soil decreased for year 2, especially for the non-inoculated plants (**Table 2**). However, it significantly increased between year 1 and year 2 for bacterial communities in roots. For both root and soil biotopes, there was no significant difference between non-inoculated and inoculated treatments, whatever the year.

No significant difference was observed for Shannon and Inverted Simpson diversity indexes for soil bacteria ($P = 0.6$); they were similar between the soil before plantation (initial state) and year 1, but they decreased in year 2 with a significant reduction for non-inoculated plants ($P = 0.002$). However, there was no significant difference between non-inoculated and inoculated treatments, regardless the year of sampling and the diversity index.

In root samples, bacterial α -diversity was significantly different between year 1 and year 2 (Shannon index only) for non-inoculated plants ($P = 0.008$). In addition, during year 1, diversity was significantly increased in inoculated plants in comparison with the non-inoculated ones ($P = 0.03$), and that for both Shannon and Inv. Simpson indexes. However, no significant difference was found between inoculated and non-inoculated treatments for year 2 ($P = 0.9$). In comparison with the soil biotope, all the indicators in roots displayed significantly lower ($P = 0.001$) values, resulting in lower bacterial richness and diversity.

TABLE 2 | Richness and diversity indexes from the 5 studied conditions for soil bacterial communities and the 4 studied conditions for root bacterial communities.

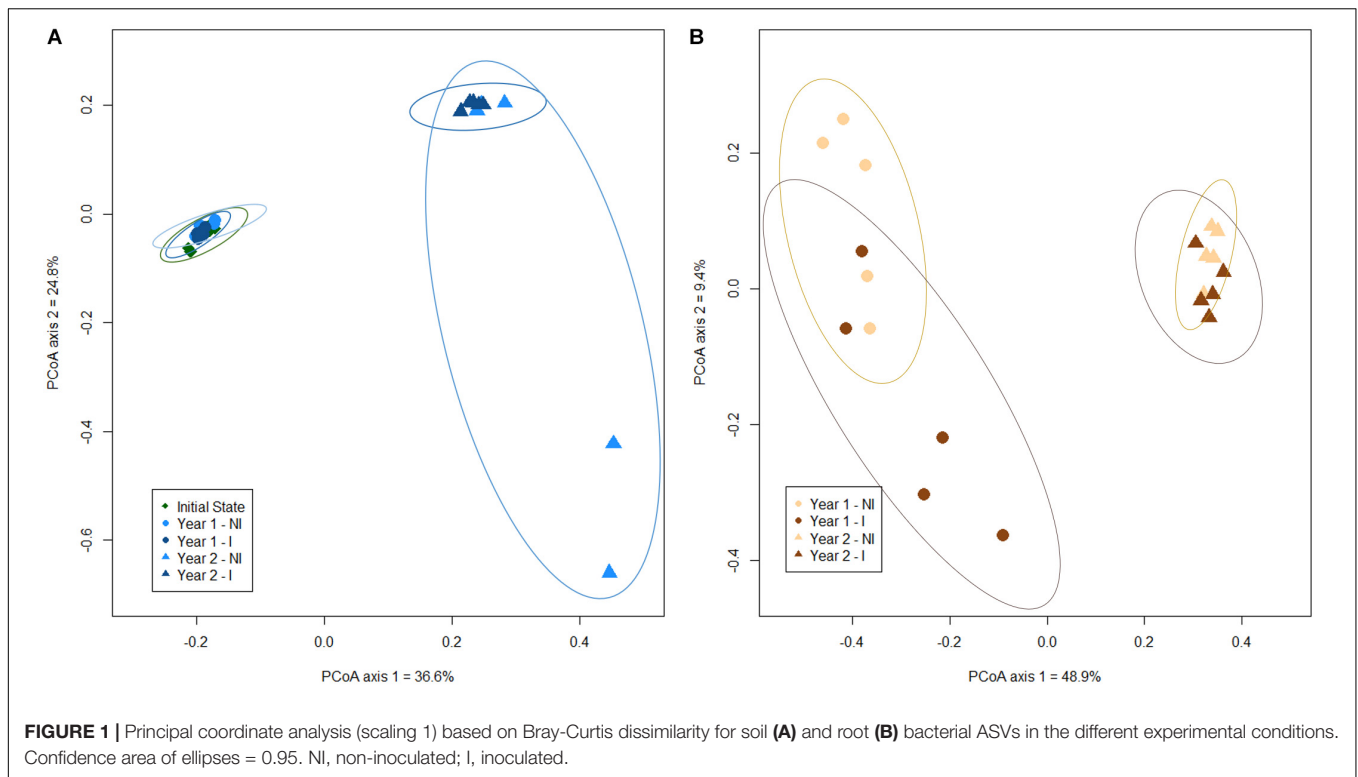
	Soil					Roots			
	Year 1		Year 2		I	Year 1		Year 2	
	NI	I	NI	I		NI	I	NI	I
Chao1	1772 ± 37 ^a	1781 ± 63 ^a	1847 ± 30 ^a	1781 ± 63 ^a	1315 ± 33 ^{ab}	659 ± 70 ^{bc}	908 ± 81 ^b	908 ± 81 ^b	908 ± 81 ^b
Shannon	6.7 ± 0.05 ^{ab}	6.7 ± 0.09 ^a	6.7 ± 0.08 ^a	6.7 ± 0.09 ^a	6.0 ± 0.14 ^{ab}	4.4 ± 0.68 ^b	4.7 ± 0.33 ^b	4.6 ± 0.5 ^b	4.7 ± 0.33 ^b
Inv. Simpson	335.0 ± 41.0 ^a	346.3 ± 83.3 ^a	346.3 ± 83.3 ^a	387.3 ± 74.4 ^a	117.6 ± 30.9 ^{ab}	36.8 ± 26.5 ^{bc}	17.0 ± 3.3 ^c	19.2 ± 8.9 ^c	17.0 ± 3.3 ^c

Data are means ± SD (n = 5) for each condition. Significant differences between experimental conditions in both soil and root biotopes are indicated by different letters. NI, non-inoculated; I, inoculated.

Evaluation of the Potential Effects of Sampling Time and Mycorrhizal Inoculation on the Bacterial Community Structure

To investigate the influence of the sampling time (before plantation, year 1 and year 2) and mycorrhizal inoculation (NI or I) on root and rhizospheric soil bacterial communities, a principal coordinate analysis (PCoA) ordination was conducted. Our results have shown a net clustering of bacterial communities, in both rhizospheric soil and root biotopes, according to the sampling time. Of the total variance in the dataset, the first two principal components together explained 61.4% of the total soil bacterial communities and 58.3% of the total root bacterial communities. For soil bacterial communities, a grouping between soil before plantation and year 1 communities was operated, highlighting a time effect (Figure 1A). However, there was no distinction between soil bacterial communities in non-inoculated or inoculated treatments, regardless of the sampling time. Similarly, bacterial communities in roots from year 1 and year 2 were clustered separately, depicting a time effect, but no distinction was made between non-inoculated and inoculated treatments, whatever the year (Figure 1B). However, one should note a greater heterogeneity in soil bacterial communities for year 2, with the inoculated treatment displaying a higher between-sample variation than the non-inoculated one. The opposite trend is visible for root bacterial communities, with a higher heterogeneity for the inoculated treatment during year 1. In addition, the results of the PERMANOVA analysis indicated that the composition of the bacterial communities was significantly different overtime ($P < 0.001$), in both rhizospheric soil and roots, but that inoculation's effect was not significant in both biotopes ($P > 0.05$ – Supplementary Figure 2).

In total, 2397, 2465, 2377, 1610, and 1988 bacterial ASVs were obtained in the rhizospheric soil before plantation, year 1 non-inoculated or inoculated and year 2 non-inoculated or inoculated, respectively. Besides, a total of 816, 1152, 1400, and 1222 bacterial ASVs were present in the root biotopes and for year 1 non-inoculated or inoculated and year 2 non-inoculated or inoculated treatments, respectively (Figure 2). The number of shared ASVs between all treatments was 897 in the rhizospheric soil biotope and 306 in the root biotope, respectively, corresponding to 19 and 11% of the total ASVs. Among them, the soil before plantation and at the first sage harvest (year 1) shared the highest number of ASVs in soil, whatever the treatment (NI or I), representing a number of 464 shared ASVs (Figure 2A). Conversely, soil before plantation and at the second sage harvest (year 2), regardless of the treatment (NI or I), shared a number of 17 ASVs, while year 1 and year 2 only have 27 ASVs in common, depicting a net shift between year 1 and year 2. In roots, the highest number of ASVs is shared between I and NI conditions, whatever the year, representing 125 shared ASVs for year 1 and 240 for year 2 (Figure 2B). On the contrary, year 1 and year 2 NI treatments only shared 5 ASVs, while year 1 and year 2 I treatments have 13 ASVs in common, depicting a change in time of the bacterial community composition. In addition, the percentage of ASVs



specific to each condition is comparable and ranged from 22 to 29% for soil bacterial communities and from 31 to 44% for root bacterial communities.

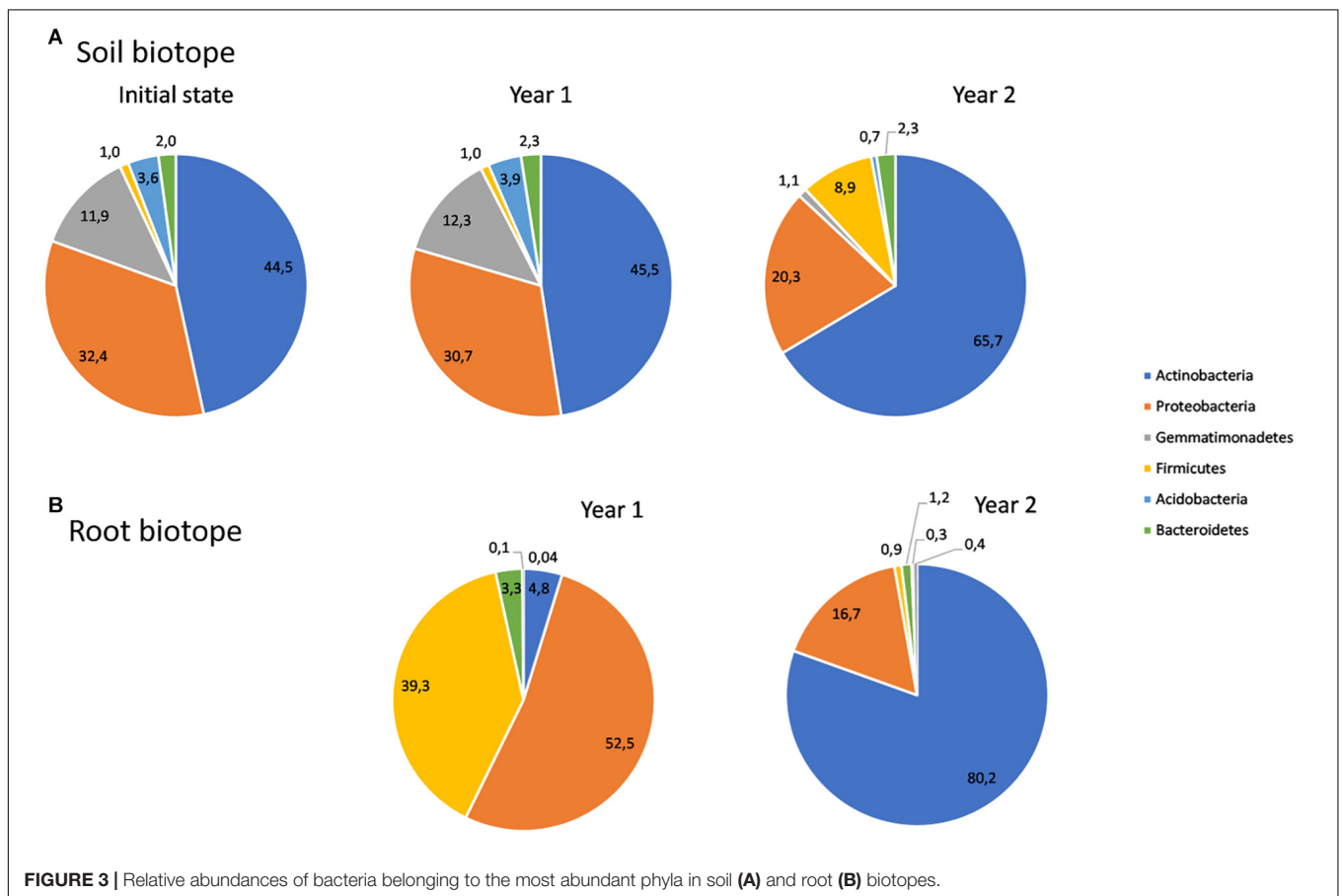
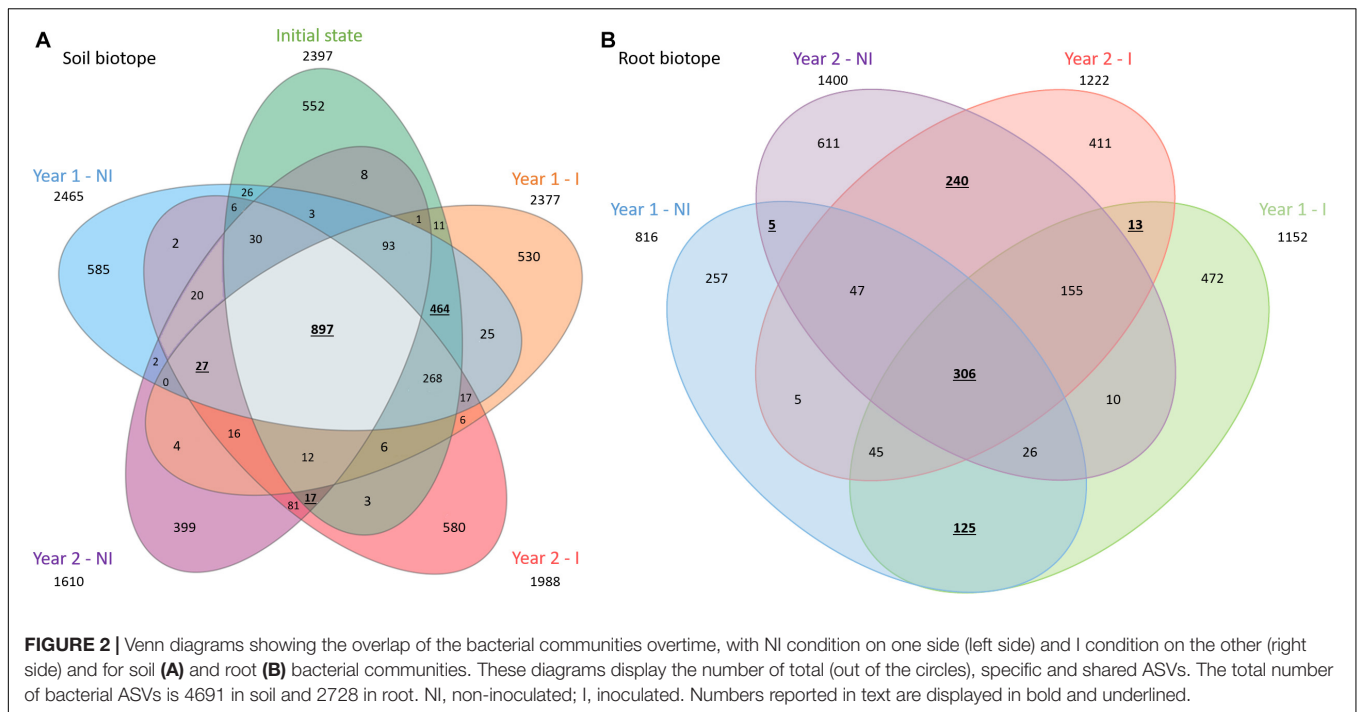
Changes Induced at the Phylogenetic Level by the Cultivation of Clary Sage and Mycorrhizal Inoculation on the Bacterial Community's Composition

To assess the community composition, each bacterial ASV was assigned to phyla, classes, families and genera. Soil bacterial ASVs were assigned to 24 phyla, while root ASVs were assigned to 20 phyla. *Actinobacteria* are the most represented one in soil, whatever the treatment. In particular, it represented 45% of bacterial ASVs for the soil before plantation (initial state) and year 1, increasing to 66% for year 2, regardless of the treatment (Figure 3A). *Proteobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Bacteroidetes*, and *Acidobacteria* are also among the most represented phyla in soil, as combined together, alongside with *Actinobacteria*, they represent nearly 95% of the soil bacterial communities. The *Firmicutes* phylum follows the same trend as *Actinobacteria* and its proportion increased in year 2. *Bacteroidetes* have shown a stable trend overtime, while an opposite trend was observed for the other three phyla, whose proportion consistently decreased in year 2. Regarding root bacterial communities, the same six phyla have been found as the most represented in the communities, containing more than 99% of the ASVs. In comparison to the soil biotope, *Actinobacteria* were poorly represented in year 1, accounting for less than 10% of the ASVs. However, they significantly increased to 80% in year 2.

Proteobacteria and *Firmicutes* have undergone the opposite trend between year 1 and year 2, decreasing from 53 to 17% and 39 to 1%, respectively (Figure 3B).

At the genus level, the dominant genera among soil bacteria included *Sphingomonas*, *Pseudarthrobacter*, *Nocardioideis*, *Streptomyces*, *Gaiella*, *Blastococcus*, and *Pseudonocardia* (Figure 4). In particular, *Sphingomonas*, *Pseudarthrobacter*, *Streptomyces*, and *Gaiella* were the most dominant genera. However, a high number of ASVs (approximately 40%) was not assigned to any genus. In addition, one should note that the proportions between the most represented bacterial genera in soil, under the two experimental conditions (NI or I) and the three monitoring times (soil before plantation, year 1 and year 2) were not significantly different (statistical analysis results provided in Supplementary Table 2).

Regarding root bacterial communities, at the genus level, *Clostridium sensu stricto* 1, *Lelliottia*, *Pseudomonas*, *Rahnella*, *Streptomyces*, *Pseudarthrobacter*, and *Nocardioideis* are the most dominant genera (Figure 4). However, the proportion of dominant bacterial genera was significantly different ($P < 0.05$), especially between the two monitoring times (year 1 and year 2) (Supplementary Table 2). In particular, *Clostridium sensu stricto* 1, *Lelliottia* and *Pseudomonas* that were extensively represented in year 1, with respective proportions of 37 and 30%; 26 and 13%; and 8 and 10% (in NI and I treatments, respectively), decreased in year 2 to, respectively, 0.03 and 0.04%; 0.02 and 0.01%; and 0.6 and 3.2% (for NI and I treatments, respectively). Conversely, *Streptomyces*, *Pseudarthrobacter*, and *Nocardioideis* were scarcely represented in year 1 (less than 2% for each genus in any treatment), whereas their relative abundances have significantly



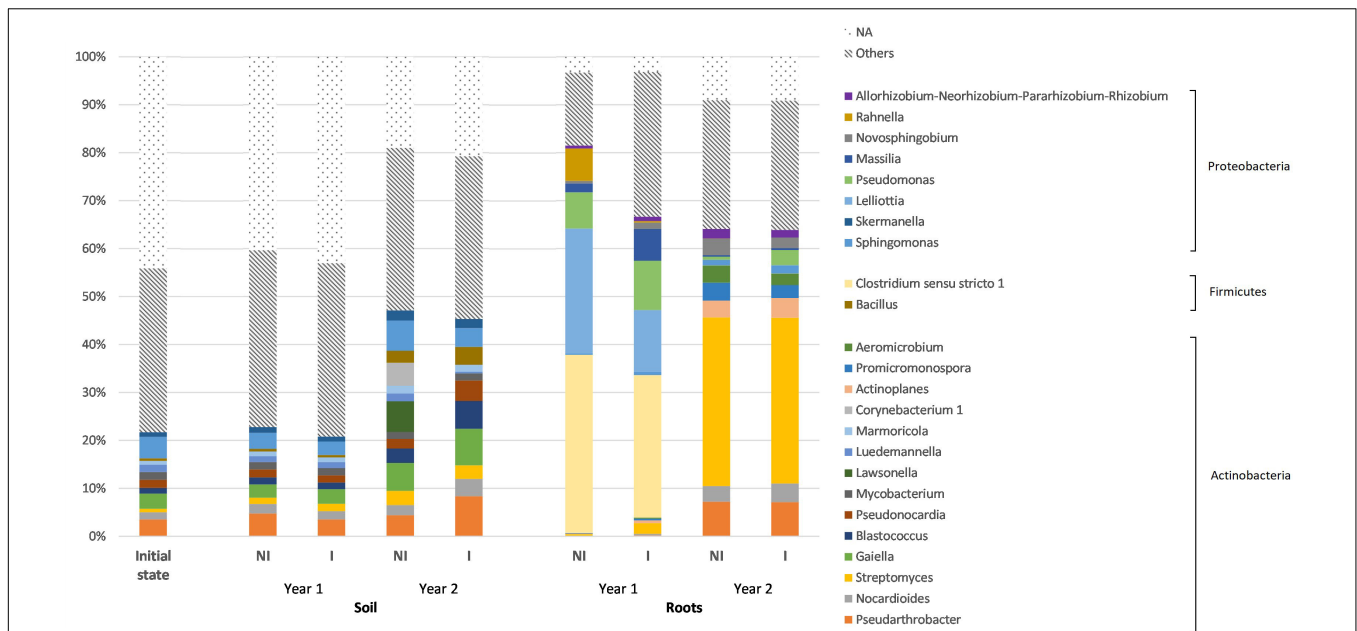


FIGURE 4 | Relative abundances of the 25 most represented bacterial genera, according to the different experimental conditions for both root and soil biotopes. The genera have been grouped under their corresponding phyla and genera with a relative abundance lower than 0.5% have been gathered in the “others” group. NI, non-inoculated; I, inoculated; NA, unassigned.

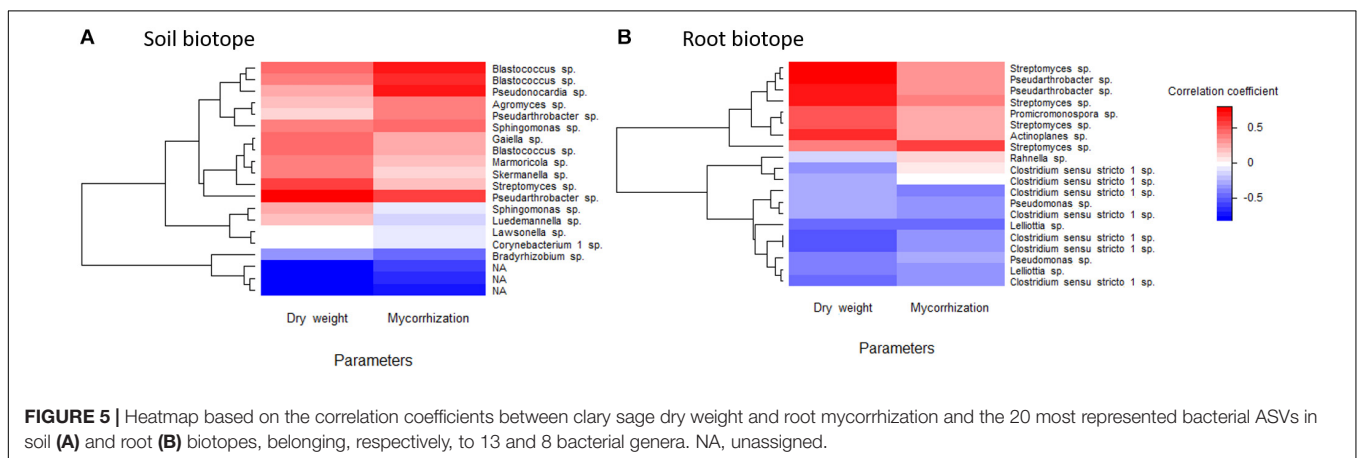


FIGURE 5 | Heatmap based on the correlation coefficients between clary sage dry weight and root mycorrhization and the 20 most represented bacterial ASVs in soil (A) and root (B) biotopes, belonging, respectively, to 13 and 8 bacterial genera. NA, unassigned.

increased in year 2, with respective proportions of 35 and 35%; 7 and 7%; and 3 and 4% (for NI and I treatments, respectively). In addition, there is no significant difference between non-inoculated and inoculated treatments, whatever the biotope and the monitoring time (Supplementary Table 2).

Correlations Between the Most Abundant ASVs and Sage Dry Weight and Root Mycorrhization

A heatmap based on Pearson correlation coefficients between the 20 most abundant ASVs and sage dry weight and root mycorrhization as explaining parameters was drawn, for rhizospheric soil and root biotopes (Figure 5). These correlation coefficients ranged from -0.68 to 0.69 and from -0.51 to

0.81 for the rhizospheric soil and root biotopes, respectively. *Pseudarthrobacter* and *Streptomyces* were the genera presenting the highest correlation coefficients regarding sage dry weight, while *Blastococcus* and *Pseudonocardia* were the strongest positively correlated ones in soil regarding root mycorrhization. Similarly in roots, *Streptomyces*, *Pseudarthrobacter*, and *Actinoplanes* are strongly and positively correlated with sage dry weight, while *Streptomyces* also displayed the highest and positive correlation coefficient regarding sage root mycorrhization. Conversely, *Corynebacterium 1* or *Lawsonella* in the soil biotope are not correlated to any of the parameters, while in the root biotope, *Clostridium sensu stricto 1* and *Lelliottia* are negatively correlated to both sage dry weight and root mycorrhization.

Bacterial ASVs that were assigned a genus were further investigated to establish whether these genera contain plant

growth promoting-rhizobacteria (PGPR) species or not, based on a literature survey. Relative abundances of ASVs that were assigned to genus level and those that comprise known PGPR species are shown in **Table 3**. In soil before plantation and at year 1, these ASVs represented about 40% of the soil bacterial communities. For year 2 and for non-inoculated plants, this proportion remained unchanged, whereas it increased to 64% for inoculated plants. In roots, PGPR-containing genera represented about 25% of the bacterial communities in year 1, increasing to 76% for year 2, regardless of inoculation treatment (**Table 3**). Nitrogen-fixing genera, such as *Rhizobium* or *Bradyrhizobium* only accounted for a small proportion of the PGPR-containing genera, whatever the treatment, the year and the biotope.

DISCUSSION

Clary sage is one of the most important aromatic plants cultivated worldwide for its EO in perfumery and cosmetics (Tibaldi et al., 2010; Angelova et al., 2016). There is a growing demand in *S. sclarea* EO and an abundant flowering leading to elevated EO yields are sought (Zutic et al., 2016). If clary sage was able to grow consistently on TE-contaminated soils, and to be established in such conditions, it could represent a valuable economic alternative for the reconversion of these marginal lands in a context of a growing arable land's demand. The present study focused on the rhizosphere bacterial community, given its contribution in ecosystem functioning and services (Delgado-Baquerizo et al., 2016; Foulon et al., 2016b; Dagher et al., 2019). It is in particular well known that the rhizosphere microbial communities are an important factor influencing plant growth and subsequently biomass and EO production (Wang X. et al., 2018; Akyol et al., 2019; Berlanas et al., 2019).

As far as we know, clary sage microbiota, however, remains unexplored and on both polluted and healthy soils, even though the microbiota of some other aromatic plant species, such as *Ocimum basilicum* L., *Mentha x piperita*, *Anethum graveolens* L., *Calendula officinalis* L., *Mentha arvensis* L., *Artemisia annua* L., and *Panax ginseng* Meyer have previously been examined in agricultural soils (Adamovic et al., 2015; Misra et al., 2019; Wei et al., 2020). Hence, the current study represents the first investigation on the effects of revegetation using clary sage, on both rhizospheric soil and roots' bacterial communities under TE-contaminated conditions. Furthermore, the potential influence of a biological amendment's introduction (AMF inoculum) on bacterial communities' structure, richness and diversity and their evolution over successive sage cultivation years has been studied.

Dominance of *Actinobacteria* and *Proteobacteria* Phyla in the Rhizosphere Microbiota of the Aged TE-Contaminated Soil

Actinobacteria, *Proteobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Bacteroidetes*, and *Acidobacteria* were found to be the most represented phyla in the aged TE-contaminated soil before

plantation and during the two successive growing seasons of clary sage. The prevailing phyla identified in the sage roots were found to be similar to those detected in the soil. These findings are in accordance with previous studies carried out in agricultural and TE-contaminated soils, involving endemic plant species such as *Silene vulgaris* (Moench) Garcke, *Bahia xylopoda* Greenm., or *Viguiera linearis* Cav. (Navarro-Noya et al., 2010; Pereira et al., 2015; Ahmad et al., 2016; Pires et al., 2017; Pacwa-Płociniczak et al., 2018). However, variations were observed between our results and previously published studies, in terms of relative abundances between the most represented phyla. One should keep in mind that changes observed between TE-contaminated sites are highly related to several factors, including the climatic conditions, the TE-contamination level, the cultivation practices as well as the soil physico-chemical characteristics, especially pH variations (Wang et al., 2019). As such, *Acidobacteria* and *Proteobacteria* have been demonstrated to decrease with increasing pH (Wang et al., 2019). In the present study, however, the soil pH was stable overtime under the different experimental conditions and was relatively high in comparison with acidic mine tailings (Navarro-Noya et al., 2010; Pereira et al., 2015). This could be responsible for the relatively low abundance of *Acidobacteria* and *Proteobacteria* observed in our conditions. As soil microbial community structure is driven by dominant sources of bioavailable C, plant species is then another determinant factor (Köberl et al., 2013; Monokrousos et al., 2019; Wang et al., 2019). In particular, plants are able to secrete specific root exudates and to modulate their nature (sugars, sterols, amino and organic acids), hence shaping the microbial communities' structure accordingly in the rhizosphere (Wang X. et al., 2018; Misra et al., 2019). Diversity indexes obtained in the present study are similar or higher in comparison with previous reports on revegetated or agricultural TE-contaminated soils (Foulon et al., 2016a; Xie et al., 2016; Tipayno et al., 2018). However, the comparison of alpha diversity between experimental sites is tricky, as it highly depends on cultivation practices amongst several parameters (soil type and physico-chemical properties, climatic conditions and plant species) (Wang X. et al., 2018; Wang et al., 2019).

Interestingly, among the identified phyla, *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* were previously demonstrated to possess TE-tolerance genes, coding for proteins involved in efflux and sequestration phenomena of TE ions, such as Cu^{2+} or $\text{Cd}^{2+}/\text{Zn}^{2+}$ -exporting ATPases, putative copper resistance protein D or copper transport protein B and in the mitigation of oxidative stress induced by the presence of TE, such as superoxide dismutase, alkyl hyperoxide reductase or mycothiol reductase (Chen et al., 2018; Cui et al., 2018; Hemmat-Jou et al., 2018). Besides, *Actinobacteria* and *Proteobacteria*, which are the two prevailing bacteria phyla in the present work whatever the biotope, were previously brought forward as reducing TE accumulation in corn. Notably, it is believed that both bacterial phyla could alter the bioavailability of heavy metals via redox reactions, precipitation, as well as sorption and desorption reactions (Cui et al., 2018). More specifically, *Bacillus*, *Pseudomonas* and *Arthrobacter*, respectively, belonging

TABLE 3 | Relative abundances of the 100 most abundant bacterial ASVs belonging to 24 genera, in which plant growth promoting-rhizobacteria (PGPR) have previously been identified.

Genus	Initial state	Soil				Roots			
		Year 1		Year 2		Year 1		Year 2	
		NI	I	NI	I	NI	I	NI	I
<i>Agromyces</i>	1.5 ± 0.3 cd	0.02 ± 0.02 e	0.1 ± 0.1 de	0.4 ± 0.1 de	0.4 ± 0.2 de	1.7 ± 0.1 cd	1.6 ± 0.3 cd	0.7 ± 1.3 cde	1.9 ± 0.4 cd
<i>Amycolatopsis</i>	nd	0.07 ± 0.19e	0.3 ± 0.5 de	2.2 ± 2.3 cd	1.3 ± 1.3 cd	nd	nd	nd	nd
<i>Bacillus</i>	0.5 ± 0.1 de	nd	nd	nd	nd	0.6 ± 0.3 de	0.6 ± 0.3 de	1.7 ± 1.4 cd	2.2 ± 1.0 cd
<i>Blastococcus</i>	3.2 ± 1.0 c	nd	nd	nd	nd	4.0 ± 0.7 c	3.9 ± 0.8 c	4.7 ± 6.7 c	12.0 ± 3.2 bc
<i>Bradyrhizobium</i>	4.0 ± 1.2 c	0.2 ± 0.1 de	0.6 ± 0.8 de	0.4 ± 0.2 de	0.9 ± 1.0 cde	4.2 ± 0.7 c	3.4 ± 0.9 c	1.1 ± 1.2 cd	0.1 ± 0.08 de
<i>Caulobacter</i>	nd	0.1 ± 0.09 de	0.3 ± 0.5 de	0.1 ± 0.1 de	0.6 ± 0.5 de	nd	nd	nd	nd
<i>Cellulomonas</i>	1.0 ± 0.5 cd	0.06 ± 0.08 e	0.3 ± 0.2 de	0.3 ± 0.3 de	0.03 ± 0.08 e	1.1 ± 0.3 cd	1.1 ± 0.2 cd	1.2 ± 3.2 cd	2.4 ± 0.7 c
<i>Flavobacterium</i>	nd	0.4 ± 0.3 de	2.2 ± 1.5 cd	nd	nd	nd	nd	nd	nd
<i>Kribbella</i>	3.0 ± 1.1 c	0.04 ± 0.04	0.2 ± 0.1 de	0.7 ± 0.3 cde	1.1 ± 0.3 cd	3.3 ± 1.4 c	3.1 ± 0.8 c	0.3 ± 0.8	1.1 ± 0.2 cd
<i>Massilia</i>	nd	1.3 ± 0.2 cd	7.2 ± 2.8 c	0.1 ± 0.03 de	0.4 ± 0.1 de	nd	nd	nd	nd
<i>Microvirga</i>	0.9 ± 0.3 cde	nd	nd	nd	nd	1.0 ± 0.1 cd	0.7 ± 0.1 cde	0.04 ± 0.1 e	0.1 ± 0.1 de
<i>Nocardioides</i>	1.4 ± 0.6 cd	0.05 ± 0.02 e	0.2 ± 0.1 de	1.6 ± 0.2 cd	2.4 ± 0.4 cd	1.9 ± 0.5 c	2.1 ± 0.5 c	1.5 ± 5.0 c	3.5 ± 1.7 c
<i>Novosphingobium</i>	nd	0.3 ± 0.2 de	0.7 ± 0.3 de	4.0 ± 2.1 c	2.2 ± 0.7 cd	nd	nd	nd	nd
<i>Pantoea</i>	nd	0.9 ± 0.9 cd	0.2 ± 0.2 de	0.03 ± 0.06 e	nd	nd	nd	nd	nd
<i>Pseudarthrobacter</i>	11.2 ± 4.3 bc	0.08 ± 0.05 e	0.3 ± 0.1 de	10.8 ± 2.2 c	10.4 ± 3.0 c	14.9 ± 5.0 bc	11.1 ± 3.3 bc	7.7 ± 13.2 c	20.6 ± 5.6 b
<i>Pseudomonas</i>	nd	6.4 ± 0.8 c	11.1 ± 1.5 bc	0.4 ± 0.05 de	4.6 ± 1.2 c	nd	nd	nd	nd
<i>Pseudonocardia</i>	4.4 ± 0.5 c	nd	nd	nd	nd	4.3 ± 0.4 c	4.0 ± 0.7 c	2.7 ± 3.5 c	8.6 ± 2.9 c
<i>Rahnella</i>	nd	7.8 ± 3.6 c	0.4 ± 0.2 de	0.006 ± 0.005 e	nd	nd	nd	nd	nd
<i>Rhizobium</i>	nd	0.3 ± 0.2 de	0.8 ± 0.5 cde	2.0 ± 0.8 cd	1.7 ± 0.4 cd	nd	nd	nd	nd
<i>Serratia</i>	nd	1.7 ± 1.1 cd	0.5 ± 0.3 de	0.02 ± 0.03 e	nd	nd	nd	nd	nd
<i>Shinella</i>	nd	0.07 ± 0.07 e	0.3 ± 0.4 de	0.2 ± 0.1 de	0.2 ± 0.2 de	nd	nd	nd	nd
<i>Sphingomonas</i>	8.6 ± 3.1 c	nd	nd	0.3 ± 0.3 de	0.8 ± 1.0 de	6.0 ± 1.9 c	5.4 ± 1.2 c	9.0 ± 10.2 c	7.5 ± 11.0 c
<i>Streptococcus</i>	nd	nd	nd	nd	nd	nd	nd	2.3 ± 1.8 cd	nd
<i>Streptomyces</i>	0.4 ± 0.06 de	0.4 ± 0.03 de	3.2 ± 0.3 c	52.5 ± 1.5 a	50.4 ± 1.0 a	1.3 ± 0.7 cd	1.2 ± 0.5 cd	3.6 ± 3.6 c	3.9 ± 1.8 c

Values are means ± SD (n = 5). Significant differences (Kruskal–Wallis non-parametric test, $\alpha = 0.05$) between conditions are indicated by different letters. NI, non-inoculated; I, inoculated; nd, not-detected.

to *Firmicutes*, *Proteobacteria*, and *Actinobacteria* phyla, are among the most widely spread and frequently listed bacterial genera in TE-contaminated soils, and at the same time display a high TE tolerance (Pires et al., 2017; Chellaiah, 2018; Chen et al., 2018; Cui et al., 2018). Nonetheless, *Sphingomonas*, member of the *Proteobacteria* (Xie et al., 2010; Chen et al., 2014; Jaafar, 2019), as well as *Pseudarthrobacter* (Chen et al., 2014; Fekih et al., 2018; Park et al., 2019), *Nocardioideis* (Bagade et al., 2016; Cui et al., 2018), and *Streptomyces* (Álvarez et al., 2013; Cui et al., 2018; Durand et al., 2018; Lasudee et al., 2018; Timková et al., 2018; Álvarez-López et al., 2020), belonging to the *Actinobacteria*, were also highlighted for displaying TE-tolerance and even studied for potential remediation applications. Indeed, these bacterial genera are known to accumulate Cd, Ni, Cr or Pb from TE-contaminated soils and to induce changes in the oxidation state of the TE (Pires et al., 2017; Chellaiah, 2018; Timková et al., 2018).

Significant Effect of Sampling Time on the Bacterial Community Structures in the Root and Soil Biomes

Regarding richness and diversity in soil, a significant drop has been observed between the two successive years of growth, whatever the index (Chao1 estimator, Shannon and Inv. Simpson). However, it is noteworthy that an increase in both richness and diversity was observed in roots in year 2. The results from PERMANOVA and PCoA analyses have clearly indicated a year-to-year variability of the bacterial community structures in both soil and root biomes with a high significance at $P < 0.001$, displaying in soil a clear clustering with the soil before plantation and year 1 on one side and year 2 on the other, while in root year 1 and year 2 were clustered separately. These results fall into line with the Venn-diagrams, displaying a high number of shared ASVs between the soil before plantation and year 1 (soil biome), as well as a drop in the total number of ASVs in soil in year 2, and a scarcer number of shared ASVs between year 1 and year 2, for both rhizospheric soil and root biomes.

Similar results were observed in the soil at the phylum level between the soil before plantation and year 1, whereas there was a clear shift between sampling year 1 and year 2. Indeed, the *Gemmatimonadetes* and *Proteobacteria* phyla decreased between years 1 and 2, with respective declines of 90 and 34%, while the *Firmicutes* phylum was more represented in year 2, increasing of about 90% in comparison with year 1. One should note that *Actinobacteria* was the prevailing phylum at any of the sampling times, in addition displaying a significant increase of about 30% between years 1 and 2. A major shift has also been observed in roots between year 1 and year 2, with *Firmicutes* and *Proteobacteria* representing more than 90% of the ASVs in year 1, while *Actinobacteria* was the prevailing phyla in year 2, accounting for 80% of the ASVs. At the genus level, a major shift was also observed especially in the root biome between year 1 and year 2, with the prevalence of *Clostridium sensu stricto* 1, *Lelliottia* and

Pseudomonas for year 1, respectively, belonging to *Firmicutes* and *Proteobacteria* phyla, while year 2 was clearly dominated by *Pseudarthrobacter*, *Nocardioideis*, and *Streptomyces*, all belonging to the *Actinobacteria*.

Thus, the shift observed with the culture duration in both biomes but particularly pronounced in the case of roots, at the phylum and genus phylogenetic levels, could be explained by different factors. First, the prevalence in year 2 for both root and rhizospheric soil biomes of *Actinobacteria*, previously demonstrated as TE-tolerant, could support the idea that the phylotypes possessing TE tolerance are susceptible to persist overtime, while sensitive ones become less frequent (Cui et al., 2018). In addition, it was previously evidenced for wheat grown in uncontaminated agricultural fields that *Actinobacteria* abundance was increasing with root age, which is possibly explained by their ability to degrade secondary cell walls containing complex molecules (Donn et al., 2015). Furthermore, environmental factors influencing root response and exudation, such as temperature or precipitation, could be responsible for this year-to-year variation (Wagner et al., 2016; Berlanas et al., 2019). In fact, it was previously demonstrated in the case of Eucalyptus or grapevine, that distinct bacterial communities were observed at different plantation ages (Berlanas et al., 2019; Qu et al., 2020). In particular, changes in exudation profiles are susceptible to occur with plant age, at distinct growth stages (Akyol et al., 2019). Similarly, in the case of aromatic plant species, such as *M. arvensis* or *A. annua*, the long-term mono-cultivation was brought forward as an explaining factor of bacterial communities' diversity and evenness (Misra et al., 2019). Moreover, aromatic plant species like clary sage are susceptible to release secondary metabolites in soil. These metabolites, due to their potential antimicrobial nature, are likely to exert adverse effects and hence affect both richness and diversity by shaping specifically microbial communities (Köberl et al., 2013; Adamovic et al., 2015; Misra et al., 2019). A previous study on cultivated aromatic plants (*M. arvensis* and *A. annua*), demonstrated that the long-term monoculture of aromatic species could durably affect the bulk soil and rhizosphere microbial communities (Misra et al., 2019). In particular, it was suggested that menthyl acetate and menthol (monoterpenes), present in *M. arvensis*, as well as artemisinin (sesquiterpene) in *A. annua*, known for their antibacterial properties and found in the rhizospheric and bulk soil of these plant species, could greatly affect soil bacterial communities, notably through a reduction of the total soil microbial diversity (Misra et al., 2019). In the case of clary sage, linalool (terpene alcohol) and linalyl acetate (ester) have been identified as EO major compounds, and have frequently been reported for their antibacterial properties against several bacterial genera, such as *Staphylococcus*, *Pseudomonas*, *Bacillus*, *Listeria*, *Enterococcus*, *Mycobacterium*, or *Escherichia* (Kuzma et al., 2009; Angelova et al., 2016; Zutic et al., 2016; Blaskó et al., 2017; Aćimović et al., 2018). In that regard, the prevalence of secondary metabolites during the second year of sage growth and cultivation (Tibaldi et al., 2010) may then be another key driver (Köberl et al., 2013; Adamovic et al., 2015; Misra et al., 2019). However, there is no specific pattern regarding the previously mentioned genera

in the current study. In soil, *Pseudomonas* and *Mycobacterium* were found stable during the successive cultivation years, while *Bacillus* and *Staphylococcus* were demonstrated increasing in year 2, whereas in roots, only *Pseudomonas* significantly decreased in year 2. Interestingly, our results have shown that sage was capable of growing consistently in TE-contaminated conditions, which is in accordance with previous works reporting the TE-tolerance of this aromatic plant species (Chand et al., 2015; Angelova et al., 2016).

Mitigated Effects of AMF Inoculation in Shaping Bacterial Communities

Surprisingly, AMF inoculation, which is likely to result in a shift of the bacterial communities in soil and in root of the cultivated plants did not lead to tremendous changes in our experimental conditions (Lioussanne et al., 2010; Rodríguez-Caballero et al., 2017; Akyol et al., 2019; Monokrousos et al., 2019). No significance of AMF inoculation either in the PERMANOVA analysis or in the Venn diagram representation was observed, with a high number of shared ASVs between inoculated and non-inoculated treatments, whatever the biotope and the year of sampling. In addition, no striking difference was highlighted regarding richness and diversity indexes, between non-inoculated and inoculated treatments, whatever the cultivation year. Interestingly, the decrease of richness and diversity indexes observed in soil between successive cultivation years is less marked in the case of inoculated plants, suggesting that mycorrhizal symbiosis is likely to improve the resilience of the plant and its associated microbial communities against biotic and abiotic stresses (Bi et al., 2018). In previous studies, the mycorrhizal symbiosis was also highlighted to result in changes in the host plant metabolism and hence in the root exudation profile, further modifying and shaping the bacterial communities, in both TE-contaminated and uncontaminated conditions (Harrison, 1999; Hause and Fester, 2005; Ferrol et al., 2016; Iffis et al., 2017). In addition, the exudation of carbohydrates and citric acid, detected in AMF hyphal exudates, and their quantitative and qualitative patterns were brought forward as potential key drivers in the changes of the soil bacterial communities as well (Lioussanne et al., 2010; Iffis et al., 2016; Qin et al., 2016). However, in the present work carried out on agricultural contaminated soils, despite the presence of TE, the soil physicochemical properties were considered sufficient for low demanding crops such as clary sage (Denoroy et al., 2004; Sharma and Kumar, 2012; Abbaszadeh et al., 2017). Consequently, sage nutritional needs were probably met. Thus, even though the mycorrhizal colonization was successfully established, even displaying an increase for the inoculated treatment in year 2, it did not result in an improved plant growth in comparison with the non-inoculated plants. Hence, considering this limited contribution it may not have resulted in changes in bacterial communities in both rhizospheric soil and root biotopes or to a very limited extent. Nonetheless, as sage was effectively colonized by AMF, the monitoring of the symbiosis' functionality through the expression of phosphate transporters genes, such as MtPT4,

LePT1, or StPT3 (Paszkowski et al., 2002; Karandashov and Bucher, 2005; Voß et al., 2018), might validate this hypothesis.

Prevalence of PGPR in Soil and Root Bacterial Communities

Although it is difficult to infer the ecological function of a bacterium based on its taxonomic assignment (Akyol et al., 2019), Pearson's correlation analysis revealed significant and positive correlations between the most abundant ASVs in soil and root biotopes with sage dry weight and root mycorrhizal rates as explaining parameters. More specifically, *Pseudarthrobacter*, *Streptomyces*, and *Actinoplanes* genera were found strongly correlated with sage dry weight in either one or both biotopes. It was in fact previously demonstrated that the rhizosphere bacterial community could be of major importance regarding the suppression of soilborne diseases (Chen et al., 2020). In particular, *Streptomyces* spp. are known to produce a wide range of secondary metabolites with antimicrobial properties and hence considered as antagonists of both plant pathogenic or non-pathogenic fungi and bacteria (Golinska et al., 2015; Al_husnan and Alkahtani, 2016; Vurukonda et al., 2018; Al-Ansari et al., 2019). They could play an important role in shaping the bacterial communities, especially since their abundance in the present work is relatively elevated. Interestingly, our results have shown that *Streptomyces* sp. were strongly correlated to both sage dry weight and mycorrhizal rates in the root biotope, which falls in line with previous studies describing *Streptomyces* spp. as promoting the growth of numerous plant and microbial species (Foulon et al., 2016a; Iffis et al., 2016; Dias et al., 2017; Durand et al., 2018; Vurukonda et al., 2018; Wang C. et al., 2018). It hence could be considered of great interest as both PGPB and mycorrhiza helper bacteria, depending on the species and the strain. Similarly, *Pseudarthrobacter* which was found well correlated with sage biomass in the present work was recently demonstrated as a bacterial genus containing PGPB species of several plants, including other aromatic species such as *Pellaea calomelanos* Sw. (Tshishonga and Serepa-Dlamini, 2020), or *Dodonaea viscosa* Jacq., *Fagonia indica* L., *Caralluma tuberculata* N.E. Brown and *Calendula arvensis* L. (Iqar et al., 2019). Interestingly, among the most abundant ASVs correlated with sage biomass in the roots' biotope, two of them were assigned to *Promicromonospora* and *Actinoplanes* genera. These 2 genera were previously highlighted as increasing plant growth promotion when belonging to microbial consortia, playing a role as "activators" and enhancing the effects of other PGPB microorganisms when present in combination (El-Tarabily et al., 2010; Golinska et al., 2015). *Pseudonocardia*, which was the highest correlated ASV with sage root mycorrhization in the rhizospheric soil biotope, was previously described as a mycorrhiza helper bacteria isolated from the AMF *Funneliformis mosseae* spores (Lasudee et al., 2018). Conversely, *Clostridium* genus which was in our experimental conditions negatively correlated to both sage dry weight and root mycorrhization, is rather known for causing bacterial soft rots, especially in potato and sweet potato (da Silva and Clark, 2013; Kõiv et al., 2015). Similarly, *Lelliottia* has so far only been reported as responsible

for onion bulb decay (Bonasera et al., 2014; Liu and Tang, 2016). Nevertheless, none of these two genera has previously been evidenced as a clary sage pathogen.

CONCLUSION

In this study, we have investigated the effects of sampling time over a period of 2 years of growth and mycorrhizal inoculation on rhizosphere and root bacterial communities in the case of the aromatic plant, clary sage, cultivated on the aged TE-contaminated soils. *Actinobacteria* and *Proteobacteria* were found to be the predominant phyla during the 2 years of clary sage cultivation in our aged TE-contaminated soil. Altogether, our findings have demonstrated that the most important factor shaping the bacterial community in our experimental setup was the sampling time, with a significant year-to-year change in terms of bacterial α -diversity and communities' structure in both root and rhizospheric soil biotopes. Conversely, AMF inoculation did not lead to remarkable changes in our experimental conditions. Only a less pronounced decrease in terms of bacterial richness and diversity in soil between successive cultivation years was observed in the inoculated treatment. Our results have also shown that some bacterial genera, such as *Pseudarthrobacter*, *Streptomyces* and *Actinoplanes*, were strongly and positively correlated to both sage dry weight and mycorrhizal rates in the root biotope. They hence could be considered of great interest in a phytomanagement context, since among them some bacterial species have previously been reported as both PGPR and highly tolerant to TE.

Thus, a more comprehensive study aiming at deciphering the underlying mechanisms in the shaping of the bacterial communities by clary sage and the specific interactions between the plant and the microbiota would facilitate the development of phytomanagement methods based on clary sage cultivation. In the same perspective, it may provide new insights to isolate culturable bacteria associated to clary sage grown on TE-contaminated soils and to investigate their potential as PGPR species as well as their ability to tolerate or even to mitigate TE-toxicity. Nonetheless, a similar approach targeting the fungal community is also relevant and could help gaining more insights into the complex interactions within the rhizosphere microbiota. In fact, both fungal and bacterial communities are in constant interaction in the rhizosphere (Bourceret et al., 2016; Akyol et al., 2019), and beside their substantial involvement in biogeochemical cycles, they have been demonstrated to behave differently in response to environmental and external factors (Bourceret et al., 2016; Foulon et al., 2016a). Notably, plant

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identity, mycorrhizal inoculation and the presence of pollutants in soils were demonstrated as key structuring parameters, responsible for shaping the fungal community (Bourceret et al., 2016; Akyol et al., 2019; Dagher et al., 2019). Besides, a long-term *in situ* monitoring aiming at evaluating the soil functioning as well as its productivity and promoting the ecosystem function, could provide additional information toward an optimization of the method.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found in NCBI, under accession PRJNA648983.

AUTHOR CONTRIBUTIONS

RR, JF, MH, and AL-HS contributed to conceptualization, methodology, and validation and wrote, reviewed, and edited the manuscript. RR wrote the original draft preparation. AL-HS contributed to supervision. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.586050/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplemental Data

Table S1: Descriptive results of Illumina MiSeq sequencing, followed by the step-by-step bioinformatic processing.

	BACTERIA-SOIL	BACTERIA-ROOT
NO SEQUENCES (SUM)	1794423	1332221
NO SEQUENCES (MEAN)	71777	66611
NO SEQ. FILTERED (MEAN)	52312	49617
NO SEQ. MERGED (MEAN)	27053	39682
NO SEQ. MERG. NO CHIMERAS (MEAN)	21770	25415
NO SAMPLES	25	20
NO ASV (SUM)	4691	2728
ASV PER SAMPLE (MEAN)	1261	516

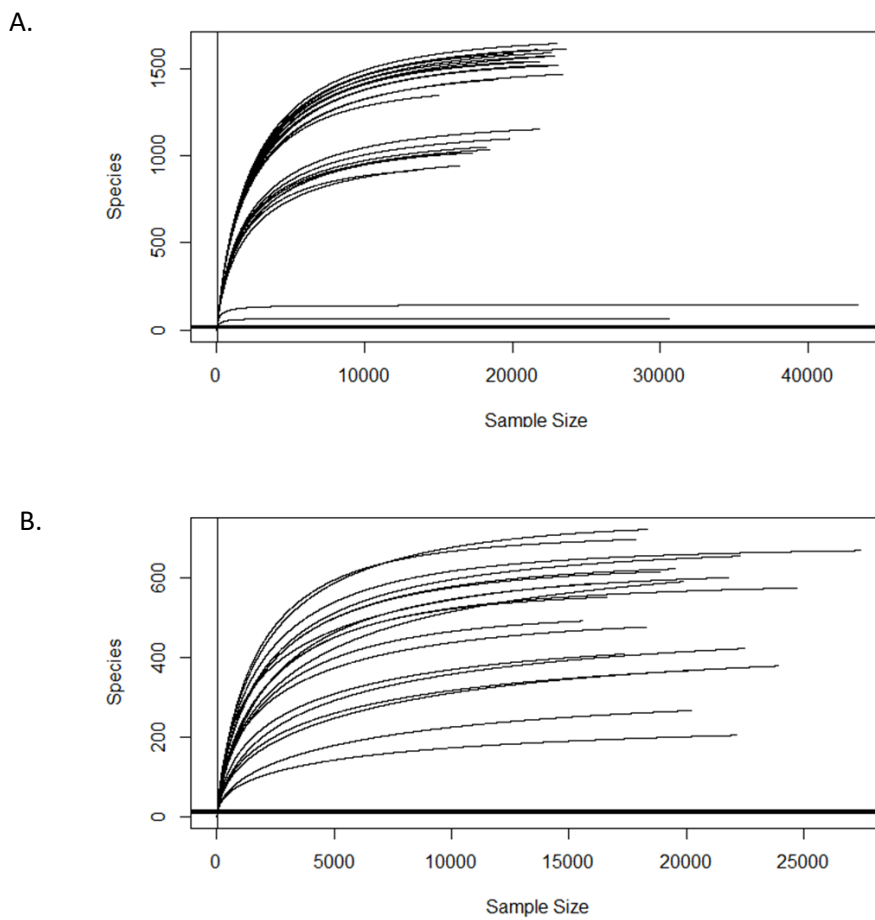


Figure S1: Rarefaction curves obtained for the soil (A.) and root (B.) biotopes, involving respectively 25 and 20 samples.

A.

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B.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Inoculation	1	0.1828	0.18282	1.6518	0.04739	0.153
Time	1	1.1016	1.10160	9.9527	0.28554	0.001 ***
Inoculation:Time	1	0.2492	0.24925	2.2519	0.06461	0.062 .
Residuals	21	2.3243	0.11068		0.60247	
Total	24	3.8580			1.00000	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Inoculation	1	0.2503	0.25029	1.9088	0.05232	0.169
Time	1	2.2381	2.23805	17.0679	0.46782	0.001 ***
Inoculation:Time	1	0.1976	0.19761	1.5070	0.04131	0.256
Residuals	16	2.0980	0.13113		0.43855	
Total	19	4.7840			1.00000	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure S2: Results from the PERMANOVA analysis, generated using a model constrained for inoculation and time parameters, for soil (A.) and root (B.) bacterial communities.

Table S2: Results from Kruskal-Wallis and Tukey HSD tests for the relative abundances of the 25 most represented bacterial genera in the different experimental conditions, for both soil and root biotopes. NI: non-inoculated; I: inoculated.

Genus	Initial state	Soil				Roots			
		Year 1		Year 2		Year 1		Year 2	
		NI	I	NI	I	NI	I	NI	I
Actinoplanes	a	a	a	a	a	ab	ab	b	b
Aeromicrobium	a	a	a	a	a	ab	ab	b	b
Allorhizobium- Neorhizobium- Pararhizobium-Rhizobium	a	a	a	a	a	ab	ab	b	b
Bacillus	ab	ab	ab	b	b	a	a	a	a
Blastococcus	b	b	b	b	b	a	a	a	a
Clostridium sensu stricto 1	a	a	a	a	a	d	d	ab	ab
Corynebacterium 1	a	a	a	b	a	a	a	a	a
Gaiella	b	b	b	bc	bc	a	a	a	a
Lawsonella	a	a	ab	c	ab	a	a	a	a
Lelliottia	a	a	a	a	a	d	c	ab	ab
Luedemannella	b	b	b	b	ab	a	a	a	a
Marmoricola	ab	b	b	b	b	a	a	a	a
Massilia	a	a	a	a	a	bc	c	ab	ab
Mycobacterium	b	b	b	b	b	a	a	a	a
NA	e	e	e	d	d	b	b	c	c
Nocardioides	b	b	b	b	b	ab	ab	b	b
Novosphingobium	a	a	a	a	a	ab	bc	b	b
Others	e	e	e	e	e	cd	de	de	de
Promicromonospora	a	a	a	a	a	ab	ab	bc	b
Pseudarthrobacter	b	b	b	b	bc	ab	ab	c	c
Pseudomonas	a	a	a	a	a	c	c	ab	b
Pseudonocardia	b	b	b	b	bc	a	a	a	a
Rahnella	a	a	a	a	a	bc	ab	ab	a
Skermanella	ab	b	b	b	b	a	a	a	a
Sphingomonas	bc	b	b	bc	b	ab	ab	b	b
Streptomyces	ab	b	b	b	b	ab	bc	e	e

Publication 4

-

Clary sage cultivation and mycorrhizal inoculation influence rhizosphere fungal community structure over time in a trace-element polluted site

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Présentation de la Publication 4

Au sein du microbiote tellurique, les communautés fongiques occupent aussi une place essentielle dans les écosystèmes naturels ou anthropisés (Liang et al., 2020; Lin et al., 2020). En effet, ces communautés représentent une biomasse importante et jouent un rôle fondamental dans le fonctionnement des sols et en particulier dans la dégradation de la matière organique et le recyclage des nutriments (carbone, azote, phosphore ...), mais également dans l'agrégation du sol (Jia et al., 2018; Lin et al., 2020). Parmi elles, les CMA sont des champignons capables d'établir une relation symbiotique avec la majorité des végétaux supérieurs (Smith and Read, 2008). Ils sont largement décrits pour leur contribution positive à la santé, la croissance et la productivité de leur plante hôte (Dagher et al., 2020; Janoušková et al., 2017). Leur intérêt au sein des agroécosystèmes a notamment été exploré de manière approfondie (Klaubauf et al., 2010), en raison des nombreux bénéfices qu'ils procurent à leur hôte végétal en améliorant leur nutrition minérale et hydrique ainsi que leur tolérance vis-à-vis des stress environnementaux, comme ceux induits par la présence de polluants dans le sol (Dagher et al., 2020; Ferrol et al., 2016).

Comme cela a été décrit dans le cadre de la publication précédente au sujet des communautés bactériennes, la présence dans le sol de polluants tels que les ET est susceptible d'affecter également durablement la structure des communautés fongiques et le fonctionnement du sol (Chen et al., 2014a; Lin et al., 2019). En revanche, il est aussi rapporté dans la littérature que les communautés bactériennes et fongiques répondent différemment aux facteurs biotiques et abiotiques. En règle générale, les communautés bactériennes présentent une réactivité moindre en comparaison aux champignons vis-à-vis de ces facteurs (Bourceret et al., 2016; Zappellini et al., 2015; Zhao et al., 2019).

Ainsi, en complément de l'étude précédente portant sur les communautés bactériennes, nous proposons d'étudier, dans le cadre de cette publication, l'impact de la revégétalisation du sol par la sauge sclérée et de l'inoculation mycorhizienne sur la structure et l'évolution des communautés fongiques des deux biotopes (sol rhizosphérique et racines) pendant deux années de culture, et ce via une approche de métagénomique par séquençage grâce à la technologie Illumina Miseq. Il est à noter que les communautés de CMA sont souvent délicates à identifier d'un point de vue taxonomique, notamment avec des amorces génériques ciblant la région de l'ITS2 fongique (Stefani et al., 2020). Afin de palier à ce problème, nous avons en plus identifié plus spécifiquement les communautés mycorhiziennes présentes au sein du sol rhizosphérique et des racines, en appliquant une PCR nichée à l'aide de deux paires d'amorces successives, ciblant successivement la région V3-V4-V5 de l'ARNr 18S eucaryote puis plus spécifiquement la région V4 (Stefani et al., 2020).

Nos résultats mettent en évidence la prévalence avant végétalisation du phylum fongique *Ascomycota* (72 %) et dans une moindre mesure des phyla *Basidiomycota* et *Mortierellomycota*, représentant respectivement 20 et 4 %. *Gibellulopsis*, *Cercophora*, *Lasiochaeris*, *Fusicolla*, *Mortierella* et *Tetracladium* sont les genres majoritaires présents dans le sol avant végétalisation. Il est à signaler également que 12 ASV appartenant au phylum *Glomeromycota* et plus spécifiquement aux familles *Acaulosporaceae* et *Glomeraceae* ont été identifiés dans le sol avant végétalisation par la sauge sclérée. En termes de richesse et de diversité, les indices de Chao1, Shannon et Simpson mesurés sont respectivement de 398 ; 4,3 et 0,97 pour la communauté fongique totale et de 6 ; 1,1 et 0,5 pour la communauté mycorhizienne de façon spécifique.

Les résultats obtenus en termes de diversité, de richesse, d'abondance relative et de nombre d'ASV sont très proches entre l'état initial avant végétalisation et l'année 1 et évoluent de façon plus marquée entre l'année 1 et l'année 2 ($P < 0,001$). Nos résultats montrent également que les communautés présentes au sein du sol rhizosphérique et des racines tendent à devenir semblables au cours du temps. La revégétalisation du sol par la culture de sauge sclérée a donc un impact fort sur la structure des communautés fongiques du sol rhizosphérique et des racines. Cette évolution se traduit notamment par une chute significative des indices de richesse et de diversité et par une diminution du nombre d'ASV, en particulier au niveau du sol. Au niveau des racines, malgré la stabilité des indices de richesse et de diversité au cours du temps, des différences qualitatives en termes de composition de la communauté et d'abondances relatives entre les genres ont toutefois été observées.

Plus spécifiquement, des genres tels que *Botrytis* ou *Plectosphaerella*, faiblement représentés avant végétalisation et en année 1 (abondance inférieure à 1 %), ont vu leur abondance augmenter significativement en année 2, dans le sol rhizosphérique ainsi que dans les racines. A l'inverse, l'abondance du genre *Tetracladium* identifié dans les racines en année 1, à hauteur de 20 %, a fortement diminué en année 2 (4 – 5 %).

Par ailleurs, l'inoculation mycorhizienne a uniquement impacté les communautés fongiques au niveau du sol rhizosphérique ($P < 0,05$). En effet, en comparaison avec la condition non inoculée, les abondances relatives des genres *Gibellulopsis* et *Acrostalagmus* sont significativement réduites, tandis que les genres *Botrytus*, *Plectosphaerella* ou *Fusicolla* sont davantage représentés.

Enfin, l'assignation fonctionnelle à l'aide de FUNGuild (Nguyen et al., 2016) a permis d'attribuer un groupe fonctionnel avec un niveau de confiance satisfaisant pour 50 % des ASV et cela quel que soit le biotope. Les résultats de cette assignation fonctionnelle ont révélé qu'une majorité de champignons dans les deux biotopes étaient saprotrophes (jusqu'à 54 %) ou phytopathogènes (19 à 60 %). La proportion de ces derniers augmente uniquement dans le sol entre l'année 1 et l'année 2 ($P < 0,05$). Par ailleurs, une très faible proportion d'endophytes a été observée, quasi nulle dans le sol et comprise entre 3 et 10 % dans les racines. Enfin, les CMA ont pu être détectés avec les amorces ciblant la région de l'ITS2 fongique, mais ne représentaient qu'une très faible proportion (0,05 %) de la communauté fongique.

Grâce à l'utilisation des amorces spécifiques, un nombre plus important d'espèces de CMA a été détecté au niveau des racines par rapport au sol rhizosphérique. Néanmoins, les tendances sont similaires à celles observées pour l'ensemble des communautés fongiques. Alors qu'un effet important de la végétalisation a été observé sur la structure et l'évolution des communautés mycorhiziennes, l'effet de l'amendement mycorhizien reste limité.

Pour cette étude, je me suis chargé à part entière des premières étapes de prélèvement des échantillons jusqu'à la mise au point des conditions d'amplification de l'ADN par PCR, en passant par l'extraction de l'ADN et la vérification de sa qualité, à l'UCEIV. Le séquençage a été réalisé par Génôme Québec. Le traitement bioinformatique a été réalisé sous la supervision de M. Hijri, lors d'un séjour de formation à l'Institut de Recherche en Biologie Végétale de Montréal. L'ensemble des analyses statistiques a été réalisé par mes soins. L'exploitation des données est issue d'un travail collectif. La publication a été rédigée avec l'aide des co-auteurs.

Clary sage cultivation and mycorrhizal inoculation influence rhizosphere fungal community structure over time in a trace-element polluted site

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Abstract

Fungal community in the soil plays a central role in natural systems and agroecosystems, therefore it attracted much research interests. However, the fungal microbiota of aromatic plants, such as *Salvia sclarea* L., especially in trace-element (TE) polluted conditions and within the framework of phytomanagement approaches, remain unexplored. The presence of high concentrations of TE in the soil can negatively affect not only microbial diversity and community structures, but also plant establishment and growth. The objective of this study is to investigate the soil fungal and arbuscular mycorrhizal fungi (AMF) communities structure and their changes over time in TE-polluted soils in the vicinity of a former lead smelter and under the cultivation of clary sage. We used Illumina MiSeq amplicon sequencing to evaluate the effects of *in situ* clary sage cultivation during two successive years, combined or not with an exogenous AMF inoculation, on the rhizospheric soil and root fungal communities. We obtained 1239 and 569 fungal amplicon sequence variants (ASV) respectively in the rhizospheric soil and roots of *S. sclarea* in TE-polluted conditions. Remarkably, 69 AMF species were detected in our experimental site, belonging to 12 AMF genera. Besides, the inoculation treatment significantly shaped the fungal communities in soil, and increased the number of AMF ASVs in clary sage roots. In addition, successive years of clary sage cultivation also significantly shaped both fungal and AMF communities in the soil and root biotopes. Our data provide new insights on fungal and AMF communities in the rhizospheric soil and roots of clary sage grown in TE-polluted agricultural soil.

Keywords: Trace Elements-polluted soils, fungal microbiota, *Salvia sclarea*, arbuscular mycorrhizal fungi

1. Introduction

Microbial communities play major roles in soil ecosystems and provide a wide range of services, notably they are involved in nutrients' biogeochemical cycles, carbon sequestration and in the provisioning of clean air and water (Adamovic et al., 2015; Bodelier, 2011; Ding et al., 2017; Klaubauf et al., 2010). Among soil microbial communities, fungi represent an important biomass and provide key function of organic matter decomposition leading to nutrient cycling, and play a crucial role in humification, mineralization and in the soil aggregation processes (Jia et al., 2018; Lin et al., 2020). Hence, they play a central role in most natural and managed ecosystems' function and fertility (Jia et al., 2018; Klaubauf et al., 2010; Liang et al., 2020; Lin et al., 2020). Some fungi, such as arbuscular mycorrhizal fungi (AMF), belonging to the phylum *Glomeromycota*, are also able to form symbiotic associations with more than 80% of the terrestrial plants (Smith and Read, 2008). Ubiquitous microorganisms from the soil and obligate biotrophs, they contribute to plant health, growth and productivity and in that regard are particularly valuable and well characterized in agricultural soils (Dagher et al., 2020a; Janoušková et al., 2017; Klaubauf et al., 2010). Their contribution to agroecosystems' functioning has in particular been well studied (Klaubauf et al., 2010), because they provide many benefits to their host plant through an improved water and mineral supply and can mitigate the adverse effects of biotic and abiotic stresses, including soil pollutant damages (Dagher et al., 2020a; Ferrol et al., 2016; Gong et al., 2018; Op De Beeck et al., 2015).

Soil pollution by trace elements (TE) is furthermore a worldwide concern, because of the major threat they represent not only to human, but also to environmental health and ecosystem functioning, owing to their non-biodegradability, bioaccumulation and potent toxicity (Gong et al., 2018; Lin et al., 2020). Notably, TE can accumulate in cultivated plants, causing growth and productivity issues as well as a possible contamination of the food chain and are responsible for various disorders and diseases to human beings (Chen et al., 2014; Gong et al., 2018; Järup, 2003; Lin et al., 2019). It has previously been shown that revegetation of TE-polluted areas could help towards a restoration of the disturbed ecosystem functioning and could also provide aesthetic value and a reduction of wind and water erosion phenomena (Bourceret et al., 2016; Burges et al., 2018; Liang et al., 2020). In that regard, among other potential remediation options, phytomanagement has attracted much attention. Based on phytotechnologies relying on the ability of plants and their associated microorganisms to naturally immobilize and sequester in their rhizosphere inorganic pollutants such as TE, phytomanagement combines such risk-mitigation method with a sustainable and profitable land use to reclaim TE-polluted sites (Burges et al., 2018; Cundy et al., 2016; Mench et al., 2018). Towards a sustainable and economically viable process, biomass valorisation is a major component of phytomanagement strategies. At the present time, the research primarily focuses on energy crops, such as willow or miscanthus, intended for bioenergy production (Bauddh et al., 2017; Evangelou et al., 2015; Pandey et al., 2016). Nonetheless, the cultivation of aromatic plants, destined for essential oil production, could represent both an innovative and economically viable alternative for reclaiming TE-polluted areas. Essential oils, that are high-added values products, may be valuable in various fields such as perfumery and cosmetics, medicine or food industries. As an example, *Salvia sclarea* L. (*Lamiaceae*) also known as clary sage, is a xerophytic aromatic plant native from the Mediterranean area, able to grow relatively quickly and to develop a sustainable vegetative cover over two or three successive years, thanks to its short-lived perennial life cycle (Angelova et al., 2016; Grigoriadou et al., 2020; Lydakakis-Simantiris et al., 2016; Tibaldi et al., 2010). It is also able to produce large amounts of biomass especially in its second cultivation year, resulting in a significant EO production, valuable in food industry and cosmetics, but

also well known for its antimicrobial and antiseptic properties (Aćimović et al., 2018; Angelova et al., 2016; Tibaldi et al., 2010; Zutic et al., 2016).

The cautious selection of the plant species, based on its ability to grow in conditions that are toxic to most of the higher plants, is another key criterion towards a success of the phytomanagement (Burges et al., 2018; Evangelou et al., 2015). Therefore, the use of exogenous amendments, either mineral, organic or microbial, could support plant growth and health. Thus, microbial inoculants such as AMF or ectomycorrhizal fungi have previously been evaluated in field trials (Dagher et al., 2020b) and improved plants' fitness as well as contributed to TE immobilization, by precipitation or complexation phenomena occurring in the rhizosphere (Antoniadis et al., 2017; Burges et al., 2018; Gong et al., 2018). However, the impact of exogenous AMF amendments on the fungal and AMF community structure, even though it has previously been investigated, remains unclear. Contrasting results have indeed been published, with some reports indicating a clear shaping of the microbial communities in response to the AMF inoculation (Akyol et al., 2019; Dagher et al., 2020a; Janoušková et al., 2017; Koch et al., 2011; Lioussanne et al., 2010). Indeed, AMF affect the soil microorganisms in the rhizosphere of their host plant, leading to the formation of a specific area in soil called the mycorrhizosphere, as they compete for nutrients and space and exchange complex chemical signalling dialogues (Iffis et al., 2016; Trabelsi and Mhamdi, 2013). Some other reports yet demonstrated no influence of the inoculation on the native fungal or AMF communities in the rhizosphere (Janoušková et al., 2013; Renaut et al., 2020). In the same perspective, even though the short-term effects of TE pollution on the microbial communities are well known and usually lead to a decrease in both richness and abundance of fungal species (Chen et al., 2014; Lin et al., 2019; Op De Beeck et al., 2015), the assessment of an aged-TE pollution in the long-term has poorly been investigated (Bourceret et al., 2016). Besides, plant identity has also been demonstrated as a key factor in the shaping of the microbial communities and as such should be carefully evaluated (Bourceret et al., 2016; Dagher et al., 2019; Iffis et al., 2016). In these regards, AMF inoculation and plant identity are likely to result in a shift of the fungal and AMF communities in the rhizosphere of the cultivated plant and their respective structuring impact on the communities should be evaluated.

Hence, studying fungal community structure in such conditions could be of interest towards the understanding of the belowground ecosystem's functioning and to potentially identify unique and TE-well adapted fungal species. From a wider perspective, the identification of fungal species capable of growing in TE-polluted conditions could provide a knowledge basis for the development of remediation methods. Thus, this study aimed at (i) characterizing the indigenous fungal and AMF communities' structure in TE-aged polluted soil, (ii) evaluating and monitoring the potential structural changes induced in the plant rhizosphere communities by the cultivation of *S. sclarea* over its whole life cycle and (iii) assessing the effects of exogenous AMF inoculation on the native fungal communities including AMF over clary sage life cycle.

2. Material and methods

2.1. Experimental site

This study was carried out on an aged TE-polluted site (50°25'55.5" N, 3°02'25.5" E; altitude 23 m) in the north of France. The site is a former agricultural field of 1.3ha. It is located 600 m away from a former lead and zinc smelter, Metaleurop Nord, whose activity during more than a century generated significant amounts of TE-containing dusts, which led to a topsoil (0-30 cm) TE pollution (Labidi et al., 2015; Sterckeman et al., 2002). The topsoil is characterised by high amounts of Cd, Pb and Zn (7 ppm, 394 ppm and 443 ppm, respectively – Table 1). These correspond to concentrations approximately 17,

11 and 6 fold higher, respectively, than those reported in regional background levels for agricultural soils (Sterckeman et al., 2007). In particular, the soil texture consists of a silt loam, with a slightly alkaline water pH (7.9). The soil physicochemical properties were homogenous across the experimental plot.

Table 1: TE-polluted soil initial physico-chemical properties. Data are mean values \pm standard deviation ($n=19$).

Physical characterization			Organic status		
Clay (%)	Silt (%)	Sand (%)	C _{org} (g.kg ⁻¹)	N _{tot} (g.kg ⁻¹)	OM (g.kg ⁻¹)
26 \pm 2	53 \pm 10	18 \pm 3	17 \pm 2	1.6 \pm 0.2	29 \pm 4
Available P – Joret-Hebert (g.kg ⁻¹)	pH and carbonates		TE pseudo total		
	pH	CaCO ₃ (%)	Cd (mg.kg ⁻¹)	Pb (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
0.17 \pm 0.02	7.9 \pm 0.1	0.5 \pm 0.1	7 \pm 0.4	394 \pm 16	443 \pm 12

C_{org}: total organic carbon content; N_{tot}: total nitrogen; OM: organic matter.

The area is characterised by a warm temperate humid climate with cool summers. The climatic conditions are marked by average annual precipitation amounts of 742.5 mm and a mean annual temperature of 12 °C, ranging from 3.6 °C in January to 20.3 °C in July (Infoclimat, 2020).

2.2. Biological material

Clary sage (*Salvia sclarea* L.), a biennial herbaceous aromatic plant species was cultivated for 2 years in the TE-polluted site. The sowing took place in late April 2017, with a sowing density of 300 000 seeds per hectare. Seeds were provided by the ITEIPMAI, a French research institute. A commercial mycorrhizal inoculum kindly provided by Premier Tech (AGTIV® Specialty Crops, PremierTech, QC, Canada) and containing the AMF *Rhizophagus irregularis* (isolate DAOM-197198, 12000 viable spores per gram of product, 125 g/ha) has also been introduced during sowing, directly mixed with sage seeds.

2.3. Experimental design

The experiment was carried out on two successive years, from April 2017 to July 2018, with an experimental design consisting of two treatments, as described here below:

The two-year assessment covered three different monitoring times (namely original state of the plot before sowing – state at first sage harvest, year-1 – state at second sage harvest, year-2) and two treatments, non-inoculated (NI) and inoculated with the mycorrhizal inoculum (I). The completely randomized design included five replicates for each condition.

2.3.1. Soil and plant sampling procedure

Five soil samples have randomly been collected in April 2017 on the polluted experimental site, in order to assess the site's original condition before sowing. Sampling was carried out from the surface horizon (0-30 cm), using a manual auger. Two other sampling campaigns, totalizing 10 soil samples each, have been successively conducted in August 2017 and in July 2018, to respectively assess the state at first and second sage harvests (after 21 and 66 weeks of cultivation). All the sampling points have been geo-referenced. In order to characterize *in situ* grown sage, the three closest growing plants with their root systems were collected at each sampling point. For each condition, fifteen plants were used as biological replicates. The plant height was measured from the collar to the top of the highest inflorescence. All samples were collected over a half-day period and transported within 2 hours. After sampling, sage roots were vigorously shaken to remove loosely attached soil (bulk), which was

discarded. The careful collection of the rhizospheric soil was done using sterile brush and spatula tip (Sun et al., 2018; Yi et al., 2019). Sage roots were finally rinsed three more times with sterilized water to get rid of the potentially remaining soil particles before DNA extraction. Soil and sage roots were frozen and stored at -20 °C for further use.

2.3.2. Determination of *arbuscular* mycorrhizal root colonisation

Fresh roots were washed with sterile water and stained using Trypan blue (0.5 g.L⁻¹), according to the method described by Mustafa et al., (2016). Root colonisation was estimated by microscopic observation (Nikon Eclipse E600, x100 magnification) using the grid line intersect method (McGonigle et al., 1990). To do so, root fragments of 1 cm from three replicates per sampling point (45 fragments in total) were observed and 3 intersections per root fragment were examined to detect the presence of specific mycorrhizal structures: arbuscules, vesicles and hyphae. In total, more than 600 observations per condition were analysed to assess arbuscular mycorrhizal colonisation.

2.4. DNA extractions

2.4.1. Soil samples

Genomic DNA was extracted in triplicate directly from 250 mg of soil using Nucleospin Soil® kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. DNA quality was verified using 1 % (w/v) agarose gels and by measuring 260/280 nm and 260/230 nm ratios. DNA extracts' quantification was carried out on a SpectraMax® iD3 device (Molecular Devices LLC, Sunnyvale, California, USA). The concentration of all samples was determined and DNA extracts were diluted to 25 ng.µL⁻¹ for further analyses. The extracted DNA was stored at -20 °C until use.

2.4.2. Plant root samples

The fine roots were kept in Eppendorf tubes and frozen at -20 °C. Genomic DNA from clary sage roots was extracted in triplicate using a method adapted from Abu-Romman, 2011 and Aleksić et al., 2012. Briefly, sage roots were beforehand frozen in liquid nitrogen (-196 °C) in a sterilized mortar and ground into fine powder. 500 mg of the ground roots were then subject to Cetyltrimethylammonium bromide (CTAB – 1.4 M NaCl, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 2 % CTAB), Polyvinylpyrrolidone (PVP 1 % w/v), β-Mercaptoethanol (5 % v/v) and activated charcoal (0.5 % w/v) extraction (30 min – 55 °C). After this incubation period, a centrifugation step was carried out (10 min – 16000 g) and the lysate extraction was then performed using two successive steps with chloroform:isoamylalcohol (24:1). DNA precipitation then occurred in the presence of isopropanol (1 h incubation – 25 °C), followed by another centrifugation (10 min – 700 g). The DNA pellet was next washed three times in a row by addition of ice-cold ethanol (70 %) and centrifuged (10 min – 900 g), before air drying at room temperature (approx. 1h30 – 20 °C). Finally, DNA pellet was dissolved in 50 µL of TE buffer (10 mM Tris-HCl, pH 8.0; 1.0 mM EDTA, pH 8.0). The quality of the extracted DNA, normalization to 25 ng.µL⁻¹ and storage, were performed as described above.

2.5. PCR and sequencing

2.5.1. Fungal ITS

The fungal ITS region was amplified by PCR using a PCR thermal cycler (Agilent Surecycler 8800) with CS1 and CS2 barcoded degenerated primers as follows:

forward primer: **CS1_ITS₃_Kyo2_F** **ACACTGACGACATGGTTCTACAGATGAAGAACGYAGYRAA**, reverse primer: **CS2_ITS₄_Kyo3_R** **TACGGTAGCAGAGACTTGGTCTCTBTTCVCKCTTCACTCG**, with an expected length of 327 bp (Toju et al., 2012).

Triplicate reactions per DNA sample were performed. The reaction mixtures (25 µL) contained 5 µL of Q5 (5X) reaction buffer, 3 µL of Q5 (5X) High GC enhancer and 0.25 µL of Q5[®] High-Fidelity DNA Polymerase (New England Biolabs France, Evry, France), 0.8 µL of each primer (0.4 µM), 1 µL of dNTPs (0.2 mM), 1 µL of DMSO, 1 µL of Bovine Serum Albumin (BSA – 100 µg.mL⁻¹) and 1 ng of DNA template. The PCR conditions were as follows: initial denaturation at 95 °C for 10 min followed by thirty-five cycles of 94 °C for 20 s, 47 °C for 30 s and 72 °C for 20 s, and a final elongation step at 72 °C for 7 min.

2.5.2. 18S rRNA gene of AMF

The DNA of AMF was amplified using Nested-PCR (Agilent Surecycler 8800). The first PCR amplification was performed using a set of primers AML₁ (ATCAACTTTCGATGGTAGGATAGA) and AML₂ (GAACCCAAACACTTTGGTTTCC), targeting the small 18S subunit of rRNA gene, which generates amplicons of about 800 bp (Lee et al., 2008; Renaut et al., 2020). PCR conditions were as follows: initial denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 1 min, and a final elongation step at 72 °C for 5 min. PCR reactions were performed in triplicates with a reaction volume of 25 µL and conditions as follows: 5 µL of Q5 (5X) reaction buffer and 0.25 µL of Q5[®] High-Fidelity DNA Polymerase (New England Biolabs France, Evry, France), 0.8 µL of each primer (0.4 µM), 1 µL of dNTPs (0.2 mM), 1 µL of DMSO, 1 µL of BSA (100 µg.mL⁻¹) and 1 ng of DNA template.

The second PCR amplification used an identical reaction mixture as described above, with the set of primers nu-SSU-0595-5-F (**ACACTGACGACATGGTTCTACAC**GGTAATTCCAGCTCCAATAG) and nu-SSU-0948-3-R (**TACGGTAGCAGAGACTTGGTCTTTGATTAATGAAAACATCCTTGGC**) (Stefani et al., 2020), complemented with CS1 and CS2 adapters (displayed in bold) and the following conditions: initial denaturation at 94 °C for 3 min followed by thirty-five cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min, and a final elongation step at 72 °C for 5 min. This second amplification generates an amplicon of about 400 bp in length (Renaut *et al.*, 2020).

2.5.3. Illumina MiSeq sequencing

The triplicates for each PCR product ($n = 25$ for soil; $n = 20$ for roots) were pooled together and sent for sequencing at the Genome Quebec Innovation Centre (Montreal, QC, Canada) using Illumina MiSeq paired-end 2x300 bp.

2.6. Bioinformatic processing

Bioinformatic and statistical analyses were performed using R 3.6.1 (R Core Team, 2019). One should note that a bioinformatic analysis was run separately for each fungal (root or soil) and AMF (root or soil) datasets, as these were separately sequenced and therefore a specific error model for each dataset was calculated. Paired-end sequences for both ITS and 18S rRNA gene datasets were filtered, denoised, dereplicated and filtered for chimeras using the sequencing pipeline DADA2 (v. 1.12), following the described procedure (<https://benjjneb.github.io/dada2/tutorial.html>). At the end of the workflow, amplicon sequent variants (ASV) were inferred. A variation was performed for the ITS dataset, due to the highly variable length of the ITS region, which may compromise the reliability of the filtering and trimming steps (https://benjjneb.github.io/dada2/ITS_workflow.html). A truncation using “Cutadapt” tool was then conducted for the removal of primers, prior to trimming and filtering (Martin, 2011).

Taxonomic assignment for the ITS dataset was performed through the DADA2 pipeline, using the Ribosomal Database Project (RDP) naïve Bayesian classifier (Wang *et al.*, 2007). The Unite fasta release (Unite Community, 2019) was used to assign fungal taxa, from kingdom to genus. Each fungal ASV was further assigned to functional groups of fungi using FUNguild (Nguyen et al., 2016). For each

assignment, a confidence ranking was attributed (“possible”, “probable” and “highly probable”), reflecting the likelihood that a taxon belongs to a given guild, based on previously peer reviewed data (Nguyen et al., 2016). Functional assignment with a probable or higher confidence ranking only were taken into account.

For AMF taxonomy, a two-step workflow was followed. The Silva v132 database formatted for DADA2 (Callahan, 2018) was used as a first step to assign fungal taxa from kingdom to genus (minimum bootstrap 80) and compared to the results obtained with a nucleotide BLAST. The first step’s aim was to detect and exclude ASVs identified as non-*Glomeromycota* at the phylum level. As a second step, a phylogenetic tree was constructed, based on reference sequences from well-identified AMF cultures, to support and refine the taxonomic identification of each ASV (Stefani et al., 2020). A multiple alignment using 86 consensus sequences (Krüger et al., 2012) and the ASVs identified as *Glomeromycota* was first conducted using Kalign (Lassmann et al., 2009). A maximum likelihood tree was then calculated using RaxML v8.2.10 (Stamatakis, 2014) via the CIPRES web-portal (Miller et al., 2010), providing online bioinformatic tools.

2.7. Diversity and richness indexes

Alpha richness (Chao1) and diversity (Shannon and Simpson) indexes were calculated with the help of Phyloseq R package. Alpha diversity is assessed locally, within a specific ecosystem or area (Thukral, 2017). Both Shannon and Simpson indexes are diversity measurement, while Chao1 is a non-parametric richness estimator, based on an estimation of the species’ number in a community by considering rare species represented by singletons and doubletons (Kim et al., 2017). Both Shannon and Simpson indexes are estimators of alpha diversity, based on relative abundances, but Shannon index puts emphasis on species richness, while Simpson index gives a greater weight to species evenness (Kim et al., 2017; Shannon, 1948; Simpson, 1949).

2.8. Nucleotide Sequence Accession Number

The ITS and 18S rRNA gene sequences of the whole dataset have been deposited in NCBI Sequence Read Archive (SRA) database and can be reached under project accession number PRJNA665726.

2.9. Statistical analysis

Prior to any statistical analysis, Shapiro and Bartlett tests were conducted to respectively assess the normality and homoscedasticity of the data. If both conditions were verified, ANOVA analysis was carried out. Otherwise, a Kruskal-Wallis non-parametric test (“kruskal.test” function in R) was used. The significance of the statistical analyses was considered at $\alpha = 0.05$.

Based on Bray-Curtis dissimilarity, permutational multivariate analyses of variance (PERMANOVA) and principal coordinate analyses (PCoA) were conducted with the help of the Vegan package from R. PERMANOVA was run with 1000 permutations using “adonis” function, with a constrained model for inoculation and time parameters. Venn diagrams were constructed to highlight the numbers of shared ASVs between the experimental conditions, with the help of Vegan package. Correlation heatmaps, based on Pearson’s correlation matrix, were plotted using the “heatmap2” function from gplots R package. Rarefaction curves were created with the “rarecurve” function of the Vegan package in R, to verify whether the sequencing depth was adequate and reflected the original diversity or not.

3. Results

3.1. Raw sequences bioinformatic processing

A total of 1 558 856 and 1 403 165 fungal (ITS) and of 1 553 389 and 1 193 261 AMF (18S) raw MiSeq reads were obtained for soil and root biotopes respectively, from 25 soil samples and 20 root samples. After quality filtering and chimera removal, 25 241 and 29 877 fungal sequences and 19053 and 32241 AMF sequences remained for the community analyses in soil and root biotopes respectively. The complete procedure with the number of sequences at each step is shown in Table S1.

The results from the rarefaction analyses have shown that both datasets (ITS and 18S) tend to reach a saturation plateau (Figure S1). As such, this suggests that the sampling procedure was adequate to explain most of the fungal and AMF diversity either in the rhizospheric soil or associated with the roots of clary sage.

3.2. *In situ* clary sage development and arbuscular mycorrhizal root colonisation

Evaluated before harvest at year-1 and year-2, sage height significantly increased for both NI and I treatments between year-1 and year-2, with respective gains of 62 and 65 % ($P < 0.05$, Table 2). No significant differences were however observed between NI and I treatments, whatever the year. Regarding arbuscular mycorrhizal colonisation rates, they were stable between year-1 and year-2 for the NI treatment. Yet, a significant increase has been observed for the I treatment, rising from 4 % in year-1 to 16 % in year-2. In addition, in year-2, the I treatment displayed a significantly higher arbuscular mycorrhizal rate, with an increase of 75 % in comparison with the NI treatment ($P < 0.05$).

Table 2: Sage height and root arbuscular mycorrhizal colonisation in NI and I conditions. Values are means \pm SD ($n=5$). Significant differences are indicated by different letters ($\alpha = 0.05$).

	Year-1		Year-2	
	NI	I	NI	I
Height (cm.plant⁻¹)	55 \pm 2 ^A	51 \pm 8 ^A	145 \pm 10 ^B	156 \pm 12 ^B
Arbuscular mycorrhizal rate (%)	4 \pm 5 ^a	0 ^b	4 \pm 2 ^a	16 \pm 5 ^c

NI: non-inoculated; I: inoculated.

3.3. Fungal α -diversity in root and soil biotopes

The fungal (ITS and 18S datasets) richness and α -diversity were evaluated separately for each experimental condition, *ie* culture duration (soil before sowing, year-1 and year-2) and mycorrhizal inoculation (NI or I), and accordingly for root and soil biotopes.

According to the Chao1 estimator, the fungal richness in soil was stable between the soil before sowing and year-1, but significantly decreased between year-1 and year-2 for both NI and I treatments (Figure 1A.). No significant difference was observed between I and NI treatments. It is noteworthy that the values measured in roots, whatever the treatment, are similar to those obtained in soil in year-2, and hence are lower than those measured in soil in year-1. In addition, in roots, the fungal richness was found equivalent whatever the treatment and the monitoring time. Regarding AMF richness, the results have shown that it was similar in soil, regardless of the treatment and the monitoring time. In roots, a significant richness increase between year-1 and year-2 has only been measured for the I treatment ($P < 0.05$). However, no significant difference was observed between NI and I treatments. In addition, similar values were obtained in soil and roots, excepted for the I treatment in year-2.

Regarding diversity estimators (Shannon and Simpson) in soil, our results have shown that they were stable between the soil before sowing and year-1 and dropped between year-1 and year-2 for the fungi (ITS) dataset (Figure 1B. and C.). Similar results were observed between NI and I treatments. In roots, the fungal diversity was found equivalent to what was measured in soil in year-2, and that whatever the treatment and the monitoring time. For the AMF dataset, the diversity was found equivalent, whatever the biotope (soil or root), the treatment (NI or I) and the monitoring time (soil before sowing, year-1 and year-2). It is noteworthy that the highlighted trends for diversity estimators are similar between the two indexes and for both fungi and AMF datasets.

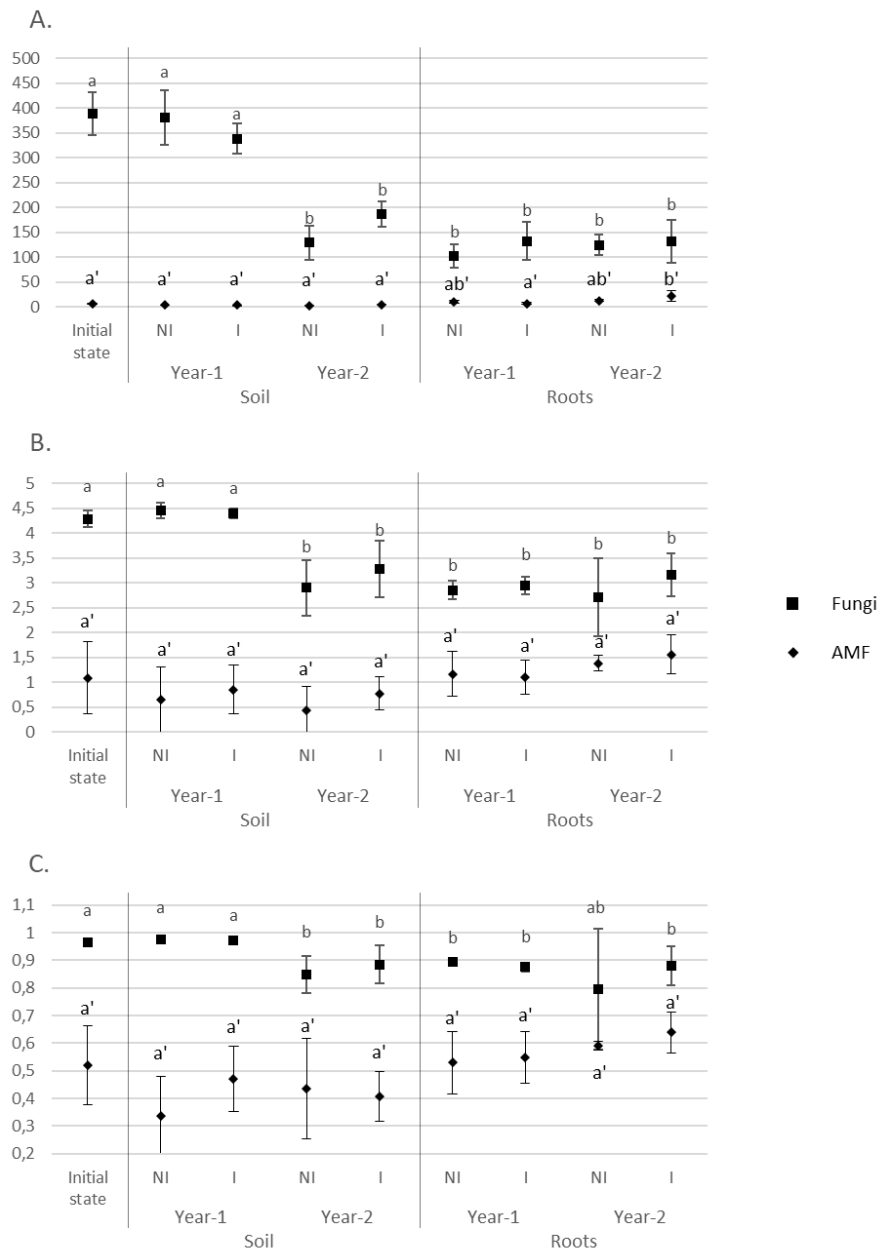


Figure 1: Richness (Chao 1 estimator – A.) and diversity (Shannon and Simpson – B. and C. respectively) indexes from the 5 studied conditions for soil fungal (ITS) and AMF (18S) communities and the 4 studied conditions for root fungal and AMF communities. Data are means \pm SD (n=5) for each condition. Significant differences between conditions are indicated by different letters (Kruskal-Wallis test, $\alpha = 0.05$). NI: non-inoculated; I: inoculated.

3.4. Influence of mycorrhizal inoculation and cultivation duration on the fungal communities' structure

The effect of the experimental parameters, namely mycorrhizal inoculation and cultivation duration, on soil and root fungal (ITS and 18S datasets) communities was evaluated by the use of a principal coordinate analysis (PCoA), complemented with a PERMANOVA analysis. For the ITS dataset, a clear clustering of the fungal communities has been demonstrated in response to the cultivation duration in both soil and root biotopes, (Figure 2A. and B.). In particular, the fungal communities in soil before sowing and at year-1 were clustered together and separated from those of year-2. In addition, an effect bound to the mycorrhizal inoculation was highlighted in soil, with a distinction of fungal communities in year-2 between I and NI treatments (Figure 2A.). In roots, fungal communities from year-1 and year-2 were clustered separately, depicting a time effect (Figure 2B.). However, no difference was observed between I and NI treatments, whatever the year. Of the total variance in the dataset, the first two principal components together explained 46 % of the total soil fungal communities and 38.3 % of the total root fungal communities.

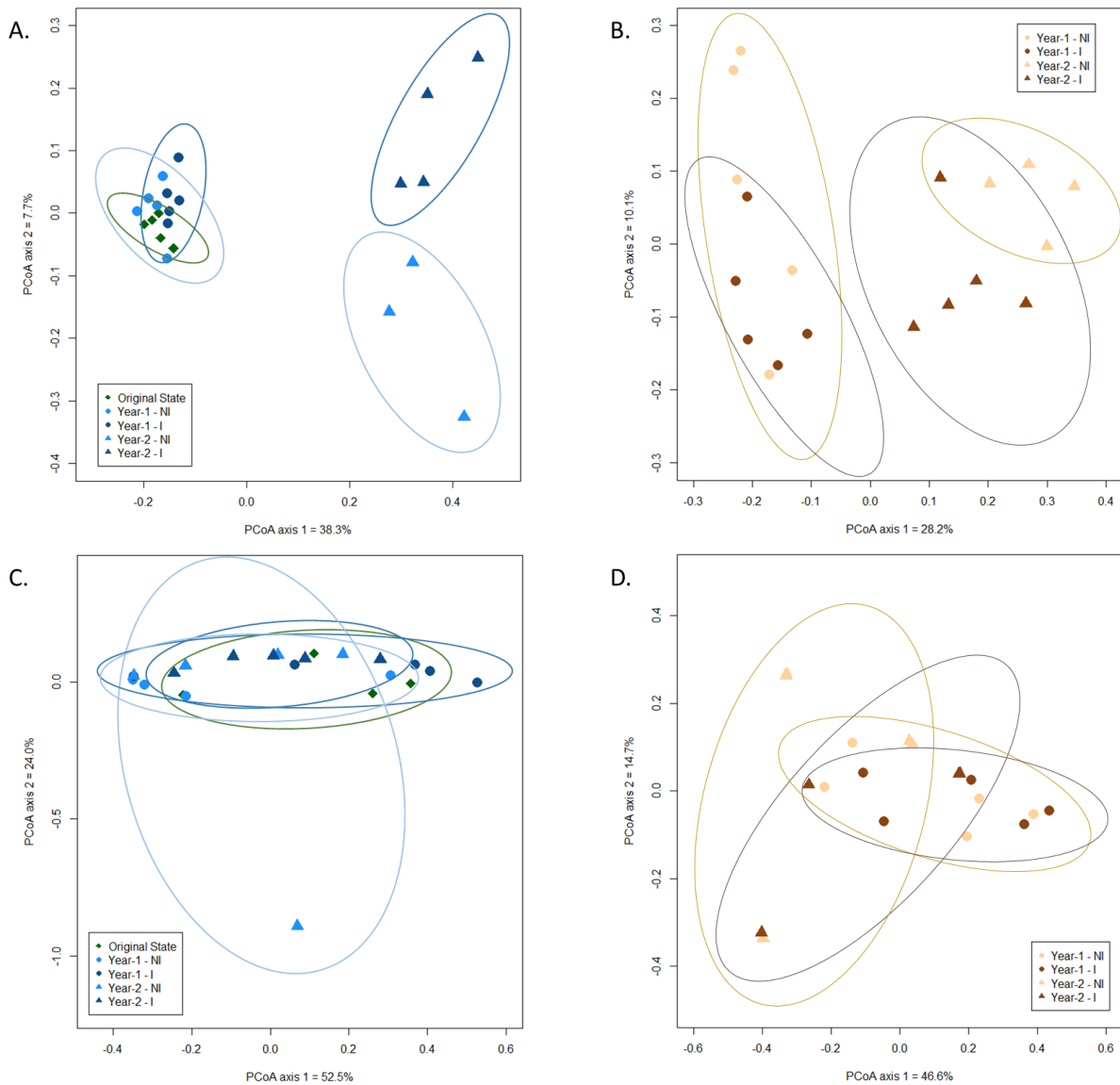


Figure 2: Principal coordinate analysis (scaling 1) based on Bray-Curtis dissimilarity for soil (A. and C.) and root (B. and D.) fungal ASVs from the ITS (A. and B.) and 18S (C. and D.) datasets in the different experimental conditions. Confidence area of ellipses = 0.95. NI: non-inoculated; I: inoculated.

These results are confirmed by the PERMANOVA analyses, displaying significant effects of the mycorrhizal inoculation in soil ($p < 0.05$) and of the cultivation duration in both soil and root biotopes ($p < 0.001$ in both cases).

For the 18S dataset, the first two principal components of the PCoA ordination together explained 76.5 % of the total soil AMF communities and 61.3 % of the total root AMF communities (Figure 2). Our results have demonstrated that neither cultivation duration nor mycorrhizal inoculation significantly affected AMF communities in soil (Figure 2C). In the root biotope, despite a significant effect of the cultivation duration showed by the PERMANOVA analysis ($p < 0.001$), no clear clustering between AMF communities in year-1 and year-2 is visible on the PCoA ordination (Figure 2D.).

For the ITS dataset, a total of 761, 696, 644, 206 and 343 fungal ASVs were identified in the soil before sowing, year-1 NI and I and year-2 NI and I treatments, respectively, while a total of 221, 294, 214 and 251 fungal ASVs were present in the root biotope and for year-1 NI and I and year-2 NI and I treatments respectively (Figure 3A. and B.).

The number of shared ASVs between the five conditions (soil before sowing, year-1 NI and I treatments and year-2 NI and I treatments) was 116 (9 % of the total ASV number) and 76 (13 % of the total), for the soil and root biotopes, respectively. Among them, the soil before sowing and at year-1 harvest shared the highest number of ASVs in soil, whatever the treatment (NI or I), representing a number of 192 shared ASVs (Figure 3A.). Conversely, the soil before sowing and at year-2 harvest, regardless of the treatment (NI or I), shared a number of 4 ASVs, while year-1 and year-2 only have 6 ASVs in common, depicting a net shift between year-1 and year-2. In addition, the total number of ASVs significantly decreases of about 60 % in year-2, regardless of the treatment. It is noteworthy that in year-2, for the I treatment, the drop in the total number of ASVs is lower in comparison with the NI treatment.

Besides, the proportion of specific ASVs is equivalent between all the treatments, ranging from 22 to 29 % of the total ASVs number, exception made for the NI treatment in year-2 displaying a significantly lower proportion of specific ASVs (11 %). In roots, equivalent numbers of ASVs are shared between I and NI treatments and between the successive years, representing 20 to 23 % of the total ASV number (Figure 3B.). However, higher numbers of total and specific ASVs have been highlighted for the I treatment in comparison with the NI one, whatever the year.

Regarding the 18S dataset, a total of 12, 8, 9, 5 and 11 AMF ASVs were identified in the soil before sowing, year-1 NI and I treatments and year-2 NI and I treatments respectively, while a total of 22, 14, 21 and 52 AMF ASVs were present in the roots and for year-1 NI and I and year-2 NI and I treatments respectively (Figure 3C. and D.). The number of shared ASVs between the five treatments (soil before sowing, year-1 NI and I treatments and year-2 NI and I treatments) was 3 (15 % of the total ASV number) and 6 (10 % of the total), for the soil and root biotopes respectively. Because of the relatively low number of AMF ASVs in the soil, there is no clear trend between the different experimental treatments (Figure 3C.). However, in the roots, there is a significant increase of about 70 % in terms of ASV number for the I treatment, between year-1 and year-2 (Figure 3D.). This same trend is not observed for the NI treatment between year-1 and year-2. In addition, there is no significant difference in year-1 between NI and I treatments, whereas the numbers – total or specific – of AMF ASVs in the I treatment are significantly higher in year-2, in comparison with the NI one.

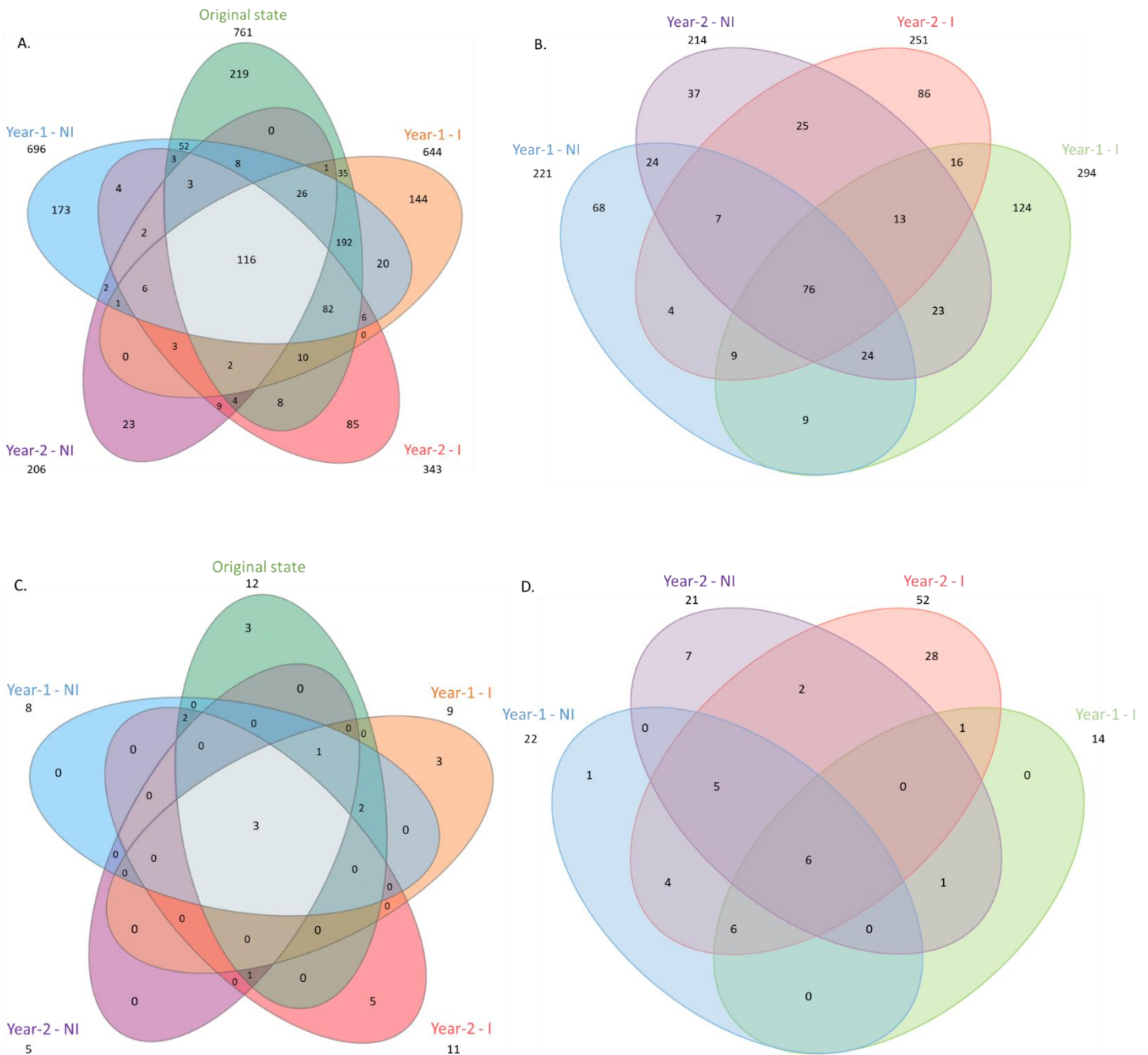


Figure 3: Venn diagrams showing the overlap of the fungal (ITS dataset – A. and B. and 18S dataset – C. and D.) community overtime for soil (A. and C.) and root (B. and D.) biotopes. These diagrams display the number of total (out of the shapes), specific and shared ASVs. The total numbers of fungal ASVs for the ITS and 18S datasets are respectively 1239 and 20 in soil and 569 and 61 in roots. NI: non-inoculated; I: inoculated

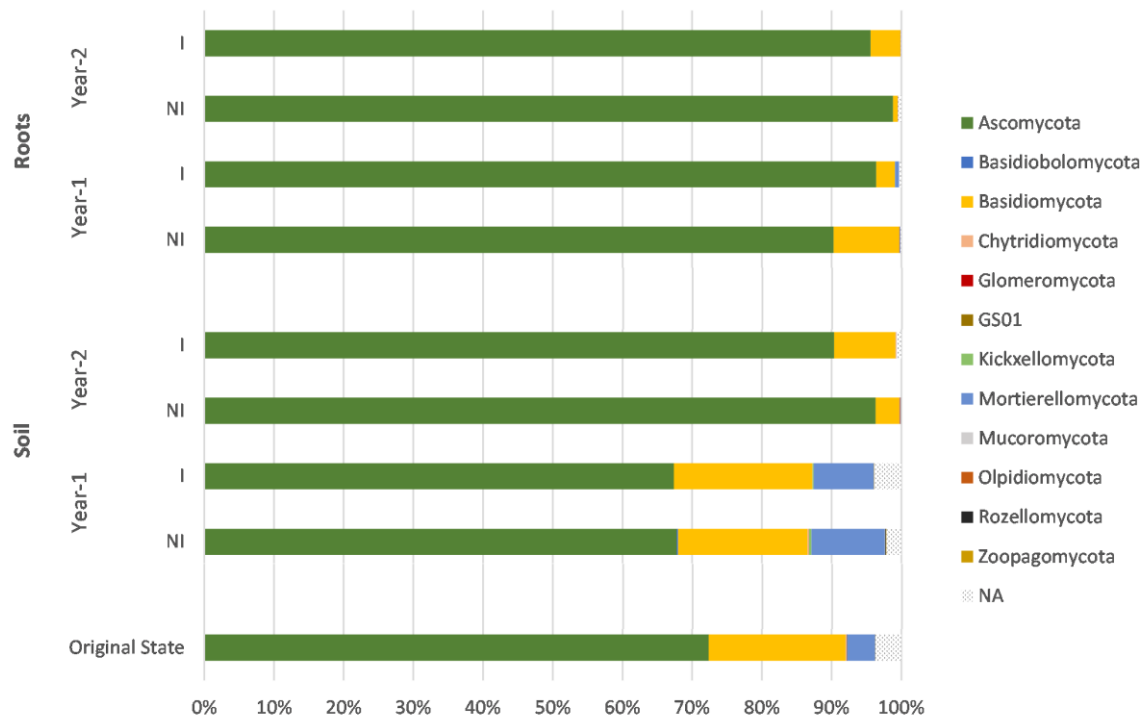
3.5. Taxonomic variations in the fungal communities’ in response to mycorrhizal inoculation and cultivation time

The fungal diversity and the potential changes induced by the cultivation time and mycorrhizal inoculation were assessed after a taxonomic assignment at phyla, classes, orders, families and genera levels. In that regard, soil fungal ASVs were assigned to 12 different fungal phyla, while root fungal ASVs were assigned to 4 different ones (Figure 4A.). Whatever the experimental condition and the biotope, *Ascomycota* was the most represented phylum. More specifically, it represented about 70 % of the total fungal ASVs in soil before sowing and at year-1, increasing to more than 90 % in year-2.

Basidiomycota and *Mortierellomycota* also are among the most represented phyla, especially in the soil before sowing and year-1, but decreased in year-2. All the other identified phyla displayed significantly lower relative abundances, below 0.5 %, whatever the condition and the biotope. In roots, the relative abundance of *Ascomycota* exceeded 90 %, whatever the condition. *Basidiomycota* and *Mortierellomycota* were also present in roots, but in significantly lower proportions, ranging from 0.005 to 9.4 %. In addition to these 3 phyla, *Glomeromycota* was the only other identified fungal phylum in roots, with a relative abundance of about 0.05 %.

At the genus level, the dominant fungal taxa in soil were *Gibellulopsis*, *Mortierella*, *Cercophora*, *Fusicolla* and *Lasiospheris* (Figure 4B.). *Gibellulopsis* was well represented in all samples regardless of the experimental condition. This genus was found particularly dominant in year-2 in the non-inoculated treatment, with a relative abundance of 42 %. In addition, while most of the genera were stable whatever the treatment and the cultivation time, some variations were observed. In particular, *Mortierella*'s abundance was about 4 % in the soil before sowing, increased to 10 % in year-1 and dropped to lower than 0.1 % in year-2, regardless of the treatments. Besides, *Plectosphaerella* and *Botrytis* were poorly represented in the soil before sowing and at year-1, with relative abundances lower than 1 %, and significantly increased in year-2, up to 13 %. Finally, *Acrostalagmus* was specifically present in the NI treatment at year-2.

A.



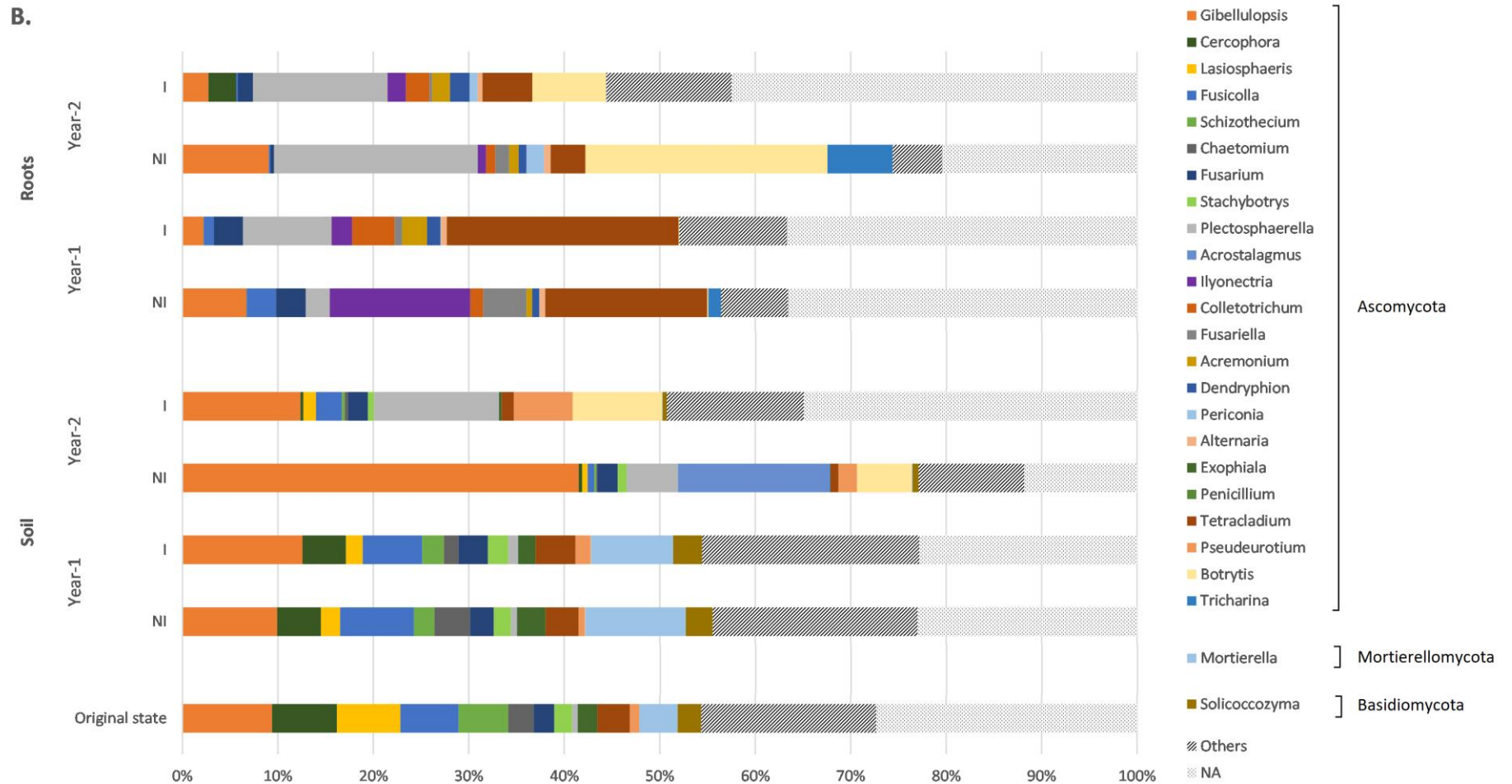


Figure 4: Relative abundances of major phyla (A.) and genera (B.) of the fungal ITS rRNA gene dataset, according to the different experimental conditions for both root and soil biotopes. The genera have been grouped by phylum and those with a relative abundance lower than 0.5% have been gathered in the "Others" group. NI: non-inoculated; I: inoculated; NA: non-assigned.

In roots, similarly to the soil biotope, *Gibellulopsis* was among the most represented genera, but along with *Tetracladium*, *Plectosphaerella* and *Botrytis* (Figure 4B.). The proportions of the dominant genera were significantly different, especially between the two sampling times (year-1 and year-2). In particular, *Tetracladium* was extensively represented in year-1 and decreased in year-2, with abundances of 17; 24; 4 and 5 %, for NI and I treatments respectively ($P < 0.05$). Conversely, *Plectosphaerella* and *Botrytis* were poorly represented in year-1 but significantly increased in year-2, with respective evolutions of about 66 and 99 % ($P < 0.01$). One should note that a relatively high number of fungal ASVs was not assigned to genus, depicted by the proportion of “Not-Assigned” (“NA”) ranging from 12 up to 40 %.

3.6. Taxonomic identification of ASVs belonging to the 18S rRNA gene dataset

The results from the phylogenetic analysis (Figure S2) allowed an assignment of 69 out of the 81 identified ASVs from the 18S rRNA gene dataset to eight genera, namely *Acaulospora*, *Ambispora*, *Archeospora*, *Claroideoglomerus*, *Funneliformis*, *Glomus*, *Rhizophagus* and *Scutellospora*. Twelve ASVs could not be assigned to the genus level with a sufficient confidence level, as they did not cluster with any of the consensus sequences. The 69 other ASVs represented 6 different families (*Acaulosporaceae*, *Ambisporaceae*, *Archeosporaceae*, *Claroideoglomeraceae*, *Gigasporaceae* and *Glomeraceae*). Notably, 39 (57 %) out of the 69 ASVs were assigned to the *Glomeraceae* family and among them, *Funneliformis* and *Glomus* genera were the two most represented ones with respectively 19 and 17 members each, while only 3 ASVs were belonging to the genus *Rhizophagus*. It is noteworthy that among the 69 ASVs assigned to the genus level, 4 of them were present in both root and soil biotopes. This includes ASVs S1 and R1, S2 and R3, S3 and R2, S4 and R43, and S5 and R33, which were two by two identical.

A heatmap representation was consecutively drawn, based on the abundance of the 10 and 15 most represented ASVs in the rhizospheric soil and the root biotopes, respectively (Figure S3). This analysis revealed that the most abundant ASV in both soil and root, which is identical, was present in the soil initial state but significantly decreased in year-1 and similarly was scarcely represented in year-1 in the root biotope. It however consistently increased and was most represented in year-2, in both biotopes. Specifically, in soil, the two most abundant ASVs attributed to the *Funneliformis* genus were particularly abundant in inoculated treatment, in both year-1 and year-2, but were scarcely represented otherwise. Conversely, ASVs attributed to *Acaulospora* spp. were more abundant in the soil initial state and decreased in year-1 and year-2. *Funneliformis* spp. were also present in roots, specifically in year-2, with no distinction between I and NI treatments. Besides, ASVs identified as *Rhizophagus irregularis* were present in the root biotope in year-2, in both I and NI treatments. From a global perspective, it is noteworthy that ASVs are more abundant in year-2 and especially in the inoculated treatment.

3.7. Prediction of functional assignments of ASVs

Among the 1239 and 569 fungal ASVs, recorded in the soil and root biotopes for the ITS dataset respectively, 48 and 53 % were successfully assigned to a functional guild with at least a “probable” confidence ranking using FUNGuild (Nguyen et al., 2016). This functional assignment revealed a majority of saprotrophic fungi, up to 54 % of the total abundance, as well as variable proportions of putative fungal plant pathogens, ranging from 19 up to 60 % (Figure 5). It is noteworthy that the relative abundance of plant pathogenic fungi significantly increased in soil in year-2 ($P < 0.05$), in comparison with the soil before sowing and at year-1. However, in roots, no significant difference was observed between year-1 and year-2. Conversely, the proportions of saprotrophic fungi decreased overtime in both biotopes and was at lowest during year-2. Besides, endophytic fungi were less represented, especially in soil, with relative abundances close to 0 excepted for the NI treatment at

year-2, whereas in roots relative abundances ranged from 3 to 10 % regardless of the treatment and the monitoring time. In addition, AMF were present but only accounted for less than 0.05 % of the total abundance, as 4 and 5 ASVs only were assigned to AMF for soil and root biotopes, respectively.

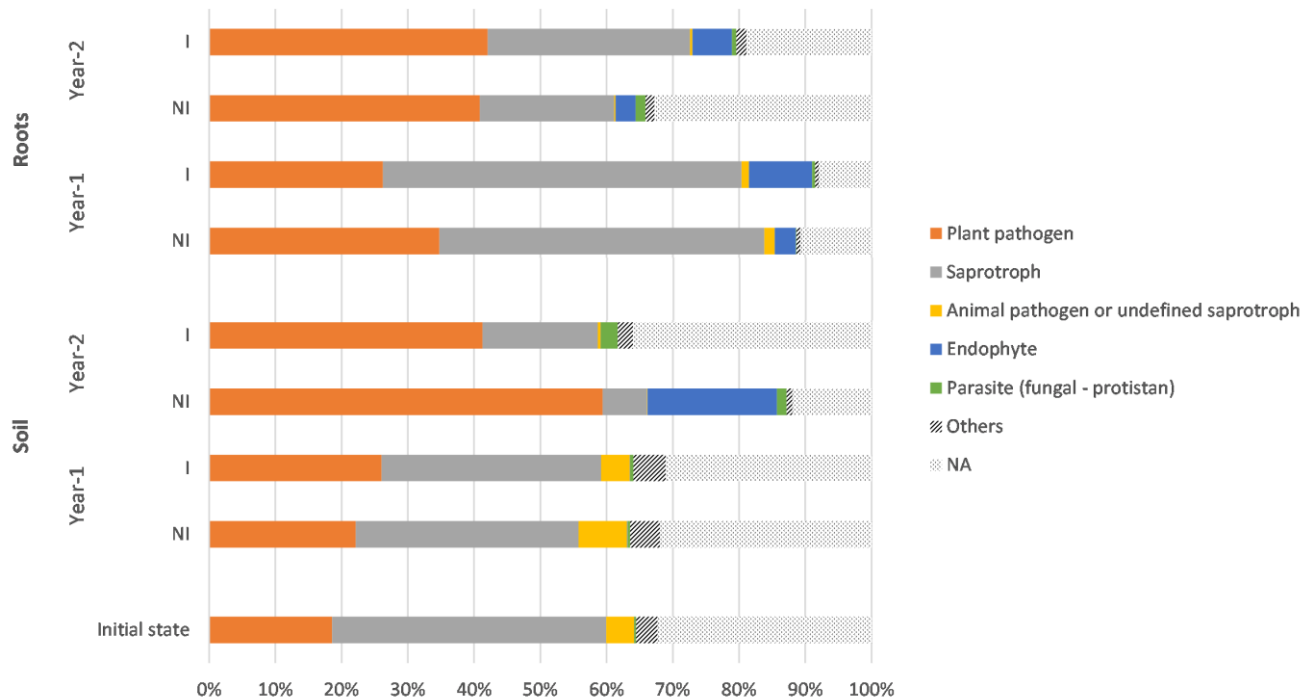
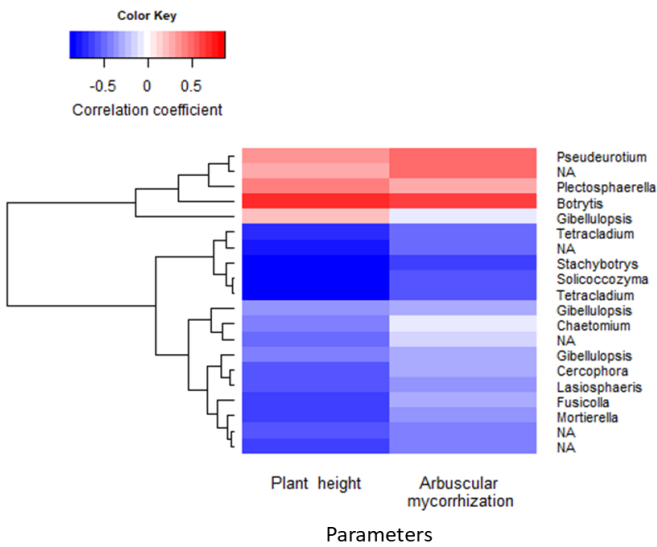


Figure 5: Relative abundances of major fungal functional groups of the ITS rRNA gene dataset, according to the different experimental conditions for both root and soil biotopes. The guilds with a relative abundance lower than 0.5 % have been gathered in the "Others" group. NI: non-inoculated; I: inoculated; NA: non-assigned.

In addition to the functional assignment, a heatmap based on Pearson coefficients between the 20 most abundant ASVs and sage height and root arbuscular mycorrhizal rates as explaining parameters was drawn, so as to identify potential correlations between each other, in both soil and root biotopes (Figure 6).

These correlation coefficients ranged from 0.88 to 0.69 and from 0.55 to 0.63 for the soil and root biotopes respectively. *Botrytis* is the fungal genus in soil displaying the highest positive correlation coefficients regarding both sage height and root arbuscular mycorrhizal rates, while *Pseudeurotium* also display a high correlation regarding root arbuscular mycorrhization (Figure 6A.). Similarly, in roots, the *Botrytis* genus was strongly and positively correlated to sage height, and so was *Plectosphaerella* (Figure 6B.). However, regarding arbuscular mycorrhization, the fungal ASVs displayed either low or null correlation coefficients. Conversely, *Tetracladium*, *Stachybotrys* and *Solicoccozyma* displayed the highest negative correlation in soil, regarding both plant height and root mycorrhization, while *Tetracladium* also was strongly but negatively correlated to both parameters in the root biotope (Figure 6B.).

A. Soil biotope



B. Root biotope

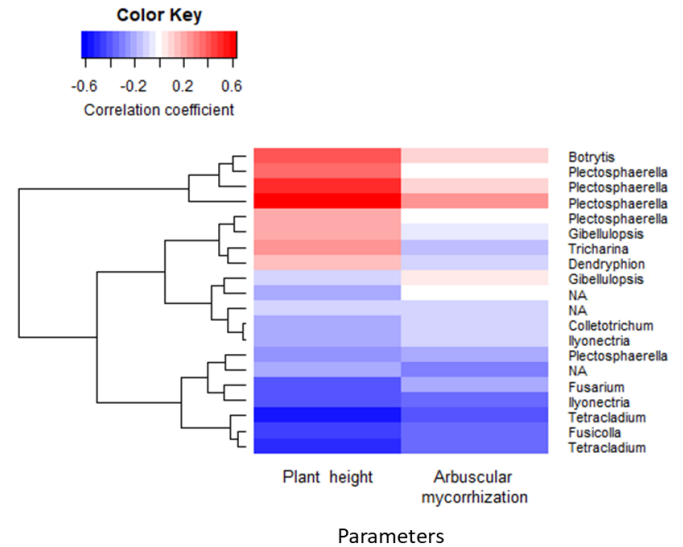


Figure 6: Heatmap representation based on the correlation coefficients between clary sage height or arbuscular mycorrhization and the 20 most represented fungal ASVs from the ITS dataset in soil (A.) and root (B.) biotopes. They respectively belong to 12 and 10 fungal genera. NA: non-assigned.

For the 18S rRNA gene dataset, correlation coefficients ranged from -0.35 to 0.30 and from 0.19 to 0.76 for the soil and root biotopes respectively. In soil, despite the positive correlation coefficients measured for the most abundant ASVs, no clear correlation was established between the 10 most represented ASVs and the explaining parameters, namely plant height and arbuscular mycorrhization, given the low value determined for the correlation coefficients (Figure S4). Conversely, in the roots, all the ASVs displayed positive correlation coefficients. In particular, ASVs assigned to *Funneliformis* spp. displayed a similar behaviour regarding plant height, with correlation coefficients equal to or slightly above 0.52, depicting a correlation between each other (Figure S4). Similarly, regarding arbuscular mycorrhization, *Glomus* spp. displayed positive correlation coefficients of about 0.55.

4. Discussion

In view of the growing demand for arable lands, the reclaiming of marginal lands such as TE-polluted fields, for the production of biomass intended for non-food valorisation could represent an attractive alternative. Clary sage has been evaluated as part of a phytomanagement assay, aiming at cultivating the aromatic plant on a TE-polluted soil and producing essential oil from the biomass, intended for use as potential biopesticides. Under field conditions, the effects of clary sage cultivation and of mycorrhizal inoculation were determined on the fungal community structure with a particular focus on AMF community structure in rhizospheric soil and root biotopes.

4.1. *Ascomycota* phylum dominates the fungal community in a TE-aged polluted soil before clary sage cultivation

Our results have shown that *Ascomycota* and to a lower extent *Basidiomycota* and *Mortierellomycota* were the dominant fungal phyla in our *in situ* experimental conditions, before clary sage sowing. Notably, *Ascomycota* and *Basidiomycota* were predominant, since they accounted together for more than 90 % of the relative abundance. This observation is consistent with recently published studies, carried out on both agricultural and on aged-TE polluted soils (Bourceret et al., 2016; Dagher et al., 2019; Liang et al., 2020; Lin et al., 2019; Misra et al., 2019; Shi et al., 2020; Torres-Cruz et al., 2018).

Ascomycota have in particular been depicted as the prevalent fungal phylum in various polluted or unpolluted environments, due to their diversity in terms of metabolic capacities, owing to the wide variety of enzymes they produce (Liang et al., 2020; Lin et al., 2019; Misra et al., 2019). They were for instance reported as key decomposers of crop residues in agricultural soils (Blackwood et al., 2007; Lin et al., 2019). Interestingly, among *Ascomycota* and *Basidiomycota* members, many were shown to display a strong tolerance to TE, even in highly polluted conditions (Lin et al., 2020, 2019; Luo et al., 2014; Torres-Cruz et al., 2018). In these particular environmental conditions, some fungal communities have indeed exhibited a capacity to adapt overtime and to develop a tolerance to TE (Bourceret et al., 2016; Op De Beeck et al., 2015). In the case of *Ascomycota* and *Basidiomycota*, previous reports pointed towards an induction of the expression of genes coding for metallothionein or phytochelatin synthase, involved in fungal cell protection against TE-harmful effects (Kalsotra et al., 2018; Shine et al., 2015; Sudhakara Reddy et al., 2014). Regarding the fungal diversity in the TE-polluted site, it also appears similar to or even slightly higher in comparison with previous results published elsewhere in the literature, from revegetated or agricultural TE-polluted soils, whatever the richness or diversity index (Bourceret et al., 2016; Chen et al., 2014; Foulon et al., 2016; Klaubauf et al., 2010; Liang et al., 2020). It could notably be explained by a water pH close to neutrality and a soil organic matter content considered as slightly higher than average in comparison with reference indicators in our experimental conditions. These parameters have in fact been demonstrated as key drivers in the richness and diversity of fungi in soils (Lin et al., 2020). Interestingly, 12 ASVs identified as belonging to the phylum *Glomeromycota* and split among 4 different AMF families were also detected in the TE-polluted soil, before clary sage sowing. Notably, a predominance of *Acaulosporaceae* and *Glomeraceae* was found, in accordance with previously published results on TE-polluted soils (Lopes Leal et al., 2016; Yang et al., 2015).

4.2. Clary sage significantly shaped the rhizosphere fungal community over its life cycle

The results from the PERMANOVA and PCoA analyses have indicated a temporal variability of the fungal communities in both rhizospheric soil and root biotopes with a high significance ($P < 0.001$), following sage growth. Regarding diversity indexes, similar results were obtained between the soil before sage sowing and the soil sampled before the harvest at year-1. Our results have also demonstrated a significant drop in terms of both richness and diversity between year-1 and year-2, especially in soil. The same trend was observed for ASV numbers, with a clear decrease between year-1 and year-2. Beside this decrease observed in the rhizospheric soil between year-1 and year-2, a shift should also be highlighted in roots, depicted by stable richness and diversity indexes over time, but changes in terms of fungal relative abundances. Interestingly, our results suggest that the fungal communities in the rhizospheric soil and in the roots of the cultivated plant tend to become similar over time. Notably, after two years of cultivation, the fungal richness and diversity were found equivalent in the rhizospheric soil and in the roots of clary sage and a similar pattern was observed between the two biotopes at the phylum level, which is consistent since it is well known that fungi associated with the roots are recruited from the surrounding soil community (Goldmann et al., 2016). Furthermore, despite significant differences of relative abundances at the genus level, it appears clearly that the pattern is identical between the state before sowing and at year-1 in soil and differs in year-2, becoming more similar to what was observed in the roots. Notably, several fungal genera, such as *Botrytis* or *Plectosphaerella* that were very poorly represented in the soil before sowing and at year-1 simultaneously increased in year-2, in both root and rhizospheric soil biotopes, which is expected, since both genera are necrotrophic plant pathogens.

It is well described in the literature that although the impact of revegetation on soil microbial communities depends on both the growing season and the plant identity (Bourceret et al., 2016;

Dagher et al., 2019, 2020), fungal diversity usually decreases in the rhizospheric soil in comparison with the bulk (loosely associated with the root) or unplanted soil (Wang et al., 2017). This fact is notably attributable to the selective pressure exerted by the plants' root exudates on the fungal communities and that even more in the vicinity of the root, in the rhizosphere (Bourceret et al., 2016; Dagher et al., 2019; Iffis et al., 2016; Misra et al., 2019). First, plants' root exudates may support the growth of rhizosphere microbial communities by providing carbohydrates, acting as substrates for microbial growth (Misra et al., 2019; Na et al., 2019; Qu et al., 2020). However, in the case of aromatic plants in particular, such consistent shifts in the fungal communities have been suggested bound to the release of various aromatic compounds in their rhizosphere, exerting antifungal properties (Adamovic et al., 2015; Misra et al., 2019; Wei et al., 2020). Notably, the involvement of various mono- and sesquiterpenes, such as limonene or menthol, was brought forward (Adamovic et al., 2015; Misra et al., 2019). This trait has been well documented for various plant species, such as *Eucalyptus* spp., *Artemisa annua* and *Ocimum basilicum* or from the *Lamiaceae* family, such as *Mentha arvensis* (Adamovic et al., 2015; Misra et al., 2019; Qu et al., 2020), but remains unknown for clary sage. Nonetheless, the antifungal activity of clary sage EO and of its major compounds, namely Linalool and Linalyl acetate, has repeatedly been demonstrated (Aćimović et al., 2018; Pitarokili et al., 2002; Puškárová et al., 2017; Zutic et al., 2016). In particular, their activity against some fungal genera which in this work have been highlighted as significantly decreasing over time, such as *Chaetomium* or *Fusarium*, has previously been established (Aćimović et al., 2018; Pitarokili et al., 2002; Puškárová et al., 2017). Regarding the other fungal genera for which the biggest decreases have been observed, such as *Cercophora* or *Tetracladium*, they are saprotrophic and not phytopathogenic fungi and as such might not have been subject to antifungal assessment. One should note that clary sage, as depicted in this work and in accordance with previously published results, consistently grows in the second year of cultivation, corresponding to the best harvest year for the EO (Tibaldi et al., 2010). In that regard, the drastic shift observed between year-1 and year-2 could be due to the release in the rhizosphere of aromatic compounds produced in high amounts in that period. Nonetheless, the influence of environmental factors on the fungal community structure has previously been raised (Bourceret et al., 2016; Lin et al., 2020), and thus should not be neglected.

The results from the functional assignment revealed a majority of saprotrophic and plant pathogenic taxa (up to 80 %), whereas endophyte and symbiotic taxa were poorly represented in both rhizospheric soil and root biotopes. This goes along with previous findings, reporting a dominance of fungal guilds attributed to pathogens and saprotrophs, accounting for up to 90 % of the community (Y. Li et al., 2020; Na et al., 2019). On another note, the proportion of saprotrophic taxa decreased over time, replaced by fungal genera assigned to plant pathogenic, such as *Gibellulopsis*, *Plectosphaerella* or *Botrytis*, especially between year-1 and year-2. It is not too surprising, since mono-cropping often results in modifications in the soil microbial abundance and diversity and usually increases the abundance of plant pathogenic microorganisms in soil (Li and Liu, 2019; X. Li et al., 2020; Na et al., 2019). Moreover, no fungicidal intervention was carried out during this field trial, which could worsen the previously described trait as well as potentially increasing the competition between endophytic and pathogenic fungi. Nonetheless, it should be remembered that plant pathogenic fungal genera, such as *Fusarium*, *Gibellulopsis* or *Botrytis*, even though they have a wide range of host plants, could be harmless for clary sage. Notably, regarding the correlation heatmaps, most of the strong positive correlations between sage biomass (height) or root mycorrhizal rates have been brought to light with potentially phytopathogenic genera, which have to the best of our knowledge not been identified as pathogens towards clary sage.

4.3. Inoculation favoured the establishment of the mycorrhizal symbiosis and consistently shaped the rhizosphere fungal community

Altogether, our results support a successful establishment of the mycorrhizal symbiosis in our experimental conditions, with a low spontaneous root colonization by native AMF depicted by non-zero values for the mycorrhizal rates, even in the NI treatment. Furthermore, a significant progression of the mycorrhizal rates in response to exogenous AMF inoculation during the second year of cultivation was assessed. Regarding plants' growth however, in spite of the consistent clary sage growth in TE-polluted conditions, similar results were obtained between I and NI treatments. Given the fact that clary sage is considered as a xerophytic plant species and requires low nutrient supplies, its nutrient requirements were probably met in our experimental conditions, considering the soil physico-chemical properties (Abbaszadeh et al., 2017; Denoroy et al., 2004; Sharma and Kumar, 2012; Tibaldi et al., 2010). Besides, it has been suggested that AMF inoculation could result in an increased competition between AMF communities (native and exogenous) and could lead to a limited efficiency or to a suppression of the mycorrhizal symbiosis establishment and functioning (Janoušková et al., 2017, 2013). In our case, no such exclusion of native AMF is visible. In fact, the introduced inoculum contained *Rhizophagus irregularis*, while the most abundant ASVs present in both the roots and rhizospheric soil of the inoculated plants have been identified as belonging to AMF genera such as *Glomus* or *Funneliformis*. In that regard, it is not clear whether the root colonization is due to the presence of the introduced mycorrhizal inoculant or to native AMF present in the TE-polluted site. In particular, it should be noted that even though during the second year of cultivation ASVs identified as *R. irregularis* were detected with a low abundance in the clary sage roots, it could be a different strain than the inoculated isolate. In addition, the question arises whether the mycorrhizal symbiosis was functional or not, since clary sage was successfully colonized by AMF. In that regard, a monitoring of the expression for instance of phosphate transporters genes, such as *MTPT4*, *LEPT1* or *STPT3*, often pointed out as markers of the symbiosis function (Karandashov and Bucher, 2005; Paszkowski et al., 2002; Voß et al., 2018), could help addressing this issue.

In our experimental conditions, inoculation clearly shaped the soil fungal communities. In fact, a clear clustering in year 2 between I and NI treatments was highlighted by the PERMANOVA analysis ($P < 0.05$) and observed on the PCoA ordination. It however did not significantly influence the root fungal community. In the same way, similar diversity and richness indexes were determined between I and NI treatments, whatever the monitoring time and the biotope. As previously highlighted, the root exudation of carbon compounds may significantly affect the abundance of soil fungi (Dagher et al., 2019). Moreover, in the presence of the mycorrhizal symbiosis, the host-plant metabolism has been found modified, which may result in variations of the root exudation pattern (Akyol et al., 2019; Ferrol et al., 2016). Thus, a stimulation or a regulation of specific fungal communities through the release of carbonated or chemical compounds by the plants colonized by AMF is likely to occur and hence to shape the fungal communities (Akyol et al., 2019; Misra et al., 2019). Likewise, the direct release of compounds by the AMF hyphae can result in a shift of the associated fungal communities. It is hence not surprising to observe an effect of inoculation on soil fungal communities' structure. In particular, some fungal taxa belonging to *Ascomycota*, such as *Nectria* or *Leptosphaeria* genera, were previously established to be associated with AMF mycelia, which could go along with the prevalence of *Ascomycota* observed in our experimental conditions (Hijri et al., 2002; Iffis et al., 2016, 2014). It is also noteworthy that the drop in terms of both ASV number and of fungal richness and diversity, observed in soil between year-1 and year-2, was also less pronounced under inoculation. This may support the idea that in the presence of the mycorrhizal symbiosis, the host plant as well as the associated microbial communities could be more resilient regarding both biotic and abiotic stresses (Bi et al., 2018; Griffiths and Philippot, 2013).

4.4. The AMF community changes over time under clary sage cultivation but was not altered by exogenous inoculation

It should first be noted that a very limited proportion (< 0.5 %) of the identified fungal ASVs using the primers targeting the ITS region were attributed to the *Glomeromycota* phylum, which demonstrates that ITS primers are not appropriate for determining AMF diversity. Thus, the use of specific primer pairs targeting the 18S rRNA gene (SSU) appeared relevant to increase the accuracy of the AMF species' detection and identification.

No significant effect of the mycorrhizal inoculation as an explaining parameter of the community structure was highlighted, whatever the biotope. A similar result has previously been demonstrated using the same mycorrhizal inoculant as in this work, under unpolluted field conditions, where the authors suggested a co-evolution of the AMF community in the presence of *R. irregularis* (Renaut et al., 2020). However, in their experimental conditions, *R. irregularis* was naturally present in the soil, which is not the case in our TE-polluted site. In fact, contrasted results regarding the effects of AMF inoculation on the native AMF community have been brought forward, depicting a context-dependant explanation, particularly in stressful or disturbed conditions (Hart et al., 2017). As previously mentioned, in our case, the introduction of the mycorrhizal inoculant did not result in a complete replacement or modification of the native AMF community and hence may not have resulted in drastic community changes.

Interestingly however, our results have demonstrated that clary sage cultivation significantly shaped the AMF community in the plant roots ($P < 0.01$). Our results also support the same trend as previously reported, namely the prevalence of the AMF taxa *Glomeraceae* and a higher number of AMF ASVs identified in roots and specific to this biotope (Saks et al., 2014; Stefani et al., 2020; Varela-Cervero et al., 2015). This is notably explained by a lower AMF biomass in soil, even in the rhizosphere, compared to that in root tissues, depicting the importance of screening both root and soil biotopes (Stefani et al., 2020; Varela-Cervero et al., 2015). Remarkably, 69 distinct AMF ASVs belonging to 12 AMF genera were identified in our conditions, with the prevalence of *Funneliformis* and *Glomus* genera. Hence, in spite of the TE-pollution and the past agricultural practices, demonstrated as key drivers of the microbial community structure, a relatively high number of AMF species was detected in our TE-polluted site (Chen et al., 2014; Njeru et al., 2015; Wang et al., 2019). The prevalence in terms of relative abundance of native AMF species could even suggest a long-term adaptation of these species to the TE-polluted context, which could hence be more suited to survive and colonize plants' roots in comparison with the exogenous species. Besides, while the effects of the AMF community on the plant diversity have been well covered, the reverse effect of plant identity on the AMF community structure and temporal evolution has been less explored (Johnson et al., 2003). Nonetheless, several reports pointed out the influence of the host plant on the composition of the AMF community, especially in the roots (Helgason et al., 2002; Husband et al., 2002; Johnson et al., 2003). Notably, our results support a strong temporal shift. This trend is in accordance with earlier reports, as previously demonstrated, since the AMF ASVs present in the newly germinated seedlings were almost entirely replaced the following year by previously scarcely represented ones (Husband et al., 2002). As previously suggested for the fungal community, the presence of aromatic compounds in the rhizosphere of clary sage could also exert a selective pressure on the AMF community, resulting in the observed temporal shift.

5. Conclusion

The main purposes of this field study were to analyse the fungal communities' structure in the rhizosphere of clary sage cultivated on a TE-polluted soil, in the presence of an introduced mycorrhizal

inoculum, and to monitor their temporal evolution over a two-year cultivation cycle, starting from an unvegetated soil.

Our results indicate that in our experimental conditions, both clary sage cultivation and mycorrhizal inoculation, investigated as structuring factors, significantly shaped the fungal communities in the aromatic plant's rhizosphere. Notably, it is believed that the cultivation of aromatic plants such as clary sage, releasing root exudates and in particular aromatic compounds in their rhizosphere, may be responsible for the temporal evolution of the rhizospheric fungal communities, along with environmental and edaphic parameters. Furthermore, beside shaping the fungal communities, the introduction of an exogenous mycorrhizal inoculum favoured the *in situ* colonization of clary sage roots by AMF. However, it is not clear whether the exogenous inoculum was directly responsible for the root colonization or if it exerted a stimulating effect on the native AMF community. Hence, one of the future prospects is the specific monitoring of the introduced inoculum isolate, as developed by Badri et al., (2016). In more details, a qPCR assay was designed, by using specific primers and probes to detect and quantify spores of the *R. irregularis* isolate, hence providing a robust and reliable molecular detection toolkit. Our findings also show the prevalence of *Ascomycota* and *Basidiomycota* in the TE-polluted soil, which were previously demonstrated to withstand the presence of TE in soils. In addition, given the number of identified AMF species in our TE-polluted site, our data suggest that quite a few AMF species could be isolated for further prospects. Notably, if it is well-known that AMF can bear the presence of TE in soils and contribute to their immobilization, their specific response to increasing levels of TE could be relevant to investigate, in view of future potential applications as part of phytomanagement approaches. Further investigations are also required regarding the long-term evolution of the fungal communities. In fact, if the ecosystem functioning may not be affected or in a positive way in response to aromatic plant cultivation and mycorrhizal inoculation, owing to the microbial function redundancy, it might also result in important functional losses. Thus, a long-term assessment of structural and functional evolution over time could be a useful tool to assess and ensure both the success of the phytomanagement channel as well as the ecosystem functioning.

Author Contributions

Conceptualization – methodology – validation, R. Raveau, J. Fontaine, M. Hijri, A. Lounès-Hadj Sahraoui; writing – original draft preparation, R. Raveau; writing – review and editing, R. Raveau, J. Fontaine, M. Hijri, A. Lounès-Hadj Sahraoui; supervision, A. Lounès-Hadj Sahraoui. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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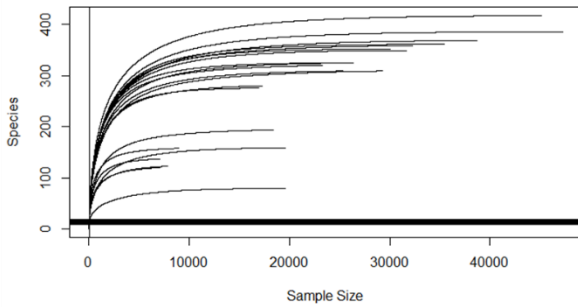
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Supplemental Data

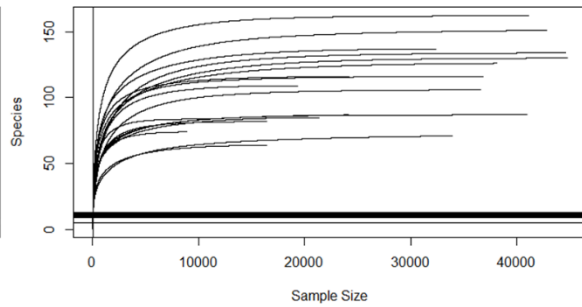
Table S1: Descriptive results of Illumina MiSeq sequencing, followed by the step-by-step bioinformatic processing, for the fungal ITS and 18S rRNA genes datasets.

	Fungi		AMF	
	Soil	Root	Soil	Root
No sequences (sum)	1558856	1403165	1553389	1193261
No sequences (mean)	70857	73851	62136	62803
No seq. filtered (mean)	39754	48470	50240	50838
No seq. merged (mean)	26342	32428	39178	46879
No seq. merg. no chimeras (mean)	25241	29877	19053	32241
No samples	25	20	25	20
No ASV (sum)	1239	569	20	61
ASV per sample (mean)	279	107	4	13

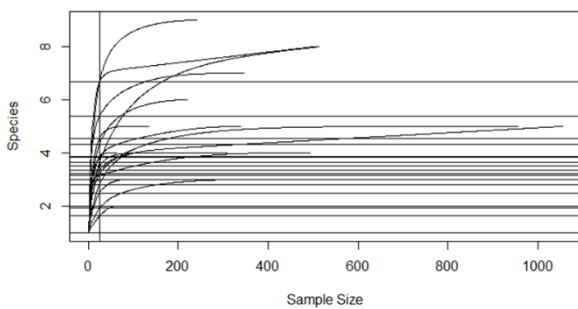
A. Soil biotope (ITS)



B. Root biotope (ITS)



C. Soil biotope (18S)



D. Root biotope (18S)

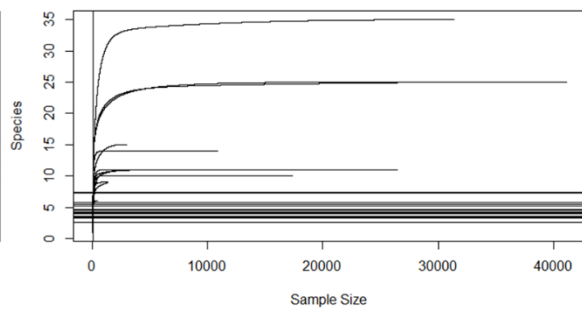
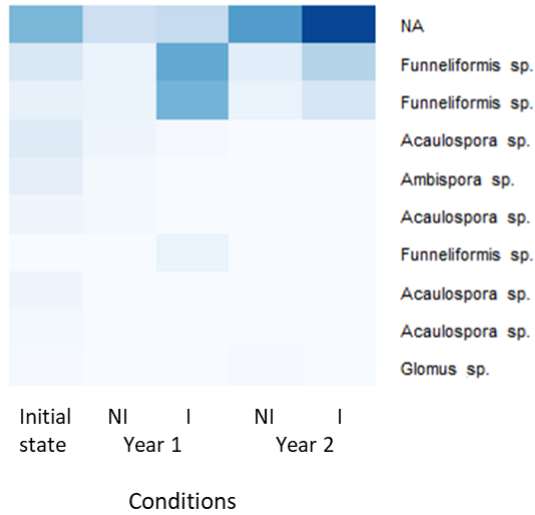


Figure S1: Rarefaction curves obtained for the ITS marker (fungi - A. and B.) and for the 18S rRNA gene (AMF - C. and D.), for the soil (A. and C.) and root (B. and D) biotopes, involving respectively 25 and 20 samples.

A. Soil biotope



B. Root biotope

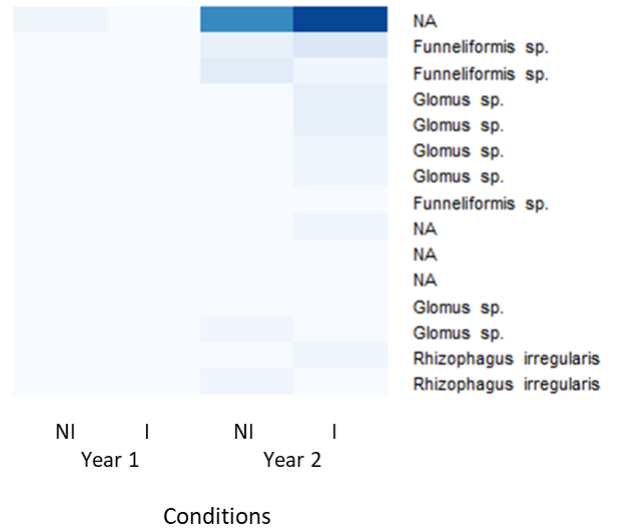
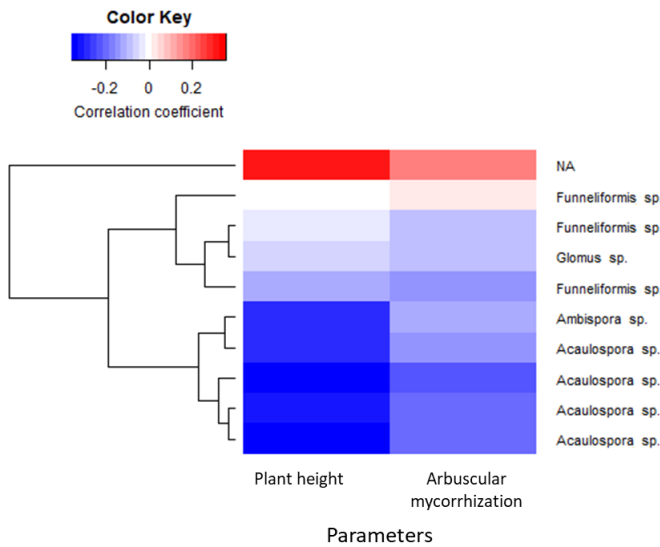


Figure S3: Heatmap representation based on the abundance of the 10 and 15 most represented ASVs from the 18S rRNA gene dataset identified in the rhizospheric soil (A.) and root (B.) biotopes respectively. NI: non-inoculated; I: inoculated; NA: non-assigned.

A. Soil biotope



B. Root biotope

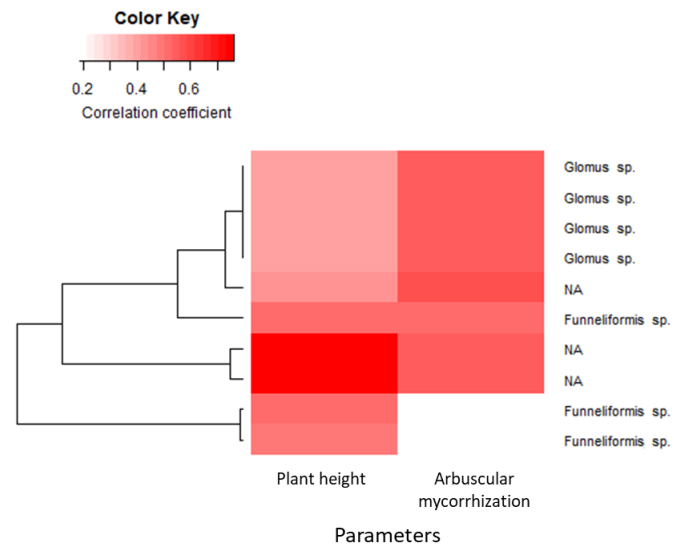


Figure S4: Heatmap representation based on the correlation coefficients between clary sage height or arbuscular mycorrhization and the 10 most represented ASVs from the 18S rRNA gene dataset in soil (A.) and root (B.) biotopes respectively. NA: non-assigned.

Discussion générale
et
perspectives

Discussion générale et perspectives

I. Enjeux de la filière PPAM-HE dans un contexte de phytomanagement de sols pollués par les ET

1. Caractère innovant de l'approche de phytomanagement via la filière PPAM-HE

L'ensemble des travaux réalisés lors de ma thèse et synthétisés dans ce manuscrit s'inscrivent dans le cadre du projet PhytEO (APR Graine 2016, financé par l'ADEME). Ce dernier vise à proposer un mode de gestion durable des sols pollués dans un contexte de phytomanagement, basé sur l'utilisation d'une phytotechnologie, la phytostabilisation aidée, combinée à une valorisation de la biomasse produite, afin d'engager la reconversion de sols agricoles pollués par les ET et de pérenniser l'agriculture locale. L'originalité de ce projet, qui s'appuie sur un dispositif expérimental *in situ* (à l'échelle de l'hectare), repose sur le développement d'une filière éco-innovante et non-alimentaire de culture de PPAM pour la production d'HE à activités biologiques.

Ma contribution au projet PhytEO se situe dans (i) la participation à la mise en place du dispositif expérimental *in situ* (deux parcelles agricoles polluée ou non à l'échelle de l'hectare), (ii) la caractérisation des sites notamment d'un point de vue biologique, (iii) le choix et le suivi de la croissance des différentes espèces de PPAM ainsi que dans les étapes de semis et de récolte, (iv) l'étude de la composition des HE distillées, (v) la détermination de certaines de leurs propriétés biologiques, en vue d'usages en protection des cultures ou en santé humaine, (vi) l'étude de l'impact de la culture de PPAM sur le microbiote rhizosphérique et (vii) la réflexion sur la viabilité économique de la filière PPAM-HE.

A l'heure actuelle, la majorité des filières de valorisation de la biomasse produite sur des sols pollués par les ET se tourne vers une utilisation à des fins énergétiques (combustion, méthanisation, pyrolyse, ...) ou pour l'élaboration d'écomatériaux (Bernal et al., 2019; Bert et al., 2017; Chalot et al., 2020). Dans la même optique, la culture de PPAM peut être suggérée comme une nouvelle option qui pourrait présenter une rentabilité élevée, grâce à la haute valeur ajoutée que présentent certaines HE (Pandey et al., 2019). Par ailleurs, de par la diversité de leurs propriétés biologiques dues à la richesse et à la nature de leurs composés, ces HE peuvent être utilisées dans de nombreux domaines à vocation non-alimentaire, tels que les industries cosmétiques, pharmaceutiques et phytosanitaires (Muchembled et al., 2017; Raveau et al., 2020a). A notre connaissance, l'utilisation des PPAM et la production d'HE dans le cadre d'une approche de phytomanagement ont rarement été décrites. Aucune étude de faisabilité technico-économique de la filière PPAM-HE n'a jusque-là fait l'objet de travaux approfondis.

2. Mobilisation de deux parcelles agricoles expérimentales à l'échelle de l'hectare

Dans le cadre du projet PhytEO, deux parcelles expérimentales de 2 ha chacune, toutes deux situées en Hauts-de-France, ont été mobilisées. La première, localisée à Evin-Malmaison, se trouve à 600 m environ de l'ancienne usine métallurgique Metaleurop Nord et est située dans la zone 3, à la limite supérieure de la zone 2 du périmètre défini autour du site Metaleurop (Figure 2). La seconde parcelle, située à Rodelinghem, est non polluée (d'après les données de la base ASPITET de l'INRA).

Dans le cadre de ce travail, un amendement biologique commercial à base d'un CMA, *R. irregularis*, a été utilisé (inoculum mycorhizien AGTIV®, Premier Tech). Son intérêt a été notamment évalué en termes d'effets sur la croissance et la biomasse des PPAM testées, sur la quantité et sur la qualité des HE produites. Le choix de cet inoculum a été motivé par l'existence d'amorces spécifiques, conçues afin de pouvoir suivre cet isolat en particulier (Badri et al., 2016) et par sa formulation sous forme de

poudre bien adaptée aux grandes cultures, puisqu'il peut être mélangé directement à la semence au moment des semis (poudrage). Ainsi, le dispositif s'appuie pour chaque site expérimental (pollué ou non par les ET) sur deux conditions, non-inoculée ou inoculée par le CMA. Dans le cadre du projet PhytEO, les effets de l'inoculum sur la mobilité et la biodisponibilité des ET dans le sol ainsi que leur transfert dans les plantes ont également été évalués en collaboration avec Valérie Bert (INERIS).

II. Etat des lieux et conditions préliminaires pour la mise en place d'une démarche de phytomanagement sur un sol pollué par les ET

1. Caractérisation physico-chimique initiale des sites expérimentaux

La première étape incontournable pour tout projet de phytomanagement passe par une caractérisation des conditions climatiques, physico-chimiques et biologiques du site. Ainsi, la nature des polluants, leur concentration, l'hétérogénéité éventuelle de la pollution au sein de la parcelle ainsi que les caractéristiques pédo-climatiques du site sont des éléments indispensables à intégrer dans le choix de la méthode de gestion d'un site pollué.

Tout d'abord, il est à noter que les deux parcelles expérimentales choisies pour notre étude sont localisées dans la même zone climatique (Csb – classification de Köppen-Geiger), caractérisée par un climat tempéré avec un été doux.

La caractérisation physico-chimique initiale des sites a notamment permis de vérifier l'absence de pollution par les ET sur la parcelle localisée à Rodelinghem, avec des teneurs respectives en Cd, Pb et Zn de 0,4; 22 et 48, similaires au bruit de fonds géochimique des sols agricoles régionaux (Sterckeman et al., 2007). Elle a aussi confirmé l'ampleur de la pollution en ET sur le site d'Evin-Malmaison, avec les teneurs suivantes : 7 ppm de Cd, 394 ppm de Pb et 443 ppm de Zn. En comparaison avec le fonds géochimique régional, ces teneurs sont respectivement 17, 11 et 6 fois supérieures (Sterckeman et al., 2007). La réalisation d'une cartographie détaillée du site, permettant de visualiser la répartition de la pollution sur le site en fonction des différents ET analysés (As, Cd, Cr, Cu, Ni, Hg, Pb, Sb et Zn) reste à réaliser. Une bonne fertilité chimique des sols a également été démontrée en comparaison avec des valeurs de référence (Denoroy et al., 2004), et ce en particulier sur la parcelle polluée par rapport à la parcelle non polluée, avec notamment des valeurs supérieures pour la capacité d'échange cationique (resp. 183 et 99 me.Kg⁻¹), la teneur totale en azote (resp. 1,62 et 1,18 g.Kg⁻¹), le carbone organique total (resp. 17 et 10,2 g.Kg⁻¹), la teneur en matière organique (resp. 29 et 17 g.Kg⁻¹) ainsi que le ratio C/N (resp. 10,5 et 8,6). La teneur en phosphore est quant à elle supérieure sur la parcelle non polluée (0,65 g.Kg⁻¹) par rapport à la parcelle polluée (0,17 g.Kg⁻¹), représentant une teneur élevée en comparaison à des valeurs de référence pour les cultures peu exigeantes (Comifer, 2019; Denoroy et al., 2004) (*Publication 1 - Raveau et al., 2020, à soumettre*).

2. Caractérisation de la flore native du site pollué par les ET

Un inventaire phytosociologique a également été réalisé sur la parcelle polluée selon la méthode décrite par Delassus, (2015). Cet inventaire a permis 1) d'identifier des espèces végétales se développant spontanément sur notre site pollué et donc probablement tolérantes aux ET, 2) de déceler la présence d'autres espèces aromatiques et 3) d'orienter le choix des espèces végétales à cultiver afin de limiter certaines contraintes liées aux pratiques culturales. En raison du spectre d'action des herbicides, ce dernier paramètre était important à vérifier afin de pouvoir contrôler le développement des espèces adventices présentes tout en évitant des effets non-intentionnels sur les plantes cultivées choisies.

Ainsi, 24 espèces végétales, appartenant à 14 familles botaniques différentes, ont été identifiées sur la parcelle polluée par les ET. Parmi elles, *Erigeron canadensis*, *Matricaria recutita*, *Senecio vulgaris*, *Erysimum* sp., *Lolium perenne*, *Festuca* sp., *Urtica dioica* L. et *Rumex* sp. avaient précédemment été retrouvées sur d'autres sites présentant une pollution par les ET (Dazy et al., 2008; Hajiboland and Manafi, 2007; Jeannin et al., 2020; Masarovičová et al., 2010). De façon intéressante, *M. recutita* avait également été caractérisée pour son comportement accumulateur vis-à-vis du Cd (Masarovičová et al., 2010). Cet inventaire pourrait s'avérer particulièrement intéressant pour sélectionner des espèces végétales non cultivées et potentiellement adaptées à la gestion des sites pollués par les ET. Certaines espèces natives pourraient en effet posséder un comportement « exclure », intéressant en phytostabilisation, tandis que des accumulateurs, voire hyperaccumulateurs pourraient s'avérer utiles pour la phytoextraction des ET.

Il est aussi important de signaler que parmi les espèces végétales identifiées, seule la matricaire camomille (*M. recutita*) est une PPAM et pourrait poser des problèmes de contamination des HE si elle est récoltée en même temps que la PPAM cultivée (modification de la composition des HE, couleur anormale de l'HE). Ainsi, des précautions au moment de la récolte ont été observées, notamment en élevant la barre de coupe, afin d'éviter la contamination des HE par les composés aromatiques de la matricaire. Par ailleurs, aucune des plantes identifiées n'appartient à la même famille botanique que les PPAM sélectionnées dans le projet PhytEO (*Apiaceae* ou *Lamiaceae*). En termes de richesse, les indices de Menhinick et Margalef mesurés indiquaient respectivement des valeurs de 1,09 et 3,72. Les indices de diversité calculés, de Shannon & Weaver et de Simpson respectivement, étaient eux de 2,15 et 0,85. Ces indices montrent que, malgré la présence des ET dans le sol, le site d'Evin-Malmaison possède une biodiversité végétale relativement élevée en comparaison avec d'autres sols agricoles, pollués ou non, décrits dans la littérature (Chateil et al., 2013; Dazy et al., 2008; Fédoroff et al., 2005; Koptsik et al., 2003; Zhou et al., 2018). En effet, la biodiversité est souvent négativement corrélée avec des teneurs croissantes en ET (Hernández and Pastor, 2008; Koptsik et al., 2003; Vangronsveld et al., 1996), ce qui ne semble pas être le cas dans nos conditions. Il est toutefois suggéré qu'au-delà de la teneur en ET des sols, les caractéristiques pédo-climatiques ainsi que l'activité microbienne du sol jouent également un rôle important dans la biodiversité végétale (Hernández and Pastor, 2008; Koptsik et al., 2003). Dans le cas de nos sites expérimentaux, une bonne fertilité chimique des sols a en effet été établie, avec notamment une bonne disponibilité des nutriments.

3. Caractérisation microbiologique du sol

La caractérisation de la structure et de la diversité des communautés bactériennes et fongiques, naturellement présentes dans le sol de la parcelle expérimentale polluée par les ET, a été réalisée avant végétalisation par les PPAM. Nos résultats ont montré que les phyla bactériens *Actinobacteria* et *Proteobacteria* et les phyla fongiques *Ascomycota* et *Basidiomycota* sont omniprésents dans le sol de cette parcelle (*Publications 3 et 4 - Raveau et al., 2020*), corroborant ce qui a été obtenu dans le cadre d'autres études conduites sur des sols agricoles pollués ou non (Bourceret et al., 2016; Foulon et al., 2016b; Liang et al., 2020; Pacwa-Płociniczak et al., 2018). A noter également que les indices de richesse et de diversité déterminés dans le cadre de cette étude sont similaires voire supérieurs en comparaison à d'autres études sur la revégétalisation de sols pollués, dans un contexte agricole ou non (Foulon et al., 2016a; Tipayno et al., 2018; Xie et al., 2016). Toutefois, la comparaison de la diversité alpha entre des sites expérimentaux éloignés est délicate, car elle est fortement dépendante des conditions environnementales du site et en particulier du contexte climatique, des paramètres édaphiques et des pratiques agricoles (Wang et al., 2019b, 2018). Parmi les phyla majoritaires identifiés, de nombreux genres ont précédemment été décrits pour leur tolérance aux ET dans les sols et il n'est donc pas surprenant de les retrouver dans ces conditions particulières. C'est notamment le cas pour les genres

bactériens *Bacillus*, *Pseudomonas*, *Streptomyces* ou *Sphingomonas* (Álvarez et al., 2013; Chellaiah, 2018; Chen et al., 2014a; Cui et al., 2018) et les genres fongiques saprotrophes *Gibellulopsis*, *Fusarium* et *Tetracladium*, dont la présence a été montrée même dans des niveaux de pollution par les ET très élevés (Bukharina et al., 2018; Lin et al., 2020). Il est aussi intéressant de noter qu'avant végétalisation de ce sol par les PPAM, 12 ASV de CMA appartenant au phylum *Glomeromycota* et notamment aux familles *Acaulosporaceae* et *Glomeraceae* ont été identifiés. Cette diversité peut être considérée comme relativement importante eu égard à la présence des ET dans le sol (Chen et al., 2014a; Njeru et al., 2015; Wang et al., 2019a) et peut dénoter une adaptation de ces souches à la pollution historique du sol (Bourceret et al., 2016).

Cependant, l'isolement et le dénombrement des spores de CMA présentes dans les sols (143 et 157 spores/ 100 g de sol sur les parcelles polluée et non polluée respectivement) ainsi que la détermination des taux de colonisation racinaire des espèces végétales qui se développent spontanément sur le site ont toutefois révélé des valeurs relativement faibles (taux de mycorhization de 4 et 7 % sur les parcelles polluée et non polluée respectivement), comparativement à d'autres sols agricoles pollués ou non. En effet, les valeurs de densités de spores de CMA dans les sols varient habituellement entre 300 et 1400 spores/100 g de sol (Oehl et al., 2004; Yang et al., 2015; Zak et al., 1982). Ainsi, les valeurs relativement faibles obtenues et similaires entre les deux parcelles expérimentales, pourraient être attribuées aux pratiques culturales des sols agricoles précédant la mise à disposition des parcelles (travail du sol, utilisation de produits phytosanitaires et d'intrants chimiques) et qui auraient provoqué une diminution de la richesse et probablement de la diversité des populations mycorhiziennes (Bünemann et al., 2006; Tilston et al., 2010).

Ainsi, ces premiers résultats justifient l'intérêt de l'ajout d'un inoculum mycorhizien, décrit pour sa capacité à stimuler la croissance des plantes en améliorant leur nutrition, en réduisant l'impact des stress environnementaux et en participant dans certains cas à l'immobilisation des ET dans le sol (Renaut et al., 2020; Smith and Read, 2008).

4. Choix des espèces végétales

L'installation d'un couvert végétal, adapté au contexte de la pollution des sols, est un enjeu important dans la gestion d'un site pollué. Le choix des espèces végétales candidates pour le phytomanagement est donc essentiel et doit s'appuyer sur un certain nombre de critères (Mench et al., 2010) :

- Adaptation aux conditions pédo-climatiques
- Capacité à se développer rapidement et à établir un couvert végétal pérenne
- Capacité à tolérer la présence des ET, voire à les immobiliser dans le cas de la phytostabilisation
- Stratégie de gestion du site en fonction de l'usage à moyen et long terme
- Considérations économiques (coût de mise en œuvre, potentiel de valorisation de la biomasse végétale produite) et pratiques (prix et disponibilité de la semence et du matériel agricole, itinéraire technique et quantité d'intrants à apporter, etc)

Dans le cadre de ces travaux, trois PPAM ont été retenues : il s'agit de la coriandre (*Coriandrum sativum* L. - Apiacées), de la sauge sclarée (*Salvia sclarea* L. - Lamiacées) et de l'angélique (*Angelica archangelica* – Apiacées). Le choix s'est porté sur trois espèces végétales présentant des cycles de développement distincts. La coriandre est une espèce annuelle à croissance rapide et donc capable d'établir en quelques semaines un couvert végétal sur le site (Ghobadi and Ghobadi, 2010). La sauge sclarée possède un cycle de vie bisannuel, fleurit abondamment la deuxième année et peut rester trois ans sur la parcelle (Grigoriadou et al., 2020; Tibaldi et al., 2010). L'angélique est pérenne, fleurissant

également à partir de la deuxième année de culture et peut être cultivée pendant une dizaine d'années sur la parcelle (Chauhan et al., 2016). Alors que dans le cas de la sauge, ce sont les inflorescences (ou sommités florales) qui sont récoltées et distillées (Aćimović et al., 2018; Kumar et al., 2013), dans le cas de la coriandre, ce sont les graines ou les parties aériennes (tiges et feuilles) qui peuvent être utilisées (Satyal and Setzer, 2020). Pour l'angélique, ce sont aussi les graines qui sont récoltées et distillées (Chauhan et al., 2016). La valorisation économique occupant une place importante dans une démarche de phytomanagement et la finalité étant de proposer aux agriculteurs une filière de reconversion adaptée à leur système d'exploitation, le choix des plantes a également tenu compte du coût et de la disponibilité des semences, des contraintes culturales associées à la plante mais aussi de l'attractivité de ces HE pour le producteur d'HE (Ferrant PHE), partenaire du projet PhytEO. Les besoins nutritionnels des plantes ainsi que l'éventuel impact des ET sur le développement de ces espèces végétales ont également guidé notre réflexion sur le choix des PPAM à retenir pour notre étude. Il a en effet été précédemment rapporté que la réponse vis-à-vis du stress induit par la présence des ET au niveau du sol ainsi que la capacité à immobiliser les ET et par conséquent à réduire leur biodisponibilité dépendait fortement de l'espèce végétale (Burgess et al., 2018; Mench et al., 2010). L'intérêt des deux familles de plantes et en particulier des Lamiacées, avec notamment les genres *Mentha*, *Ocimum*, *Lavendula* ou encore *Rosmarinus*, pour la gestion des sols a déjà été mis en avant (Affholder et al., 2013; Angelova et al., 2015; Anwar et al., 2016; Zheljazkov et al., 2008), principalement en raison de leur tolérance aux ET. Toutefois, les PPAM considérées dans le cadre de ce projet ont rarement été étudiées dans une approche de phytomanagement. En effet, si plusieurs travaux se sont intéressés à la capacité de la sauge sclarée ou de la coriandre à accumuler les ET, les résultats sont contrastés et ne permettent pas de conclure quant à leur potentiel pour la phytostabilisation (Angelova et al., 2016; Chand et al., 2015; Petrescu et al., 2015; Zheljazkov et al., 2008).

III. La culture des PPAM, une option prometteuse pour le phytomanagement des sols pollués par les ET

1. La coriandre et la sauge sclarée, deux PPAM capables de se développer sur des sols fortement pollués par les ET

En premier lieu, le pouvoir germinatif des semences a été vérifié au laboratoire sur différents substrats, avant semis. Alors que les taux de germination étaient relativement élevés pour la sauge et la coriandre (90 et 70 % respectivement), ils étaient inférieurs à 40 % pour l'angélique.

Une fois semées, les PPAM ont ensuite fait l'objet d'un suivi régulier *in situ*. Pour rappel, la coriandre cultivée pour ses parties aériennes ainsi que la sauge sclarée ont été semées au printemps 2017 sur les deux parcelles expérimentales, polluée ou non par les ET. La coriandre a également été semée en avril 2018 pour une seconde année de culture donnant lieu à la récolte des graines. L'inoculum commercial testé dans le cadre de cette étude, sous forme de poudre, a été introduit au moment du semis, directement mélangé avec la semence.

Concernant l'angélique, le faible pouvoir germinatif de la semence ainsi que des conditions climatiques particulières, avec notamment un été 2017 plus chaud et plus sec que la normale (Archives climatiques – Infoclimat, 2018), ont conduit à un très faible taux de levée. Cette espèce végétale a par conséquent été ressemée au printemps 2018 en utilisant de nouvelles semences présentant un taux de germination, plus élevé (supérieur à 50 % - résultats non montrés). La levée a cependant été avortée en raison de conditions climatiques à nouveau exceptionnelles pour l'été 2018 (Archives climatiques – Infoclimat, 2019). Un dernier semis a été réalisé en septembre 2018, avec les mêmes semences, et a

donné lieu à des taux de levée satisfaisants, compris entre 30 et 40 % (résultats non montrés). L'angélique s'est ensuite bien développée. Cependant, du fait de sa floraison en deuxième année, la récolte des graines n'a pu avoir lieu que fin juin 2020. De par son caractère pérenne et un marché porteur pour la commercialisation de l'HE, cette PPAM semble prometteuse. Les données, en cours d'analyse, n'ont toutefois pu être traitées dans le cadre de ce manuscrit et feront l'objet de futurs travaux.

Concernant la coriandre, nos résultats ont montré que les taux de levée variaient entre 50 et 84 %, sans différence significative entre les deux parcelles expérimentales polluée et non polluée, quelle que soit l'année de culture et la modalité inoculée ou non. En termes de croissance, la hauteur des plants de coriandre avant récolte des parties aériennes (2017), atteignait en moyenne 60 cm, quelle que soit la parcelle, pour des poids secs de 10 et 4 g/plante, sur les parcelles non polluée et polluée respectivement, quelles que soient les modalités inoculées ou non. Au stade graine (2018), la hauteur des plantes était de 77 cm en moyenne, tandis que les poids secs s'élevaient à 11 et 7 g/plante, sur les parcelles non polluée et polluée respectivement, quelles que soient les modalités inoculées ou non (*Publication 1 - Raveau et al., 2020, à soumettre*). La quantité de biomasse produite sur les parcelles expérimentales était en moyenne de 22 m³/ha lors de la récolte des parties aériennes, et de 4 m³/ha lors de la récolte des graines (résultats non montrés). Ces estimations ont été établies lors de la distillation des HE. Une pesée de la biomasse n'a pu être réalisée, représentant un transport supplémentaire de la biomasse pour le distillateur.

⇒ **La germination ainsi que la levée de la coriandre ne sont pas affectées par la présence des ET dans le sol.**

Cependant, la croissance de la plante ainsi que la production de biomasse étaient légèrement plus faibles sur la parcelle polluée par les ET. Ces différences observées sont davantage le résultat de la présence d'espèces adventices envahissantes ayant affecté le développement et la survie des plantes cultivées et de la coriandre en particulier, ainsi que des caractéristiques physico-chimiques des sols entre les deux sites expérimentaux.

En ce qui concerne la sauge sclarée, des taux de levée plus importants de 30 % ont été mesurés sur la parcelle polluée, par rapport à la parcelle non polluée. Ces valeurs plus élevées peuvent s'expliquer notamment par des différences pédo-agronomiques entre les deux parcelles ou de capacité de rétention en eau. Sur la parcelle non polluée, le sol se sature notamment plus rapidement en eau, ce qui peut bloquer la germination des graines de sauge sclarée, de très petite taille, en les asphyxiant. La sauge sclarée affichait une hauteur moyenne de 35 cm la première année, de 153 cm la deuxième année et de 107 cm la troisième année, sans différence significative entre les deux parcelles ni entre les modalités inoculées ou non. En termes de poids secs, les valeurs mesurées étaient comprises entre 50 et 160 g/plante et entre 52 et 170 g/plante sur les parcelles non polluée et polluée respectivement. Des valeurs plus faibles ont été observées sur la parcelle polluée en deuxième année de culture, ce qui peut s'expliquer par la densité plus importante de plantes au mètre carré, dénotée par les taux de levée supérieurs, ce qui a pu limiter le développement en largeur de la plante (*Publication 1 - Raveau et al., 2020, à soumettre*). En termes de biomasse, 15, 106 et 42 m³/ha ont été récoltés en moyenne la première, deuxième et troisième année, respectivement (résultats non montrés).

⇒ **La sauge sclarée est capable de se développer sur des sols pollués, de la même façon que sur des sols non pollués par les ET. La germination des graines, la levée, le développement ainsi que la production de biomasse n'ont en effet pas été affectés par la présence des ET dans le sol.**

Ces résultats montrent que la sauge sclarée et la coriandre, testées dans le cadre de cette étude, semblent bien adaptées pour une culture sur des sols pollués par les ET, suggérant leur tolérance aux ET. Cependant les mécanismes moléculaires impliqués dans la tolérance de ces PPAM aux ET n'ont pas été étudiés.

C'est pourquoi, une étude transcriptomique ciblant l'expression de gènes codant par exemple pour des méthallothionéines ou des phytochélatines, agents de chélation pouvant jouer un rôle important dans la séquestration des ET et la tolérance des plantes aux ions métalliques (Chen et al., 2014b; Jain et al., 2018), pourrait être conduite dans le futur. Certaines enzymes clés du système antioxydant, telles que les superoxyde dismutases ou les peroxydases, dont l'induction peut être causée par la présence des ET (Dazy et al., 2009; Lin et al., 2015; Ling et al., 2011), pourraient également être suivies. Le cas de l'angélique sera aussi évalué a posteriori, en fonction des données collectées au cours des deux années de culture et des résultats en cours de traitement.

2. Intérêt de l'apport d'un inoculum mycorhizien pour la croissance des PPAM et la production de biomasse

De nombreux travaux ont démontré l'utilité de la symbiose mycorhizienne dans les agroécosystèmes, pour sa capacité à promouvoir la croissance des plantes en améliorant leur nutrition minérale et hydrique, en augmentant la tolérance des plantes aux stress environnementaux et en contribuant à l'immobilisation des ET au niveau de la rhizosphère (Begum et al., 2019; Ferrol et al., 2016; Garg et al., 2017).

Dans nos conditions expérimentales *in situ*, une mycorhization spontanée des espèces végétales natives et cultivées a été observée, sur les deux sites expérimentaux. Cette mycorhization spontanée est toutefois relativement faible, comprise entre 3 et 7 % de colonisation racinaire. L'ajout d'un inoculum mycorhizien a permis d'augmenter significativement les taux de colonisation racinaire des deux espèces de PPAM testées. En effet, les taux de mycorhization mesurés sur la coriandre au stade « parties aériennes », cultivée en l'absence de l'inoculum mycorhizien, étaient de 8 et 2 % sur les parcelles non polluée et polluée respectivement et passent à 22 et 9 % en conditions inoculées ([Publication 1 - Raveau et al., 2020, à soumettre](#)). Pour la coriandre au stade « graine », une augmentation des taux de mycorhization en conditions inoculées a seulement été observée sur la parcelle non polluée, avec des valeurs de 3 et 13 % pour les conditions non-inoculée et inoculée respectivement. Pour la sauge sclarée, une amélioration significative des taux de mycorhization pour la modalité inoculée par rapport à celle non inoculée a été observée en deuxième (augmentation de 50 %) et troisième (augmentation de 25 %) années de culture. De plus, une augmentation progressive de la colonisation racinaire a été observée au cours du temps pour la modalité inoculée, avec des taux de mycorhization qui ont atteint 40 % en 3^{ème} année de culture alors qu'ils étaient de l'ordre de 5 % en première année.

Cependant, l'augmentation des taux de mycorhization chez ces deux PPAM n'a pas permis d'observer une amélioration de la croissance des plantes et de la production de biomasse. Plusieurs hypothèses peuvent être émises pour expliquer les résultats observés. En premier lieu, la mycorhization spontanée des PPAM par les CMA indigènes peut masquer l'apport de l'inoculation mycorhizienne. Par ailleurs, s'il est connu que les CMA sont capables d'établir une relation symbiotique avec la majorité des végétaux supérieurs (Smith and Read, 2008) et qu'ils possèdent une spécificité d'hôte végétal relativement faible (Lee et al., 2013; Muneer et al., 2019; Smith and Read, 2008), il a toutefois été montré que la réponse de la plante hôte à la colonisation racinaire par les CMA dépendait fortement de l'espèce mycorhizienne (Lee et al., 2013). Ainsi, il est possible que l'espèce de CMA contenue dans

l'inoculum commercial utilisé ne soit pas la mieux adaptée aux PPAM cultivées. Il est également décrit dans la littérature qu'un inoculum exogène peut entrer en compétition avec les espèces indigènes naturellement présentes dans le sol, exerçant une pression sélective sur les espèces de CMA autochtones, réduisant ainsi la colonisation racinaire (Janoušková et al., 2017). Enfin, la souche de CMA contenue dans l'inoculum mycorhizien utilisé a été isolée à l'origine à partir de sols agricoles non pollués par les ET, puis cultivée *in vitro* à partir de racines modifiées de carottes (Badri et al., 2016). A cet égard, la pollution du sol de la parcelle d'Evin-Malmaison par les ET est susceptible d'avoir affecté négativement la souche de *R. irregularis*.

*Afin de vérifier ces hypothèses, le suivi moléculaire de l'inoculum commercial introduit, à la fois au niveau du sol et des racines de sauge sclarée, permettrait d'apporter des éléments de réponse quant à la capacité de cette souche à survivre dans le sol pollué par les ET et à coloniser les racines de la sauge sclarée. Ce suivi pourrait être réalisé par qPCR, comme décrit par Badri et al., (2016), grâce à l'utilisation d'amorces spécifiques développées pour le suivi de cette souche de *R. irregularis* (DAOM 197198) et d'une sonde comportant un fluorophore, émettant un signal fluorescent lors de son hybridation à la séquence complémentaire.*

*Il serait également intéressant de tester d'autres inoculums mycorhiziens à base d'autres espèces et souches de CMA. Les inoculums SYMBIVIT (composé de 4 espèces du genre *Glomus*, de *Funneliformis mosseae* et de *Rhizophagus irregularis*), commercialisé par INOCULUMplus, ou à base de *Funneliformis mosseae*, développé par MycAgro Lab, pourraient par exemple être testés. Cependant, leur formulation n'est a priori pas aussi bien adaptée que celle du produit AGTIV® pour un emploi à l'échelle agricole. Il pourrait toutefois être envisagé d'incorporer l'inoculum à tester dans la couche de sol superficielle, comme ce qui est réalisé pour l'application des engrais minéraux, en ayant recours à des disques à engrais montés sur le semoir.*

Par ailleurs, un isolement de souches indigènes à partir du sol d'Evin-Malmaison pourrait être réalisé afin de produire un inoculum mycorhizien mieux adapté à un contexte de sol pollué par les ET. En effet, il est admis que les espèces microbiennes indigènes bénéficient à long-terme d'une adaptation aux conditions spécifiques du site. La mise en place d'un dispositif en microscopie, permettant le piégeage des espèces mycorhiziennes indigènes de la parcelle polluée pourrait alors être envisagée grâce à la culture d'espèces mycotrophes (ex : poireau, trèfle, luzerne) sur le sol pollué d'Evin-Malmaison. L'isolement des spores à partir de la culture de piégeage permettrait finalement de produire un inoculum mycorhizien autochtone.

*La coriandre comme la sauge sclarée sont par ailleurs des cultures peu exigeantes en éléments minéraux (Sharma and Sharma, 2012; Tibaldi et al., 2010). Ainsi, il est possible que la disponibilité relativement élevée de certains éléments minéraux (Mg sur les deux parcelles, P sur la parcelle non polluée - Denroy et al., 2004) sur les sites expérimentaux ait atténué la contribution effective de la symbiose mycorhizienne. Pour vérifier cette hypothèse, il faudrait évaluer la fonctionnalité de la symbiose mycorhizienne, grâce à la mesure de l'expression de certains gènes, codant par exemple pour des transporteurs de phosphate, tels que *GINTPT*, *MTPT4*, *LEPT1* ou *STPT3* (Mohanta and Bae, 2015; Voß et al., 2018; Wang et al., 2017a) ou de nitrate, tels que *AMT2* (Mohanta and Bae, 2015).*

3. Capacité des PPAM testées pour la phytostabilisation aidée des sols pollués par les ET

La question de la mobilité et de la biodisponibilité des ET, sous l'influence de la culture de sauge sclarée ou de la coriandre, ainsi que les aspects de transferts des ET vers les parties aériennes, ont été traités en partenariat avec Valérie Bert (INERIS) dans le cadre du projet PhytEO. Ainsi, bien que ces aspects n'aient pas été présentés dans la partie résultats de ce manuscrit, car ne faisant pas partie directement

des objectifs de mon travail de thèse, certains éléments seront abordés dans cette discussion pour mieux appréhender la filière PPAM-HE dans sa globalité. Ces résultats ont déjà fait l'objet de plusieurs présentations orales (Bert et al., 2018; Raveau et al., 2019, 2020b) et donneront lieu à une publication commune entre l'INERIS et l'ULCO (*Perlein et al., en cours de rédaction*).

Concernant la mobilité des ET, il a été observé qu'après deux années de culture, la sauge sclarée avait tendance à réduire la fraction mobile en Zn. En revanche, la culture de sauge sclarée comme celle de coriandre n'ont pas eu d'impact significatif sur la fraction mobile en Cd et en Pb, quelle que soit l'année de culture (*Perlein et al., en cours de rédaction*). Les deux PPAM testées, sauge sclarée et coriandre, se comportent par ailleurs différemment vis-à-vis des ET. En effet, la coriandre a montré un comportement accumulateur pour le Cd, présentant un facteur de bioconcentration supérieur à 1, tandis que pour la sauge, un comportement « exclure » a été mis en évidence (facteur de bioconcentration inférieur à 1, quel que soit l'ET considéré). De manière intéressante, les teneurs en Cd et en Pb mesurées dans les graines de coriandre étaient inférieures à celles mesurées dans les parties aériennes. Ce résultat est en accord avec la littérature où il a été montré que l'accumulation des ET dans les graines de coriandre était moins élevée en comparaison avec les parties comestibles (tiges et feuilles) de la plante (Kisku et al., 2011).

Il est à noter que la coriandre possède un système racinaire très superficiel et peu volumineux, impliquant que si la stabilisation via les racines a lieu, elle ne sera *a priori* effective que dans l'horizon de surface et dans les premiers centimètres du sol en particulier, ce qui pourrait être problématique pour une pollution plus profonde.

Par ailleurs, la contribution de l'inoculum mycorhizien vis-à-vis de l'immobilisation des ET dans un contexte de phytostabilisation aidée a également été évaluée en collaboration avec Valérie Bert (INERIS). Les résultats démontrent que durant la première année de culture de sauge sclarée, l'inoculation mycorhizienne réduit significativement la fraction mobile de Pb dans le sol. Par ailleurs, le transfert de Pb et de Zn dans les parties végétatives de la sauge lors de la première année de culture, ainsi que de Cd et de Pb dans les inflorescences lors de la seconde année de culture a été significativement réduit sur les parcelles amendées à l'aide de l'inoculum mycorhizien.

Pour compléter ce travail, il serait intéressant d'étudier les mécanismes impliqués dans l'immobilisation des ET présents dans le sol. Par exemple, il est connu que la glomaline, glycoprotéine produite par les CMA, est impliquée dans la chélation des ET de même que les hyphes qui participent à l'adsorption des ET sur leurs parois (Cornejo et al., 2008; Wang et al., 2019d), réduisant par conséquent leur mobilité dans le sol et leur transfert vers les parties aériennes de la plante (Ferrol et al., 2016). Pour vérifier cela, la glomaline facilement extractible (fraction labile) ou totale (fraction récalcitrante et labile) pourrait être quantifiée par une méthode spectrophotométrique à l'aide du réactif de Bradford (Cornejo et al., 2008; Wang et al., 2019d).

IV. Production et contrôle de la qualité des HE issues de la biomasse de PPAM cultivées sur un pollué par les ET

1. Rendements de distillation des HE satisfaisants

Les HE étudiées dans le cadre de cette étude ont été distillées par entraînement à la vapeur à partir de la biomasse produite sur les deux sites expérimentaux. La distillation a été réalisée par la société Ferrant PHE (Patrice Ferrant) à partir des inflorescences (sommités fleuries) de la sauge sclarée, des parties aériennes de coriandre ou des graines de coriandre (*Publication 1 - Raveau et al., 2020, à soumettre*).

Dans le cas de la sauge sclarée, les rendements en HE sont généralement plus élevés la deuxième année de culture par rapport ceux de la première et troisième année (Carrubba et al., 2002; Zutic et al., 2016). Cela a été vérifié dans le cadre de cette étude puisque les rendements ont atteint 0,5 à 2,7 kg/ha la première et la troisième année de culture, et 13 à 34 kg/ha, la deuxième année. En comparant ces chiffres à des valeurs de référence (de l'ordre de 10 à 30 kg d'HE/ha), les rendements obtenus la deuxième année se situent donc dans une fourchette haute (CRIEPPAM ; Yaseen et al., 2015). Si les chiffres affichés étaient attendus pour la première année, ils sont en revanche plus surprenant pour la troisième année, où des rendements en HE à hauteur de 50 à 60 % de ceux obtenus en deuxième année étaient espérés (Carrubba et al., 2006). Ceci peut s'expliquer par le fait que la sauge a notamment fait l'objet d'une récolte tardive en raison des conditions climatiques et que les pratiques culturales n'étaient pas optimales (absence de broyage de la partie aérienne au printemps, difficultés dans le contrôle des adventices). Au vu des chiffres affichés dans la littérature, la marge d'amélioration des rendements semble importante.

Ainsi, nos résultats montrent que :

- ⇒ **Les rendements en HE de sauge sclarée étaient satisfaisants en 2^{ème} année et similaires entre la parcelle polluée ou non par les ET.**

Dans le cas de la coriandre, des rendements de 10 et de 6 kg/ha ont été obtenus, pour l'HE issue des parties aériennes et des graines respectivement, quelle que soit la parcelle expérimentale et la modalité, inoculée ou non. Les rendements obtenus pour l'HE issue des parties aériennes de coriandre sont comparables à ceux décrits dans la littérature, de l'ordre de 10 kg/ha (Smallfield et al., 1994). En revanche, les rendements obtenus à partir des graines de coriandre sont deux à trois fois plus faibles que ceux attendus, ces derniers pouvant atteindre jusqu'à 18 kg/ha (Priyadarshi and Borse, 2014). Ce faible rendement peut en partie s'expliquer par un été très sec qui a pu impacter la taille des graines, une récolte tardive ayant entraîné une chute importante des graines lors de la récolte et une distillation des graines sans broyage au préalable.

Ainsi :

- ⇒ **Les HE de coriandre issues des parties aériennes ou des graines ont été obtenues avec des rendements similaires entre les parcelles polluée et non polluée et entre les modalités inoculées ou non.**

Il est à noter également que la teneur en HE des graines est supérieure à celle des parties aériennes, comme rapporté dans la littérature (Bhuiyan et al., 2009; Sharma and Sharma, 2012). Plusieurs pistes pourraient toutefois permettre d'optimiser les rendements obtenus.

D'un point de vue technique, le broyage des graines de coriandre avant distillation pourrait contribuer à améliorer les rendements de distillation. Il serait aussi pertinent d'être vigilants sur la date de récolte afin d'obtenir une quantité optimale de graines ou d'inflorescences à maturité, sans qu'elles n'aient commencé à tomber ou à faner.

De même, une réflexion sur la rotation des cultures pourrait être menée afin d'éviter un appauvrissement du sol et le recours aux amendements chimiques ou l'augmentation au cours du temps du nombre d'agents pathogènes. Il serait par conséquent intéressant de tester successivement plusieurs espèces possédant différents cycles de vie et ainsi de déterminer les systèmes de rotation culturaux les plus adaptés. Par ailleurs, au vu du cycle de développement rapide de certaines espèces végétales comme la coriandre, il pourrait par exemple être envisagé de semer et/ou de récolter la biomasse deux fois par an. Deux mois après la récolte de la sauge, un nombre important

d'inflorescences a également été observé et pourrait donner lieu à une deuxième récolte, augmentant ainsi la rentabilité de la filière. De la même façon, lorsque la récolte d'une PPAM est précoce, une deuxième espèce pourrait être cultivée pour ses HE, pour enrichir le sol avant d'implanter une nouvelle espèce (interculture d'hiver à vocation d'engrais vert) ou pour être intégrée à une autre filière de valorisation telle que la méthanisation, qui est en cours de développement dans le cadre du projet de territoire du bassin minier de Metaleurop.

2. Qualité satisfaisante des HE issues de la coriandre et de la sauge sclarée cultivées sur un sol pollué

Dans une perspective de valorisation non-alimentaire des HE produites à partir de la biomasse obtenue sur des sols pollués, les résultats obtenus en termes de qualité des HE sont encourageants. Il a en effet été montré que :

- ⇒ **L'accumulation des ET dans les HE est très limitée. Les ET ont été détectés à l'état de traces ou à des concentrations inférieures aux limites de quantification de la méthode suivie.**

Ces valeurs sont aussi très proches entre les conditions polluées ou non par les ET (*Publication 1 - Raveau et al., 2020, à soumettre*). Les résultats obtenus montrent par ailleurs que l'inoculation à l'aide du CMA contribue à réduire les teneurs en ET détectés à l'état de trace dans les HE (*Perlein et al., en cours de rédaction*).

Une analyse multi-résidus de pesticides a également été menée, afin d'évaluer la qualité globale des HE vis-à-vis de polluants divers. Il a ainsi été établi que :

- ⇒ **Les HE de sauge sclarée et de coriandre (parties aériennes et graines) présentaient une bonne qualité au regard de l'analyse des résidus de pesticides, avec des molécules détectées à l'état de traces et en faible nombre.**

Toutefois, il existe à l'heure actuelle un vide réglementaire résidant dans l'absence de norme établie pour des usages non-alimentaires comme alimentaires des HE et relatif à la limite maximale en ET tolérée dans les HE, aussi bien en France que plus largement en Europe. Concernant les résidus de pesticides, les HE à vocation non-alimentaires n'ont en pratique pas non plus de limite maximale de résidus définie (Quarré, 2018; Tascone et al., 2013). Afin de tenter de trouver des éléments de réponse, la qualité des HE obtenues dans le cadre de cette étude a donc été comparée à celle d'échantillons commerciaux, ou à des résultats d'analyses de pesticides fournis par Ferrant PHE et relatifs à des usages alimentaires. Ainsi, il a notamment été démontré que :

- ⇒ **Les teneurs en ET observées dans les HE de notre étude étaient inférieures à celles mesurées dans des échantillons d'HE trouvés sur le marché.**

Compte tenu des concentrations à l'état de traces des ET et des résidus de pesticides détectées dans les HE produites sur la parcelle d'Evin-Malmaison, comparables à celles obtenues sur la parcelle non polluée et à des échantillons commerciaux, la commercialisation des HE produites sur sols pollués dans des filières alimentaires ou non-alimentaires semble envisageable, d'autant plus que les HE sont généralement utilisées dans les procédés industriels en très petites quantités (Quarré, 2018).

Il est à noter toutefois que la présence d'ET dans la biomasse peut générer une contrainte notamment dans la gestion des résidus de distillation. C'est pourquoi, l'étude de la contamination éventuelle des résidus de distillation par les ET a été menée par l'INERIS dans le cadre du projet PhytEO. En fonction de ces résultats, une réflexion portant sur la méthode de valorisation la mieux adaptée (combustion, compostage ou méthanisation notamment) a été engagée

En outre, malgré la très faible contamination des HE par les ET et par les résidus de pesticides, la production de ces HE sur des sols pollués peut engendrer des réserves au niveau des industriels ou des consommateurs finaux. C'est pourquoi, une étude a été réalisée dans le cadre du projet PhytEO afin d'évaluer l'acceptabilité sociale de l'utilisation des HE produites sur des sols pollués pour l'élaboration de produits d'hygiène et de beauté ou utilisés dans la protection des cultures (en collaboration avec Hervé Flanquart, TVES-ULCO).

3. La composition chimique des HE : conforme aux attentes et non altérée ni par la pollution du sol, ni par l'inoculation

Outre la qualité des HE en termes de contaminations potentielles par les ET ou les résidus de pesticides, la composition chimique des HE peut également être modifiée par la présence des ET dans le sol et par l'inoculation mycorhizienne. La composition chimique des HE de sauge sclarée et de coriandre (parties aériennes ou graines) a donc été établie pour l'ensemble des conditions expérimentales (site expérimental pollué ou non par les ET, condition inoculée ou non à l'aide de l'amendement biologique). Nos résultats indiquent que l'HE de sauge sclarée est composée majoritairement de monoterpènes (jusqu'à 85 %) dont font partie le linalol (10-17 %) et l'acétate de linalyle (51-63 %), ainsi que des sesquiterpènes, tels que le germacrène-D (7-17 %) et corroborent les données décrites dans la littérature (Aćimović et al., 2018; Kumar et al., 2013; Sharopov and Setzer, 2012; Zutic et al., 2016). Par ailleurs, les HE issues des graines et des parties aériennes de coriandre présentent des compositions distinctes. Les HE issues des graines de coriandre sont majoritairement composées de monoterpènes, tandis que celles provenant des parties aériennes sont essentiellement constituées d'alcools et d'aldéhydes, comme décrit dans la littérature (Bhuiyan et al., 2009; Freires et al., 2014; Matasyoh et al., 2009; Satyal and Setzer, 2020). Ainsi, l'HE issue de la distillation des graines de coriandre présente une très forte proportion de linalol (75-83 %), accompagnée d' α -pinène et de γ -terpinène variant respectivement de 3 à 5 % et de 6 à 9 %, tandis que l'HE issue des parties aériennes présente une majorité de 2-décénal (38-51 %), du linalol, du décanal ainsi que du 2-dodécénal.

Par ailleurs, nos résultats montrent que les compositions chimiques des HE de sauge sclarée et de coriandre (parties aériennes et graines) étaient similaires entre les différentes conditions expérimentales (parcelle polluée ou non et modalité inoculée ou non avec le CMA - [Publication 2 - Raveau et al., 2020, soumise](#)).

⇒ **Les compositions chimiques des différentes HE testées n'étaient affectées ni par la présence des ET dans le sol, ni par l'inoculation mycorhizienne.**

Ces résultats sont en accord avec la littérature qui rapporte que les PPAM de la famille des Lamiacées, telles que *Ocimum basilicum*, *Anethum graveolens*, *Mentha piperita* et plus généralement les différentes espèces des genres *Mentha*, *Rosmarinus* ou *Ocimum* sont capables de se développer sur des sols pollués par des ET et d'afficher une composition chimique inchangée en termes de quantité et de qualité (Biswas et al., 2011; Pandey et al., 2019). En revanche, certains travaux ont montré que l'introduction d'un inoculum mycorhizien pouvait modifier la quantité et la qualité de l'HE produite, notamment en affectant les voies de biosynthèse de certains métabolites secondaires (Golubkina et al., 2020; Kapoor et al., 2002; Khaosaad et al., 2006). Il a par exemple été souligné que l'inoculation de la coriandre avec *Glomus macrocarpum* et *Glomus fasciculatum* provoquait respectivement une augmentation des teneurs en géraniol et en linalol (Kapoor et al., 2002). De manière similaire, la quantité d'acétate de linalyle présente dans l'HE de *Lavandula angustifolia* a été significativement augmentée en présence d'un inoculum composé de *R. irregularis* (Golubkina et al., 2020).

En s'appuyant sur les conclusions précédemment tirées de l'analyse des taux de mycorhization présentés dans le cadre de la publication 1, bien que les taux de mycorhization diffèrent, toutes les plantes, inoculées ou non par l'amendement biologique, présentaient une colonisation racinaire par les CMA, ce qui pourrait expliquer qu'il n'y ait pas de différence de composition entre les conditions inoculée et non-inoculée. Par ailleurs, la question de la fonctionnalité de la symbiose se pose à nouveau et mériterait d'être traitée.

V. La production d'HE à activités biologiques, une filière de valorisation à haute valeur ajoutée avec des débouchés potentiels dans le domaine de la protection des cultures et de la santé humaine

Un autre axe de travail de mon projet de thèse a consisté à étudier les activités biologiques des HE distillées à partir de la sauge sclarée (inflorescences) et de la coriandre (graines, parties aériennes) cultivées sur sols pollués par les ET. En s'appuyant sur des prévisions d'évolution, le marché des HE et des produits naturels à base de plante est en constante augmentation et pourrait atteindre 16 milliards de dollars en 2026 (Allied Market Research, 2020; CBI, 2020). Leur valorisation à des fins non-alimentaires dans des secteurs tels que la protection des cultures (Raveau et al., 2020a) ou la santé humaine (Kostić et al., 2017) pourrait permettre de diversifier leurs domaines d'application et d'accroître le potentiel de la filière en augmentant l'offre et en stimulant la demande.

1. Des propriétés biologiques des HE avec un potentiel d'application en protection des cultures

En vue d'applications en protection des cultures, les pouvoirs antifongique, anti-germinatif et herbicide des HE, distillées à partir de la sauge sclarée et de la coriandre (graines, parties aériennes) cultivées sur sols pollués par les ET, ont été évalués *in vitro* (Publication 2 - Raveau et al., 2020, soumise).

Un effet fongistatique a été montré vis-à-vis de deux champignons phytopathogènes testés (*F. culmorum* et *Z. tritici*), quelle que soit l'HE. Des valeurs de CI_{50} comprises entre 0,20 et 2,83 g.L⁻¹ et entre 0,001 et 0,08 g.L⁻¹ ont été obtenues pour les tests antifongiques contre *F. culmorum* et *Z. tritici* respectivement. En termes d'efficacité antifongique et en comparaison avec un témoin positif (fongicide Aviator), les HE testées peuvent être classées dans l'ordre suivant : Aviator ≥ HE de coriandre parties aériennes ≥ HE de coriandre graines ≥ HE de sauge sclarée.

Des effets anti-germinatifs et herbicides ont également été mis en évidence sur deux espèces végétales, la laitue (*L. sativa*) et le ray-grass (*L. perenne*), communément utilisées dans des tests de phytotoxicité. Pour l'effet anti-germinatif, des valeurs de CI_{50} comprises entre 0,05 et 44,0 g.L⁻¹ et entre 0,15 et 145 g.L⁻¹ ont été déterminées, contre la laitue et le ray-grass respectivement. Pour le second test visant à évaluer l'effet des HE sur la croissance de la plantule (effet herbicide), les valeurs de CI_{50} variaient entre 0,011 et 1,17 g.L⁻¹ et entre 0,044 et 0,77 g.L⁻¹ contre la laitue et le ray-grass respectivement. L'efficacité anti-germinative et herbicide des HE testées, en comparaison avec un témoin positif, le glyphosate, peut être classée comme suit, quelle que soit la plante cible : Glyphosate (témoin positif) > HE de coriandre parties aériennes > HE de coriandre graines ≥ HE de sauge sclarée.

Nous avons ainsi démontré grâce à ces tests *in vitro* que :

- ⇒ Les HE issues de la sauge sclarée et de la coriandre présentaient un réel potentiel, antifongique (par contact direct et par volatilité), anti-germinatif et herbicide.
- ⇒ L'HE issue des parties aériennes de la coriandre présente une efficacité plus prononcée.

2. Des propriétés biologiques des HE avec un potentiel d'application en santé humaine

Les activités antioxydantes et anti-inflammatoires ont également été recherchées, en vue d'applications dans le domaine de la santé humaine.

Les différents résultats obtenus soulignent un effet antioxydant des trois HE, toutefois moins marqué (3 à 10 fois inférieur) que celui du Trolox[®], analogue de la vitamine E (témoin positif). Les CI_{50} obtenues varient entre 0,009 et 0,038 g.L⁻¹. En comparaison avec le Trolox[®], les effets antioxydants des HE peuvent être classés comme suit : Trolox[®] > HE de coriandre parties aériennes > HE de sauge sclarée > HE de coriandre graines.

Par ailleurs, un effet anti-inflammatoire de l'HE issue des parties aériennes de coriandre en particulier a été mis en évidence, diminuant l'intensité de la réponse inflammatoire au sein de la lignée cellulaire bronchiale, induite par la présence de particules atmosphériques. En revanche, l'HE de sauge sclarée ou de coriandre graines a produit l'effet inverse, renforçant l'intensité de la réponse inflammatoire. Il est possible qu'un ou plusieurs des composés présents exclusivement au sein des HE de sauge sclarée et de coriandre graines présentent un effet pro-inflammatoire. Toutefois, le limonène, le trans-acétate de géranyle et l'oxyde de caryophyllène, présents au sein de ces deux HE, présentent une activité anti-inflammatoire avérée, de même que le linalol (Chavan et al., 2010; Gonçalves et al., 2012; Yu et al., 2017).

L'étude du potentiel antioxydant et anti-inflammatoire des HE issues de la sauge sclarée et de la coriandre (graines et parties aériennes) a mis en évidence que :

⇒ **L'ensemble des HE testées présentent des activités antioxydantes et anti-inflammatoires, avec des effets plus marqués de l'HE issue des parties aériennes de coriandre.**

Il est à souligner que dans l'ensemble, les activités biologiques des différentes HE testées étaient similaires, que les plantes aient été cultivées en conditions polluée ou non et en présence ou non de l'inoculum mycorhizien. Au vu des compositions chimiques établies, n'ayant présenté aucune modification quelles que soient les modalités testées, ces résultats étaient attendus.

Par ailleurs, quelle que soit la propriété biologique étudiée, l'HE issue des parties aériennes de coriandre présentait des effets plus marqués que les deux autres HE, en témoignent les CI_{50} (tests antifongiques, anti-germinatifs ou herbicides) ou CE_{50} (tests anti-oxydants) plus faibles obtenues. Ses effets biologiques sont d'ailleurs comparables *in vitro* à ceux des produits homologués et employés comme témoins positifs, à l'exception de l'effet herbicide contre le ray-grass où le glyphosate (inhibition de la biosynthèse des acides aminés aromatiques) possède une efficacité nettement supérieure. Ceci peut en particulier être dû à l'abondance et aux proportions relatives des différents principes actifs et aux effets synergiques qui peuvent exister entre eux. Il est notamment établi que les terpènes sont majoritairement responsables des activités biologiques des HE, et dans une moindre mesure les flavonoïdes, les isoflavonoïdes et les alcools (Lagrouh et al., 2017; Nazzaro et al., 2017). S'il est connu que les mécanismes d'action antifongiques des HE ciblent majoritairement l'intégrité et le fonctionnement des membranes (Lagrouh et al., 2017; Nazzaro et al., 2017), ils peuvent varier d'une HE à l'autre : l'HE de coriandre pourrait notamment se lier à un ergostérol membranaire et affecter la perméabilité membranaire (Freires et al., 2014; Silva et al., 2011a, 2011b).

3. Une efficacité limitée de l'encapsulation des HE par la β -CD

En raison de leur volatilité, la stabilité des HE ainsi que la persistance de leurs effets biologiques au cours du temps sont souvent présentées comme limitées. Pour surmonter ces problèmes, l'encapsulation des HE à l'aide de la β -CD, décrite pour sa capacité à complexer les composés des HE,

pourrait ainsi contribuer à réduire les phénomènes de dégradation et de volatilisation des HE, tout en permettant une diffusion progressive (Abarca et al., 2016; Kfoury et al., 2019). Ces différentes propriétés biologiques ont donc été comparées, en présence et en absence de la β -CD.

Bien que nos résultats aient montré une capacité de rétention importante des HE en présence de la β -CD, les activités obtenues étaient, dans la majorité des cas, plus faibles lorsque les HE étaient encapsulées (*Publication 2 - Raveau et al., 2020, soumise*). En effet, dans nos conditions expérimentales, au vu de la capacité de rétention de la β -CD, les composés encapsulés des HE étaient probablement moins disponibles. Dans le cadre des tests antifongiques par volatilité notamment, les HE encapsulées étant moins volatiles, l'effet inhibiteur exercé sur le développement du champignon était moindre.

*En revanche, dans un système plus ouvert qui nécessite une diffusion progressive des HE, la β -CD pourrait trouver une application. Ainsi, il serait intéressant de s'assurer d'un relargage progressif au cours du temps des composés des HE lorsqu'elles sont complexées avec à la β -CD afin de proposer l'utilisation de ces HE dans diverses applications telles que la protection des cultures. Afin de vérifier la persistance des propriétés biologiques au cours du temps, la croissance fongique d'une espèce comme *F. culmorum* en milieu ouvert (volatilité accrue des HE) pourrait être évaluée lorsque les HE sont encapsulés ou non. Cette méthodologie pourrait également être utilisée pour les tests herbicides.*

*Par ailleurs, afin de valider les résultats obtenus in vitro et de proposer l'utilisation des HE dans le cadre de la protection des cultures, le passage à un modèle in planta pour tester les propriétés biologiques des différentes HE est nécessaire. Dans le contexte actuel du plan national d'action EcoPhyto II+, nous suggérons d'étudier l'apport des HE dans la protection d'une grande culture, le blé, contre deux champignons phytopathogènes : *Blumeria graminis* f. sp. *tritici* et *Mycosphaerella graminicola*, responsables de l'oïdium et de la septoriose, respectivement. Le recours à un dispositif en conditions contrôlées et s'appuyant sur un modèle in planta (interaction champignon/plante/HE) en microcosme semble pertinent. L'étude herbicide, n'ayant à l'heure actuelle qu'été conduite sur des plantes modèles, celle-ci pourrait aussi être élargie à des espèces adventices. Dans la région des Hauts-de-France, le liseron (*Convolvulus arvensis*), le rumex (*Rumex crispus*) ou la matricaire (*M. recutita*) affichent une nuisibilité importante et pourraient par exemple être ciblés.*

Enfin, sur le plan fondamental mais également pour la mise sur le marché de nouvelles molécules dans le cadre de la protection des cultures, il est important de connaître les mécanismes d'action impliqués dans certaines propriétés biologiques. Par exemple, pour les propriétés antifongiques, il serait dans un premier temps envisageable d'observer les hyphes au microscope électronique afin d'identifier les structures cellulaires affectées par la présence des HE (Wang et al., 2019c).

*Par ailleurs, il serait intéressant d'étudier l'impact des HE sur le métabolisme stérolique des champignons traités. En effet, certaines HE sont capables d'inhiber la biosynthèse des stérols et de modifier la teneur en ergostérol de la membrane plasmique, causant ainsi un déséquilibre osmotique et métabolique ainsi que des fuites ioniques au niveau de la membrane (Lagrouh et al., 2017; Nazzaro et al., 2017). Ainsi, un dosage de la teneur en ergostérol en présence et en absence d'HE chez un champignon comme *F. culmorum*, semble pertinent (Tian et al., 2012).*

VI. Impacts de la filière de phytomanagement PPAM-HE sur le microbiote d'un sol pollué par les ET

Les communautés microbiennes jouent un rôle majeur au sein des écosystèmes, en raison de la diversité des services écosystémiques qu'elles offrent et des nombreuses fonctions qu'elles

remplissent (Chen et al., 2020; Klaubauf et al., 2010; Lin et al., 2019). Elles sont notamment impliquées dans la fertilité des sols, les cycles géochimiques du carbone et de l'azote ainsi que dans la croissance des plantes (Adamovic et al., 2015; Bodelier, 2011; Lin et al., 2019). Dans un contexte de pollution du sol par les ET, conduisant à des changements au niveau de la structure des communautés microbiennes ainsi que de leur diversité fonctionnelle, le microbiote du sol rhizosphérique et racinaire peut être fortement affecté et le fonctionnement de l'écosystème perturbé (Ding et al., 2017).

De nombreux travaux ont montré que la revégétalisation des sols pouvait s'accompagner d'une refunctionalisation, en contribuant à la séquestration du carbone, à un enrichissement du sol en matière organique et en carbone organique en particulier et enfin à la respiration du sol (Burges et al., 2018; Buta et al., 2019; Durand et al., 2018). Dans cette optique, la dernière partie de ma thèse a été consacrée au suivi des communautés microbiennes du sol et des racines de la sauge sclarée, cultivée sur le sol d'Evin-Malmaison historiquement pollué par les ET. En effet, l'impact de la culture de sauge sclarée sur la structure et l'évolution du microbiote d'un sol, en particulier pollué par les ET, n'a jamais été investigué.

Dans le cadre de cette thèse, nous avons donc procédé, via une approche de métagénomique par séquençage grâce à la technologie Illumina Miseq, à l'étude des communautés microbiennes du sol rhizosphérique et des racines de la sauge sclarée. Le choix s'est porté sur la sauge sclarée qui possède un cycle de vie bisannuel et qui permettait ainsi de réaliser un suivi du microbiote associé à la plante, dans le sol et les racines, sur deux années consécutives et à trois temps de prélèvement (état initial de la parcelle avant végétalisation – année 1 avant récolte, à 21 semaines de culture – année 2 avant récolte, à 66 semaines de culture). Nos travaux ont porté sur les communautés bactériennes ([Publication 3 - Raveau et al., 2020](#)) et fongiques ([Publication 4 - Raveau et al., 2020, soumise](#)) du sol rhizosphérique et des racines de la sauge sclarée, avec un focus sur les CMA grâce à l'utilisation d'amorces spécifiques ciblant la région V4 de l'ARNr 18S (Stefani et al., 2020).

1. Influence de la végétalisation par la sauge sclarée, au cours de son cycle de développement, sur le microbiote tellurique et racinaire

Nos résultats indiquent une forte évolution au cours du temps des communautés bactériennes et fongiques, en réponse à la végétalisation du sol pollué par la sauge sclarée. Un changement notable est visible entre la première et la deuxième année de culture, que ce soit au niveau du sol rhizosphérique ou des racines, aussi bien pour les communautés bactériennes que fongiques. Ce changement se traduit par une chute du nombre d'ASV entre les deux années et des modifications en termes d'abondances relatives au niveau des phyla ou des genres bactériens et fongiques. Ces changements s'accompagnent d'une chute de la diversité entre ces deux années de culture et sont particulièrement marqués au niveau de la racine ([Publications 3 et 4 - Raveau et al., 2020](#)). Il est connu que la nature des exsudats racinaires exerce une pression sélective importante sur les communautés microbiennes rhizosphériques (Bourceret et al., 2016; Dagher et al., 2019; Iffis et al., 2016). Par exemple, la libération de composés carbonés sous forme d'exsudats racinaires peut stimuler la croissance de certaines communautés microbiennes, qui les utilisent comme source de carbone et d'énergie (Na et al., 2019; Qu et al., 2020). Ainsi, des changements métaboliques engendrant une modification des profils d'exsudation racinaire peuvent modifier durablement la structure des communautés microbiennes (Wang et al., 2017b). Ces changements métaboliques sont susceptibles de se produire suivant les différents stades de développement, avec l'évolution de l'âge de la plante (Akyol et al., 2019; Qu et al., 2020). De nombreux travaux ont par ailleurs montré que la concentration et la composition des exsudats racinaires ainsi que l'architecture racinaire varient en fonction des espèces végétales et ont un rôle structurant sur les communautés microbiennes (Dagher et al., 2019;

Iffis et al., 2017). Dans le cas particulier des PPAM, parmi les exsudats racinaires, la synthèse et la libération de composés aromatiques pourrait jouer un rôle particulier et important dans la structuration des communautés microbiennes rhizosphériques (Adamovic et al., 2015; Misra et al., 2019). En effet, en raison de leurs propriétés antimicrobiennes et probablement de leur abondance en deuxième année de culture, correspondant à la maturité de la plante et du développement de son système racinaire, leur libération et leur présence dans le sol pourrait être importante et se répercuter sur l'abondance et la diversité microbienne dans les sols.

Il est aussi intéressant de noter dans nos conditions expérimentales que les communautés microbiennes et en particulier fongiques tendent à s'équilibrer au cours du temps entre le sol rhizosphérique et les racines de la sauge sclarée ([Publication 4 - Raveau et al., 2020, soumise](#)). En effet, après deux années de culture, la richesse et la diversité des espèces fongiques étaient équivalentes entre les deux biotopes. De manière similaire, une évolution analogue au niveau du phylum mais également du genre a été observée entre les deux biotopes. Cette observation ne semble pas surprenante et est en accord avec l'hypothèse émise par Goldmann et al., (2016) qui rapporte que les champignons associés avec les racines sont recrutés à partir du sol rhizosphérique environnant. Cette tendance est toutefois moins marquée au niveau des communautés bactériennes, ce qui pourrait s'expliquer par la plus forte dépendance des communautés fongiques vis-à-vis des facteurs biotiques et en particulier de la disponibilité de la matière organique (Bourceret et al., 2016; Chen et al., 2017; Wang et al., 2020).

2. L'introduction d'un inoculum mycorhizien commercial exogène induit des réponses contrastées sur la structure et l'évolution des communautés microbiennes telluriques et racinaires

Les réponses des communautés bactérienne et fongique suite à l'introduction d'un inoculum mycorhizien divergent. Alors que la structure de la communauté fongique du sol rhizosphérique a été significativement modulée par l'introduction de l'inoculum commercial, se traduisant par une baisse des indices de diversité et une modification des abondances relatives des genres fongiques ([Publication 4 - Raveau et al., 2020, soumise](#)), aucune réponse n'a été observée au niveau de la communauté bactérienne, dans le sol ou les racines ([Publication 3 - Raveau et al., 2020](#)).

Il est à noter que les communautés bactériennes présentent souvent une réactivité moindre par rapport aux communautés fongiques, vis-à-vis des facteurs biotiques et abiotiques (Bourceret et al., 2016; Yung, 2020; Zappellini et al., 2015; Zhao et al., 2019). Parmi ces facteurs, l'établissement de la symbiose mycorhizienne, connue pour modifier le métabolisme de son hôte végétal et par là même les profils d'exsudats, est ainsi susceptible d'affecter indirectement la structure des communautés microbiennes rhizosphériques (Akyol et al., 2019; Monokrousos et al., 2019), corroborant ce qui a été observé dans le cadre de nos travaux vis-à-vis des communautés fongiques du sol rhizosphérique. Comme les racines des plantes, les hyphes des CMA sont également capables de sécréter des exsudats (Qin et al., 2016; Toljander et al., 2006). Ces exsudats pourraient aussi stimuler la croissance de certaines espèces microbiennes et donc exercer un effet direct sur la structure des communautés microbiennes rhizosphériques (Qin et al., 2016). Par ailleurs, l'introduction d'une nouvelle espèce de CMA au moyen de l'inoculation peut également exercer un effet direct sur les communautés microbiennes du sol. En effet, si les espèces microbiennes natives peuvent présenter une meilleure adaptations aux conditions environnementales et ainsi ne pas être affectées par l'introduction d'une nouvelle espèce, il a aussi été démontré que les espèces indigènes de CMA en particulier pouvaient être fortement réprimées (en termes de diversité et d'efficacité de colonisation racinaire) en présence d'une souche exogène exerçant une forte pression sélective (Janoušková et al., 2017).

En revanche, si dans nos conditions un impact de l'inoculation sur les communautés fongiques a en particulier été observé, les processus (directs ou indirects) impliqués dans la modulation de la structure des communautés demeurent flous.

Afin de compléter ces travaux, il serait intéressant d'étudier dans un premier temps, la nature des exsudats racinaires présents dans la rhizosphère de la sauge sclarée qui seraient impliqués dans la structuration et la modulation des communautés microbiennes, au moyen d'une analyse métabolomique globale. Une analyse non ciblée par LC-ToF-MS, couvrant un large spectre d'exsudats racinaires (acides organiques, acides aminés, sucres simples ou complexes, phytohormones, composés volatiles) pourrait en ce sens être adapté (van Dam and Bouwmeester, 2016; Dundek et al., 2011).

En résumé :

- ⇒ **La végétalisation du sol par la sauge sclarée ainsi que l'inoculation mycorhizienne modulent les communautés microbiennes dans le sol rhizosphérique et les racines.**

3. Aspects fonctionnels

Afin d'appréhender les aspects fonctionnels des groupes microbiens identifiés, une étude exploratoire reposant sur une assignation taxonomique a été menée. Elle indique une forte proportion de genres bactériens susceptibles de contenir des espèces PGPR, bénéfiques pour la croissance et le développement des plantes auxquelles elles sont associées. L'analyse des corrélations entre les ASV majeurs et la biomasse ou la colonisation racinaire a notamment démontré que les ASV les plus fortement corrélés appartenaient à des genres incluant des espèces PGPR, tels que *Pseudarthrobacter*, *Streptomyces* ou *Actinoplanes* ([Publication 3 - Raveau et al., 2020](#)). *Streptomyces* en particulier est reconnu pour de nombreuses fonctions, telles que la production de métabolites secondaires présentant des propriétés antimicrobiennes (Álvarez-López et al., 2020; Vurukonda et al., 2018; Zappelini et al., 2018) ou sa contribution à la croissance des plantes, grâce à sa capacité à synthétiser des hormones de croissance végétale telles que l'acide indole acétique ou à solubiliser le phosphate (Dias et al., 2017; Zappelini et al., 2018). Ainsi, il pourrait être d'un grand intérêt dans le cadre d'une approche de phytostabilisation aidée.

En revanche, les résultats de l'assignation fonctionnelle fongique à l'aide de l'outil FUNGuild (Nguyen et al., 2016) montrent une dominance des champignons saprotrophes et phytopathogènes (atteignant jusqu'à 80 % en proportion relative), avec un accroissement au cours du temps de la proportion des seconds, tandis que la proportion d'espèces symbiotiques et endophytes est très limitée ([Publication 4 - Raveau et al., 2020, soumise](#)). Ces observations sont en accord avec de précédentes études rapportant une dominance des groupes de champignons saprotrophes et phytopathogènes, pouvant constituer jusqu'à 90 % de la communauté fongique d'un sol (Li et al., 2020; Na et al., 2019). Il est à souligner qu'en dépit de la forte proportion de champignons phytopathogènes, tels que *Fusarium*, *Gibellulopsis* ou *Botrytis*, possédant un large spectre de plantes hôtes, ils pourraient s'avérer sans danger pour la sauge sclarée.

Afin d'approfondir cette première étude exploratoire fonctionnelle reposant sur des données bibliographiques, il serait pertinent de réaliser une analyse métatranscriptomique permettant de déterminer spécifiquement le rôle fonctionnel des communautés microbiennes au sein du sol rhizosphérique pollué et des racines de la sauge sclarée. Une approche basée sur le séquençage de l'ARNr 16S (bactéries) ou 18S (eucaryotes), complétée par une assignation fonctionnelle des gènes surexprimés et/ou une analyse de l'expression de l'ARNr codant pour des gènes impliqués dans la tolérance aux ET pourrait ainsi être adaptée (Chung et al., 2020; Sato et al., 2019). Afin de sélectionner des gènes d'intérêts, la base de donnée établie par Pal et al., (2014), contenant 470 gènes de résistance

vérifiés, pourrait être utilisée. Des gènes tels que *YCF1* et *ZRC1*, codant respectivement pour un transporteur de Cd et de Zn, impliqués dans la séquestration de ces ET dans les vacuoles, ou du gène *YAP1*, codant pour un facteur de transcription régulant les gènes induits lors de stress, pourraient par exemple être ciblés (Lehembre et al., 2013).

VII. L'étude de faisabilité technico-économique indique un réel potentiel de la sauge sclarée, mais des résultats mitigés pour la coriandre

L'un des aspects importants pour tout projet de phytomanagement est finalement la réalisation d'une étude de faisabilité technico-économique, prenant en compte les coûts de production (charges d'approvisionnement et opérationnelles, amortissement) et de distillation ainsi que la valeur marchande des HE, dans le cas de la filière PPAM-HE.

1. Coûts de production des espèces cultivées

Les coûts de production des PPAM ont été estimés sur la base d'une utilisation de matériel agricole et en suivant les préconisations relatives à l'itinéraire technique propre à chaque espèce. Ils sont donc exprimés séparément pour chaque espèce de PPAM et en fonction de l'organe récolté. Il est à noter que les coûts de production étaient similaires entre les parcelles polluées ou non, puisque le matériel utilisé et la méthode de récolte étaient équivalents. Excepté le prix de l'inoculum (170 €/ha), les coûts de production étaient également équivalents entre parcelles inoculées ou non.

Les coûts de production s'élèvent à 706 et 772 €/ha pour la coriandre (parties aériennes et graines respectivement) et à 386 €/ha et par an pour la sauge sclarée, cultivée sur 3 ans. Les coûts de production de cultures agricoles de référence, telles que l'orge d'hiver, le blé ou le colza, sont respectivement de 669, 720 et 761 €/ha (Projet Casdar, 2015). Par conséquent, les coûts de production de ces PPAM et ceux de grandes cultures sont relativement proches.

Ces coûts de production ont été comparés à ceux affichés dans le cadre d'autres filières de phytomanagement. Wan et al., (2016) décrivent l'utilisation de deux espèces hyperaccumulatrices, *Pteris vittata* et *Sedum alfredii*, en combinaison avec une interculture commerciale (cane à sucre) pour la remédiation d'un sol pollué par les ET, et affichent un coût opérationnel à hauteur de 10 000 €/ha à l'année, en raison d'intrants très coûteux, nettement supérieur aux coûts de production et de fonctionnement affichés dans notre étude. Une autre étude menée par Włóka et al., (2019) sur des sols pollués par des hydrocarbures aromatiques polycycliques et s'appuyant sur l'utilisation de *Phalaris arundinacea* L. et *Brassica napus* L. pour la biodégradation des hydrocarbures, a indiqué des coûts moyens de 700 €/ha pour la culture et la récolte des deux espèces végétales.

Il est intéressant de noter qu'en raison de la surface de la parcelle agricole polluée (2 ha) et de la pollution superficielle du sol, au niveau des horizons de surface (0-30 cm - Sterckeman et al., 2002), la remédiation du site en utilisant des techniques physico-chimiques représenterait un coût supérieur à 1 000 000 € (plus de 500 000 €/ha). Ces chiffres sont donnés sur la base d'une estimation récente du coût moyen (180 €/m³) des méthodes physico-chimiques de traitement (appliquées aux sols pollués par les ET, Zhang, 2019) et semblent réhibitores pour ce type de remédiation, au vu de la vaste surface polluée mais également de leurs effets délétères sur la vie microbienne du sol (Burges et al., 2018; Gong et al., 2018). Par conséquent, en comparaison des méthodes conventionnelles de traitements des sols pollués par les ET, notre étude démontre que l'utilisation des PPAM dans le cadre de la phytostabilisation des ET offre des perspectives intéressantes en raison d'un coût de mise en œuvre relativement bas.

2. Viabilité économique des trois filières PPAM-HE testées

L'étude de la viabilité économique des trois filières repose sur le coût de distillation des HE, estimé à 300 € par chargement (14 m³ de biomasse à distiller), sur les rendements en HE obtenus dans nos conditions expérimentales (dans certains cas anormalement faibles) et sur le prix de vente des HE affichés en 2020 sachant que le marché des HE est connu pour être particulièrement versatile.

En raison du prix de vente des HE de coriandre (graines ou parties aériennes) relativement faible et inférieur à 100 €/kg d'HE, les marges brutes dégagées pour la filière « coriandre » sont en moyenne de 150 €/ha pour les parties aériennes et négatives pour la coriandre graine (- 450 €/ha en moyenne).

Pour la sauge sclarée, la marge brute dégagée est en moyenne de 1600 €/ha et s'élève jusqu'à 4485 €/ha sur la parcelle polluée par les ET, en raison des rendements élevés en HE obtenus. Si ces résultats obtenus pour la sauge sclarée, en termes de coût de production et de marge brute dégagée notamment, sont comparés au blé, utilisée comme référence agricole, la sauge sclarée affiche une marge brute moyenne inférieure de 20 % à celle du blé (2100 €/ha – Projet Casdar, 2015).

⇒ **La sauge sclarée, qui présente une marge brute relativement importante apparaît comme un candidat potentiel.**

Comme cela a été précédemment suggéré, la perspective d'inclure la culture des PPAM dans un système de rotation culturale, intégrant différentes espèces de PPAM et/ou des intercultures à vocation bioénergétique ou d'engrais verts, pourrait permettre d'abaisser les coûts de gestion de la parcelle et de production, en réduisant par exemple les quantités d'intrants apportées et/ou les interventions techniques, ou en améliorant la rentabilité de la filière. Il pourrait aussi être envisagé de récolter la sauge sclarée une seconde fois la deuxième année. Grâce à son cycle de développement rapide, il pourrait également être possible de récolter la coriandre deux fois par an, ce qui pourrait augmenter la quantité d'HE produite et la marge brute ainsi dégagée par an.

Il est par ailleurs important de noter qu'au-delà du simple bénéfice économique potentiel, le phytomanagement, en intégrant la démarche de gestion durable du site, contribue aussi à restaurer la fonction du sol et à assurer des bénéfices écologiques ainsi que sociaux, qui sont certes difficilement monétisables, mais qu'il est désormais important de prendre en compte (Burges et al., 2018; Cundy et al., 2016).

Conclusions générales

Conclusions générales

Tableau 10: Principaux critères évalués dans le cadre de la filière PPAM-HE.

		Sauge sclarée <i>Salvia sclarea</i> L.	Coriandre <i>Coriandrum sativum</i> L.	
			Parties aériennes 	Graines 
Germination des graines		+	+	
Taux de levée <i>in situ</i>		+	+	
Croissance des PPAM		+	+	
Production de biomasse		+	+	
Rendement en HE		Elevé	Moyen	Faible
Qualité des HE produites	Contamination par les ET	Traces	Traces	
	Contamination par les résidus de pesticides	Traces	Traces	
	Composition chimique des HE	Acétate de linalyle (51-63 %), linalol (10-17 %), germacrène D (7-17 %)	2-decenal (38-51 %), linalol (22-39 %), décanal (7-8 %) and 2-dodécenal (3-6 %)	Linalol (75-83 %), γ -terpinène (6-9 %), α -pinène (3-5 %)
		Non affectée ni par les ET, ni par l'inoculation mycorhizienne		
	Propriétés biologiques - Protection des cultures (antifongiques, anti-germinatives, herbicides) - Santé humaine (anti-inflammatoires, anti-oxydantes)	+	++	+
		+	++	+
Capacité de phytostabilisation	Phytostabilisation	Réduction de la mobilité du Zn dans le sol (tendance)	Pas d'effet de la plante sur la mobilité des ET (sol)	
	Phytostabilisation aidée par l'inoculum mycorhizien	Mobilité réduite du Pb	Pas d'effet sur la mobilité des ET	
	Comportement des PPAM vis-à-vis des ET	« Excluer » pour le Cd, Pb et Zn BCF << 1	Accumulateur pour le Cd – BCF > 1 Accumulation moindre de Cd et de Pb dans les graines par comparaison aux parties aériennes	
	Effet de l'inoculation sur le transfert des ET	Transfert réduit de Cd et de Pb dans les parties aériennes	Pas d'effet sur le transfert	
	Contamination des résidus de distillation par les ET	Traces	Traces de Pb et Zn – teneurs relativement élevées en Cd	
Impact de la culture de PPAM sur le microbiote racinaire et tellurique		- Diminution du nombre d'ASV dans le sol et les racines - Chute de la diversité, plus marquée au niveau racinaire - Modulation des abondances relatives (phyla et genres) - Prévalence des phyla bactériens <i>Actinobacteria</i> et <i>Proteobacteria</i> et fongiques <i>Ascomycota</i> et <i>Basidiomycota</i> - Pas d'effet sur la communauté de CMA	Non étudié	
Faisabilité technico-économique		++	+	-

Conclusions générales

Les travaux réalisés dans le cadre de cette thèse s'inscrivent dans le projet PhytEO, dont la principale ambition était de développer une filière éco-innovante de valorisation non alimentaire de biomasse produite sur les sols agricoles du site atelier de Metaleurop, pollués par des ET. Reposant sur des démonstrateurs *in situ* à l'échelle de l'hectare, ce projet avait pour vocation d'évaluer, en intégrant les aspects techniques, environnementaux et socio-économiques, l'intérêt d'un mode de gestion combinant la phytostabilisation aidée par des PPAM et la production d'HE dans un contexte de phytomanagement des sols historiquement pollués par des ET. Ma contribution à ce projet a permis d'évaluer la pertinence d'un mode de gestion reposant sur la culture, notamment, de deux espèces de PPAM, la sauge sclarée et la coriandre, en présence ou non d'un amendement mycorhizien. Les résultats obtenus (synthésisés dans le Tableau 10) permettent de dresser un bilan sur le potentiel de ces deux PPAM utilisées dans le cadre général du phytomanagement d'un site pollué par les ET :

La coriandre ainsi que la sauge sclarée ont toutes deux montré une bonne capacité à s'installer, à se développer et à produire une quantité importante de biomasse sur des sols pollués par les ET, soulignant l'aptitude de ces deux PPAM à tolérer des concentrations en ET six à dix-sept fois supérieures au fonds géochimique des sols agricoles régionaux. Par ailleurs, l'étude de la capacité de ces PPAM pour la phytostabilisation des ET, traitée en collaboration avec l'INERIS, a montré un comportement « exclure » de la sauge sclarée vis-à-vis des ET et sa capacité à immobiliser les ET (Pb et Zn) dans le sol. La coriandre, quant à elle, a tendance à accumuler les ET, en particulier le Cd, dans ses parties aériennes et a peu d'effets sur la mobilité et la biodisponibilité des ET dans le sol.

Bien que l'ajout d'un amendement biologique à base de CMA ait permis d'augmenter de façon significative les taux de mycorhization des racines de coriandre et de sauge sclarée, aucun gain n'a été observé ni au niveau de la croissance de ces deux PPAM, ni au niveau de la quantité et de la qualité des HE. Un effet positif en termes d'immobilisation des ET dans le sol, en particulier du Pb, et une réduction des transferts des ET (Cd et Pb) dans les parties aériennes a toutefois été montré chez la sauge sclarée (résultats INERIS).

D'autre part, les HE distillées à partir de la biomasse de sauge sclarée (inflorescences) ou de coriandre (parties aériennes ou graines) produite sur sols pollués présentent une qualité très satisfaisante, caractérisée par une très faible contamination en ET (résultats INERIS) ou en résidus de pesticides (traces), compatible avec un usage non alimentaire, voire même alimentaire. Leurs compositions chimiques, non altérée ni par la pollution en ET ni par l'inoculation mycorhizienne, sont conformes à celles décrites dans la littérature et mettent en évidence divers principes actifs dont le linalol, l'acétate de linalyle, le germacrène, l' α -pinène, le γ -terpinène, le 2-décenal, le décanal et le 2-dodécenal.

De nombreuses propriétés biologiques potentiellement valorisables dans des domaines à vocation non-alimentaire, tels que la protection des cultures (effets fongistatiques, anti-germinatifs et herbicides) ou la santé humaine (effets anti-inflammatoires et antioxydants) ont été mises en évidence pour l'ensemble des HE produites lors de ce travail. L'HE issue des parties aériennes de coriandre se distingue par des activités biologiques, plus marquées, souvent équivalentes à certains produits phytosanitaires commerciaux et antioxydants de référence. En revanche, en dépit de son fort pouvoir complexant des HE, la β -CD utilisée pour éviter la dégradation des HE et en améliorer les effets biologiques, n'a montré aucun effet positif sur les propriétés testées dans nos conditions expérimentales.

Par ailleurs, la restauration écologique des sols pollués grâce aux phytotechnologies est également un élément important à considérer dans l'évaluation d'une méthode de phytomanagement. Ainsi, nos

résultats ont mis en évidence une modulation de la diversité et de la composition des communautés bactériennes et fongiques, telluriques et racinaires par la revégétalisation du sol à l'aide de la sauge sclarée, ainsi qu'un effet moins prononcé de l'inoculation mycorhizienne. Nos résultats suggèrent aussi que la culture de sauge sclarée favorise le recrutement de genres bactériens spécifiques, tels que *Streptomyces*, au sein desquels des espèces PGPR ont été identifiées. En revanche, la proportion de champignons endophytes et symbiotiques demeure relativement faible au cours du temps, masquée par la prévalence des groupes de champignons saprotrophes et phytopathogènes.

Enfin, compte tenu de l'ensemble des réponses obtenues et en se basant sur les différents critères évalués lors de cette étude, rappelés dans le Tableau 10, il apparaît que la coriandre est un candidat modérément satisfaisant, alors que la sauge sclarée, elle, semble être un candidat plus attractif pour la phytostabilisation aidée et la valorisation de la biomasse par la production d'HE, dans le cadre du phytomanagement de sols pollués par les ET. Elle pourrait donc être envisagée comme une nouvelle option de reconversion pour les agriculteurs. En effet, l'analyse technico-économique préliminaire réalisée dans le cadre de ce travail a montré que la valeur ajoutée de la sauge sclarée résidait notamment dans la marge brute bénéficiaire dégagée, grâce à des coûts de production relativement faibles expliqués en partie par le caractère pérenne de cette PPAM, des rendements élevés en HE et une valeur marchande satisfaisante des HE de sauge sclarée. En revanche, cette étude économique a révélé un seuil de rentabilité assez faible dans le cas de la coriandre au vu du coût de production, des rendements en HE modérés et du faible prix de vente de son HE. Dans le cadre de la reconversion des sols pollués par les ET sur le site Metaleurop, cette espèce végétale apporte donc de faibles garanties économiques aux agriculteurs, notamment en comparaison à des cultures de référence telles que le blé dont l'itinéraire technique est bien connu.

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Annexe

Liste des valorisations scientifiques

Publications parues :

- R. Raveau, J. Fontaine, M. Hijri and A. Lounès-Hadj Sahraoui (2020). The culture of clary sage shaped the rhizospheric bacterial communities more strongly than mycorrhizal inoculation in the trace element-contaminated soil -a two-year monitoring field trial. *Frontiers in Microbiology* 11. 10.3389/fmicb.2020.586050
- I. El-Alam, R. Raveau, J. Fontaine, A. Verdin, F. Laruelle, S. Fourmentin, R. Chahine, H. Makhlof and A. Lounès-Hadj Sahraoui (2020). Antifungal and Phytotoxic Activities of Essential Oils: In Vitro Assays and Their Potential Use in Crop Protection. *Agronomy* 10, 825. <https://doi.org/10.3390/agronomy10060825>
- R. Raveau, J. Fontaine and A. Lounès-Hadj Sahraoui (2020). Essential Oils as Potential Alternative Biocontrol Products against Plant Pathogens and Weeds: A Review. *Foods* 9, 365. <https://doi.org/10.3390/foods9030365>

Publications soumises/à soumettre :

- R. Raveau, J. Fontaine, V. Bert, A. Perlein, B. Tisserant and A. Lounès-Hadj Sahraoui. *In situ* cultivation of aromatic plant species for the phytomanagement of an aged-trace element contaminated soil: plant biomass improvement options and techno-economic assessment of the essential oil production channel. *A soumettre dans "Science of the Total Environment"*.
- R. Raveau, J. Fontaine, A. Verdin, L. Mistrulli, P. Ferrant, F. Laruelle, S. Fourmentin and A. Lounès-Hadj Sahraoui. Biological activities of clary sage and coriander essential oils: *in vitro* screening towards their potential use as crop protection and healthcare products. *Soumise dans "Industrial Crops and Products" (2020)*.
- R. Raveau, A. Lounès-Hadj Sahraoui, M. Hijri, J. Fontaine. Clary sage cultivation and mycorrhizal inoculation influence rhizosphere fungal community structure over time in a trace-element polluted site. *Soumise dans "Mycorrhiza" (2020)*.
- R. Raveau, A. Lounès-Hadj Sahraoui, J. Fontaine. Recent advances in microbial aided phytostabilisation of trace element contaminated soils. *Dans "Advances in Microbe-assisted Phytoremediation of Polluted Sites"*, eds. K. Baudh et Y. Ma (Elsevier 2021).

Communications orales :

- R. Raveau, J. Fontaine, V. Bert, A. Perlein, A. Lounès-Hadj Sahraoui. Combiner la phytostabilisation aidée avec la culture de plantes aromatiques productrices d'huiles essentielles pour le phytomanagement des sols contaminés par les ETM (Projet PhytEO). Intersoil 2020, Belgique, Bruxelles, 2 et 3 mars 2020.
- R. Raveau, V. Bert, J. Fontaine, A. Perlein, A. Lounès-Hadj Sahraoui. Projet PhytEO : Développement d'une filière éco-innovante de valorisation non alimentaire de phytomasse produite sur des sols contaminés par des éléments traces métalliques : production d'huiles essentielles. 4èmes rencontres nationales de la recherche sur les sites et sols pollués (ADEME), France, Paris, 26 et 27 novembre 2019.
- R. Raveau, J. Fontaine, A. Lounès-Hadj Sahraoui. Apports d'un inoculum mycorrhizien dans le cadre d'une filière de phytomanagement des sols contaminés par des éléments traces métalliques. Mycorrhizes 2019, Canada, Québec, 30 octobre 2019.
- R. Raveau, J. Fontaine, V. Bert, A. Lounès-Hadj Sahraoui. La production d'huiles essentielles à action « bio-pesticide » comme filière de valorisation de la phytomasse produite sur des sols historiquement pollués par les éléments traces métalliques. Workshop « transition écologique », France, Beauvais, 18 octobre 2019.

- R. Raveau, J. Fontaine, A. Lounès-Hadj Sahraoui. Arbuscular mycorrhizae for green and sustainable phytomanagement of aged contaminated soils? A feedback from laboratory to field scale experiments. 16th International Phytotechnology Conference (IPC), China, Changsha, 23 – 27 September 2019.
- R. Raveau, J. Fontaine, V. Bert, A. Lounès-Hadj Sahraoui. Essential oil production, an innovative phytomanagement approach to value plant biomass produced on Metal Trace -aged contaminated soils: a field experiment. 16th International Phytotechnology Conference (IPC), China, Changsha, 23 – 27 September 2019.
- R. Raveau, J. Fontaine, A. Lounès-Hadj Sahraoui. Valorisation de la phytomasse produite dans le cadre d'une stratégie de phytomanagement de sols contaminés par des ETM : production d'huiles essentielles à activités biologiques. Journées Jeunes Chercheurs Transfrontalières, France, Reims, 2 et 3 avril 2019.
- R. Raveau, J. Fontaine, A. Lounès-Hadj Sahraoui. PhytEO project: The production of essential oils, an innovative eco-sector to reclaim historically contaminated soils – Initial results. Congrès Intersol 2019, France, Lille, 26, 27 et 28 mars 2019.
- A. Lounès-Hadj Sahraoui, R. Raveau, J. Fontaine. PhytEO project: The production of essential oils, an innovative eco-sector to reclaim historically contaminated soils – Comprehensive approach. Congrès Intersol 2019, France, Lille, 26, 27 et 28 mars 2019.
- V. Bert, A. Perlein, A. Papin, A. Lounès-Hadj Sahraoui, J. Fontaine, R. Raveau, R. Gaucher. Metal transfer in aromatic medicinal plants, essential oil and residues focusing on valorization pathways. 15th International Phytotechnologies Conference (IPC), Serbia, Novi Sad, 1-5 October 2018.

Communications sous forme de posters :

- R. Raveau, J. Fontaine, V. Bert, A. Lounès-Hadj Sahraoui. La production d'huiles essentielles à action « bio-pesticide » comme filière de valorisation de la phytomasse produite sur des sols historiquement pollués par les éléments traces métalliques. Journées SFR Condorcet, Reims, 25 et 26 Juin 2019.
- V. Bert, A. Perlein, J. Fontaine, R. Raveau, A. Lounès-Hadj Sahraoui. Etude de la faisabilité technique du phytomanagement d'un site agricole pollué par les éléments potentiellement toxiques à l'aide de plantes à parfum aromatiques et médicinales (Projet PhytEO). 4èmes rencontres nationales de la recherche sur les sites et sols pollués (ADEME), France, Paris, 26 et 27 novembre 2019.
- R. Raveau, J. Fontaine, A. Lounès-Hadj Sahraoui. Phytomanagement des sols historiquement contaminés par les ETM : production d'huiles essentielles à activités biologiques. Congrès de la société française de Toxicologie, Lille, 27 et 28 Novembre 2018.
- R. Raveau, J. Fontaine, A. Lounès-Hadj Sahraoui. Apport d'un inoculum mycorhizien sur la croissance de plantes aromatiques et la production d'huiles essentielles sur un sol historiquement contaminé par les éléments traces métalliques. 5èmes Journées Francophones des mycorhizes, Dunkerque, 27, 28 et 29 juin 2018.
- R. Raveau, J. Fontaine, A. Lounès-Hadj Sahraoui. La production d'huiles essentielles : une nouvelle voie de reconversion des sols agricoles historiquement contaminés par les éléments traces métalliques. Journées SFR Condorcet, Calais, 14 et 15 Juin 2018.
- R. Raveau, J. Fontaine, A. Lounès-Hadj Sahraoui - Production d'huiles essentielles à partir de plantes aromatiques cultivées *in situ* sur les sols historiquement pollués de Metaleurop. Journées jeunes chercheurs J2C2, Amiens, 18 et 19 janvier 2018.
- R. Raveau, J. Fontaine, A. Verdin, S. Fourmentin, A. Lounès-Hadj Sahraoui. La production d'huiles essentielles à activités biologiques : une filière éco-innovante de valorisation non alimentaire de biomasse végétale produite sur sols pollués. Colloque Santé-Environnement, Dunkerque, 23 et 25 octobre 2017.
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* **Lorsque j'ai présenté les communications (orales ou posters) en tant qu'orateur principal, le nom est souligné.**

Contribution au développement d'une filière éco-innovante de phytomanagement de sols pollués par les ET : culture de plantes aromatiques et production d'huiles essentielles

Le phytomanagement compte parmi les méthodes innovantes de gestion des sites et sols pollués en raison de son adéquation avec le développement durable. Pour répondre à la double exigence de performances environnementales et économiques, les phytotechnologies sont désormais combinées à la valorisation de la biomasse produite sur sols pollués. Parmi les filières éco-innovantes et non-alimentaires de valorisation de cette biomasse, la culture de plantes à parfums, aromatiques et médicinales (PPAM) pour la production d'huiles essentielles (HE), substances biosourcées à haute valeur ajoutée, a été proposée dans le cadre du projet PhytEO, financé par l'ADEME. Ainsi, ma contribution à ce projet a permis d'évaluer la pertinence d'un mode de gestion reposant sur la culture de la sauge sclarée et de la coriandre, en présence ou non d'un amendement mycorhizien, en s'appuyant sur des démonstrateurs *in situ* à l'échelle de l'hectare, parcelles historiquement polluées ou non par les éléments traces (ET). La coriandre ainsi que la sauge sclarée ont toutes deux montré une bonne capacité à s'installer, à se développer et à produire une quantité importante de biomasse sur des sols présentant une forte pollution historique par les ET. Bien que l'ajout d'un amendement biologique à base de champignons mycorhiziens à arbuscules ait permis d'augmenter de façon significative les taux de mycorhization des racines de coriandre et de sauge sclarée, aucun gain n'a été observé ni au niveau de la croissance de ces deux PPAM, ni au niveau de la quantité et de la qualité des HE. Un effet positif en termes d'immobilisation des ET dans le sol, en particulier du Pb, et une réduction des transferts des ET (Cd et Pb) dans les parties aériennes a toutefois été montré chez la sauge sclarée. D'autre part, les HE distillées à partir de la biomasse de sauge sclarée (inflorescences) ou de coriandre (parties aériennes ou graines) cultivées sur sols pollués présentent une qualité très satisfaisante, caractérisée par leur non contamination en ET ou en résidus de pesticides (traces). Leurs compositions chimiques, non altérées par la pollution en ET et l'inoculation mycorhizienne, affichent divers principes actifs dont le linalol, l'acétate de linalyle, le germacrène, l' α -pinène, le γ -terpinène, le 2-décénal, le décanal et le 2-dodécénal. Ainsi, plusieurs propriétés biologiques potentiellement valorisables dans des domaines à vocation non-alimentaire, tels que la protection des cultures (effets fongistatiques, anti-germinatifs et herbicides) ou la santé humaine (effets anti-inflammatoires et antioxydants) ont été mises en évidence. En revanche, en dépit de son fort pouvoir complexant des HE, le β -cyclodextrine n'a montré aucun effet positif sur les propriétés biologiques testées. Par ailleurs, la végétalisation du sol pollué par la sauge sclarée permet une modulation de la structure des communautés bactériennes et fongiques, telluriques et racinaires. Enfin, l'ensemble des résultats de cette étude et de l'analyse technico-économique de la filière PPAM-HE soulignent le haut potentiel de la sauge sclarée dans une démarche de phytomanagement de sols pollués par les ET.

Mots-clés : Pollution des sols – éléments traces – phytomanagement – plantes à parfum, aromatiques et médicinales – sauge sclarée – coriandre – huiles essentielles – inoculation mycorhizienne – microbiote rhizosphérique

Contribution to the development of an eco-innovative phytomanagement channel of TE-polluted soils: aromatic plants' cultivation and essential oil production

To cope with the soil trace element (TE) pollution, phytomanagement was brought forward as an eco-friendly and feasible approach. To meet the dual requirements of environmental and economic performances, phytotechnologies are now combined with the valorisation of biomass produced on polluted soils. Among the eco-innovative channels intended for the non-food valorisation of the produced biomass, the cultivation of aromatic and medicinal plants producing essential oils (EO), high-added value biosourced products, has been suggested within the framework of PhytEO project, funded by ADEME. Thus, this thesis contributed to evaluate the relevance of a management approach based on clary sage and coriander cultivation, combined or not with a mycorrhizal inoculum, on *in situ* experimental plots polluted or not with TE. Both clary sage and coriander have shown a good ability to settle in, to grow and to produce high amounts of biomass, despite the presence of high TE concentrations in the soil. The addition of an amendment based on arbuscular mycorrhizal fungi enhanced the mycorrhizal colonisation rates of both coriander and clary sage roots. However, no significant improvement was observed in terms of plants' growth, EO amounts and quality. Moreover, mycorrhizal inoculation allowed TE immobilisation in soils, in particular Pb, and reduced TE (Cd and Pb) transfers in aerial parts of clary sage. Besides, the EO distilled from clary sage inflorescences and coriander (aerial parts or seeds) grown on TE polluted soils, displayed a highly satisfactory quality, regarding the absence of contamination by TE or pesticide residues (trace amounts). Their chemical compositions were not altered by both TE pollution and mycorrhizal inoculation and were characterized by several active principles, such as linalool, linalyl acetate, germacrene, α -pinene, γ -terpinene, 2-decenal, decanal and 2-dodecenal. Furthermore, several biological properties with potential applications in non-food fields such as crop protection (antifungal, anti-germinative and herbicidal) or human healthcare (antioxidant and anti-inflammatory) have been brought forward. However, despite its ability to form inclusion complexes with EO components, β -cyclodextrin has shown no significant improvement of the tested EO biological activities. On another note, the soil revegetation with clary sage displayed a clear shaping of the bacterial and fungal communities, in both the rhizospheric soil and the roots of the aromatic plant species. Altogether, our results combined with the techno-economic feasibility assessment of the channel "aromatic and medicinal plants/EO" have emphasised clary sage as a good candidate for the phytomanagement of TE-polluted soils.

Keywords: Soil pollution – trace elements – phytomanagement – aromatic and medicinal plants – clary sage – coriander – essential oils – mycorrhizal inoculation – rhizospheric microbiota