



Étude comparative de trois biomoniteurs (conifère, escargot et miel) pour évaluer la variabilité spatio-temporelle de polluants organiques dans l'atmosphère au Liban

Firas Baroudi

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**Étude comparative de trois biomoniteurs
(conifère, escargot et miel) pour évaluer la
variabilité spatio-temporelle de polluants
organiques dans l'atmosphère au Liban**

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Liste des abréviations et notations

AAS	Spectrométrie d'absorption atomique
ACN	Acétonitrile
AE	Acétate d'éthyle
ANT	Anthracène
ASE	Extraction accélérée par solvant
BaA	Benz[a]anthracène
BaP	Benzo[a]pyrène
BeP	Benzo[e]pyrène
C₁₈	Octadécylsilane
CHR	Chrysène
DDT	Dichlorodiphenyltrichloroethane
FL	Fluorène
FLA	Fluoranthène
GC	Chromatographie en phase gazeuse
GC-MS	Chromatographie en phase gazeuse couplée à la spectrométrie de masse
GC-MS/MS	Chromatographie en phase gazeuse couplée à la spectrométrie de masse en tandem
HAPs	Hydrocarbures aromatiques polycycliques
HClO₄	Acide perchlorique
HMW	Somme des HAPs à quatre et cinq cycles
HNO₃	Acide nitrique
HPLC	Chromatographie en phase liquide à haute performance
LC	Chromatographie en phase liquide
LC-MS/MS	Chromatographie en phase liquide couplée à la spectrométrie de masse en tandem
LLE	Extraction liquide-liquide
LMW	Somme des HAPs à deux et trois cycles
LOD	Limite de détection
LOQ	Limite de quantification
MAE	Extraction assistée par micro-ondes
MgSO₄	Magnésium anhydre
MS-MS	Spectrométrie de masse en tandem
NaCl	Chlorure de sodium
OCPs	Pesticides organochlorés
PA	Polyacrylate
PbNO₃	Nitrate de plomb
PCBs	Polychlorobiphényles
PDMS	Polydiméthylsiloxane
PHE	Phénanthrène
POPs	Polluants organiques persistants
PSA	Amine primaire secondaire
PYR	Pyrène
SPE	Extraction en phase solide
SPME	Microextraction en phase solide

Liste des publications

Articles publiés

Baroudi, F., Al Alam, J., Fajloun, Z., Millet, M., 2020a. Snail as sentinel organism for monitoring the environmental pollution; a review. *Ecological Indicators* 113, 106240. <https://doi.org/10.1016/j.ecolind.2020.106240>

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Baroudi, F., Al-Alam, J., Delhomme, O., Chimjarn, S., Al-Ghech, H., Fajloun, Z., Millet, M., 2020. Liquid-liquid extraction procedure for non-volatile pesticides determination in Acacia honey as environmental biomonitors. *Journal of Environmental Science and Health, Part B (JESHB-2020-0267)*.

Introduction

La pollution environnementale due aux émissions de divers types de polluants dans l'air ou le sol constitue à l'heure actuelle un des axes de recherches les plus importants. Malgré l'intérêt que porte l'usage de certaines molécules chimiques telles que les médicaments sur le développement de l'humanité et son mode de vie, celles-ci peuvent être à l'origine de perturbations de l'équilibre écologique dont l'homme sera affecté de manière plus ou moins importante. En effet, les activités anthropiques qu'elles soient industrielles ou agricoles sont à l'origine de l'émission dans l'environnement d'une multitude de composés organiques qui peuvent être considérés comme des polluants chimiques. Ces composés du fait de leur résistance à la dégradation et leurs propriétés physico-chimiques peuvent persister dans l'environnement et s'accumuler jusqu'aux niveaux des organismes humains conduisant parfois à des effets néfastes inéluctables. Ainsi, l'homme est fréquemment exposé à des tonnes de ces substances chimiques via ses pratiques journalières mais également via sa nourriture. Parmi ces polluants figurent les pesticides, y compris les organochlorés, les hydrocarbures aromatiques polycycliques et les polychlorobiphényles.

En effet, le travail dans des secteurs qui utilisent régulièrement les pesticides, comme l'agriculture et la lutte antiparasitaire, ainsi que la vie à proximité de zones traitées par ces molécules, augmentent l'exposition globale des personnes évoluant au contact ou à proximité de ce genre de polluants. En outre, la consommation d'aliments contaminés par des résidus de pesticides reste la source majeure d'exposition humaine aux produits phytosanitaires mais elle est fortement influencée par l'âge des personnes et leurs préférences alimentaires. Quant aux polluants organiques persistants, ces produits sont connus pour leur persistance élevée, leur transport à longue distance, leur bioaccumulation et leur toxicité. En effet, les caractéristiques de ces polluants conduisent à leur présence de manière ubiquiste dans tous les compartiments de l'environnement.

L'atmosphère est un excellent vecteur pour la mondialisation de la contamination des milieux et des denrées. En effet, ces nombreux polluants, quand ils sont émis, transitent à un moment ou à un autre par l'air qui assurera leur transport souvent loin de leurs zones d'émissions. Ainsi, les cultures sont non seulement contaminées par les applications mais

également par les dépôts d'origine atmosphérique. De ce fait, la caractérisation de la qualité de l'air semble, de nos jours, une approche fondamentale afin d'évaluer l'impact potentiel des divers polluants sur l'environnement. Deux techniques principales d'échantillonnage ont été couramment utilisées permettant une évaluation qualitative et quantitative précise de la pollution de l'air : les échantillonneurs d'air actif et les échantillonneurs d'air passif. Néanmoins, vu les contraintes électriques, chimiques et pratiques des échantillonneurs d'air actif, les études récentes sont plutôt orientées vers l'usage des échantillonneurs d'air passif. Parmi ces échantillonneurs les plus disponibles et les plus utilisés, figure l'usage de certains éléments biologiques naturels comme capteurs fiables de polluants émis dans l'atmosphère. Il s'agit de la technique dite de « biomonitoring » ou de « biosurveillance » environnementale, généralement définie comme « l'utilisation systématique des organismes vivants ou de leurs réponses pour déterminer l'état et / ou les changements de l'environnement ».

Dans cette thèse de doctorat, différentes méthodes analytiques ont été utilisées pour la détermination des polluants organiques dans l'air et les biomoniteurs retenus.

La partie I de ce manuscrit de thèse est consacrée à une étude bibliographique centrée sur la problématique de la large utilisation des pesticides et la présence des polluants organiques persistants dans l'air et la nécessité de développer des méthodes analytiques appropriées pour les quantifier. Ainsi, la chromatographie couplée à la spectrométrie de masse est également évoquée dans cette partie.

La partie II présente les travaux expérimentaux réalisés et les résultats obtenus au cours de ces travaux de thèse et ils sont répartis en six chapitres différents selon la matrice étudiée et la technique chromatographique utilisée :

- Le chapitre 1 concerne une revue scientifique publiée dans le journal « *Ecological Indicators* » sur l'usage des escargots en tant que biomoniteur de la pollution de l'air. L'intérêt de ce travail était d'explorer la possibilité d'utiliser différents types d'escargots, en raison de leur sensibilité à divers contaminants et de leur capacité à les accumuler dans leurs tissus, comme matrice de surveillance potentielle et idéale pour évaluer la pollution de l'air et détecter la contamination en métaux lourds, en pesticides et en polluants organiques persistants par différentes techniques

d'extraction, notamment l'extraction au Soxhlet, l'extraction accélérée par solvant (ASE), l'extraction en phase solide (SPE), l'extraction assistée par micro-ondes (MAE) et la digestion acide par micro-ondes ;

- Le chapitre 2 est consacré à un travail publié dans « *Journal of Chromatography A* » qui porte sur le développement d'une méthode multirésidus permettant l'analyse de pesticides, d'hydrocarbures aromatiques polycycliques (HAPs) et de polychlorobiphényles (PCBs) dans des escargots utilisés comme biomoniteurs environnementaux. Ainsi, une stratégie d'extraction a été élaborée et est basée sur la procédure d'extraction QuEChERS et sur la microextraction en phase solide pour le criblage simultané de 120 pesticides, 16 HAPs et 22 PCBs à partir de l'escargot terrestre *Helix aspersa*. Cette méthode a été validée et elle a montré une sensibilité, une exactitude et une précision élevées, avec des limites de détection (LOD) et de quantification (LOQ) inférieures à 20 ng g⁻¹ pour la plupart des polluants considérés. Les analyses inter et intra-journalières ont révélé un faible écart-type relatif qui était inférieur à 20% pour la plupart des composés ciblés. De plus, le coefficient de régression (R²) obtenu était supérieur à 0.98 et les taux de récupérations étaient supérieurs à 60% pour la majorité des polluants évalués ;
- Le chapitre 3 présenté sous forme d'article publié dans « *Microchemical Journal* », décrit le développement d'une autre méthode multi-résidus pour la quantification concomitante, dans des aiguilles de conifères (*Pinus nigra*), de 134 pesticides, 16 HAPs et 22 PCBs, utilisant les analyses chromatographiques en phase liquide (LC) et gazeuse (GC) couplées à la spectrométrie de masse en tandem après une extraction avec la technique QuEChERS et pré-concentration par microextraction sur phase solide (SPME). Cette méthode présente l'avantage d'utiliser un faible volume de solvants organiques tout en permettant l'extraction d'une large gamme de polluants (172 polluants) et cela avec un temps de préparation des échantillons court. Les performances obtenues par cette méthode ont révélé un taux de récupération compris entre 60% et 121% pour tous les composés ciblés, une sensibilité et précision élevées, avec des LOD et des LOQ inférieures à 20 ng g⁻¹ pour la plupart des composés cibles avec un RSD inférieures à 20 % ;
- Le chapitre 4 présenté sous forme d'un article publié dans « *Environmental Science and Pollution Research* » présente une application directe de la méthode d'extraction des aiguilles de conifères prélevés dans quinze régions différentes au nord du Liban.

Les principaux objectifs étaient de déterminer les sept sites les plus pollués à choisir comme les sites principaux pour l'étude finale, qui consistera en une campagne d'évaluation de la qualité de l'air utilisant à la fois des escargots et des conifères ;

- Le chapitre 5, soumis dans « *Environmental Chemistry Letters* » est consacré à la campagne d'évaluation de la qualité de l'air au voisinage des cultures par une approche basée sur le biomonitoring grâce aux escargots et aiguilles de conifères. Au cours de ce travail et en utilisant les deux méthodes analytiques mentionnées précédemment, il a été possible d'étudier des échantillons d'escargots et de conifères prélevés dans sept sites différents au nord du Liban et cela pendant six mois consécutifs (de Juin à Décembre 2019). L'analyse des échantillons collectés a montré une persistance des différents polluants étudiés et ceci en fonction des caractéristiques de chaque site. Les résultats obtenus à l'issue de ce travail ont montré que les échantillons des aiguilles de conifères et des escargots collectés révèlent une variation non similaire des différents types de polluants pour chaque matrice. L'analyse des données météorologiques au cours de cette période suggère que les résultats obtenus dans ce cadre étaient en corrélation avec les conditions climatiques qui varient considérablement au cours de cette période de l'année ;
- Le chapitre 6, soumis dans « *Journal of Environmental Science and Health, Part B* » contribue au développement d'une méthode multi-résidus pour l'analyse de pesticides dans du miel. La méthode développée consistait à l'extraction liquide-liquide (LLE) des pesticides par l'acétate d'éthyle (AE) et leur analyse par chromatographie en phase liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS). Le coefficient de régression obtenu était supérieur à 0.99, les récupérations étaient supérieures à 60% et l'écart type obtenu sur des échantillons dopés à plusieurs niveaux de concentration était inférieur à 5% dans toutes les extractions.

Enfin, la partie III du manuscrit est consacrée à la discussion générale de tous les travaux entrepris et des résultats obtenus au cours de ces années de recherche. Une conclusion générale menant à quelques perspectives est présentée à la fin du manuscrit.

Chapitre I : Synthèse bibliographique

I. Pollution de l'air

1. Généralités

La pollution de l'air est une préoccupation majeure du nouveau monde civilisé, qui a un grave impact toxicologique sur la santé humaine et l'environnement (Manisalidis et al., 2020). Elle est définie comme l'ensemble des effets destructeurs de toute source contribuant à la pollution de l'air et / ou à la détérioration de l'environnement. La pollution de l'air est causée à la fois par des phénomènes naturels et / ou par des interventions humaines. De nombreux types de polluants, y compris des matériaux en phases solide, liquide et gazeuse, sont présents dans l'air au-dessus de leurs niveaux ambiants normaux et à une concentration suffisamment élevée pour produire ainsi un effet mesurable et indésirable pour les animaux, les hommes et les végétaux (Manisalidis et al., 2020 ; Ghorani-Azam et al., 2016).

L'organisation mondiale de la santé (OMS) estime que l'exposition à la pollution de l'air ambiant provoque chaque année environ 3,7 millions de décès prématurés dans le monde (WHO, 2013). Cette pollution de l'air a donc été identifiée comme la principale cause environnementale de ces décès prématurés (Yin et al., 2020 ; Babatola, 2018). Dans un scénario socioéconomique, il a été estimé que la contribution de la mortalité prématurée à l'air extérieur pollué pourrait doubler d'ici 2050 et qu'elle resterait ainsi la cause environnementale principale de mortalité dans le monde (Vandyck et al., 2018).

Selon le degré d'exposition, l'impact de l'air pollué sur la santé humaine s'étend à partir d'événements symptomatiques et subcliniques jusqu'à une mortalité accrue et / ou une morbidité (Hamanaka et Mutlu, 2018). Les preuves scientifiques confirment que les expositions à court et à long terme aux polluants de l'air ambiant sont associées à un large éventail de conséquences néfastes pour la santé (Domingo et Rovira, 2020 ; Kelly et Fussell, 2015), comme des taux de mortalité plus élevés et des admissions à l'hôpital plus importantes (Achilleos et al., 2019) (Figure 1).

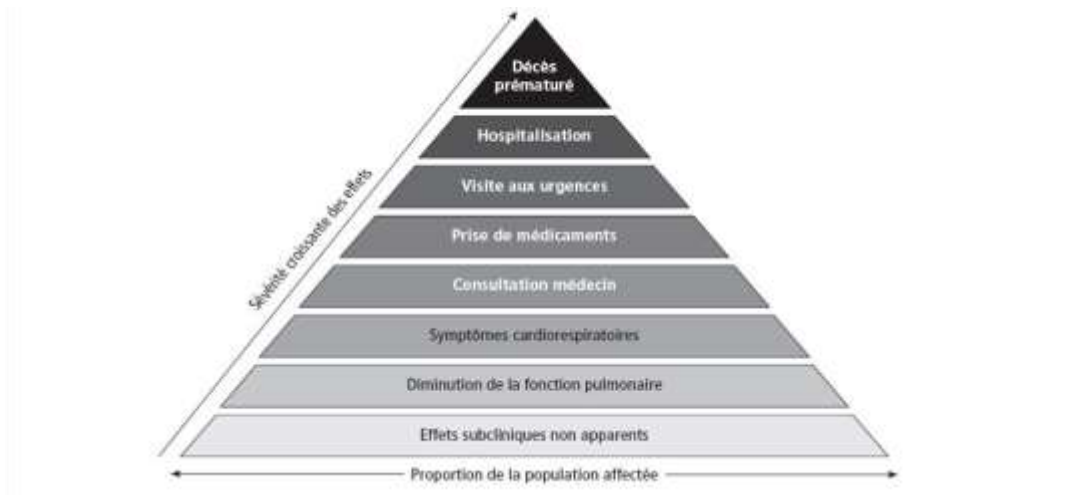


Figure 1. Pyramide des effets associés à la pollution de l'air sur la santé (Melody et Johnston, 2015)

Les expositions à la pollution de l'air ambiant affectent de grandes populations du monde entier. Plus de 90% de la population mondiale est exposée à des niveaux néfastes de pollution de l'air dépassant les recommandations de l'OMS (WHO, 2019). Les personnes à tous les stades de la vie, en particulier les personnes âgées et les enfants, peuvent être affectés par l'exposition à des niveaux élevés de pollution de l'air ambiant et dont l'exposition à long terme représente le plus grand risque (Lelieveld et al., 2018). Du point de vue santé humaine, l'exposition aiguë et chronique aux polluants organiques, aérosols et gaz est positivement associée aux maladies cardiovasculaires, respiratoires et au cancer du poumon (Beelen et al., 2014). Les effets respiratoires et cardiovasculaires de l'exposition à la pollution de l'air sont bien démontrés tant dans la population professionnelle que dans la population générale (Lee et al., 2018). La plupart de la littérature disponible sur les relations entre l'exposition à la pollution de l'air et le cancer porte sur les cancers du poumon (Santibanez-Andrade et al., 2017 ; Raaschou-Nielsen et al., 2016) et les cancers infantiles (Pacitto et al., 2018 ; Shmuel et al., 2017). Des études ont révélé que l'exposition à plusieurs polluants tels que les gaz d'échappement des moteurs diesel et les HAPs était liée à un risque accru de maladies (Peters et al., 2018).

La pollution de l'air nuit non seulement à la santé humaine, mais aussi à l'environnement dans lequel nous vivons. Un large éventail d'écosystèmes est actuellement menacé par la pollution de l'air et ceci à travers une variété des processus, tels que l'acidification et l'eutrophisation. Les précipitations humides (brouillard, pluie, neige) ou sèches (particules et gaz) impliqués dans la formation des pluies acides sont capables d'acidifier

les environnements aquatiques et terrestres et d'endommager les arbres et les plantations (De Vries et al., 2014). Concernant les particules en tant que polluant atmosphérique, son impact sur la productivité alimentaire, le rendement des cultures et sur les organismes aquatiques a été signalé (Zuhara et Isaifan, 2018).

2. Méthodologie et évolution des émissions de polluants

La qualité de l'air dépend des émissions de polluants atmosphériques (anthropiques et naturels), des conditions météorologiques (qui régissent la dispersion, le dépôt et les transformations physiques et chimiques de ces polluants dans l'atmosphère) et de la topographie (qui module les modes de circulation et de transport des polluants) (Abdel-Shafy et Mansour, 2016).

Au niveau local, les émissions naturelles dépendent de l'humidité et de la température. Le transport des polluants repose sur les caractéristiques du vent et des turbulences. Les précipitations influencent le dépôt de ces polluants (dépôts humide) et la topographie contrôle la dynamique à méso-échelle, comme les courants de vallée en montagne et les brises terre-mer. De plus, la circulation atmosphérique à l'échelle synoptique affecte le transport de la pollution à l'échelle régionale (Liao et al., 2020 ; Tibbetts, 2015). En conséquence et afin de caractériser la qualité de l'air dans un territoire donné, il est essentiel de saisir le rôle de la circulation synoptique contrôlant sa dynamique régionale et locale (Pineda-Martínez et al., 2014).

Divers polluants émis dans l'atmosphère peuvent être transportés par le vent, parfois vers des zones loin du lieu d'émission. Sous l'influence du rayonnement solaire et de la dynamique atmosphérique, les polluants dits primaires peuvent être convertis en polluants secondaires par des réactions physiques (coagulation, nucléation, condensation) et chimiques (réactions acido-basiques, réactions photochimiques, réactions d'oxydo-réductions). Par conséquent, de nouvelles particules telles que le nitrate d'ammonium, par exemple, se forme au cours de ce processus. Ces polluants ont parfois une demi-vie plus longue et peuvent être plus toxiques et nocifs que les polluants dits primaires (Juodis et al., 2016 ; Manisalidis et al., 2020).

Le transport des polluants dans l'air varie selon leurs propriétés et comprend trois niveaux : local (accumulation des polluants autour de leur zones d'émission), régional (les

polluants sont observés à plusieurs centaines de kilomètres de la zone d'émission) et global (les polluants atteignent la planète entière) (Gheorghe et Ion, 2011).

Enfin, le processus final provoqué par la pollution de l'air est le dépôt. Deux types de dépôts peuvent se produire, les dépositions humides liées aux précipitations et les dépositions sèches dues à l'influence de la gravité terrestre. Le dépôt humide réunit tous les mécanismes de transfert de matériaux de l'atmosphère au sol par précipitation qui peut se présenter sous forme diverses (pluie, neige, brouillard). Le dépôt sec se forme lorsque de fines particules acides et des gaz se déposent à la surface du sol. Les gaz tels que l'oxyde nitrique et le dioxyde de soufre se convertissent en acides au contact de l'eau (Arinaitwe et al., 2016) (Figure 2).

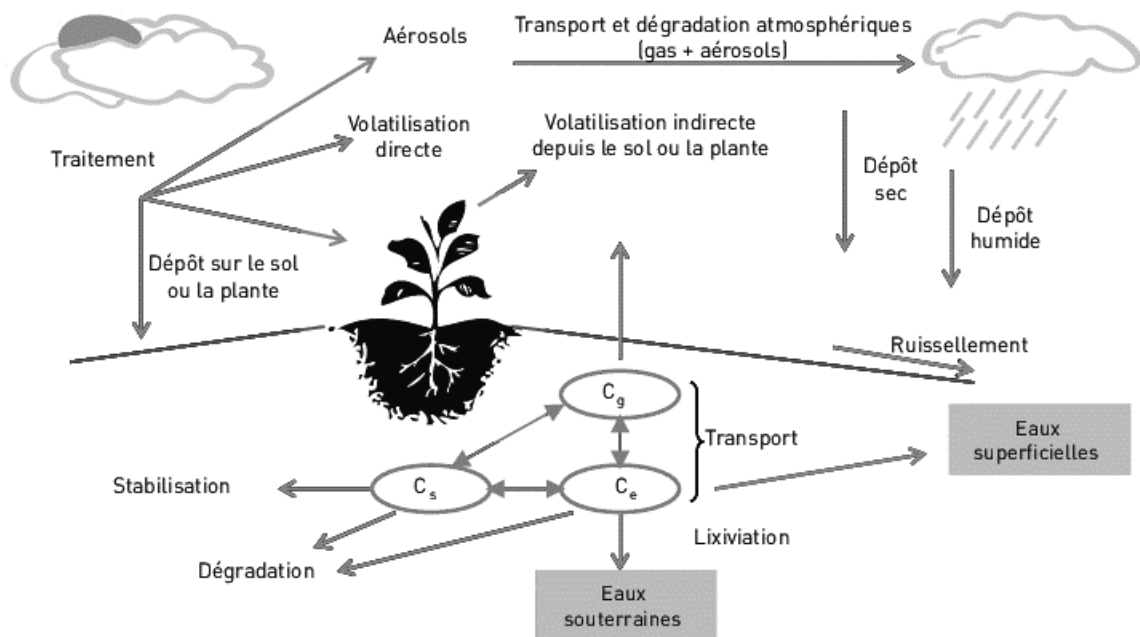


Figure 2. Processus et voies de dispersion des polluants dans l'environnement (Blanchoud et al., 2011)

3. Sources d'émission des polluants dans l'air

Les inventaires des sources d'émissions pour les polluants de l'air prennent en compte les principales catégories telles que le chauffage domestique, le brûlage des déchets, la production d'électricité, la consommation de carburant industriel et le transport. Il existe donc différentes sources de pollution atmosphérique qui diffèrent selon leur composition, leur caractéristique et les conditions dans lesquelles elles sont produites. Les sources

courantes de pollution de l'air sont les sources industrielles et agricoles ainsi que la combustion des combustibles fossiles provenant du trafic. Les polluants préoccupants sont les composés organiques volatils, les particules et les gaz comme le monoxyde de carbone et le dioxyde de soufre. Ce sont les polluants dits primaires, qui sont rejetés directement dans l'air alors que les polluants secondaires s'y forment principalement à partir des polluants primaires exposés au rayonnement ultra-violet solaire. Les particules ambiantes sont l'une des composantes de la pollution de l'air ambiant, qui est produite soit par des processus naturels soit par l'activité humaine. La pollution par les particules est un mélange complexe de particules et de gouttelettes extrêmement petites et se compose d'un certain nombre de composants, notamment des produits chimiques organiques, des métaux, des acides et des poussières ou des particules de sol (D'Amato et al., 2016 ; Schikowski et Altuğ, 2020).

Les sources principales de pollution de l'air sont réparties en sources naturelles qui existent certainement dans l'air et en sources anthropiques dues aux activités humaines.

- Les sources naturelles : parmi ces sources, il existe le volcanisme, libérant une grande quantité de cendres, de soufre et d'oxydes de carbone dans l'air. De plus, les volcans et les tempêtes de sable et de poussières constituent une source principale de petites particules dans l'environnement. La foudre est également considérée comme la source d'émissions d'oxyde d'azote, en particulier d'acide nitrique dans la haute troposphère. D'autres processus naturels peuvent également entraîner une augmentation de cette pollution naturelle comme la décomposition bactérienne de la matière organique, les incendies naturels et divers processus d'érosion (D'Amato et al., 2016).
- Les sources anthropiques : diverses sources sont classées comme sources anthropiques; les sources domestiques, les sources industrielles et les émissions d'échappement des automobiles. En plus de ces sources, il existe des sources agricoles qui sont responsables du rejet de nombreux polluants dans l'air et qui sont particulièrement dues aux applications d'engrais et de pesticides. Ces sources sont soit fixes telles que les centrales thermiques, les chaudières industrielles et les émissions provenant du chauffage domestique, ou des sources mobiles provenant de l'évaporation des essences, des effluents d'échappement des moteurs et des pulvérisations de pesticides (Manisalidis et al., 2020).

4. Pollution de l'air au Liban

Le Liban est situé sur la côte de la Méditerranée entre les latitudes 33° 20 - 34° 38 et les longitudes 35° 16 - 35° 16. D'est en ouest, le Liban peut être divisé géographiquement en quatre zones: la fertile plaine de la Bekaa (l'est du Liban), la chaîne de montagnes du Mont-Liban (centre ouest du Liban), une plaine côtière le long de l'ouest et la chaîne de montagnes qui longent la frontière orientale avec la Syrie (nord du Liban) (Ministère Libanais de l'Environnement, 2006). La surface du Liban est de 10 452 km² et sa population, est de 6 millions de personnes (CIA, 2018); par conséquent, le Liban a une densité de population élevée de 638.09 habitants / km². En 2018, l'agriculture qui employait plus de 39% de la population (Chaza et al., 2018) et l'industrie sont considérés comme les principaux contributeurs à la contamination par les pesticides et les polluants organiques persistants (POPs) au Liban (Chen, 2007).

Le Liban, membre de la Convention de Stockholm en 2003, a créé des inventaires nationaux des polluants organiques et en 2006 a formulé un aperçu national de mise en œuvre, afin de développer des cadres juridiques efficaces pour éliminer ou réduire la libération des polluants dans l'environnement. Malheureusement, le Liban est caractérisé par un secteur privé non réglementé de générateurs diesels (Shihadeh et al., 2013 ; Ghanem, 2018 ; Bouri et El Assad, 2016), un secteur de transport routier non durable (Haddad et al., 2019 ; Daher et al., 2013) et une gestion médiocre de la qualité de l'air. Le Liban connaît une augmentation des événements liés à la pollution avec un effet néfaste plus important dans les quartiers urbains due aux émissions élevées des polluants dans ces zones densément peuplées, comme la ville de Beyrouth (Mokalled et al., 2018 ; Massoud et al., 2011) (Figure 3).



Figure 3. Effet de la pollution atmosphérique sur la visibilité: panorama de la ville de Beyrouth par temps clair (à gauche) et pollué (à droite)

La pollution au Liban par les particules en suspension (PM_{10} et $PM_{2.5}$) a dépassée de 100% le seuil recommandé par l'OMS en 2005 (Roumie et al., 2011 ; Waked et al., 2013). Le Liban, comme de nombreux pays méditerranéens, connaît une croissance démographique croissante avec plus de 6 millions d'habitants en 2018. Environ 70% de la population libanaise vit sur un littoral étroit qui accueille un taux croissant d'urbanisations, d'industries, de polluants produits par les activités agricoles et transportés directement ou indirectement vers l'eau, l'air, et le sol.

Les pesticides, une des causes de la contamination de l'air, ne sont pas produits au Liban. Leur utilisation locale dépend des importations légales répondant aux besoins (Youssef et al., 2015). Au Liban, malheureusement, il existe de nombreux obstacles à la protection de l'agriculture malgré l'existence d'une législation, tels que la manque d'exigences de conformité pour l'utilisation et l'identification des polluants ainsi que la manque d'installations de matériels de recherche pour détecter leurs résidus et leurs effets, tandis que des mesures de contrôle législatives et politiques doivent encore être établies. Plusieurs études ont montré que la plupart des travailleurs agricoles ne connaissaient pas le nom des pesticides et la limite de toxicité à ne pas dépasser. Par ailleurs, plus de 66% ne pouvaient nommer aucun pesticide dangereux et semblaient ignorer les risques réels des pesticides, par manque de connaissance sur la sécurité. La liste des pesticides interdits publiée par le ministère de l'agriculture était auparavant connue par moins de 10% des travailleurs agricoles (Maddah et al., 2019 ; Salameh et al., 2004).

II. Principaux polluants dans l'air

L'OMS recense six principaux polluants atmosphériques, à savoir la pollution par l'ozone troposphérique, le plomb, le monoxyde de carbone, les particules, les oxydes de soufre et les oxydes d'azote. La pollution atmosphérique peut avoir un effet désastreux sur toutes les composantes de l'environnement, y compris l'air, le sol et les eaux souterraines. De plus, il constitue une menace sérieuse pour les organismes vivants. Un polluant atmosphérique est une matière dans l'air qui peut avoir des effets néfastes sur l'écosystème et les humains. La substance peut être des gouttelettes de liquide, des particules solides ou des gaz présents en suspension dans l'air. Ces polluants peuvent être d'origines naturelles ou d'origines anthropiques et sont classés comme primaires ou

secondaires, entraînant une augmentation du niveau de la pollution dans l'environnement naturel (Manisalidis et al., 2020).

1. Pesticides

1.1. Généralités

Les pesticides sont des produits chimiques, appliqués dans les terres agricoles, les jardins privés et autres zones publiques. Leurs rôles sont de détruire, repousser ou atténuer les parasites tels que les insectes, les acariens, les nématodes et les mauvaises herbes. Selon l'OMS, environ un million de personnes sont touchées par une intoxication aiguë au contact des pesticides, avec un taux de mortalité compris entre 0,4 et 1,9% enregistré chaque année (Jia et al., 2020 ; Qiu et al., 2017). La plupart des décès résultent d'expositions accidentelles ou professionnelles, qui sont généralement topiques ou par inhalation, plutôt que de l'auto-intoxication par ingestion. Les intoxications graves aux pesticides sont plus courantes dans les pays ruraux, à revenu faible ou intermédiaire, en raison de leur large utilisation (Eddleston, 2020) et en l'absence de réglementation par les ministères de l'agriculture et de la santé (Salameh et al., 2004).

De plus, de nombreux pesticides sont stables lors de leur application et, par conséquent, peuvent être transportés dans l'eau et l'air pour contaminer des zones éloignées de leur source d'application (Li et al., 2020). Il a été montré dans de nombreuses études que les pesticides sont des substances toxiques pour l'homme et l'environnement (Rasheed et al., 2019 ; Nicolopoulou-Stamati et al., 2016 ; Covert et al., 2020 ; El-Nahhal, 2020 ; Cheng et al., 2020) car leur utilisation affecte les écosystèmes et menace la faune, les animaux domestiques et sauvages (Ruiz-Suárez et al., 2015 ; Tavalieri et al., 2020). De nombreuses études ont également montré qu'une exposition persistante à ces polluants entraîne leur accumulation dans les tissus et induisent des effets néfastes sur le développement, la croissance ainsi que le métabolisme (Mahmood et al., 2015 ; Lushchak et al., 2018 ; Sabarwal et al., 2018 ; Jayaraj et al., 2016 ; La Merrill et al., 2013). Les pesticides ont été liés à plusieurs troubles, qui sont associés au système nerveux central (Mostafalou et Abdollahi, 2018), pulmonaire (Ye et al., 2013) et cardiovasculaire (Sekhatha et al., 2016).

La toxicité des pesticides dépend principalement de deux facteurs, à savoir la dose et le temps. Par conséquent, la quantité de substance impliquée (dose) et la fréquence à

laquelle l'exposition à la substance se produit (temps) donnent lieu à deux types différents de toxicité: la toxicité chronique et la toxicité aiguë. La toxicité chronique est la capacité d'un pesticide, après une exposition continue ou répétée, à provoquer des effets néfastes sur la santé pendant une période prolongée. Ce type de toxicité des pesticides est préoccupant non seulement pour ceux qui travaillent directement avec les pesticides mais aussi, pour le grand public, étant donné l'exposition potentielle aux pesticides présents dans les produits, l'eau et l'air. La toxicité aiguë désigne le degré de toxicité d'un pesticide après une seule exposition de courte durée. Un pesticide ayant une toxicité aiguë élevée est mortel même lorsqu'une très petite quantité est absorbée. Ce type de toxicité peut être mesuré en tant que toxicité aiguë par inhalation, toxicité cutanée aiguë et toxicité orale aiguë (Damalas et Koutroubas, 2016).

Les résidus de pesticides représentent un large éventail de sources de pollution agricole diffuses, qui dure longtemps et qui est difficile à dégrader, ce qui entraîne des risques d'exposition professionnelle à long terme pour les agriculteurs (Balazs et al., 2020 ; Yuan et al., 2017). En fait, un contact continu avec un pesticide avec de faibles doses était associé à un groupe de syndromes à moyen et long terme, impliquant de troubles du système nerveux et de nombreuses tumeurs (Bertero et al., 2020). Il a été démontré que l'exposition aux pesticides est liée à divers effets néfastes sur la santé, allant de la simple irritation des yeux et de la peau à des effets plus graves tels que le cancer (Lentola et al., 2017), des effets sur le système nerveux et des mutations génétiques (Marcelino et al., 2019). Des preuves substantielles existent pour d'autres résultats négatifs de l'exposition aux pesticides, comme des dommages neuronaux ainsi que des altérations neurocomportementales, qui augmentent le risque de maladie d'Alzheimer (Li et al., 2021).

De nombreuses techniques analytiques peuvent être utilisées pour déterminer la concentration de résidus de pesticides dans différentes matrices contaminées telles que sédiments, tissus de poissons, sol, plante, légumes, escargots, fruits et l'eau. Il s'agit notamment de la GC (Alghamdi et al., 2020 ; Iqbal et al., 2020 ; Issa et al., 2020), la LC (Al-Alam et al., 2020 ; Baroudi et al., 2020 ; Diuzheva et al., 2019 ; Hou et al., 2020 ; Yetim et al., 2020 ; Zhang et al., 2019) et la chromatographie en fluide supercritique (Cutillas et al., 2020 ; Wang et al., 2019).

Des techniques de préconcentration et de nettoyage doivent être utilisées avant l'analyse chromatographique des pesticides (Parrilla Vázquez et al., 2019 ; Madej et al., 2018 ; Makoś et al., 2018). Les techniques de préconcentration les plus couramment utilisées dans les échantillons comprennent la SPE (Wang et al., 2019), la SPME (Gionfriddo et al., 2018 ; Wu et al., 2016), l'extraction QuEChERS (Baroudi et al., 2020 ; Al-Alam et al., 2020), la micro-extraction dispersive liquide-liquide (Musarurwa et Tavengwa, 2020 ; Salemi et al., 2019) et l'extraction par dispersion en phase solide (Chatzimitakos et al., 2019 ; Soares et al., 2017).

1.2. Classification des pesticides

Un pesticide est un produit chimique fabriqué par l'homme et n'existant pas dans la nature. Ces pesticides varient par leurs propriétés physiques et chimiques d'une classe à l'autre et sont classés en plusieurs groupes en fonction de leur utilisation. Ils peuvent être utilisés comme insecticides, fongicides, herbicides, rodenticides en agriculture et servent à la fois à détruire et à protéger des ravageurs (Jayaraj et al., 2016).

Actuellement, il existe deux approches largement répandues de classification des pesticides (Yadav et al., 2015). Ces classifications peuvent fournir des informations utiles sur la structure chimique du pesticide, leur fonctionnement et leur cible. Les pesticides chimiques sont classés en plusieurs types selon leurs structures chimiques : les carbamates, les organophosphorés, les organochlorés, les triazines, les azolés et les pyréthriinoïdes de synthèse par exemple. Parmi les pesticides, il existe une autre classe nommés biopesticides, qui sont des matières d'origine naturelle, et reposent sur l'utilisation d'organismes vivants tels que les champignons, les plantes et les bactéries pour supprimer directement ou indirectement l'impact d'un organisme nuisible spécifique (Girard et al., 2020) (Figure 4).

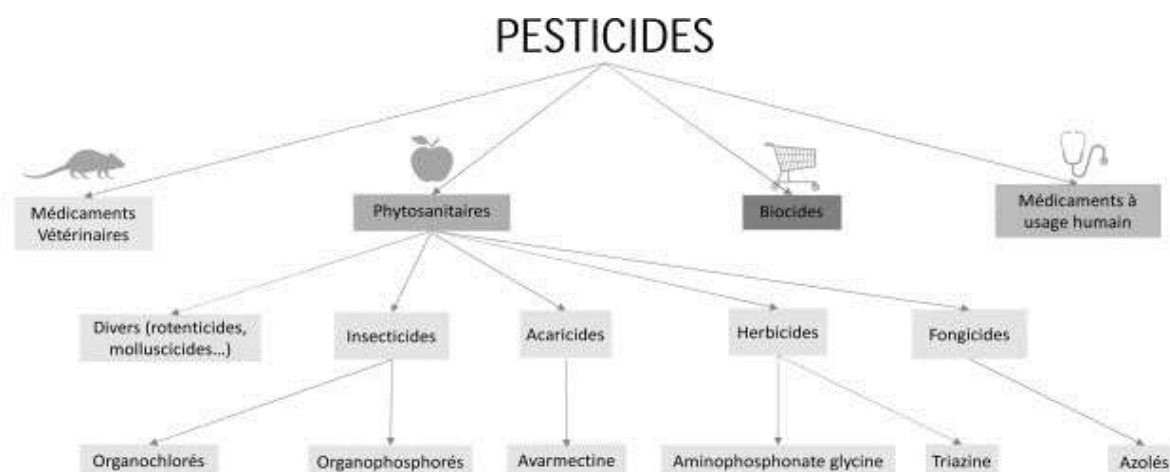


Figure 4. Classification générale des pesticides (Girard et al., 2020)

Tableau 1 Classification des pesticides sur la base de l'organisme nuisible qu'ils tuent et de la fonction des pesticides

Type de pesticide	Ravageurs / fonctions cibles	Exemples
Acaricides	Substances utilisées pour tuer les acariens et/ou perturber leur croissance ou leur développement	Chlorpyrifos, DDT
Bactéricides	Composés utilisés pour tuer ou inhiber les bactéries dans les plantes ou le sol	Streptomycine
Fongicides	Produits chimiques utilisés éliminer ou limiter le développement des champignons parasites des végétaux	Cymoxanil
Herbicides	Substances utilisées pour tuer les plantes ou pour inhiber leur croissance ou leur développement.	Alachlore, 2,4-D
Régulateur de croissance des insectes	Substance qui agit en perturbant la croissance ou le développement d'un insecte	Diflubenzuron
Algicides	Substances utilisées pour tuer ou inhiber les algues	Diuron
Insecticides	Pesticides utilisées pour tuer les insectes ou pour interrompre leur croissance ou leur développement	Chlorpyrifos, DDT
Nématicides	Produits chimiques utilisés pour lutter contre les nématodes	Chlorpyrifos
Régulateurs de croissance des plantes	Ces substances modifient le taux de croissance, de floraison ou de reproduction prévu des plantes	2,4-D
Rodenticides	Substances utilisées pour tuer les rats et les animaux apparentés	Warfarine

2. Polluants organiques persistants (POPs)

2.1. Généralités

Ces dernières années, l'industrialisation, la croissance économique et l'urbanisation ont créé d'énormes défis pour la santé humaine, les écosystèmes et l'environnement (Al-

Mulali et al., 2015). Les POPs sont des composés organiques présentant des caractéristiques de persistance, de toxicité, de bioaccumulation et un potentiel de transport environnemental à longue distance (Ong et al., 2018). Il existe diverses sources d'émission de POPs, y compris l'incinération des déchets, la production de métaux, la production de chaleur et d'électricité et le transport (Odabasi et al., 2015). Les POPs sont des composés organiques qui peuvent être classés en deux catégories, les composés chimiques issus de la synthèse chimique (intentionnellement), en particulier, les pesticides organochlorés (DDT, aldrine, endrine, heptachlore, chlordane), les polychlorobiphényles, l'hexachlorobenzène et les composés chimiques produits non intentionnellement en particulier par combustion, comme les dioxines, les furanes et les HAPs (Merhaby et al., 2019) (Figure 5 et 6).

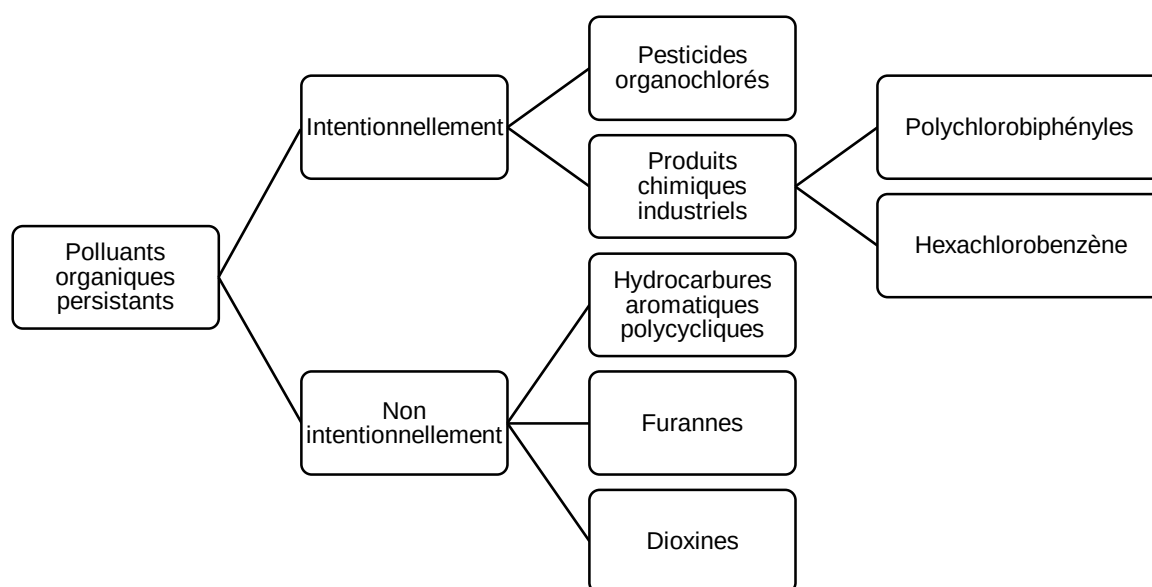


Figure 5. Classification des polluants organiques persistants (Merhaby et al., 2019)

Pendant plusieurs décennies, ces composés ont été largement utilisés en agriculture et dans l'industrie et sont actuellement largement distribués dans l'air, l'eau, les sédiments et les sols (Anim et al., 2017 ; Pariatamby et Kee, 2016). En raison de leur persistance et leur nature lipophile, ces POPs ont tendance à s'accumuler dans la chaîne alimentaire ainsi que dans les tissus riches en lipides du corps humain et des animaux (Imbeault et al., 2018 ; Fernández-Rodríguez et al., 2015 ; Govaerts et al., 2018).

En conséquence, la concentration de certains POPs, par exemple ceux utilisés comme fongicides ou insecticides, est principalement déterminée par l'exposition alimentaire (Malisch et Kotz, 2014 ; Bányiová et al., 2017). Pour d'autres, la principale voie d'exposition humaine peut être due à des sources non alimentaires, telles que l'ingestion ou l'inhalation de particules contaminées en suspension dans l'air ou de poussières intérieure (Fromme et al., 2016).

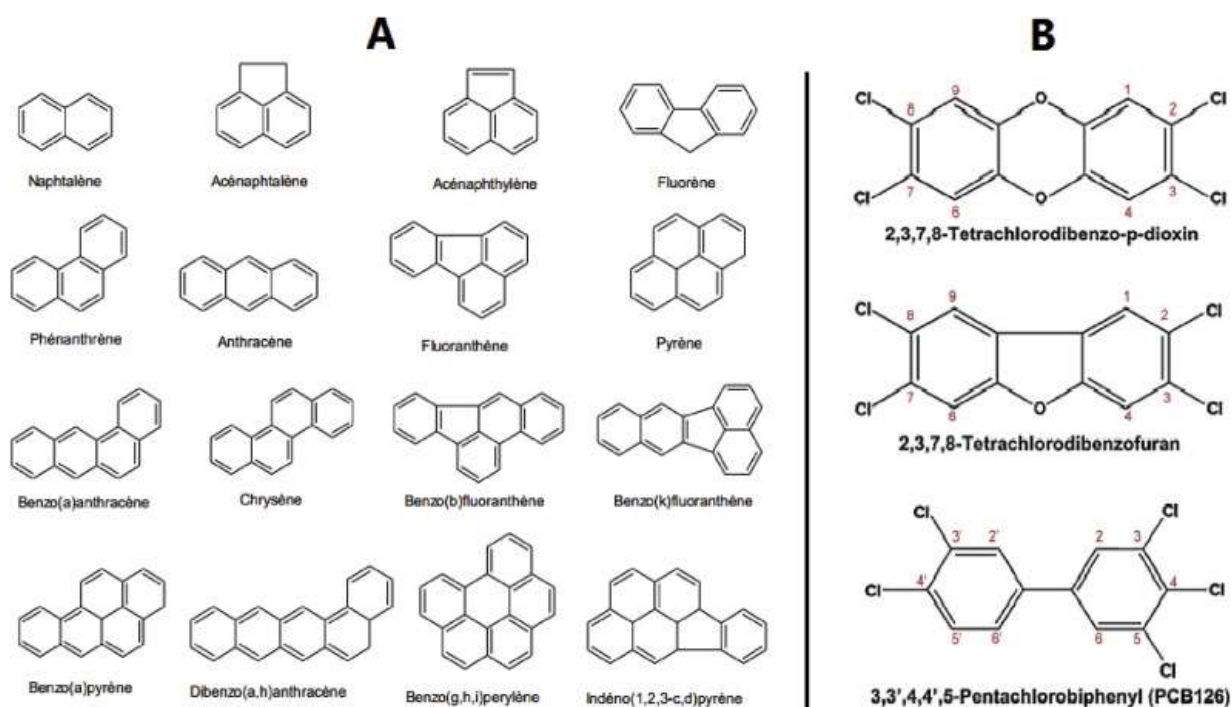


Figure 6. A: Structure des 16 HAPs répertoriés comme composés prioritaires par l'agence américaine de protection de l'environnement (US-EPA) et B: structures de base des PCBs, des dibenzofuranes chlorés et des dibenzo-p-dioxines chlorées (d'après Merhaby et al., 2019)

L'exposition aux POPs a été associée à un large éventail d'effets négatifs sur la santé, notamment un risque accru de diabète de type 2 (Han et al., 2020 ; Lee et al., 2018 ; Wolf et al., 2019), une mortalité accrue (Lind et al., 2019), l'obésité (Liang et al., 2020) et une hypertension (Lind et al., 2014). Les enfants sont extrêmement sensibles aux POPs car ils sont exposés avant la naissance par le transfert de ces composés de la mère au fœtus à travers le placenta et en tant que nourrisson via le lait maternel (Vukavic et al., 2013 ; Vizcaino et al., 2014). L'exposition prénatale aux POPs a été associée à une perturbation du système endocrinien, une altération de la croissance fœtale, des maladies endocriniennes-métaboliques avec un risque de maladies respiratoires aiguës et chroniques (Govarts et al., 2018 ; García-Villarino et al., 2018 ; Tang-Péronard et al., 2015 ; Gascon et al., 2014). Ils ont soulevé d'importantes préoccupations mondiales en raison

de leurs effets néfastes importants sur la santé humaine, de leur bioaccumulation et leur persistance dans l'écosystème (Bakirtas et Akpolat, 2018 ; Al-Mulali et al., 2015). Ils peuvent nuire considérablement aux êtres vivants et à la santé humaine par inhibition de la réponse normale du système immunitaire et la réduction simultanée de la résistance du corps aux virus (Islam et al., 2018). De plus, de nombreuses études ont montré que les organismes exposés à ces polluants peuvent entraîner des troubles de la reproduction et des malformations congénitales (Nadal et al., 2015 ; Lee et al., 2014 ; Tartu et al., 2015).

Les différentes étapes pour l'évaluation d'une vaste gamme de POPs comprennent la collecte d'échantillons dans des matrices sélectionnées, l'extraction et la purification des échantillons avec des solvants appropriés, suivie par leur l'analyse et l'interprétation des résultats. Des recherches pour obtenir des concentration détectables de ces polluants dans les plantes (Massimi et al., 2021), les aliments (Vaccher et al., 2020; Rusin et al., 2019), les lichens et les mousses (Kosior et al., 2017), ont été menées en utilisant des méthodes conventionnelles telles que la méthode d'extraction Soxhlet, l'ASE, la SPE, l'extraction liquide sous pression et la méthode QuEChERS (Chamkasem et al., 2016 ; Cloutier et al., 2017 ; Kim et al., 2019 ; Lee et al., 2020 ; Li et al., 2020 ; Sun et Wu, 2020).

Outre les différentes méthodologies d'extraction, diverses approches analytiques simples, sensibles et rapides sont développées et introduites pour une détermination précise des POPs en termes de quantité et de qualité. Les analyses chromatographiques en phase liquide et en phase gazeuse avec l'utilisation de détecteurs sensibles, tels que détecteur à photométrie de flamme, le détecteur à ionisation de flamme, le détecteur d'émission atomique, le détecteur azote-phosphore et la détection par spectrométrie de masse sont généralement classés comme techniques analytiques largement utilisées pour la séparation et la détection des POPs (Nardelli et al., 2020 ; Tang et al., 2020 ; Stader et al., 2016).

2.2. Hydrocarbures aromatiques polycycliques (HAPs)

Les HAPs sont des polluants préoccupants à l'échelle mondiale en raison de leur large distribution dans l'environnement et de leurs impacts écologique et sanitaire (Ji et al., 2019 ; Cachada et al., 2016). Pendant des décennies, la contamination par les HAPs a fait l'objet d'études environnementales, en raison des rejets provenant de la combustion, des

activités industrielles et en particulier des activités pétrolières. Les HAPs sont des cancérogènes, des mutagènes et des tératogènes qui ont tendance à s'accumuler dans l'eau et les sédiments (Bandowe et Nkansah, 2016).

En raison de leur structure moléculaire, les HAPs ont tendance à subir à la fois des processus de dégradation et de transformation (Ghosal et al., 2016). Certains de ces processus comprennent la réduction, l'oxydation, la photolyse, la biodégradation et l'hydrolyse aidées par plusieurs facteurs tels que la présence de micro-organismes et la lumière du soleil (Achten et Andersson, 2015 ; Jia et al., 2014). De plus, les produits de transformation et de dégradation de certains HAPs conduisent souvent à la formation de produits qui sont principalement différents des polluants émis à l'origine en termes de propriétés physico-chimiques et sont plus toxiques entraînant des effets néfastes. Certains peuvent éventuellement pénétrer dans les cours d'eau et causer des problèmes environnementaux supplémentaires en raison de leur toxicité et de leur potentiel de bioaccumulation accrus par rapport aux hydrocarbures d'origine (Achten et Andersson, 2015 ; Wei et al., 2015).

Les HAPs constituent une classe large et diversifiée de composés organiques et sont généralement décrits comme des molécules constituées de deux ou plusieurs cycles aromatiques fusionnés constitués d'atomes d'hydrogène et de carbone, fusionnés dans diverses configurations structurelles angulaires, linéaires ou groupées (Chikere et Fenibo, 2018 ; Iwegbue et al., 2020). Les principales sources qui contribuent à leur émission dans l'air ambiant et intérieur proviennent de sources anthropiques (combustion des matières fossiles, émissions industrielles, de transport et de chauffage) ou naturelles (incendies de forêt, volcans, ...). La contribution des sources anthropiques est plus élevée que celle des sources naturelles. Par conséquent, le risque associé à l'exposition humaine aux HAPs dans les zones urbaines est plus élevé que dans les zones suburbaines ou rurales, compte tenu de l'activité industrielle, de la densité de la population et de la circulation automobile plus importante (Duodu et al., 2017 ; Abdel-Shafy et Mansour, 2016).

Dès leur émission dans l'air, les HAPs de faible poids moléculaire (acénaphène, naphthalène, fluorène, anthracène, phénanthrène) seront distribués dans la phase gazeuse, tandis que ceux de haut poids moléculaire (benz [a] anthracène, pyrène, benzo (k) fluoranthène, benzo [e] pyrène, fluoranthène, chrysène) seront adsorbés sur des

particules (Han et al., 2015). Les HAPs de poids moléculaire intermédiaire (quatre anneaux) sont répartis en fonction de la température atmosphérique entre les phases vapeur et particulaire (fluoranthène, phénanthrène, anthracène, pyrène) (Srogi, 2007). Ils sont soumis à divers procédés de transformation telle que la photolyse, des réactions d'oxydation et le transfert ou l'élimination par dépôt humide et sec. Une fois déposés, ils peuvent être réactivés par les masses d'air et transportés sur de longues distances et atteindre des zones éloignées de leur site d'émissions, soit ils surviennent au milieu marin par les transferts fluviaux ainsi que le lessivage des sols et seront adsorbés sur des particules pour être transférés dans les sédiments (Abdel-Shafy et Mansour, 2016). La lipophilie des HAPs permet à ces substances d'être absorbées, accumulées ou transformées par l'organisme aux différents niveaux des chaînes alimentaires jusqu'à l'homme (Harris et al., 2013).

L'exposition humaine aux HAPs se produit également par la fumée de tabac (Orisakwe et al., 2015), la circulation automobile (Slezakova et al., 2013 ; Gong et al., 2015 ; Cui et al., 2016), les déchets électroniques et les déchets médicaux (Liu et al., 2014 ; Luo et al., 2015), le charbon de bois (Nguyen et al., 2013) et les feux de forêt (Adetona et al., 2015). Les personnes sont également exposées à ces composés via l'eau, l'air et les aliments contaminés qu'ils ingèrent. Les voies d'exposition comprennent donc le contact cutané, l'inhalation et l'ingestion dans les milieux professionnels et non professionnels et certaines expositions peuvent impliquer simultanément plusieurs voies telles que l'inhalation à partir d'air contaminé et par des expositions cutanées affectant la dose totale d'absorption.

Les HAPs dans les aliments (Lee et al., 2018), le sol (Leech et al., 2020), l'air (Szulejko et al., 2014), les plantes (Baroudi et al., 2020), l'eau (Zhou et Gao, 2014), les abeilles et les escargots (Kargar et al., 2017; Al-Alam et al., 2020) sont généralement extraits dans un solvant organique approprié avant l'étape d'analyse. Les méthodes d'extraction utilisées pour la quantification des HAPs dans l'environnement comprennent : la SPE (Xu et al., 2015 ; Zheng et al., 2016), l'extraction par fluide supercritique (Gbeddy et al., 2020), l'extraction assistée par ultrasons (De Barros Caetano et al., 2019), la microextraction dispersive liquide-liquide (Agus et al., 2020), la microextraction dispersive en phase solide (Saburouh et al., 2020 ; Sajid et al., 2020) et la technique d'extraction QuEChERS (Sun et Wu, 2020 ; Garbalo-Rubio et al., 2020). L'analyse des composés est réalisée par

différentes techniques chromatographiques couplées à des détecteurs sensibles : détecteur à fluorescence (Zhang et al., 2020), détecteur à ionisation de flamme (Olatunji et al., 2014) et détecteur par spectrométrie de masse (Martinefski et al., 2019) sont les plus fréquemment utilisés.

Les ratios entre différentes HAPs sont couramment utilisés comme outil d'identification et d'évaluation des sources d'émission de pollution (Tableau 2). Les ratios sont applicables aux HAPs déterminés dans différents milieux environnementaux: sol, eau, air (particules + phase gazeuse), sédiments ainsi que des organismes biomoniteurs tels que les escargots ou les aiguilles de conifères. Ces ratios distinguent la pollution aux HAPs provenant des émissions d'essence et des véhicules, des produits pétroliers et de la combustion du charbon et du pétrole (Tobiszewski et Namiesnik, 2012).

Tableau 2 Rapports de diagnostic utilisés avec leurs valeurs généralement rapportées pour des processus particuliers.

PAH ratio	Valeurs	Source	Référence
$\Sigma\text{LMW}/\Sigma\text{HMW}$	<1	Pyrogénique	Zhang et al., 2008
	>1	Pétrogénique	
FL/(FL + PYR)	<0.5	Émissions d'essence	Ravindra et al., 2008b
	>0.5	Émissions diesel	
ANT/(ANT + PHE)	<0.1	Pétrogénique	Pies et al., 2008
	>0.1	Pyrogénique	
FLA/(FLA + PYR)	<0.4	Pétrogénique	De La Torre-Roche et al., 2009
	0.4–0.5	Combustion de combustibles fossiles	
	>0.5	Combustion du charbon	
BaA/(BaA + CHR)	0.2–0.35	Combustion du charbon	Akyüz et Çabuk, 2010
	>0.35	Émissions des véhicules	
	<0.2	Pétrogénique	Yunker et al., 2002
	>0.35	Combustion	
BaP/(BaP + BeP)	~0.5	Particules	Oliveira et al., 2011
	<0.5	Photolyse	

ΣLMW : somme des HAPs à deux et trois cycles, ΣHMW : somme des HAPs à quatre et cinq cycles
FL: Fluorène, PYR: Pyrène, ANT: Anthracène, PHE: Phénanthrène, FLA: Fluoranthène, BaA: Benzo [a] anthracène, CHR: Chrysène, BaP: Benzo [a] pyrène, BeP: Benzo [e] pyrène

2.3. Polychlorobiphényles (PCBs)

Les PCBs classés parmi les POPs sont identifiés par l'OMS (WHO, 2019) comme étant des composés hautement néfastes pour l'environnement et la santé humaine en raison de leur capacité de bioamplification et de bioaccumulation tout au long de la chaîne alimentaire (Omwoma et al., 2019 ; Nourira et al., 2013). Structuellement, les propriétés physico-chimiques des PCBs dépendent du nombre et de la position des atomes de chlore dans les cycles biphényles (Wahlang et al., 2019). Les PCBs faiblement chlorés sont souvent métabolisés et rapidement éliminés des organismes, y compris les humains, tandis que ceux fortement chlorés sont lentement absorbés et accumulés dans le tissu adipeux et la peau et ont tendance à entraîner des troubles de santé (Helou et al., 2019). Leurs propriétés diélectriques et leur résistance au feu ont conduit à une large utilisation des PCBs depuis des décennies dans les équipements électriques, tels que les condensateurs et les transformateurs, les lubrifiants, les peintures et comme plastifiants. La présence environnementale des PCBs provient d'une élimination incorrecte, des fuites des décharges, de l'incinération et des activités industrielles. Néanmoins, l'exposition aux PCBs est associée à un risque accru de certains cancers du tube digestif, de la peau et du foie. Cette exposition est également associée à des déficiences de la reproduction (développement retardé, taux de croissance réduits) et du système immunitaire (augmentation des taux d'infection, des modifications cutanées) (Iwegbue et al., 2019).

Les PCBs sont persistants dans les environnements biotiques et abiotiques, en raison de leur large éventail d'applications et de leur stabilité chimique excessive. Les propriétés physico-chimiques distinctes ainsi que la propriété diélectrique exceptionnelle des PCBs les ont certifiés comme des composés indispensables dans un large domaine d'applications industrielles. En outre, leur capacité thermique élevée et leur faible conductivité électrique ont prouvé qu'ils étaient idéaux comme réfrigérants dans les équipements électriques. Par conséquent, les PCBs ont été détectés presque dans tous les compartiments de l'écosystème mondial au moins à l'état de traces et sont distribués à travers les régions où ils n'ont jamais été utilisés auparavant due à leur facilité de transport sur de longues distances via l'atmosphère (Randoll et al., 2014; Zeng et al., 2013).

Les PCBs résistent à la dégradation par les bases, les acides et la chaleur dans une large mesure en raison de leur propriété hydrophobe et peuvent pénétrer pratiquement dans tous les écosystèmes et se maintenir pendant des années sans subir de réduction, d'oxydation ou de biodégradation. De plus, ces PCBs peuvent se lier aux segments lipidiques des tissus animaux et tout au long de la chaîne alimentaire vu leur solubilité élevée dans les lipides (Tang et al., 2015; Wu et al., 2018).

Compte tenu de leur toxicité aiguë et de leur persistance dans l'environnement, plusieurs stratégies analytiques ont été élaborées pour l'échantillonnage, le prétraitement et l'analyse des PCBs dans le sol, l'eau, l'air et les sédiments contaminées. Récemment, plusieurs études ont déterminé la concentrations de ces composés dans les sols (Song et al., 2018 ; Francisco et al., 2017), les eaux de surface (Cerasa et al., 2020; Habibullah-Al-Mamun et al., 2019), l'air (Melymuk et al., 2017 ; Sakin et al., 2017), ainsi que dans différents échantillons tels que les aiguilles de conifères (Al-Alam et al., 2017 ; Silva et al., 2015), le miel (Chiesa et al.2016), les escargots (Baroudi et al., 2020) et les poissons (Li et al., 2019 ; Visha et al., 2018).

III. Principaux biomoniteurs de la qualité de l'air

La pollution de l'air doit être surveillée afin d'évaluer son impact sur l'homme et l'environnement. Deux techniques d'échantillonnage sont couramment utilisées permettant une évaluation quantitative et qualitative précise de la pollution de l'air. Les échantillonneurs d'air passif et actif font partie des méthodes couramment utilisées pour la détermination des polluants dans l'air. De même, une évaluation qualitative et quantitative des polluants peut être réalisée par biosurveillance, permettant la détermination des polluants organiques avec une substance naturelle (Al-Alam et al., 2017; Xu et al., 2013).

La biosurveillance peut être définie comme une série d'étapes de détermination et d'identification des polluants atmosphériques accumulés dans des biomoniteurs / bioindicateurs sur une période donnée. C'est une excellente alternative aux échantillonneurs passifs et actifs en estimant la pollution de l'air avec une substance naturelle et en permettant ainsi une évaluation qualitative et quantitative de la présence de polluants (Van der Wat et Forbes, 2015).

1. Analyse des polluants dans l'air par échantillonnage passif

Les études de surveillance atmosphérique des polluants sont généralement réalisées à l'aide de technique d'échantillonnage passif et actif (Karásková et al., 2018 ; Newton et al., 2016 ; Tuduri et al., 2012). Les échantillonneurs actifs aspirent l'air ambiant à travers un filtre et un matériau absorbant à l'aide d'une pompe. L'échantillonnage passif de l'air est la collecte de polluants atmosphériques par la diffusion de gaz et, dans une moindre mesure, l'entraînement de particules, sur et dans un milieu d'échantillonnage. L'échantillonnage passif complète l'échantillonnage actif de l'air en fournissant des méthodes moins coûteuses, nécessitant moins de sécurité, silencieuses et ne nécessitant pas d'électricité (Ellickson et al., 2017 ; Lee et al., 2017). Les principales concentrations de polluants dans l'air peuvent être obtenues directement avec des échantillonneurs d'air actifs. Ceux-ci présentent également l'avantage d'utiliser des données de concentration quantitatives fiables, d'évaluer les effets des facteurs contrôlant la variabilité de la concentration à court terme et de déterminer la répartition des particules de gaz, la distribution des particules dans l'air et la température. Ces systèmes présentent l'inconvénient de la nécessité d'une alimentation électrique et de coûts d'équipement élevés (Sari et al., 2021 ; Hayward et al., 2011; Xu et al., 2013 ; Tuduri et al., 2012).

Les échantillonneurs d'air passifs sont utilisés comme un choix efficace par rapport aux échantillonneurs d'air actifs pour la mesure des polluants organiques. Les avantages des échantillonneurs d'air passifs comprennent la facilité d'utilisation , les faibles coûts et l'absence d'électricité (McLagan et al., 2018 ; Okeme et al., 2016). Cependant, les longues périodes d'échantillonnage nécessaires constituent un des inconvénients majeur de ce type d'échantillonnage (Lai et al., 2018) avec la calibration.

L'utilisation de dispositifs d'échantillonnage passifs s'est rapidement développée, ce qui a permis une surveillance continue des contaminants environnementaux pendant la période où ils sont exposés sur le terrain. L'usage de certaines substances ou organismes naturelles (biomoniteurs) comme capteurs fiables de polluants dans l'air, figure parmi les échantillonneurs passifs les plus utilisés et les plus disponibles. Il s'agit en fait de la technique de biosurveillance environnementale dite de biomonitoring (Baroudi et al., 2020 ; Ghosh et al., 2014 ; Mukhopadhyay et al., 2020 ; Roll et Halden, 2016).

2. Organismes pour la surveillance de la pollution : Biomoniteurs

2.1. Généralités

La biosurveillance est définie comme l'acte d'observer et d'évaluer l'état et les changements en cours dans les composants de la biodiversité et les écosystèmes, y compris les populations, les habitats naturels et les espèces.

La biosurveillance est au cœur de la gestion, de la restauration et de la conservation des écosystèmes. Etant donné que la biosurveillance est devenue une obligation, leurs programmes sont élaborés par des organisations gouvernementales. La biosurveillance consiste à enregistrer l'abondance des espèces et la diversité à différents moments et à différents endroits à l'aide d'une gamme de techniques de recensement écologique et d'identification taxonomique. La plupart des méthodologies d'échantillonnage de biosurveillance ont été développées au milieu du 20^e siècle et ont été sélectionnées pour des raisons entièrement pragmatiques reflétant l'état actuel des connaissances, la simplicité et le coût (Bohan et al., 2017).

Parmi les échantillonneurs passifs très connus, figurent les biomoniteurs. Un biomoniteur est décrit comme une espèce d'animaux, de plantes ou de champignons fournissant des informations sur l'environnement dans lequel il réside. Il est généralement caractérisé par sa large distribution spatio-temporelle, son mode de vie sédentaire, son pouvoir cumulatif, sa sensibilité élevée aux différents types de contaminants, et par sa facilité d'identification et de collecte (Al Alam et al., 2019 ; Parmar et al., 2016). Le biomoniteur est une alternative peu coûteuse pour analyser la qualité de l'air et obtenir des informations relatives à l'exposition de la population aux polluants atmosphériques, et permettent ainsi d'évaluer simultanément plusieurs sites d'échantillonnage différents (Giampaoli et al., 2016 ; Capozzi et al., 2016).

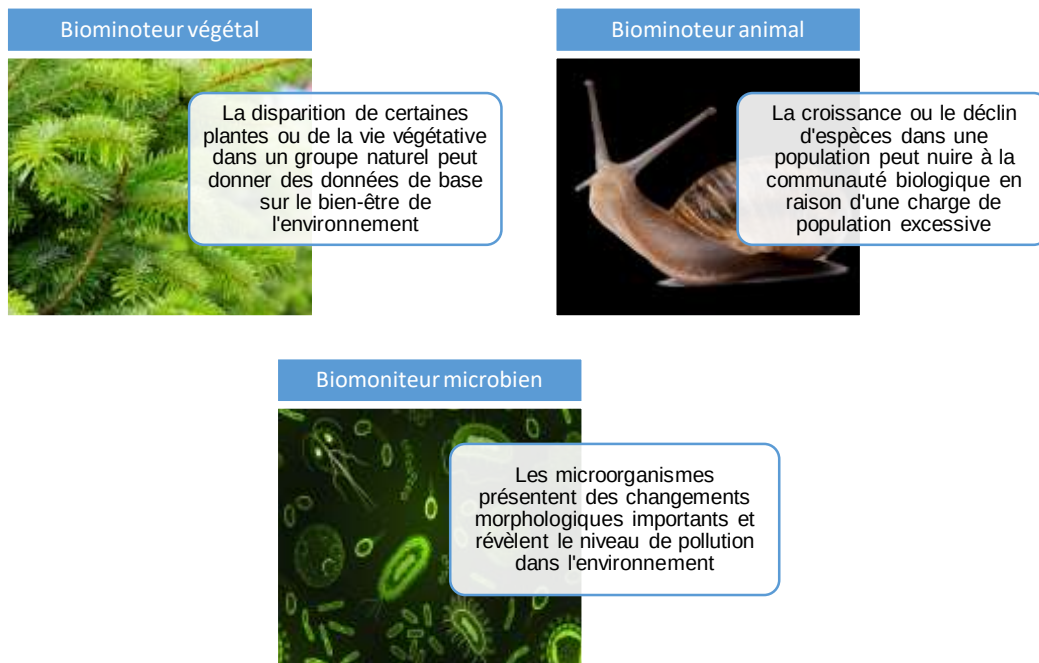


Figure 7. Types de biomoniteurs avec des réponses spécifiques (Parmar et al., 2016)

Les espèces d'invertébrés (escargots et abeilles) et les espèces végétales (aiguilles de conifères et lichens) (Varga et al., 2020 ; Girones et al., 2020 ; Ji et al., 2019 ; Luo et al., 2020) sont reconnues comme des indicateurs biologiques appropriés, en raison de leur accumulation potentielle de polluants organiques et d'oligo-éléments métalliques, et sont également utilisées comme espèce modèle pour évaluer l'effet de ces polluants sur leur développement (Krupnova et al., 2018 ; Cossi et al., 2018) (Figure 7).

2.2. Biomoniteur à base végétale

L'utilisation de la végétation pour la surveillance de l'environnement peut être considérée comme une technique simple, efficace et rentable pour détecter et évaluer la pollution de l'environnement. L'importance de la biosurveillance de la pollution de l'air par les plantes offre des résultats importants pour différentes raisons (Parmar et al., 2016 ; Ratola et al., 2014 ; Cuny, 2012) :

- Les plantes montrent une réponse intégrée au climat de la pollution, pouvant même donner des informations sur la puissance des mélanges de polluants complexes. Elles sont plus ou moins sensibles et ne réagissent qu'à une partie d'une situation de pollution donnée ;

- Différents niveaux de la plante, allant de la plante unique à l'association végétale et à l'écosystème peuvent être utilisés pour la biosurveillance. La réponse obtenue est le résultat d'une intégration de différents facteurs sur une période relativement longue vécue par des espèces végétales compétitives ;
- Certains polluants sont difficiles à être mesuré avec précision par des méthodes chimiques et physiques du fait de leur concentrations ambiantes très faibles. Ces polluants peuvent être accumuler par les plantes à un niveau plus facile à analyser ;
- Les effets sont exprimés sous forme de lésions visibles dans les espèces végétales sensibles (lésions foliaires ou changements d'habitude) et dans les espèces moins sensibles dans l'accumulation de polluants (même les espèces tolérantes à la pollution). Les deux fournissent un outil important pour reconnaître les effets de la pollution de l'air et le transfert des traces de polluants au niveau de la chaîne biologique.

En raison des problèmes liés à l'environnement et à la santé humaine, les processus de surveillance et de contrôle des polluants étroitement liés à la contamination de l'air deviennent essentiels. Les échantillonneurs actifs ont été largement utilisés dans la surveillance atmosphérique mais ne peuvent pas être appliqués dans les zones de couverture éloignées ou étendues en raison du manque d'alimentation électrique. Au cours des dernières décennies, le développement de méthodes utilisant des algues, des plantes, des lichens et des mousses comme biomoniteurs a suscité un intérêt croissant en raison de leur rapidité, de leur faible coût et de leur facilité d'utilisation (Vitali et al., 2019 ; Massimi et al., 2019).

2.2.1. Biomonitor à base végétale : les aiguilles de conifères

Des plantes vasculaires persistantes, telles que les aiguilles de conifères, ont été suggérées pour surveiller les polluants, comme les métaux, les pesticides et d'autres polluants organiques (Luo et al., 2020 ; Tang et al., 2014 ; Ratola et al., 2014). L'un des avantages de l'utilisation de conifères qui sont de bons indicateurs de pollution au cours de l'année est la capacité à accumuler tout au long de l'année des polluants liés au trafic et qui sont à l'origine de la pollution de l'air (Turkyilmaz et al., 2018). De plus, les espèces à feuilles larges peuvent avoir une accumulation de polluants plus élevée que les feuilles plus grandes et plus plates du fait que les particules en suspension dans l'air peuvent être plus

facilement accumulés par les aiguilles étroites et longues (Alexandrino et al., 2020; Chen et al., 2017). La présence d'une couche cireuse et la teneur élevée en lipides permettent l'accumulation et l'adsorption de polluants organiques de l'air au fil des années dans leurs tissus (Al Alam et al., 2019 ; Bertolotti et Gialanella, 2014). La biosurveillance avec ces biomoniteurs passifs est économiquement pratique pour étudier divers polluants organiques, y compris les polybromodiphényléthers (Ratola et al., 2011), les PCBs (Al Dine et al., 2015), les polychlorodibenzo-p-dioxines, les polychlorodibenzofuranes (Chen et al., 2017) et les HAPs (Oishi, 2018 ; Kargar et al., 2017), ainsi que la détection de leur accumulation à long terme dans l'air à une large échelle spatiale.

2.2.2. Biomonitor à base végétale : les lichens

En raison de leur capacité à accumuler de nombreux polluants différents, les lichens sont considérés comme des outils efficaces pour un échantillonnage à long terme des polluants organiques (Srivastava et Bhattacharya, 2016). Plusieurs facteurs, tels que les conditions atmosphériques au cours de la période d'échantillonnage, la concentration des polluants dans l'air ainsi que leurs propriétés physico-chimiques affectent l'accumulation de quantités détectables de polluants de l'air dans les lichens. La campagne de biosurveillance peut être menée en utilisant des lichens indigènes présents dans la zone étudiée ou transplantés vers la zone d'étude (Van der Wat et Forbes, 2015 ; Augusto et al., 2013). Néanmoins, les greffes de lichen sont les plus appropriées pour réaliser un vaste réseau d'observation qui permet d'étudier la répartition spatiale des polluants atmosphériques (Massimi et al., 2021 ; Gallo et al., 2017 ; Vannini et al., 2016 ; Conti et Tudino, 2016).

Les polluants organiques toxiques présents dans l'air qui pénètrent dans les lichens peuvent influencer la structure anatomique des thalles (Osyczka et al., 2018). Certaines caractéristiques anatomiques semblent être liées à l'accumulation de métal, et des différences significatives de structure anatomique ont été observées entre des thalles de la même espèce prélevés dans des localités dont le niveau de pollution diffère. Les changements structurels susceptibles résultant de la pollution ont été fréquemment rapportés en ce qui concerne la composante des algues, et ces changements dépendent de facteurs climatiques et peuvent différer d'une espèce de lichen à l'autre (Rola et Osyczka, 2018). Les lichens sont couramment utilisés comme biomoniteurs passifs pour étudier les

métaux lourds (zinc, cuivre, plomb) (Rola et al., 2021) et divers polluants organiques, y compris, les HAPs (Cappozi et al., 2021 ; Ji et al., 2019), les PCBs (Massimi et al., 2021 ; Vitali et al., 2019), les polychlorodibenzodioxines et les polychlorodibenzofuranes (Massimi et al., 2021).

2.2.3. Biomoniteur à base végétale : les mousses

Les mousses sont considérés comme des biomoniteurs de surveillance et de gestion de la qualité de l'air (Ogunkunle et al., 2016). Ils permettent d'évaluer la pollution de l'air par les polluants accumulés ou déposés directement dans leurs tissus (Salo et al., 2012 ; Boquete et al., 2014).

Les mousses, fournissant des informations sur la contamination temporelle et spatiale de divers polluants, sont régulièrement utilisées comme biomoniteurs des polluants présentes dans l'air par deux processus méthodologiques tels que la biosurveillance active ou passive. Dans le cas de la biosurveillance active, des échantillons de mousse sont prélevés dans des zones relativement peu polluées, où ils sont nettoyés et traités avant d'être exposés à différents environnements pollués. Pour la biosurveillance passive, les mousses poussant dans une zone particulière sont collectées directement et analysées (Gallego-Cartagena et al., 2021 ; Baltrenaite et al., 2014 ; Boquete et al., 2013).

Différentes techniques analytiques sont mise en œuvre pour l'étude de la qualité de l'air à l'aide d'échantillons de mousse, telles que la spectrométrie d'absorption atomique (AAS) (Lazo et al., 2019 ; Macedo-Miranda et al., 2016), l'analyse par activation neutronique (Madadzada et al., 2019), la spectrométrie de masse à plasma à couplage inductif (Gallego-Cartagena et al., 2021 ; Fackovcova et al., 2020) et la spectrométrie d'émission optique à plasma à couplage inductif (Donovan et al., 2016).

2.3. Biomoniteur à base animale

Les matrices animales comestibles ont l'avantage de démontrer le risque réel des polluants sur la population et leur passage dans la chaîne alimentaire. La bioaccumulation dans les tissus et les organes rend la surveillance biologique plus sensible que la surveillance environnementale, en particulier dans les zones où aucune pollution n'a été détectée. La biosurveillance animale présente des avantages spécifiques étant donné que

les animaux se caractérisent par une mobilité quotidienne limitée et sont moins susceptibles d'être affectés par des facteurs de confusion ce qui facilite l'identification de la source de contamination (Scaramozzino et al., 2019).

2.3.1. Les mollusques: les escargots

Un biomoniteur animal fournissant des informations sur la qualité de l'environnement dans lequel il réside, se caractérise généralement par son mode de vie sédentaire, sa large distribution et sa facilité d'identification et de collecte (Parmar et al., 2016). Les espèces d'invertébrés, comme les escargots, sont reconnues comme des indicateurs biologiques appropriés en raison de leur accumulation potentielle de POPs et d'oligo-éléments métalliques (Louzon et al., 2020) et sont également utilisées comme outil pour évaluer l'effet de ces polluants sur leur développement (Louzon et al., 2020 ; Cossi et al., 2018 ; Krupnova et al., 2018 ; De Vaufleury, 2015). Les escargots terrestres (*Helix aspersa*, *Cepaea nemoralis*, *Theba pisana* et *Eobania vermiculata*) sont d'excellents biomoniteurs de la contamination environnementale en raison de leur échantillonnage facile, de leur large distribution et de leur capacité à accumuler divers contaminants dans l'air, le sol et la flore (Baroudi et al., 2020).

Ces espèces occupent une place importante dans l'interface sol-air-végétation du milieu et intègrent de nombreuses sources de pollution (sol, atmosphère, végétaux) à travers les voies digestive, respiratoire et / ou cutanée. Plusieurs espèces d'escargots ont été étudiées, par exemple *Papillifera papillaris* (Emilia et al., 2016), *Indothais gradata* (Proum et al., 2016), *Pomacea canaliculata* (Ramli et al., 2019), *Cantareus apertus* (Mleiki et al., 2018), *Helix aspersa* (Abdel-Halim et al., 2013), *Eobania vermiculata* (El-Shenawy et al., 2012), *Cepaea nemoralis* (Boshoff et al., 2015), *Bellamya aeruginosa* (Yin et al., 2014), *Theodoxus niloticus* (Abdel Gawad, 2018) et *Achatina fulica* (Cho et al., 2019).

En raison de leur large distribution, les escargots peuvent être utilisés pour évaluer la biodisponibilité des contaminants du sol en mesurant la transmission sol-escargot ou sol-plante-escargot et pour analyser la biodisponibilité des polluants environnementaux en mesurant leur concentration dans les escargots présents dans leur biotope, dans leur torsion ou leur masse viscérale et leur coquille lors d'une période de temps spécifiée. Leur mucus les protège des menaces extérieures et des infections bactériennes et fongiques

(Gomot de Vaufleury et Pihan, 2000). Les escargots peuvent être facilement collectés, élevés, identifiés et trouvés presque partout. De plus, ils sont en contact avec différents polluants qu'ils consomment par voie transcutanée, digestive et respiratoire provenant de l'eau, des plantes et du contact avec le sol. L'analyse des polluants a montré que les escargots sont plus susceptibles de s'accumuler dans les viscères que dans le pied, ce qui indique la pertinence du choix des viscères pour étudier la biodisponibilité des polluants dans l'écosystème et illustre l'utilité d'analyser séparément le pied et les viscères (Baroudi et al., 2020).

La bioaccumulation de polluants dans les escargots dépend de la durée de l'exposition, ce qui implique que les escargots sauvages sont fortement contaminés en raison de leur exposition aux polluants environnementaux. La période d'exposition de plusieurs semaines reflète les effets nocifs et prouve que la bioaccumulation est modulée par le type des polluants par rapport à des expositions courtes qui peuvent être suffisantes pour détecter la bioaccumulation (Nica et al., 2013).

Les escargots sont largement utilisés comme détecteurs environnementaux pour les métaux lourds, le glufosinate, le glyphosate, les HAPs, les diphényléthers polybromés, les PCBs et les pesticides, y compris les pesticides organochlorés (OCPs). Plusieurs méthodes d'extraction des polluants organiques et des métaux lourds des escargots ont été publiées dans la littérature (Sturba et al., 2020 ; Girones et al., 2020 ; Louzon et al., 2020 ; Abdel-Halim et al., 2013 ; Wu et al., 2019). Par exemple, les polluants organiques ont été principalement extraits par QuEChERS (Al-Alam et al., 2020), par SPE (Beach et al., 2009), par l'extraction d'eau chaude sous pression (Cho et al., 2019) et MAE (Ramli et al., 2019). Cependant, l'extraction des métaux lourds se faisait surtout par digestion avec un mélange d'acide nitrique (HNO_3), d'eau oxygénée (H_2O_2), d'acide perchlorique (HClO_4) et de nitrate de plomb (PbNO_3) en utilisant une digestion acide assistée par micro-ondes (Abdel Gawad, 2018 ; Emilia et al., 2016). De plus, il existe de nombreuses techniques d'analyses disponibles pour l'étude des polluants émergents de ces espèces telles que la LC (Cho et al., 2019 ; Ramli et al., 2019), la GC (Wu et al., 2019), l'AAS (Abdel Gawad, 2018 ; Emilia et al., 2016) et le spectromètre de masse à plasma à couplage inductif (Emilia et al., 2016 ; Boshoff et al., 2015).

2.3.2. Les abeilles et leurs produits

Bien que les populations d'abeilles soient développées dans le monde entier, des pertes de colonies ont été provoquées par de multiples facteurs tels que le changement climatique, la réduction de la diversité florale, l'intoxication par des composés chimiques y compris les POPs et les pesticides, et l'infection par des agents pathogènes y compris des bactéries, des virus, des parasites et des champignons (Belsky et Joshi, 2019 ; Goulson et al., 2015 ; Harwood et Dolezal, 2020). La diminution des populations d'abeilles dans le monde est préoccupante compte tenu de leur rôle de biomoniteur, leur large distribution et leur importance économique et écologique (Goulson et al., 2018). La présence de la déforestation, des maladies naturelles et de l'application de pesticides sur les cultures ont été signalées comme des causes de l'effondrement des colonies d'abeilles (Wood et Goulson, 2017 ; Park et al., 2015 ; Goulson et al., 2015).

L'abeille est un pollinisateur essentiel pour l'agriculture dans le monde entier et a été largement considéré comme un biomoniteur des polluants présents dans l'air (Skorbiłowicz et al., 2018 ; Niell et al., 2017 ; Villalba et al., 2020). Au cours de leurs recherche de nourriture, les abeilles sont exposées à des polluants notamment des polluants organiques et des métaux qui sont ramenés dans les ruches et peuvent également se retrouver dans les produits de la ruche, tels que le miel et la cire (Ostiguy et al., 2019 ; Sadowska et al., 2019 ; Negri et al., 2015 ; Hooven et al., 2019). L'origine de ces polluants peut être à l'origine de l'inhalation de polluants par les stigmates de la trachée, l'exposition des particules via les poils des corps d'insectes, l'ingestion de nectar, de pollen et d'eau contaminée.

La plupart des études utilisent des corps d'abeilles adultes pour surveiller les polluants dans l'environnement. Il a été constaté que le niveau de pollution dans les corps des abeilles était significativement différent d'un environnement à l'autre et correspondait aux différents niveaux de pollution (Barganska et al., 2013). La zone d'activité associée aux colonies d'abeilles peut généralement s'étendre sur une vaste zone (Bargańska et al., 2016) autour de la colonie, et cette variabilité dans la recherche de nourriture est généralement due à des différences dans la disponibilité des sources de nourriture autour de la colonie (Garbuzov et al., 2015). Cependant, même au même endroit, les colonies peuvent différer dans leur zone d'alimentation réelle, leur activité d'alimentation et même

dans la distance parcourue. La quantité de pollution trouvée dans les corps d'abeilles et dans le pollen collecté peuvent être affecter par ces différences entre les colonies. Plusieurs auteurs (El-Nahhal, 2020 ; Larson et al., 2015 ; Zioga et al., 2020 ; Bonmatin et al., 2015 ; Sanchez-Bayo et Goka, 2014) ont révélé une contamination directe des abeilles mellifères par des acaricides, des insecticides, des herbicides et des fongicides lors de la visite des fleurs, de la collecte du pollen, du retour dans les ruches et du stockage des échantillons de miel. De plus, une contamination directe du nectar peut survenir pendant la saison de floraison et la collecte du nectar.

Le miel, fabriqué par les abeilles à partir de pollen et de nectar, contiennent une quantité relativement élevée de protéines, de matières organiques, de vitamines et d'enzymes (Donkersley et al., 2017 ; Di Bella et al., 2015 ; Da Silva et al., 2016). Des études montrent que l'analyse élémentaire des propriétés chimiques et physiques du miel est importante non seulement pour l'identification de l'origine géographique ou botanique mais aussi pour le simple contrôle de la qualité de l'air (Czipa et al., 2015 ; Louppis et al., 2017). Les changements dans la composition élémentaire du miel peuvent indiquer une pollution locale, du fait que les polluants de l'air, de l'eau et du sol peuvent être accumulés dans les produits de la ruche. La production de miel d'une zone dépend principalement des conditions climatiques dans lesquelles les plantes poussent et du type de la végétation utilisée par les abeilles (Escuredo et al., 2014).

Le miel est généralement le moins exposé aux polluants atmosphériques, et son niveau de pollution est inférieur à celui du pollen qui peut être exposé aux polluants atmosphériques pendant longtemps, et du fait de sa viscosité élevée l'amenant à accumuler de plus grandes quantités de polluants (Maragou et al., 2017). Le pollen et le nectar peuvent être contaminés par le dépôt et l'absorption de polluants organiques sur les plantes comme les métaux lourds du sol et les radionucléides (Ismael et al., 2019 ; Silva et al., 2012). Ainsi, la présence de contaminants dans le miel peut varier selon la capacité des plantes à absorber et à excréter des polluants dans le nectar produit et de la capacité de la fleur à accumuler des polluants (Zięba et al., 2020).

Les abeilles ainsi que le miel peuvent être utilisés pour surveiller l'environnement pour la distribution de divers polluants telles que les métaux lourds (Goretti et al., 2020 ; Xun et al., 2018), les HAPs (Zieba et al., 2020 ; Kargar et al., 2017), les pesticides (Moreno-

González et al., 2020 ; Sgargi et al., 2020) et les PCBs (Villalba et al., 2020 ; Sari et al., 2021). Diverses techniques et méthodes d'échantillonnage sont connues pour collecter, mesurer et analyser les niveaux de pollution sur le corps d'abeilles, des parties de corps ou d'échantillons de produits d'abeille. L'extraction des polluants dans les matrices environnementales a été principalement réalisée à l'aide de la méthode QuEChERS (Calatayud-Vernich et al., 2016 ; Liu et al., 2016 ; Martinello et al., 2017 ; Shendy et al., 2016 ; Silva et al., 2019 ; Tette et al., 2016), de l'ASE (Chiesa et al., 2016), de la SPE (Sun et al., 2016 ; Surma et al., 2015 ; Shamsipur et al., 2016) et la LLE (Zhu et al., 2019).

IV. Méthodes d'analyse des polluants

L'importance de la qualité de l'air est devenue un problème sérieux en raison de la présence importante des polluants organiques et de l'utilisation généralisée des pesticides. Au cours de la dernière décennie, il y a eu une augmentation de la consommation de pesticides en raison de l'urbanisation rapide et de la population élevée (Kopittke et al., 2019). Ces résidus dans l'air, l'eau, le sol et les aliments ont des effets potentiellement néfastes sur la santé humaine et l'environnement. Divers rapports suggèrent le risque de la contamination par différents polluants avec leur mode d'action et l'exposition continue provoquant des déficits neurologiques, des maladies respiratoires telles que la rhinite et dans les cas les plus graves, le cancer, l'avortement spontané et la mort fœtale (Lim et al., 2018).

Plusieurs méthodes spécifiques à un polluant, à une classe de polluant ou à plusieurs polluants ont été utilisées pour l'analyse des polluants dans différentes matrices. Il convient toutefois de noter que la sélection appropriée de la méthode analytique est étroitement liée à la qualité des résultats. Une méthode multirésiduelle appropriée pour quantifier et détecter les polluants d'intérêt dans un temps relativement court, comprenant des étapes minimales de préparations de l'échantillon est essentielle pour un programme de surveillance efficace.

Des exemples de méthodes de préparation d'échantillons fréquemment utilisées sont la SPE, la LLE, la SPME, l'ASE et la méthode QuEChERS. La LC et la GC sont les techniques les plus couramment utilisées pour séparer efficacement les composés étudiés.

1. Extraction des polluants

La préparation des échantillons est une étape importante de l'ensemble du processus analytique. Cependant il s'agit de la partie du protocole analytique la plus longue, la plus laborieuse et surtout la partie qui peut engendrer le plus d'erreurs et de perte des composés, entraînant une influence non négligeable sur les résultats. La préparation des échantillons a pour principaux objectifs de favoriser l'extraction et l'enrichissement des composés analysés et d'éliminer autant que possible les interférences. Une méthode de préparation d'échantillons pour l'analyse des résidus de polluants devrait avoir les propriétés suivantes: inclure le plus grand nombre possible de polluants, avoir des taux de récupération aussi proches que possible de 100%, éliminer les composés potentiellement interférents dans l'échantillon, avoir une précision et une robustesse appropriées. Ainsi, l'étape de préparation des échantillons est primordiale pour garantir une meilleure sélectivité, sensibilité de la méthode et de limiter au maximum l'effet de matrice lors de l'analyse des composés. Les méthodes d'extraction de polluants organiques dans les matrices comprennent la LLE (Cacho et al., 2018 ; Zhu et al., 2019), la SPE (Wang et al., 2019 ; Ly et al., 2020), la SPME (Panio et al., 2020 ; Grandy et al., 2019 ; Wu et al., 2016), et QuEChERS (Wang et al., 2020 ; Wurita et al., 2020 ; Rahman et al., 2018 ; Lee et al., 2020).

1.1. Extraction liquide-liquide (LLE)

La LLE également appelé extraction par solvant, implique la séparation des composés, principalement en fonction de leurs solubilités relatives dans des liquides non miscibles. La LLE, en présence de différents solvants d'extraction tels que l'acétonitrile (ACN), l'hexane et l'acétate d'éthyle (AE) est l'une des techniques les plus largement utilisées et les plus anciennes dans la préparation d'échantillons pour l'analyse quantitative et qualitative. L'AE à polarité moyenne est l'un des solvants le plus couramment utilisés pour l'extraction de pesticides à partir de matrices alimentaires (Stocka et al., 2011 ; Raina-Fulton et Xie, 2017).

Cependant, la LLE se caractérise également par l'utilisation de plusieurs étapes de manipulation des échantillons, ce qui le rend vulnérable aux erreurs et à la contamination. De plus, elle permet généralement d'extraire des composés appartenant à une seule classe

chimique. Malgré ces inconvénients, ce type d'extraction continue d'être utilisé dans l'analyse des polluants comme les pesticides (Rodrigues et al., 2018 ; Zhao et al., 2019 ; Duca et al., 2014).

Dans cette méthode d'extraction, trois étapes peuvent être distinguées. Dans la première étape précédant l'extraction, la matrice est homogénéisée dans de l'eau, méthanol-eau ou l'acétone-eau permettant une meilleure homogénéisation des échantillons. Dans la deuxième étape, les polluants sont extraits avec différents solvants non miscibles à l'eau en fonction de la polarité du polluant tels que l'eau, l'ACN, l'AE, le dichlorométhane, le tétrachlorure de carbone, ou des mélanges d'hexane-acétone et de benzène-isopropanol. Finalement, dans la troisième étape après le traitement de l'échantillon, l'extrait obtenu est nettoyé pour éviter les composés co-extraits de poids moléculaire élevé et pour réduire les composés interférents qui peuvent contaminer le système chromatographique et rendre l'analyse du chromatogramme difficile en raison de la présence de pics (Daso et Okonkwo, 2015).

Plusieurs études ont montré l'efficacité de la LLE pour la détermination des polluants organiques tels que les pesticides (Zahiri et al., 2020 ; Shamsipur et al., 2016), les HAPs (Harris et al., 2020) et les métaux lourds (Matin et al., 2016) détectées dans plusieurs matrices comme le lait (Andrade et al., 2013), les escargots (Druart et al., 2011) et le miel (Kadziński et al., 2018 ; Zhu et al., 2019).

1.2 Extraction accélérée par solvant (ASE)

L'ASE, aussi appelé extraction de fluide sous pression ou extraction de liquide sous pression (Harris et al., 2020 ; Gbeddy et al., 2020), est un procédé d'extraction solide-liquide effectué à des pressions élevées (10-15 MPa) et à des températures élevées (40-200 °C). Ses principaux avantages par rapport aux méthodes d'extraction traditionnelles se traduisent par une diminution du temps d'extraction et de la quantité de solvant utilisé. L'échantillon est placé dans une cellule d'extraction, en acier inoxydable. Après l'ajout du solvant, la cellule est mise sous pression, chauffée à la température souhaitée et l'échantillon est extrait de manière statique pendant une période de temps spécifique. L'application d'une pression permet d'effectuer une extraction à plus haute température en maintenant le solvant sous forme liquide. Cette augmentation de température entraîne

une augmentation du pouvoir de solvation et du pouvoir de diffusion. Ensuite, l'extrait est retiré de la cellule qui sera rincée avec du solvant. Une fois l'extraction terminée, l'azote comprimé déplacera tous les solvants de la cellule d'échantillon vers le flacon d'échantillon pour analyse. L'extrait est filtré avant d'être collecté dans le récepteur, aucune étape de filtrage ultérieure n'est donc nécessaire (Kinross et al., 2020 ; Mandal et al., 2015).

Aujourd'hui, l'ASE est utilisée avec succès pour l'extraction des composés organiques à partir de matrices solides environnementales, biologiques, alimentaires et de plantes médicinales. Ce type d'extraction combine les avantages du haut débit, de l'automatisation et de la faible consommation de solvants (Lavin et Hageman, 2012). Des études ont montré l'efficacité et l'applicabilité de l'ASE pour l'analyse des polluants organiques tels que les pesticides (Kinross et al., 2020 ; Lavin et Hageman, 2012), les HAPs (Tan et al., 2019), les PCBs (Al-Alam et al., 2017) et les OCPs (Duodu et al., 2016 ; Al Dine et al., 2015) détectées dans plusieurs matrices alimentaires (Ahmad et al., 2020 ; Wang et al., 2020 ; Kellogg et al., 2017), les abeilles et leur produits (Chiesa et al., 2016), les aiguilles de conifères (Noth et al., 2013) et les lichens (Kodnik et al., 2015).

1.3. Extraction en phase solide (SPE)

La SPE est la méthode la plus couramment utilisée en raison de sa rapidité, sa simplicité et sa capacité à traiter avec une récupération élevée un grand volume d'échantillons. La SPE consiste à retenir le composé sélectionné sur l'adsorbant, puis à l'éluer avec un solvant approprié. Elle combine des procédures d'extraction et de nettoyage pour fournir des extraits propres qui peuvent être analysés directement (Manesiotis et al., 2012).

Au cours de l'extraction, l'échantillon passe à travers une cartouche ou une colonne contenant un sorbant solide où les polluants sont absorbés puis élués avec un solvant organique. Cette procédure présente plusieurs avantages : elle diminue l'utilisation de solvants toxiques et prend moins de temps que la procédure d'extraction LLE. La procédure de la SPE peut être réalisée en quatre étapes qui consiste tout d'abord par le conditionnement de l'adsorbant, nécessaire afin d'assurer une interaction reproductible avec le composé, puis la percolation de l'échantillon sur le support suivie d'un lavage éventuel de l'adsorbant pour améliorer l'élution et la récupération, et enfin l'élution des

composés ciblés par percolation d'un solvant spécifique (AE, méthanol, acétone, hexane, dichlorométhane ou mélanges hexane-AE, hexane-dichlorométhane et de méthanol-eau ou méthanol-AE-dichlorométhane) pour rompre les interactions entre le support solide et les composés d'intérêt tout en évitant, autant que possible l'élution des composés interférents qui sont fortement retenus sur le support.

Les sorbants, utilisés avec succès pour l'extraction de polluants dans différentes matrices, sont la cartouche C₁₈ en phase inverse qui constitue le choix le plus couramment utilisée par les chercheurs pour l'extraction d'herbicides, de fongicides, d'acaricides et d'insecticides, et le sorbant Florisil utilisé pour les pyréthroïdes et les OCPs (Hercegová et al., 2007). Le pH de l'échantillon est essentiel pour obtenir des rendements élevés de rétention de polluants dans le matériau absorbant. Par conséquent, dans certains cas, il peut être nécessaire de modifier le pH de l'échantillon pour protonifier le polluant étudié et augmenter son absorption sur la phase solide. Le sorbant utilisé pour les extractions en phase solide et la polarité des pesticides sont directement liés au choix du meilleur sorbant d'élution. Bien que des limites de détection et des récupérations similaires aient été obtenues, l'extraction en phase solide a donné une meilleure précision et un nombre moins important de composés co-extraits lors de leur analyse par rapport à la LLE pour l'extraction de pesticides (Canbay, 2017).

La SPE a été largement utilisée dans des études de biosurveillance environnementale, telles que l'extraction des pesticides (Al-Alam et al., 2017), des HAPs et des OCPs (Al Dine et al., 2015) dans les aiguilles de conifères ainsi que dans les lichens et les mousses (Concha-Grana et al., 2015 ; Zhu et al., 2015 ; Foan et Simon, 2012), des pesticides dans les échantillons du miel (Shamsipur et al., 2016 ; Oellig, 2016) et des PCBs dans la matrice d'eau (Wang et al., 2016).

1.4. Microextraction en phase solide (SPME)

La SPME est une méthode de préparation d'échantillons rapide, simple et sans solvant qui peut être couplée facilement et avec sensibilité à la GC et la chromatographie en phase liquide à haute performance (HPLC). Dans les années 1990, la SPME a été développée pour la première fois par Pawliszyn et considérée comme une méthode de prétraitement prometteuse largement utilisée dans les domaines de l'alimentation (Dou et al., 2020), de

l'environnement (Yin et al., 2019), de la biologie (Zhang et al., 2019) et de la médecine clinique (Filipiak et Bojko, 2019).

La SPME est fondée sur la répartition des composés entre la phase stationnaire et l'échantillon liquide ou gazeux. Par conséquent, la phase stationnaire enduite sur la fibre joue un rôle important dans le développement de la répétabilité et de la sensibilité de la méthode (Piri-Moghadam et al., 2016). L'extraction consiste à placer la fibre sur un support solide en contact avec l'échantillon à extraire pour l'analyse des composés par chromatographie liquide ou gazeuse couplée à un spectromètre de masse. L'extraction est basée sur un principe similaire à la chromatographie, basé sur le partage liquide-liquide ou gaz-liquide. La cinétique du processus de la SPME dépend de nombreux paramètres comme l'épaisseur du film de phase stationnaire, l'agitation de l'échantillon et la durée d'échantillonnage (Ruiz del Castillo et al., 2019 ; Zhao et al., 2015).

L'inconvénient majeur de cette méthode est la fragilité et la durée de vie limitée des fibres commerciales telles que le polyacrylate (PA) et le polydiméthylsiloxane (PDMS). Par contre, ce procédé d'extraction respectueux de l'environnement par absence de solvants présente de nombreux avantages : simplicité, fiabilité, sensibilité, rentabilité, facilité d'automatisation et nécessitant une quantité minime d'échantillon (Zhang et al., 2016 ; Dimpe et Nomngongo, 2016).

Plusieurs procédures de SPME ont été menées par Saraji et al. en 2016, pour l'analyse des pesticides organophosphorés par GC, par Abdulra'uf et Tan en 2015, pour la détermination des pesticides dans les fruits et légumes par analyse GC-MS, par Kin et Huat en 2010, pour l'étude des résidus de pesticides dans les échantillons de fraises et de concombres et par Zhang et al. en 2017, pour la détermination des pyréthrinoïdes combinée à une GC.

1.5. QUEChERS

La méthode QuEChERS, est une technique d'extraction qui est devenu courante au sein de la communauté scientifique, lors de l'analyse des pesticides dans les matrices alimentaires (Lee et al., 2016 ; Bernardi et al., 2016 ; Tette et al., 2016 ; Golge et al., 2018). Anastassiades et al. en 2003, ont développé la technique QuEChERS qui a été nommée ainsi en raison de ces caractéristiques : être rapide, facile, bon marché, efficace, robuste

et sûr. La méthode QuEChERS a subi plusieurs modifications, ce qui est essentiel pour l'analyse multi-résidus de pesticides et l'extraction de grandes quantités de ces pesticides à partir de différentes catégories et matrices alimentaires (Musarurwa et al., 2019 ; He et al., 2015). La plupart des modifications proposées à la méthode QuEChERS se concentrent sur les étapes de nettoyage, y compris l'utilisation de différents sorbants tels que l'amine primaire secondaire (PSA) (Ferreira et al., 2016), l'octadécylsilane (C_{18}) (Dankyi et al., 2015), le noir de carbone graphité (Bernardi et al., 2016) et le Florisil (silicate de magnésium) (Kemmerich et al., 2020).

La procédure QuEChERS utilise surtout l'ACN, qui permet l'extraction des analytes polaires avec un degré élevé de détection et de sélectivité, ainsi qu'une compatibilité directe avec une analyse par LC et / ou en GC, couplée à la spectrométrie de masse (Alcântara et al., 2019 ; Lehotay et al., 2010 ; Iqbal et al., 2020 ; Kim et al., 2019). Par rapport à d'autres techniques mentionnées ci-dessus telles que la LLE et la SPE, la méthode QuEChERS minimise le nombre d'étapes en deux, dont la première consiste en une simple extraction avec de l'ACN et un mélange de sels ainsi que la deuxième est une étape de nettoyage par extraction en phase solide dispersive comprenant un ou plusieurs sorbants (Muhammad et al., 2017). Les autres avantages de la méthode QuEChERS par rapport aux autres techniques sont la faible consommation de solvant, le gain de temps pour la préparation des échantillons et leurs excellentes récupérations (Zhang et al., 2014).

En général, le PSA est utilisé comme un adsorbant ayant comme phase éthylènediamine-N-propyle ($C_5H_{14}N_2$) et qui renferme à la fois des amines primaires et secondaires ce qui lui confère une forte sélectivité (due à la présence du NH_2) ainsi qu'une capacité très élevée (due à la présence du NH). C'est un échangeur d'anions faible avec un pK_a de 10,1 et 10,9. Il a également une très forte affinité et grande capacité d'élimination des acides organiques, des acides gras, des sucres polaires et de certains pigments lors de l'analyse de pesticides multi-résidus dans les aliments (Suganthi et al., 2018). La méthode QuEChERS originale a trouvé de nombreuses applications lors de l'analyse de pesticides dans des matrices complexes telles que les échantillons de miel (Tette et al., 2016), des échantillons de tabac (Bernardi et al., 2016), des échantillons de poissons (Medeiros et al., 2020), des échantillons de sang humain (Iqbal et al., 2020) et des échantillons de fruits et végétaux (Narenderan et al., 2019).

La méthode QuEChERS modifiée utilise l'extraction par l'ACN, suivie du relargage de l'eau de l'échantillon à l'aide de chlorure de sodium (NaCl) et de sulfate de magnésium anhydre (MgSO_4) (Lee et al., 2016). Lors de cette méthode, les sels de citrate sont utilisés comme tampon afin de créer des conditions de pH appropriées pour induire un partage liquide-liquide. Une extraction en phase solide dispersive est effectuée pour le nettoyage en utilisant une combinaison de MgSO_4 pour réduire la quantité de l'eau restante dans l'extrait, C_{18} pour éliminer les lipides, PSA pour éliminer les acides gras parmi d'autres composants et le noir de carbone graphité particulièrement utile pour l'élimination de la chlorophylle qui, lorsqu'il est injecté dans un chromatographe en phase gazeuse et liquide peut également avoir un impact négatif sur l'analyse (Zheng et al., 2018). De nombreux chercheurs (Calatayud-Vernich et al., 2016 ; Garcia and Gotah, 2017 ; Lee et al., 2016 ; Lehotay et al., 2010 ; Ly et al., 2020) ont utilisé la technique QuEChERS pour analyser les pesticides dans les matrices alimentaires. Par exemple, Lee et al. en 2016, ont analysé les herbicides dans le riz, Wang et al. en 2020, détectent la présence de pesticides dans les légumes et les fruits, tandis que Zheng et al. en 2018, ont analysé les pesticides dans le miel. La technique QuEChERS est appliquée lors de l'analyse des pesticides et d'autres polluants organiques dans de nombreuses matrices différentes. Ces matrices comprennent les escargots (Al-Alam et al., 2020), les fruits et les légumes (Alcântara et al., 2019 ; Wang et al., 2020), les produits d'origine animale (Rahman et al., 2018 ; Mu et al., 2016), les aiguilles de conifères (Baroudi et al., 2020) et les céréales (Tian et al., 2020).

2. Séparation et détection

Plusieurs méthodes analytiques ont été utilisées pour séparer et détecter les polluants dans les matrices. En raison de la grande complexité de la matrice et de la faible concentration de ces composés, l'utilisation de méthodes analytiques qui offrent une sensibilité et une sélectivité élevées est essentielle. Le choix de la technique de séparation dépend particulièrement des caractéristiques des composés d'intérêts (Souza Tette et al., 2016). La détection des composés thermiquement stables (volatils, semi-volatils) se fait par GC, tandis que les composés thermiquement instables (non volatils) peuvent être analysés par LC (Dimpe et Nomngongo, 2016 ; Coskun, 2016). Les méthodes chromatographiques telles que la chromatographie en phase gazeuse ou la chromatographie liquide avec la détection par spectrométrie de masse en tandem, sont

particulièrement adaptées à la détection de nombreux polluants présents dans des matrices complexes.

2.1. Chromatographie en phase gazeuse

La GC a été la méthode la plus largement utilisée pour l'analyse des résidus de polluants organiques présentant des propriétés hydrophobes et volatiles dans les matrices complexes. Les composés analysés sont séparés à l'aide de colonnes capillaires avec différentes phases stationnaires (Madej et al., 2018). Elle a été associée à différents types de méthodes de détection, comme la détection par ionisation de flamme (Farajzadeh et al., 2015), la détection par capture d'électrons (Yu et al., 2012) et la détection photométrique de flamme (Zhu et al., 2018).

Parmi les méthodes chromatographiques en phase gazeuse adaptées à l'analyse des résidus de pesticides, la chromatographie gazeuse avec détection par spectromètre de masse (GC-MS), y compris les spectromètres de masse à simple quadripôle et à triple quadripôle, a été le plus souvent utilisée. La GC-MS fonctionnant en mode d'ionisation par impact électronique (EI) a été utilisé pour la quantification des résidus de pesticides (Shamsipur et al., 2016), des HAPs et des PCBs (Chamkasem et al., 2016 ; Issa et al., 2020 ; Tang et al., 2020).

Les composés ont été identifiés par leur temps de rétention et quantifiés à l'aide de leurs ions spécifiques donnant des résultats corrects même à de faibles niveaux de concentration pour des composés cibles. Cependant, si les ions sélectionnés sont affectés par des interférences de la matrice, l'utilisation de la chromatographie gazeuse couplée à la spectrométrie de masse en tandem (GC-MS/MS) peut être appliquée, permettant des niveaux de sensibilité plus élevés et des limites de détection plus basses (Loos et al., 2016 ; Panuwet et al., 2016).

Par rapport à la LC, la GC-MS est la technique la plus largement utilisée pour analyser les résidus dans les aliments. Cependant, les pesticides utilisés aujourd'hui sont thermiquement instables, plus polaires ou difficilement vaporisables, ils sont devenus essentiellement analysables par LC (David et al., 2017).

2.2. Chromatographie en phase liquide

La LC est une technique analytique utilisée pour la détermination de pesticides polaires, thermolabiles et / ou non volatils ainsi que des résidus des autres polluants organiques dans les matrices complexes. En raison de sa sélectivité et de sa sensibilité élevées, la LC couplée à la détection spectrophotométrie (Tuzimski et Rejczak, 2016,2014) et à la spectrométrie de masse (Wurita et al., 2020 ; Han et al., 2016) a été principalement utilisée. Cependant, le détecteur à barrette de diodes, le détecteur à ultraviolet et la spectroscopie de fluorescence sont généralement utilisés pour analyser uniquement quelques pesticides ou quelques classes de pesticides en raison de la similitude entre les spectres de différents pesticides appartenant à la même famille (Akvan et al., 2019 ; Ferreyra et al., 2021).

La méthode d'extraction QuEChERS suivie d'une analyse par LC-MS/MS est la technique la plus puissante pour l'analyse de pesticides dans les aiguilles de conifères (Baroudi et al., 2020), la détermination de carbamates (Moreno-González et al., 2014), la quantification de pesticides de différentes classes dans les matrices végétales (Rajski et al., 2013), l'analyse des pesticides dans les escargots (AL-Alam et al., 2020) et la détection du diméthoate et de la terbuthylazine dans les échantillons d'olives (Gómez-Almenar et García-Mesa, 2015).

Cependant, malgré tous les avantages de la technique de détection actuelle, des méthodes appropriées de préparation d'échantillons sont toujours cruciales, car les effets de matrice peuvent avoir un impact significatif sur la détection en créant un bruit de fond d'analyse et en modifiant l'efficacité d'ionisation, résultant en une sensibilité réduite (Souza Tette et al., 2016 ; Loos et al., 2016).

2.3. Spectrométrie de masse en mode tandem

La spectrométrie de masse couramment utilisée permet de détecter et d'identifier des molécules chargées en mesurant le rapport de leur masse au nombre de charges (m/z) en fournissant des informations quantitatives et qualitatives précises sur les composés analysés. Compte tenu de sa sélectivité et de sa sensibilité élevée, cette technique peut être couplée à différents détecteurs tels que la chromatographie en phase gazeuse et liquide (Loos et al., 2016). La spectrométrie de masse en tandem, également connue sous

le nom de MS / MS, implique plusieurs étapes de sélection par spectrométrie de masse, avec une certaine forme de fragmentation se produisant entre les étapes (Mittal, 2015). L'analyse d'échantillons biologiques complexes avec des techniques de spectrométrie de masse et de masse en tandem, a contribué à l'acquisition d'informations structurales importantes pour de nombreux types d'ions, notamment les peptides, protéines, lipides et glucides (Kailemia et al., 2014).

Dans la spectrométrie de masse en tandem, un premier analyseur est utilisé pour isoler l'ion précurseur, qui subit ensuite une fragmentation pour produire des ions produits et des fragments neutres qui sont ensuite analysés par un deuxième analyseur de masse. La détection MS / MS offre une sélectivité accrue par rapport à d'autres techniques de mesure analytiques courantes, telles que la chromatographie liquide et la spectrophotométrie ultraviolette-visible (Breemen et Martinez, 2013; Rockwood et al., 2018).

V. Objectifs

Compte tenu de la situation environnementale concernant la pollution de l'air au Liban, il est possible d'énoncer le but de la thèse. L'objectif principal de cette thèse est l'étude comparative de trois biomoniteurs (conifère, escargot et miel) pour évaluer la variabilité spatio-temporelle de polluants organiques dans l'atmosphère au Liban.

Pour y parvenir, il est nécessaire de développer les objectifs spécifiques suivants :

- développer deux méthodes d'extraction multi-résidus avec la technique du QuEChERS pour l'analyse des pesticides, des HAPs et des PCBs dans les escargots et les aiguilles de conifères ;
- caractériser la qualité de l'air au voisinage des cultures par une approche basée sur le « biomonitoring » grâce aux escargots et aiguilles de conifères, tout en étudiant l'accumulation des polluants en fonction du temps et des sites d'échantillonnage ;
- développer une méthode d'analyse des pesticides non volatiles dans le miel.

- Abdel Gawad, S.S., 2018. Acute toxicity of some heavy metals to the fresh water snail, *Theodoxus niloticus* (Reeve, 1856). *The Egyptian Journal of Aquatic Research* 44, 83–87. <https://doi.org/10.1016/j.ejar.2018.06.004>
- Abdel-Halim, K.Y., Abo El-Saad, A.M., Talha, M.M., Hussein, A.A., Bakry, N.M., 2013. Oxidative stress on land snail *Helix aspersa* as a sentinel organism for ecotoxicological effects of urban pollution with heavy metals. *Chemosphere* 93, 1131–1138. <https://doi.org/10.1016/j.chemosphere.2013.06.042>
- Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum* 25, 107–123. <https://doi.org/10.1016/j.ejpe.2015.03.011>
- Abdulra'uf, L.B., Tan, G.H., 2015. Chemometric approach to the optimization of HS-SPME/GC-MS for the determination of multiclass pesticide residues in fruits and vegetables. *Food Chemistry* 177, 267–273. <https://doi.org/10.1016/j.foodchem.2015.01.031>
- Achilleos, S., Al-Ozairi, E., Alahmad, B., Garshick, E., Neophytou, A.M., Bouhamra, W., Yassin, M.F., Koutrakis, P., 2019. Acute effects of air pollution on mortality: A 17-year analysis in Kuwait. *Environment International* 126, 476–483. <https://doi.org/10.1016/j.envint.2019.01.072>
- Achten, C., Andersson, J.T., 2015. Overview of Polycyclic Aromatic Compounds (PAC). *Polycycl Aromat Compd* 35, 177–186. <https://doi.org/10.1080/10406638.2014.994071>
- Agus, B.A.P., Hussain, N., Selamat, J., 2020. Quantification of PAH4 in roasted cocoa beans using QuEChERS and dispersive liquid-liquid micro-extraction (DLLME) coupled with HPLC-FLD. *Food Chemistry* 303, 125398. <https://doi.org/10.1016/j.foodchem.2019.125398>
- Ahmad, R., Ahmad, N., Al-Anaki, W.S., Ismail, F.A., Al-Jishi, F., 2020. Solvent and temperature effect of accelerated solvent extraction (ASE) coupled with ultra-high-pressure liquid chromatography (UHPLC-PDA) for the determination of methyl xanthines in commercial tea and coffee. *Food Chemistry* 311, 126021. <https://doi.org/10.1016/j.foodchem.2019.126021>
- Akash, M., Pawade, U., Nikam, A., 2018. CLASSIFICATION OF PESTICIDES: A REVIEW. *International Journal of Research in Ayurveda and Pharmacy* 9, 144–150. <https://doi.org/10.7897/2277-4343.094131>
- Akvan, N., Azimi, G., Parastar, H., 2019. Chemometric assisted determination of 16 PAHs in water samples by ultrasonic assisted emulsification microextraction followed by fast high-performance liquid chromatography with diode array detector. *Microchemical Journal* 150, 104056. <https://doi.org/10.1016/j.microc.2019.104056>
- Akyüz, M., Çabuk, H., 2010. Gas-particle partitioning and seasonal variation of polycyclic aromatic hydrocarbons in the atmosphere of Zonguldak, Turkey. *Science of The Total Environment* 408, 5550–5558. <https://doi.org/10.1016/j.scitotenv.2010.07.063>
- Al Dine, E.J., Mokbel, H., Elmoll, A., Massem, S., Vuilleumier, S., Toufaily, J., Hanieh, T., Millet, M., 2015. Concomitant evaluation of atmospheric levels of polychlorinated biphenyls, organochlorine pesticides, and polycyclic aromatic hydrocarbons in Strasbourg (France) using pine needle passive samplers. *Environ Sci Pollut Res* 22, 17850–17859. <https://doi.org/10.1007/s11356-015-5030-5>
- Al-Alam, J., Baroudi, F., Chbani, A., Fajloun, Z., Millet, M., 2020. A multiresidue method for the analysis of pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in snails used as environmental biomonitors. *Journal of Chromatography A* 1621, 461006. <https://doi.org/10.1016/j.chroma.2020.461006>
- Al-Alam, J., Chbani, A., Fajloun, Z., Millet, M., 2019. The use of vegetation, bees, and snails as important tools for the biomonitoring of atmospheric pollution—a review. *Environ Sci Pollut Res* 26, 9391–9408. <https://doi.org/10.1007/s11356-019-04388-8>
- Al-Alam, J., Fajloun, Z., Chbani, A., Millet, M., 2017. The use of conifer needles as biomonitors candidates for the study of temporal air pollution variation in the Strasbourg region. *Chemosphere* 168, 1411–1421. <https://doi.org/10.1016/j.chemosphere.2016.11.103>
- Alcântara, D.B., Fernandes, T.S.M., Nascimento, H.O., Lopes, A.F., Menezes, M.G.G., Lima, A.C.A., Carvalho, T.V., Grinberg, P., Milhome, M.A.L., Oliveira, A.H.B., Becker, H., Zocolo, G.J., Nascimento, R.F., 2019. Diagnostic detection systems and QuEChERS methods for multiclass pesticide analyses in different types of fruits: An overview from the last decade. *Food Chemistry* 298, 124958. <https://doi.org/10.1016/j.foodchem.2019.124958>
- Alexandrino, K., Viteri, F., Rybarczyk, Y., Guevara Andino, J.E., Zalakeviciute, R., 2020. Biomonitoring of metal levels in urban areas with different vehicular traffic intensity by using *Araucaria heterophylla* needles. *Ecological Indicators* 117, 106701. <https://doi.org/10.1016/j.ecolind.2020.106701>
- Alghamdi, B.A., Alshumrani, E.S., Saeed, M.S.B., Rawas, G.M., Alharthi, N.T., Baeshen, M.N., Helmi, N.M., Alam, M.Z., Suhail, M., 2020. Analysis of sugar composition and pesticides using HPLC and GC-MS techniques in honey samples collected from Saudi Arabian markets. *Saudi Journal of Biological Sciences*. <https://doi.org/10.1016/j.sjbs.2020.08.018>
- Al-Mulali, U., Ozturk, I., Lean, H.H., 2015. The influence of economic growth, urbanization, trade openness, financial development, and renewable energy on pollution in Europe. *Nat Hazards* 79, 621–644. <https://doi.org/10.1007/s11069-015-1865-9>
- Andrade, P.D., da Silva, J.L.G., Caldas, E.D., 2013. Simultaneous analysis of aflatoxins B1, B2, G1, G2, M1 and ochratoxin A in breast milk by high-performance liquid chromatography/fluorescence after liquid-liquid extraction with low temperature purification (LLE-LTP). *Journal of Chromatography A* 1304, 61–68. <https://doi.org/10.1016/j.chroma.2013.06.049>
- Anim, A.K., Drage, D.S., Goonetilleke, A., Mueller, J.F., Ayoko, G.A., 2017. Distribution of PBDEs, HBCDs and PCBs in the Brisbane River estuary sediment. *Marine Pollution Bulletin* 120, 165–173. <https://doi.org/10.1016/j.marpolbul.2017.05.002>
- Arihailam, E.C., Arihailam*, N.H., 2019. Impact and control of anthropogenic pollution on the ecosystem A review. *JBBD* 4, 54–59. <https://doi.org/10.31248/JBBD2019.098>
- Arinaitwe, K., Rose, N.L., Muir, D.C.G., Kiremire, B.T., Balirwa, J.S., Teixeira, C., 2016. Historical deposition of persistent organic pollutants in Lake Victoria and two alpine equatorial lakes from East Africa: Insights into atmospheric deposition from sedimentation profiles. *Chemosphere* 144, 1815–1822. <https://doi.org/10.1016/j.chemosphere.2015.10.061>
- Augusto, S., Máguas, C., Branquinho, C., 2013. Guidelines for biomonitoring persistent organic pollutants (POPs), using lichens and aquatic mosses – A review. *Environmental Pollution* 180, 330–338. <https://doi.org/10.1016/j.envpol.2013.05.019>
- Babatola, S.S., 2018. Global burden of diseases attributable to air pollution. *Journal of Public Health in Africa* 9. <https://doi.org/10.4081/jphia.2018.813>
- Bakirtas, T., Akpolat, A.G., 2018. The relationship between energy consumption, urbanization, and economic growth in new emerging-market countries. *Energy* 147, 110–121. <https://doi.org/10.1016/j.energy.2018.01.011>
- Balázs, H.E., Schmid, C.A.O., Podar, D., Hufnagel, G., Radl, V., Schröder, P., 2020. Development of microbial communities in organochlorine pesticide contaminated soil: A post-reclamation perspective. *Applied Soil Ecology* 150, 103467. <https://doi.org/10.1016/j.apsoil.2019.103467>
- Baltrėnaitė, E., Baltrėnas, P., Lietuvninkas, A., Šerevičienė, V., Zuokaitė, E., 2014. Integrated evaluation of aerogenic pollution by air-transported heavy metals (Pb, Cd, Ni, Zn, Mn and Cu) in the analysis of the main deposit media. *Environ Sci Pollut Res* 21, 299–313. <https://doi.org/10.1007/s11356-013-2046-6>

- Bandowe, B.A.M., Nkansah, M.A., 2016. Occurrence, distribution and health risk from polycyclic aromatic compounds (PAHs, oxygenated-PAHs and azaarenes) in street dust from a major West African Metropolis. *Science of The Total Environment* 553, 439–449. <https://doi.org/10.1016/j.scitotenv.2016.02.142>
- Banerjee, S., Mazumdar, S., 2012. Electrospray Ionization Mass Spectrometry: A Technique to Access the Information beyond the Molecular Weight of the Analyte [WWW Document]. *International Journal of Analytical Chemistry*. <https://doi.org/10.1155/2012/282574>
- Bányiová, K., Černá, M., Mikeš, O., Komprdová, K., Sharma, A., Gyalpo, T., Čupr, P., Scherlinger, M., 2017. Long-term time trends in human intake of POPs in the Czech Republic indicate a need for continuous monitoring. *Environment International* 108, 1–10. <https://doi.org/10.1016/j.envint.2017.07.008>
- Bargańska, Z., Ślebioda, M., Namieśnik, J., 2016. Honey bees and their products: Bioindicators of environmental contamination. *Critical Reviews in Environmental Science and Technology* 46, 235–248. <https://doi.org/10.1080/10643389.2015.1078220>
- Baroudi, F., Al Alam, J., Fajloun, Z., Millet, M., 2020a. Snail as sentinel organism for monitoring the environmental pollution; a review. *Ecological Indicators* 113, 106240. <https://doi.org/10.1016/j.ecolind.2020.106240>
- Baroudi, F., Al-Alam, J., Chimjarn, S., Delhomme, O., Fajloun, Z., Millet, M., 2020b. Conifers as environmental biomonitors: A multi-residue method for the concomitant quantification of pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls by LC-MS/MS and GC-MS/MS. *Microchemical Journal* 154, 104593. <https://doi.org/10.1016/j.microc.2019.104593>
- Beach, D.G., Quilliam, M.A., Hellou, J., 2009. Analysis of pyrene metabolites in marine snails by liquid chromatography using fluorescence and mass spectrometry detection. *Journal of Chromatography B* 877, 2142–2152. <https://doi.org/10.1016/j.jchromb.2009.06.006>
- Beelen, R., et al., 2014. Effects of long-term exposure to air pollution on natural-cause mortality: an analysis of 22 European cohorts within the multicentre ESCAPE project. *The Lancet* 383, 785–795. [https://doi.org/10.1016/S0140-6736\(13\)62158-3](https://doi.org/10.1016/S0140-6736(13)62158-3)
- Belsky, J., Joshi, N.K., 2019. Impact of Biotic and Abiotic Stressors on Managed and Feral Bees. *Insects* 10. <https://doi.org/10.3390/insects10080233>
- Bernardi, G., Kemmerich, M., Ribeiro, L.C., Adaime, M.B., Zanella, R., Prestes, O.D., 2016. An effective method for pesticide residues determination in tobacco by GC-MS/MS and UHPLC-MS/MS employing acetonitrile extraction with low-temperature precipitation and d-SPE clean-up. *Talanta* 161, 40–47. <https://doi.org/10.1016/j.talanta.2016.08.015>
- Bertero, A., Rivolta, M., Davanzo, F., Caloni, F., 2020. Suspected environmental poisoning by drugs, household products and pesticides in domestic animals. *Environmental Toxicology and Pharmacology* 80, 103471. <https://doi.org/10.1016/j.etap.2020.103471>
- Bertolotti, G., Gialanella, S., 2014. Review: use of conifer needles as passive samplers of inorganic pollutants in air quality monitoring. *Anal. Methods* 6, 6208–6222. <https://doi.org/10.1039/C4AY00172A>
- Bohan, D.A., Vacher, C., Tamaddoni-Nezhad, A., Raybould, A., Dumbrell, A.J., Woodward, G., 2017. Next-Generation Global Biomonitoring: Large-scale, Automated Reconstruction of Ecological Networks. *Trends in Ecology & Evolution* 32, 477–487. <https://doi.org/10.1016/j.tree.2017.03.001>
- Bonmatin, J.-M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A.D., Noome, D.A., Simon-Delso, N., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. *Environ Sci Pollut Res* 22, 35–67. <https://doi.org/10.1007/s11356-014-3332-7>
- Boquete, M.T., Aboal, J.R., Carballeira, A., Fernández, J.A., 2014. Effect of age on the heavy metal concentration in segments of *Pseudoscleropodium purum* and the biomonitoring of atmospheric deposition of metals. *Atmospheric Environment* 86, 28–34. <https://doi.org/10.1016/j.atmosenv.2013.12.039>
- Boquete, M.T., Fernández, J.A., Carballeira, A., Aboal, J.R., 2013. Assessing the tolerance of the terrestrial moss *Pseudoscleropodium purum* to high levels of atmospheric heavy metals: A reciprocal transplant study. *Science of The Total Environment* 461–462, 552–559. <https://doi.org/10.1016/j.scitotenv.2013.05.039>
- Boshoff, M., Jordaens, K., Baguet, S., Bervoets, L., 2015. Trace metal transfer in a soil–plant–snail microcosm field experiment and biomarker responses in snails. *Ecological Indicators* 48, 636–648. <https://doi.org/10.1016/j.ecolind.2014.08.037>
- Bouri, E., El Assad, J., 2016. The Lebanese Electricity Woes: An Estimation of the Economical Costs of Power Interruptions. *Energies* 9, 583. <https://doi.org/10.3390/en9080583>
- Breemen, R.B. van, Martinez, E.M., 2013. Best Practice in Mass Spectrometry for LC-MS, in: *Handbook of LC-MS Bioanalysis*. John Wiley & Sons, Ltd, pp. 205–216. <https://doi.org/10.1002/9781118671276.ch16>
- Büyükköroğlu, G., Dora, D.D., Özdemir, F., Hızal, C., 2018. Chapter 15 - Techniques for Protein Analysis, in: Barh, D., Azevedo, V. (Eds.), *Omics Technologies and Bio-Engineering*. Academic Press, pp. 317–351. <https://doi.org/10.1016/B978-0-12-804659-3.00015-4>
- Cachada, A., Ferreira da Silva, E., Duarte, A.C., Pereira, R., 2016. Risk assessment of urban soils contamination: The particular case of polycyclic aromatic hydrocarbons. *Science of The Total Environment* 551–552, 271–284. <https://doi.org/10.1016/j.scitotenv.2016.02.012>
- Cacho, J.I., Campillo, N., Viñas, P., Hernández-Córdoba, M., 2018. In situ ionic liquid dispersive liquid-liquid microextraction coupled to gas chromatography-mass spectrometry for the determination of organophosphorus pesticides. *Journal of Chromatography A* 1559, 95–101. <https://doi.org/10.1016/j.chroma.2017.12.059>
- Calatayud-Vernich, P., Calatayud, F., Simó, E., Picó, Y., 2016. Efficiency of QuEChERS approach for determining 52 pesticide residues in honey and honey bees. *MethodsX* 3, 452–458. <https://doi.org/10.1016/j.mex.2016.05.005>
- Canbay, H.S., 2017. Effectiveness of Liquid-Liquid Extraction, Solid Phase Extraction, and Headspace Technique for Determination of Some Volatile Water-Soluble Compounds of Rose Aromatic Water [WWW Document]. *International Journal of Analytical Chemistry*. <https://doi.org/10.1155/2017/4870671>
- Capozzi, F., Adamo, P., Spagnuolo, V., Giordano, S., 2021. Field comparison between moss and lichen PAHs uptake abilities based on deposition fluxes and diagnostic ratios. *Ecological Indicators* 120, 106954. <https://doi.org/10.1016/j.ecolind.2020.106954>
- Cerasa, M., Benedetti, P., De Stefanis, A., Guerriero, E., Mosca, S., Bacaloni, A., Rotatori, M., 2020. Validation studies on activated carbon fiber passive sampler for PCDD/Fs and PCBs in water. *Chemosphere* 239, 124666. <https://doi.org/10.1016/j.chemosphere.2019.124666>
- Chamkasem, N., Lee, S., Harmon, T., 2016. Analysis of 19 PCB congeners in catfish tissue using a modified QuEChERS method with GC-MS/MS. *Food Chemistry* 192, 900–906. <https://doi.org/10.1016/j.foodchem.2015.07.088>
- Chatzimitakos, T.G., Karali, K.K., Stalikas, C.D., 2019. Magnetic graphene oxide as a convenient nanosorbent to streamline matrix solid-phase dispersion towards the extraction of pesticides from vegetables and their determination by GC-MS. *Microchemical Journal* 151, 104247. <https://doi.org/10.1016/j.microc.2019.104247>
- Chaza, C., Sopheak, N., Mariam, H., David, D., Baghdad, O., Moomen, B., 2018. Assessment of pesticide contamination in Akkar groundwater, northern Lebanon. *Environ Sci Pollut Res* 25, 14302–14312. <https://doi.org/10.1007/s11356-017-8568-6>

- Chen, J., 2007. Rapid urbanization in China: A real challenge to soil protection and food security. *CATENA*, Influences of rapid urbanization and industrialization on soil resource and its quality in China 69, 1–15. <https://doi.org/10.1016/j.catena.2006.04.019>
- Chen, P., Xiao, X., Mei, J., Cai, Y., Tang, Y., Peng, P., 2017. Characteristic accumulation of PCDD/Fs in pine needles near an MSWI and emission levels of the MSWI in Pearl River Delta: A case study. *Chemosphere* 181, 360–367. <https://doi.org/10.1016/j.chemosphere.2017.04.098>
- Chen, S., Shahi, C., Chen, H.Y.H., McLaren, B., 2017. Economic analysis of forest management alternatives: Compositional objectives, rotation ages, and harvest methods in boreal forests. *Forest Policy and Economics* 85, 124–134. <https://doi.org/10.1016/j.forpol.2017.09.006>
- Cheng, L., Lu, Y., Zhao, Z., Hoogenboom, R.L.A.P., Zhang, Q., Liu, X., Song, Wei, Guan, S., Song, Weiguo, Rao, Q., 2020. Assessing the combined toxicity effects of three neonicotinoid pesticide mixtures on human neuroblastoma SK-N-SH and lepidopteran Sf-9 cells. *Food and Chemical Toxicology* 145, 111632. <https://doi.org/10.1016/j.fct.2020.111632>
- Chiesa, L.M., Labella, G.F., Giorgi, A., Panseri, S., Pavlovic, R., Bonacci, S., Arioli, F., 2016. The occurrence of pesticides and persistent organic pollutants in Italian organic honeys from different productive areas in relation to potential environmental pollution. *Chemosphere* 154, 482–490. <https://doi.org/10.1016/j.chemosphere.2016.04.004>
- Chikere, C.B., Fenibo, E.O., 2018. Distribution of PAH-ring hydroxylating dioxygenase genes in bacteria isolated from two illegal oil refining sites in the Niger Delta, Nigeria. *Scientific African* 1, e00003. <https://doi.org/10.1016/j.sciaf.2018.e00003>
- Cho, Y.J., Getachew, A.T., Saravana, P.S., Chun, B.S., 2019. Optimization and characterization of polysaccharides extraction from Giant African snail (*Achatina fulica*) using pressurized hot water extraction (PHWE). *Bioactive Carbohydrates and Dietary Fibre* 18, 100179. <https://doi.org/10.1016/j.bcdf.2019.100179>
- Cloutier, P.-L., Fortin, F., Groleau, P.E., Brousseau, P., Fournier, M., Desrosiers, M., 2017. QuEChERS extraction for multi-residue analysis of PCBs, PAHs, PBDEs and PCDD/Fs in biological samples. *Talanta* 165, 332–338. <https://doi.org/10.1016/j.talanta.2016.12.080>
- Concha-Graña, E., Muniategui-Lorenzo, S., De Nicola, F., Aboal, J.R., Rey-Asensio, A.I., Giordano, S., Reski, R., López-Mahía, P., Prada-Rodríguez, D., 2015. Matrix solid phase dispersion method for determination of polycyclic aromatic hydrocarbons in moss. *Journal of Chromatography A* 1406, 19–26. <https://doi.org/10.1016/j.chroma.2015.06.014>
- Conti, M.E., Tudino, M.B., 2016. Chapter 6 - Lichens as Biomonitoring of Heavy-Metal Pollution, in: de la Guardia, M., Armenta, S. (Eds.), *Comprehensive Analytical Chemistry, The Quality of Air*. Elsevier, pp. 117–145. <https://doi.org/10.1016/bs.coac.2016.02.005>
- Coppock, R.W., Dziwenka, M.M., 2020. Chapter 63 - Threats to wildlife by chemical and warfare agents, in: Gupta, R.C. (Ed.), *Handbook of Toxicology of Chemical Warfare Agents* (Third Edition). Academic Press, Boston, pp. 1077–1087. <https://doi.org/10.1016/B978-0-12-819090-6.00063-5>
- Coskun, O., 2016. Separation techniques: Chromatography. *North Clin Istanbul* 3, 156–160. <https://doi.org/10.14744/nci.2016.32757>
- Cossi, P.F., Herbert, L.T., Yusseppone, M.S., Pérez, A.F., Kristoff, G., 2018. Environmental concentrations of azinphos-methyl cause different toxic effects without affecting the main target (cholinesterases) in the freshwater gastropod *Biomphalaria straminea*. *Ecotoxicology and Environmental Safety* 162, 287–295. <https://doi.org/10.1016/j.ecoenv.2018.06.091>
- Covert, S.Alex., Shoda, M.E., Stackpoole, S.M., Stone, W.W., 2020. Pesticide mixtures show potential toxicity to aquatic life in U.S. streams, water years 2013–2017. *Science of The Total Environment* 745, 141285. <https://doi.org/10.1016/j.scitotenv.2020.141285>
- Cui, M., Chen, Y., Tian, C., Zhang, F., Yan, C., Zheng, M., 2016. Chemical composition of PM 2.5 from two tunnels with different vehicular fleet characteristics. *Science of The Total Environment* 550, 123–132. <https://doi.org/10.1016/j.scitotenv.2016.01.077>
- Cuny, D., 2012. La biosurveillance végétale et fongique de la pollution atmosphérique: concepts et applications. *Annales Pharmaceutiques Françaises* 70, 182–187. <https://doi.org/10.1016/j.pharma.2012.05.003>
- Cutillas, V., García-Valverde, M., Gómez-Ramos, M. del M., Díaz-Galiano, F.J., Ferrer, C., Fernández-Alba, A.R., 2020. Supercritical fluid chromatography separation of chiral pesticides: Unique capabilities to study cyhalothrin and metalaxyl as examples. *Journal of Chromatography A* 1620, 461007. <https://doi.org/10.1016/j.chroma.2020.461007>
- Czipa, N., András, D., Kovács, B., 2015. Determination of essential and toxic elements in Hungarian honeys. *Food Chemistry* 175, 536–542. <https://doi.org/10.1016/j.foodchem.2014.12.018>
- Da Silva, P.M., Gauche, C., Gonzaga, L.V., Costa, A.C.O., Fett, R., 2016. Honey: Chemical composition, stability and authenticity. *Food Chemistry* 196, 309–323. <https://doi.org/10.1016/j.foodchem.2015.09.051>
- Daher, N., Saliba, N.A., Shihadeh, A.L., Jaafar, M., Baalbaki, R., Sioutas, C., 2013. Chemical composition of size-resolved particulate matter at near-freeway and urban background sites in the greater Beirut area. *Atmospheric Environment* 80, 96–106. <https://doi.org/10.1016/j.atmosenv.2013.08.004>
- Damalas, C.A., Koutroubas, S.D., 2016. Farmers' Exposure to Pesticides: Toxicity Types and Ways of Prevention. *Toxics* 4. <https://doi.org/10.3390/toxics4010001>
- D'Amato, G., Vitale, C., Lanza, M., Molino, A., D'Amato, M., 2016. Climate change, air pollution, and allergic respiratory diseases: an update. *Current Opinion in Allergy and Clinical Immunology* 16, 434–440. <https://doi.org/10.1097/ACI.0000000000000301>
- Dankyi, E., Carboo, D., Gordon, C., Fomsgaard, I.S., 2015. Application of the QuEChERS procedure and LC-MS/MS for the assessment of neonicotinoid insecticide residues in cocoa beans and shells. *Journal of Food Composition and Analysis* 44, 149–157. <https://doi.org/10.1016/j.jfca.2015.09.002>
- Daso, A.P., Okonkwo, O.J., 2015. Conventional Extraction Techniques: Soxhlet and Liquid-Liquid Extractions and Evaporation, in: *Analytical Separation Science*. American Cancer Society, pp. 1437–1468. <https://doi.org/10.1002/9783527678129.assep053>
- David, F., Devos, C., Dumont, E., Yang, Z., Sandra, P., Huertas-Pérez, J.F., 2017. Determination of pesticides in fatty matrices using gel permeation clean-up followed by GC-MS/MS and LC-MS/MS analysis: A comparison of low- and high-pressure gel permeation columns. *Talanta* 165, 201–210. <https://doi.org/10.1016/j.talanta.2016.12.032>
- De Barros Caetano, V.C.L., da Costa Cunha, G., Oliveira, R.V.M., da Rosa Alexandre, M., Romão, L.P.C., 2019. Magnetic hybrid support for ultrasound-assisted magnetic solid-phase extraction of polycyclic aromatic hydrocarbons from produced water. *Microchemical Journal* 146, 1195–1203. <https://doi.org/10.1016/j.microc.2019.02.055>
- De La Torre-Roche, R.J., Lee, W.-Y., Campos-Díaz, S.I., 2009. Soil-borne polycyclic aromatic hydrocarbons in El Paso, Texas: Analysis of a potential problem in the United States/Mexico border region. *Journal of Hazardous Materials* 163, 946–958. <https://doi.org/10.1016/j.jhazmat.2008.07.089>
- De Vries, W., Du, E., Butterbach-Bahl, K., 2014. Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems. *Current Opinion in Environmental Sustainability*, SI: System dynamics and sustainability 9–10, 90–104. <https://doi.org/10.1016/j.cosust.2014.09.001>

- Di Bella, G., Lo Turco, V., Potorti, A.G., Bua, G.D., Fede, M.R., Dugo, G., 2015. Geographical discrimination of Italian honey by multi-element analysis with a chemometric approach. *Journal of Food Composition and Analysis* 44, 25–35. <https://doi.org/10.1016/j.jfca.2015.05.003>
- Dimpe, K.M., Nomngongo, P.N., 2016. Current sample preparation methodologies for analysis of emerging pollutants in different environmental matrices. *TrAC Trends in Analytical Chemistry* 82, 199–207. <https://doi.org/10.1016/j.trac.2016.05.023>
- Diuzheva, A., Dejmková, H., Fischer, J., Andruch, V., 2019. Simultaneous determination of three carbamate pesticides using vortex-assisted liquid–liquid microextraction combined with HPLC–amperometric detection. *Microchemical Journal* 150, 104071. <https://doi.org/10.1016/j.microc.2019.104071>
- Domingo, J.L., Rovira, J., 2020. Effects of air pollutants on the transmission and severity of respiratory viral infections. *Environmental Research* 187, 109650. <https://doi.org/10.1016/j.envres.2020.109650>
- Donkersley, P., Rhodes, G., Pickup, R.W., Jones, K.C., Power, E.F., Wright, G.A., Wilson, K., 2017. Nutritional composition of honey bee food stores vary with floral composition. *Oecologia* 185, 749–761. <https://doi.org/10.1007/s00442-017-3968-3>
- Donovan, G.H., Jovan, S.E., Gatzolis, D., Burstyn, I., Michael, Y.L., Amacher, M.C., Monleon, V.J., 2016. Using an epiphytic moss to identify previously unknown sources of atmospheric cadmium pollution. *Science of The Total Environment* 559, 84–93. <https://doi.org/10.1016/j.scitotenv.2016.03.182>
- Doocy, S., Lyles, E., Fahed, Z., Mkanna, A., Kontunen, K., Burnham, G., 2018. Characteristics of Syrian and Lebanese Diabetes and Hypertension Patients in Lebanon. *The Open Hypertension Journal* 10. <https://doi.org/10.2174/1876526201810010060>
- Dou, T.-X., Shi, J.-F., Li, Y., Bi, F.-C., Gao, H.-J., Hu, C.-H., Li, C.-Y., Yang, Q.-S., Deng, G.-M., Sheng, O., He, W.-D., Yi, G.-J., Dong, T., 2020. Influence of harvest season on volatile aroma constituents of two banana cultivars by electronic nose and HS-SPME coupled with GC-MS. *Scientia Horticulturae* 265, 109214. <https://doi.org/10.1016/j.scienta.2020.109214>
- Druart, C., Millet, M., Scheifler, R., Delhomme, O., Raeppe, C., de Vaulfleur, A., 2011. Snails as indicators of pesticide drift, deposit, transfer and effects in the vineyard. *Science of The Total Environment* 409, 4280–4288. <https://doi.org/10.1016/j.scitotenv.2011.07.006>
- Duca, R.-C., Salquebre, G., Hardy, E., Appenzeller, B.M.R., 2014. Comparison of solid phase- and liquid/liquid-extraction for the purification of hair extract prior to multi-class pesticides analysis. *Journal of Chromatography B* 955–956, 98–107. <https://doi.org/10.1016/j.jchromb.2014.02.035>
- Duodu, G.O., Goonetilleke, A., Ayoko, G.A., 2016. Optimization of in-cell accelerated solvent extraction technique for the determination of organochlorine pesticides in river sediments. *Talanta* 150, 278–285. <https://doi.org/10.1016/j.talanta.2015.12.049>
- Duodu, G.O., Ogogo, K.N., Mummullage, S., Harden, F., Goonetilleke, A., Ayoko, G.A., 2017. Source apportionment and risk assessment of PAHs in Brisbane River sediment, Australia. *Ecological Indicators* 73, 784–799. <https://doi.org/10.1016/j.ecolind.2016.10.038>
- Eddleston, M., 2020. Poisoning by pesticides. *Medicine* 48, 214–217. <https://doi.org/10.1016/j.mpmed.2019.12.019>
- El-Shenawy, N.S., Mohammadden, A., Al-Fahmie, Z.H., 2012. Using the enzymatic and non-enzymatic antioxidant defense system of the land snail *Eobania vermiculata* as biomarkers of terrestrial heavy metal pollution. *Ecotoxicology and Environmental Safety* 84, 347–354. <https://doi.org/10.1016/j.ecoenv.2012.08.014>
- Ellickson, K.M., McMahon, C.M., Herbrandson, C., Krause, M.J., Schmitt, C.M., Lippert, C.J., Pratt, G.C., 2017. Analysis of polycyclic aromatic hydrocarbons (PAHs) in air using passive sampling calibrated with active measurements. *Environ Pollut* 231, 487–496. <https://doi.org/10.1016/j.envpol.2017.08.049>
- Emilia, R., Debora, B., Stefania, A., Nicola, B., Roberto, B., 2016. *Papillifera papillaris* (O.F. Müller), a small snail living on stones and monuments, as indicator of metal deposition and bioavailability in urban environments. *Ecological Indicators* 69, 360–367. <https://doi.org/10.1016/j.ecolind.2016.04.024>
- Escuredo, O., Dobre, I., Fernández-González, M., Seijo, M.C., 2014. Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry* 149, 84–90. <https://doi.org/10.1016/j.foodchem.2013.10.097>
- Fačkovcová, Z., Vannini, A., Monaci, F., Grattacaso, M., Paoli, L., Loppi, S., 2020. Effects of wood distillate (pyrolygneous acid) on sensitive bioindicators (lichen and moss). *Ecotoxicology and Environmental Safety* 204, 111117. <https://doi.org/10.1016/j.ecoenv.2020.111117>
- Farajzadeh, M.A., Khorram, P., Ghorbanpour, H., 2015. Simultaneous derivatization and solid-based disperser liquid–liquid microextraction for extraction and preconcentration of some antidepressants and an antiarrhythmic agent in urine and plasma samples followed by GC-FID. *Journal of Chromatography B* 983–984, 55–61. <https://doi.org/10.1016/j.jchromb.2015.01.004>
- Fellet, G., Pošćić, F., Lilen, S., Marchiol, L., Musetti, R., Tolio, A., Barbieri, P., Zerbi, G., 2016. PAHs accumulation on leaves of six evergreen urban shrubs: A field experiment. *Atmospheric Pollution Research* 7, 915–924. <https://doi.org/10.1016/j.apr.2016.05.007>
- Fernández-Rodríguez, M., Arrebola, J.P., Artacho-Cordón, F., Amaya, E., Aragones, N., Llorca, J., Perez-Gomez, B., Ardanaz, E., Kogevinas, M., Castano-Vinyals, G., Pollan, M., Olea, N., 2015. Levels and predictors of persistent organic pollutants in an adult population from four Spanish regions. *Science of The Total Environment* 538, 152–161. <https://doi.org/10.1016/j.scitotenv.2015.07.162>
- Ferreira, J.A., Ferreira, J.M.S., Talamini, V., Facco, J. de F., Rizzetti, T.M., Prestes, O.D., Adaime, M.B., Zanella, R., Bottoli, C.B.G., 2016. Determination of pesticides in coconut (*Cocos nucifera* Linn.) water and pulp using modified QuEChERS and LC–MS/MS. *Food Chemistry* 213, 616–624. <https://doi.org/10.1016/j.foodchem.2016.06.114>
- Ferreira, S., Bottini, R., Fontana, A., 2021. Tandem absorbance and fluorescence detection following liquid chromatography for the profiling of multiclass phenolic compounds in different winemaking products. *Food Chemistry* 338, 128030. <https://doi.org/10.1016/j.foodchem.2020.128030>
- Filipiak, W., Bojko, B., 2019. SPME in clinical, pharmaceutical, and biotechnological research – How far are we from daily practice? *TrAC Trends in Analytical Chemistry* 115, 203–213. <https://doi.org/10.1016/j.trac.2019.02.029>
- Foan, L., Simon, V., 2012. Optimization of pressurized liquid extraction using a multivariate chemometric approach and comparison of solid-phase extraction cleanup steps for the determination of polycyclic aromatic hydrocarbons in mosses. *Journal of Chromatography A* 1256, 22–31. <https://doi.org/10.1016/j.chroma.2012.07.065>
- Francisco, A.P., Nardocci, A.C., Tominaga, M.Y., da Silva, C.R., de Assunção, J.V., 2017. Spatial and seasonal trends of polychlorinated dioxins, furans and dioxin-like polychlorinated biphenyls in air using passive and active samplers and inhalation risk assessment. *Atmospheric Pollution Research* 8, 979–987. <https://doi.org/10.1016/j.apr.2017.03.007>
- Fromme, H., Hilger, B., Albrecht, M., Gries, W., Leng, G., Völkel, W., 2016. Occurrence of chlorinated and brominated dioxins/furans, PCBs, and brominated flame retardants in blood of German adults. *International Journal of Hygiene and Environmental Health* 219, 380–388. <https://doi.org/10.1016/j.ijheh.2016.03.003>
- Gallego-Cartagena, E., Morillas, H., Carrero, J.A., Madariaga, J.M., Maguregui, M., 2021. Naturally growing grimmiaecae family mosses as passive biomonitors of heavy metals pollution in urban-industrial atmospheres from the Bilbao Metropolitan area. *Chemosphere* 263, 128190. <https://doi.org/10.1016/j.chemosphere.2020.128190>

- Gallo, L., Corapi, A., Apollaro, C., Vespasiano, G., Lucadamo, L., 2017. Effect of the interaction between transplants of the epiphytic lichen *Pseudevernia furfuracea* L. (Zopf) and rainfall on the variation of element concentrations associated with the water-soluble part of atmospheric depositions. *Atmospheric Pollution Research* 8, 912–920. <https://doi.org/10.1016/j.apr.2017.03.002>
- Garballo-Rubio, A., Soto-Chinchilla, J., Martín-Pozo, L., Zafra-Gómez, A., 2020. Use of Quick, Easy, Cheap, Effective, Rugged & Safe (QuEChERS) and molecular imprinted polymer followed by gas chromatography with tandem mass spectrometry for the quantitative analysis of polycyclic aromatic hydrocarbons (PAH4) in complex health supplements. *Journal of Food Composition and Analysis* 93, 103588. <https://doi.org/10.1016/j.jfca.2020.103588>
- Garbuzov, M., Schürch, R., Ratnieks, F.L.W., 2015. Eating locally: dance decoding demonstrates that urban honey bees in Brighton, UK, forage mainly in the surrounding urban area. *Urban Ecosyst* 18, 411–418. <https://doi.org/10.1007/s11252-014-0403-y>
- Garcia, C.V., Gotah, A., 2017. Application of QuEChERS for Determining Xenobiotics in Foods of Animal Origin. *J Anal Methods Chem* 2017. <https://doi.org/10.1155/2017/2603067>
- García-Villarino, M., Riaño-Galán, I., Rodríguez-Dehli, A.C., Vizcaíno, E., Grimalt, J.O., Tardón, A., Fernández-Somoano, A., 2018. Prenatal Exposure to Persistent Organic Pollutants and Anogenital Distance in Children at 18 Months. *Horm Res Paediatr* 90, 116–122. <https://doi.org/10.1159/000492236>
- Gascon, M., Sunyer, J., Martínez, D., Guerra, S., Lavi, I., Torrent, M., Vrijheid, M., 2014. Persistent organic pollutants and children's respiratory health: The role of cytokines and inflammatory biomarkers. *Environment International* 69, 133–140. <https://doi.org/10.1016/j.envint.2014.04.021>
- Gbeddy, G., Egodawatta, P., Goonetilleke, A., Ayoko, G., Jayarathne, A., Chen, L., Russell, S., 2020. Optimized simultaneous pressurized fluid extraction and in-cell clean-up, and analysis of polycyclic aromatic hydrocarbons (PAHs), and nitro-, carbonyl-, hydroxy-PAHs in solid particles. *Analytica Chimica Acta* 1125, 19–28. <https://doi.org/10.1016/j.aca.2020.05.021>
- Gheorghe, I.F., Ion, B., 2011. The Effects of Air Pollutants on Vegetation and the Role of Vegetation in Reducing Atmospheric Pollution. The Impact of Air Pollution on Health, Economy, Environment and Agricultural Sources. <https://doi.org/10.5772/17660>
- Ghorani-Azam, A., Riahi-Zanjani, B., Balali-Mood, M., 2016. Effects of air pollution on human health and practical measures for prevention in Iran. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences* 21, 65. <https://doi.org/10.4103/1735-1995.189646>
- Ghosal, D., Ghosh, S., Dutta, T.K., Ahn, Y., 2016. Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. *Front Microbiol* 7. <https://doi.org/10.3389/fmicb.2016.01369>
- Ghosh, U., Driscoll, S.K., Burgess, R.M., Jonker, M.T., Reible, D., Gobas, F., Choi, Y., Apitz, S.E., Maruya, K.A., Gala, W.R., Mortimer, M., Beegan, C., 2014. Passive sampling methods for contaminated sediments: Practical guidance for selection, calibration, and implementation. *Integrated Environmental Assessment and Management* 10, 210–223. <https://doi.org/10.1002/ieam.1507>
- Giampaoli, P., Wannaz, E.D., Tavares, A.R., Domingos, M., 2016. Suitability of *Tillandsia usneoides* and *Aechmea fasciata* for biomonitoring toxic elements under tropical seasonal climate. *Chemosphere* 149, 14–23. <https://doi.org/10.1016/j.chemosphere.2016.01.080>
- Gill, H.K., Garg, H., 2014. Pesticides: Environmental Impacts and Management Strategies. *Pesticides - Toxic Aspects*. <https://doi.org/10.5772/57399>
- Gionfriddo, E., Souza-Silva, É.A., Ho, T.D., Anderson, J.L., Pawliszyn, J., 2018. Exploiting the tunable selectivity features of polymeric ionic liquid-based SPME sorbents in food analysis. *Talanta* 188, 522–530. <https://doi.org/10.1016/j.talanta.2018.06.011>
- Girard, L., Reix, N., Mathelin, C., 2020. Impact des pesticides perturbateurs endocriniens sur le cancer du sein. *Gynécologie Obstétrique Fertilité & Sénologie* 48, 187–195. <https://doi.org/10.1016/j.gofs.2019.10.008>
- Girones, L., Arias, A.H., Oliva, A.L., Recabarren-Villalon, T., Marcovecchio, J.E., 2020. Occurrence and spatial distribution of organochlorine pesticides in the southwest Buenos Aires using the freshwater snail *Chilina parchappii* as environmental biomonitor. *Regional Studies in Marine Science* 33, 100898. <https://doi.org/10.1016/j.rsma.2019.100898>
- Golge, O., Hepsag, F., Kabak, B., 2018. Health risk assessment of selected pesticide residues in green pepper and cucumber. *Food and Chemical Toxicology* 121, 51–64. <https://doi.org/10.1016/j.fct.2018.08.027>
- Gómez-Almenar, M.C., García-Mesa, J.A., 2015. Determination of pesticide residues in olives by liquid extraction surface analysis followed by liquid chromatography/tandem mass spectrometry. *Grasas y Aceites* 66, 078. <https://doi.org/10.3989/gya.0828142>
- Gomot de Vaufléury, A., Pihan, F., 2000. Growing snails used as sentinels to evaluate terrestrial environment contamination by trace elements. *Chemosphere* 40, 275–284. [https://doi.org/10.1016/S0045-6535\(99\)00246-5](https://doi.org/10.1016/S0045-6535(99)00246-5)
- Gong, J., Zhu, T., Kipen, H., Rich, D.Q., Huang, W., Lin, W.-T., Hu, M., Zhang, J. (Jim), 2015. Urinary polycyclic aromatic hydrocarbon metabolites as biomarkers of exposure to traffic-emitted pollutants. *Environment International* 85, 104–110. <https://doi.org/10.1016/j.envint.2015.09.003>
- Goretti, E., Pallottini, M., Rossi, R., La Porta, G., Gardi, T., Cenci Goga, B.T., Elia, A.C., Galletti, M., Moroni, B., Petroselli, C., Selvaggi, R., Cappelletti, D., 2020. Heavy metal bioaccumulation in honey bee matrix, an indicator to assess the contamination level in terrestrial environments. *Environmental Pollution* 256, 113388. <https://doi.org/10.1016/j.envpol.2019.113388>
- Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science (New York, N.Y.)* 347, 1255957. <https://doi.org/10.1126/science.1255957>
- Goulson, D., Thompson, J., Croombs, A., 2018. Rapid rise in toxic load for bees revealed by analysis of pesticide use in Great Britain. *PeerJ* 6. <https://doi.org/10.7717/peerj.5255>
- Govaerts, A., Verhaert, V., Covaci, A., Jaspers, V.L.B., Berg, O.K., Addo-Bediako, A., Jooste, A., Bervoets, L., 2018. Distribution and bioaccumulation of POPs and mercury in the Ga-Selati River (South Africa) and the rivers Gudbrandsdalslågen and Rena (Norway). *Environment International* 121, 1319–1330. <https://doi.org/10.1016/j.envint.2018.10.058>
- Govarts, E., Iszatt, N., Trnovec, T., de Cock, M., Eggesbø, M., Palkovicova Murinova, L., van de Bor, M., Guxens, M., Chevrier, C., Koppen, G., Lamoree, M., Hertz-Picciotto, I., Lopez-Espinosa, M.-J., Lertxundi, A., Grimalt, J.O., Torrent, M., Goñi-Irigoyen, F., Vermeulen, R., Legler, J., Schoeters, G., 2018. Prenatal exposure to endocrine disrupting chemicals and risk of being born small for gestational age: Pooled analysis of seven European birth cohorts. *Environment International* 115, 267–278. <https://doi.org/10.1016/j.envint.2018.03.017>
- Grandy, J.J., Lashgari, M., Heide, H.V., Poole, J., Pawliszyn, J., 2019. Introducing a mechanically robust SPME sampler for the on-site sampling and extraction of a wide range of untargeted pollutants in environmental waters. *Environmental Pollution* 252, 825–834. <https://doi.org/10.1016/j.envpol.2019.06.013>
- Habibullah-Al-Mamun, Md., Kawser Ahmed, Md., Saiful Islam, Md., Tokumura, M., Masunaga, S., 2019. Occurrence, distribution and possible sources of polychlorinated biphenyls (PCBs) in the surface water from the Bay of Bengal coast of Bangladesh. *Ecotoxicology and Environmental Safety* 167, 450–458. <https://doi.org/10.1016/j.ecoenv.2018.10.052>

- Haddad, N., Kanj, S.S., Awad, L.S., Abdallah, D.I., Moghnieh, R.A., 2019. The 2018 Lebanese Society of Infectious Diseases and Clinical Microbiology Guidelines for the use of antimicrobial therapy in complicated intra-abdominal infections in the era of antimicrobial resistance. *BMC Infect Dis* 19. <https://doi.org/10.1186/s12879-019-3829-2>
- Hamanaka, R.B., Mutlu, G.M., 2018. Particulate Matter Air Pollution: Effects on the Cardiovascular System. *Front Endocrinol (Lausanne)* 9. <https://doi.org/10.3389/fendo.2018.00680>
- Han, X., Meng, L., Li, Y., Li, A., Turyk, M.E., Yang, R., Wang, P., Xiao, K., Zhao, J., Zhang, J., Zhang, Q., Jiang, G., 2020. Associations between the exposure to persistent organic pollutants and type 2 diabetes in East China: A case-control study. *Chemosphere* 241, 125030. <https://doi.org/10.1016/j.chemosphere.2019.125030>
- Han, Y., Song, L., Zou, N., Chen, R., Qin, Y., Pan, C., 2016. Multi-residue determination of 171 pesticides in cowpea using modified QuEChERS method with multi-walled carbon nanotubes as reversed-dispersive solid-phase extraction materials. *Journal of Chromatography B* 1031, 99–108. <https://doi.org/10.1016/j.jchromb.2016.07.043>
- Han, Y.M., Bandowe, B.A.M., Wei, C., Cao, J.J., Wilcke, W., Wang, G.H., Ni, H.Y., Jin, Z.D., An, Z.S., Yan, B.Z., 2015. Stronger association of polycyclic aromatic hydrocarbons with soot than with char in soils and sediments. *Chemosphere* 119, 1335–1345. <https://doi.org/10.1016/j.chemosphere.2014.02.021>
- Harris, K.J., Subbiah, S., Tabatabai, M., Archibong, A.E., Singh, K.P., Anderson, T.A., Adunyah, S.E., Ramesh, A., 2020. Pressurized liquid extraction followed by liquid chromatography coupled to a fluorescence detector and atmospheric pressure chemical ionization mass spectrometry for the determination of benzo(a)pyrene metabolites in liver tissue of an animal model of colon cancer. *Journal of Chromatography A* 1622, 461126. <https://doi.org/10.1016/j.chroma.2020.461126>
- Harris, K.L., Myers, J.N., Ramesh, A., 2013. Benzo(a)pyrene modulates fluoranthene-induced cellular responses in HT-29 colon cells in a dual exposure system. *Environmental Toxicology and Pharmacology* 36, 358–367. <https://doi.org/10.1016/j.etap.2013.04.017>
- Harwood, G.P., Dolezal, A.G., 2020. Pesticide–Virus Interactions in Honey Bees: Challenges and Opportunities for Understanding Drivers of Bee Declines. *Viruses* 12. <https://doi.org/10.3390/v12050566>
- Hayward, S.J., Lei, Y.D., Wania, F., 2011. Sorption of a diverse set of organic chemical vapors onto XAD-2 resin: Measurement, prediction and implications for air sampling. *Atmospheric Environment* 45, 296–302. <https://doi.org/10.1016/j.atmosenv.2010.10.028>
- He, Z., Wang, L., Peng, Y., Luo, M., Wang, W., Liu, X., 2015. Multiresidue analysis of over 200 pesticides in cereals using a QuEChERS and gas chromatography–tandem mass spectrometry-based method. *Food Chemistry* 169, 372–380. <https://doi.org/10.1016/j.foodchem.2014.07.102>
- Helou, K., Harmouche-Karaki, M., Karake, S., Narbonne, J.-F., 2019. A review of organochlorine pesticides and polychlorinated biphenyls in Lebanon: Environmental and human contaminants. *Chemosphere* 231, 357–368. <https://doi.org/10.1016/j.chemosphere.2019.05.109>
- Hercegová, A., Dömötöróvá, M., Matisová, E., 2007. Sample preparation methods in the analysis of pesticide residues in baby food with subsequent chromatographic determination. *Journal of Chromatography A* 1153, 54–73. <https://doi.org/10.1016/j.chroma.2007.01.008>
- Heub, S., Tscherner, N., Kehl, F., Dittrich, P.S., Follonier, S., Barbe, L., 2016. A Simple Method for Automated Solid Phase Extraction of Water Samples for Immunological Analysis of Small Pollutants. *J Vis Exp*. <https://doi.org/10.3791/53438>
- Hoooven, L.A., Chakrabarti, P., Harper, B.J., Sagili, R.R., Harper, S.L., 2019. Potential Risk to Pollinators from Nanotechnology-Based Pesticides. *Molecules* 24. <https://doi.org/10.3390/molecules24244458>
- Hou, X., Xu, X., Xu, X., Xiaoying, Han, M., Qiu, S., 2020. Application of a multiclass screening method for veterinary drugs and pesticides using HPLC-QTOF-MS in egg samples. *Food Chemistry* 309, 125746. <https://doi.org/10.1016/j.foodchem.2019.125746>
- Imbeault, P., Ravanelli, N., Chevrier, J., 2018. Can POPs be substantially popped out through sweat? *Environment International* 111, 131–132. <https://doi.org/10.1016/j.envint.2017.11.023>
- Iqbal, S., Iqbal, M.M., Javed, M., Bahadur, A., Yasien, S., Najam-ud-din, Hurr, A., Ahmad, N., Raheel, M., Liu, G., 2020. Modified QuEChERS extraction method followed by simultaneous quantitation of nine multi-class pesticides in human blood and urine by using GC-MS. *Journal of Chromatography B* 1152, 122227. <https://doi.org/10.1016/j.jchromb.2020.122227>
- Islam, A.K.M.M., Lee, H.-S., Ro, J.-H., Kim, D., Kwon, H., 2019. Application of high-surface-area graphitized carbon black with primary secondary amine as an alternative quick, easy, cheap, effective, rugged, and safe cleanup material for pesticide multi-residue analysis in spinach. *Journal of Separation Science* 42, 2379–2389. <https://doi.org/10.1002/jssc.201900066>
- Islam, R., Kumar, S., Karmaker, J., Kamruzzaman, Md., Rahman, Md.A., Biswas, N., Tran, T.K.A., Rahman, M.M., 2018. Bioaccumulation and adverse effects of persistent organic pollutants (POPs) on ecosystems and human exposure: A review study on Bangladesh perspectives. *Environmental Technology & Innovation* 12, 115–131. <https://doi.org/10.1016/j.eti.2018.08.002>
- Ismael, M.A., Elyamine, A.M., Moussa, M.G., Cai, M., Zhao, X., Hu, C., 2019. Cadmium in plants: uptake, toxicity, and its interactions with selenium fertilizers. *Metallomics* 11, 255–277. <https://doi.org/10.1039/C8MT00247A>
- Issa, M.M., M. Taha, S., El- Marsafy, A.M., Khalil, M.M.H., Ismail, E.H., 2020. Acetonitrile-Ethyl acetate based method for the residue analysis of 373 pesticides in beeswax using LC-MS/MS and GC-MS/MS. *Journal of Chromatography B* 1145, 122106. <https://doi.org/10.1016/j.jchromb.2020.122106>
- Iwegbue, C.M.A., Eyengho, S.B., Egobueze, F.E., Odali, E.W., Tesi, G.O., Nwajeri, G.E., Martincigh, B.S., 2019. Polybrominated diphenyl ethers and polychlorinated biphenyls in indoor dust from electronic repair workshops in southern Nigeria: Implications for onsite human exposure. *Science of The Total Environment* 671, 914–927. <https://doi.org/10.1016/j.scitotenv.2019.02.449>
- Iwegbue, C.M.A., Osijaye, K.O., Igbuku, U.A., Egobueze, F.E., Tesi, G.O., Basse, F.I., Martincigh, B.S., 2020. Effect of the number of frying cycles on the composition, concentrations and risk of polycyclic aromatic hydrocarbons (PAHs) in vegetable oils and fried fish. *Journal of Food Composition and Analysis* 94, 103633. <https://doi.org/10.1016/j.jfca.2020.103633>
- Jabali, Y., Millet, M., El-Hoz, M., 2019. Optimization of a DI-SPME-GC-MS/MS method for multi-residue analysis of pesticides in waters. *Microchemical Journal* 147, 83–92. <https://doi.org/10.1016/j.microc.2019.03.004>
- Jayaraj, R., Megha, P., Sreedev, P., 2016. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdiscip Toxicol* 9, 90–100. <https://doi.org/10.1515/intox-2016-0012>
- Ji, X., Abakumov, E., Polyako, V., Xie, X., Dongyang, W., 2019. The ecological impact of mineral exploitation in the Russian Arctic: A field-scale study of polycyclic aromatic hydrocarbons (PAHs) in permafrost-affected soils and lichens of the Yamal-Nenets autonomous region. *Environmental Pollution* 255, 113239. <https://doi.org/10.1016/j.envpol.2019.113239>
- Jia, H., Zhao, J., Li, L., Li, X., Wang, C., 2014. Transformation of polycyclic aromatic hydrocarbons (PAHs) on Fe(III)-modified clay minerals: Role of molecular chemistry and clay surface properties. *Applied Catalysis B: Environmental* 154–155, 238–245. <https://doi.org/10.1016/j.apcatb.2014.02.022>

- Jia, Z.-Q., Zhang, Y.-C., Huang, Q.-T., Jones, A.K., Han, Z.-J., Zhao, C.-Q., 2020. Acute toxicity, bioconcentration, elimination, action mode and detoxification metabolism of broflanilide in zebrafish, *Danio rerio*. *Journal of Hazardous Materials* 394, 122521. <https://doi.org/10.1016/j.jhazmat.2020.122521>
- Juodis, L., Filistovič, V., Maceika, E., Remeikis, V., 2016. Analytical dispersion model for the chain of primary and secondary air pollutants released from point source. *Atmospheric Environment* 128, 216–226. <https://doi.org/10.1016/j.atmosenv.2015.12.019>
- Kadziński, L., Banasiuk, R., Banecki, B., 2018. Determination of ten sulfonamides in honey using tetrahydrofuran Salting Out Liquid Liquid Extraction and monolithic silica column. *LWT* 96, 7–12. <https://doi.org/10.1016/j.lwt.2018.05.007>
- Kagaya, S., Katoh, T., Saito, M., Ohki, M., Shirota, R., Saeki, Y., Kajiura, T., Nakada, S., Miyazaki, H., Gemmei-Ide, M., Inoue, Y., 2018. A porous sintered material consisting of Presep PolyChelate as a chelating resin and particulate polyethylene as a thermoplastic binder for solid-phase extraction of trace elements. *Talanta* 188, 665–670. <https://doi.org/10.1016/j.talanta.2018.06.024>
- Kailemia, M.J., Ruhaak, L.R., Lebrilla, C.B., Amster, I.J., 2014. Oligosaccharide Analysis By Mass Spectrometry: A Review Of Recent Developments. *Anal Chem* 86, 196–212. <https://doi.org/10.1021/ac403969n>
- Karášková, P., Codling, G., Melymuk, L., Klánová, J., 2018. A critical assessment of passive air samplers for per- and polyfluoroalkyl substances. *Atmospheric Environment* 185, 186–195. <https://doi.org/10.1016/j.atmosenv.2018.05.030>
- Kargar, N., Matin, G., Matin, A.A., Buyukisik, H.B., 2017. Biomonitoring, status and source risk assessment of polycyclic aromatic hydrocarbons (PAHs) using honeybees, pine tree leaves, and propolis. *Chemosphere* 186, 140–150. <https://doi.org/10.1016/j.chemosphere.2017.07.127>
- Kellogg, J.J., Wallace, E.D., Graf, T.N., Oberlies, N.H., Cech, N.B., 2017. Conventional and accelerated-solvent extractions of green tea (*Camellia sinensis*) for metabolomics-based chemometrics. *Journal of Pharmaceutical and Biomedical Analysis* 145, 604–610. <https://doi.org/10.1016/j.jpba.2017.07.027>
- Kelly, F.J., Fussell, J.C., 2015. Air pollution and public health: emerging hazards and improved understanding of risk. *Environ Geochem Health* 37, 631–649. <https://doi.org/10.1007/s10653-015-9720-1>
- Kemmerich, M., Demarco, M., Bernardi, G., Prestes, O.D., Adaime, M.B., Zanella, R., 2020. Balls-in-tube matrix solid phase dispersion (BiT-MSPD): An innovative and simplified technique for multiresidue determination of pesticides in fruit samples. *Journal of Chromatography A* 1612, 460640. <https://doi.org/10.1016/j.chroma.2019.460640>
- Kim, L., Lee, D., Cho, H.-K., Choi, S.-D., 2019. Review of the QuEChERS method for the analysis of organic pollutants: Persistent organic pollutants, polycyclic aromatic hydrocarbons, and pharmaceuticals. *Trends in Environmental Analytical Chemistry* 22, e00063. <https://doi.org/10.1016/j.teac.2019.e00063>
- Kinross, A.D., Hageman, K.J., Doucette, W.J., Foster, A.L., 2020. Comparison of Accelerated Solvent Extraction (ASE) and Energized Dispersive Guided Extraction (EDGE) for the analysis of pesticides in leaves. *Journal of Chromatography A* 1627, 461414. <https://doi.org/10.1016/j.chroma.2020.461414>
- Kodnik, D., Candotto Carniel, F., Lichen, S., Tolloi, A., Barbieri, P., Tretiach, M., 2015. Seasonal variations of PAHs content and distribution patterns in a mixed land use area: A case study in NE Italy with the transplanted lichen *Pseudevernia furfuracea*. *Atmospheric Environment* 113, 255–263. <https://doi.org/10.1016/j.atmosenv.2015.04.067>
- Kopittke, P.M., Menzies, N.W., Wang, P., McKenna, B.A., Lombi, E., 2019. Soil and the intensification of agriculture for global food security. *Environment International* 132, 105078. <https://doi.org/10.1016/j.envint.2019.105078>
- Kosior, G., Přibylková, P., Vaňková, L., Kukučka, P., Audy, O., Klánová, J., Samecka-Cymerman, A., Mróz, L., Kempers, A.J., 2017. Bioindication of PBDEs and PCBs by native and transplanted moss *Pleurozium schreberi*. *Ecotoxicology and Environmental Safety* 143, 136–142. <https://doi.org/10.1016/j.ecoenv.2017.05.025>
- Krupnova, T.G., Mashkova, I.V., Kostyukova, A.M., Schelkanova, E.E., Gavrilkina, S.V., 2018. Gastropods as potential biomonitors of contamination caused by heavy metals in South Ural lakes, Russia. *Ecological Indicators* 95, 1001–1007. <https://doi.org/10.1016/j.ecolind.2017.12.005>
- La Merrill, M., Emond, C., Kim, M.J., Antignac, J.-P., Le Bizec, B., Clément, K., Birnbaum, L.S., Barouki, R., 2013. Toxicological Function of Adipose Tissue: Focus on Persistent Organic Pollutants. *Environ Health Perspect* 121, 162–169. <https://doi.org/10.1289/ehp.1205485>
- Lai, F.Y., Raurert, C., Gobelius, L., Ahrens, L., 2019. A critical review on passive sampling in air and water for per- and polyfluoroalkyl substances (PFASs). *TrAC Trends in Analytical Chemistry* 121, 115311. <https://doi.org/10.1016/j.trac.2018.11.009>
- Larson, J.L., Redmond, C.T., Potter, D.A., 2015. Mowing mitigates bioactivity of neonicotinoid insecticides in nectar of flowering lawn weeds and turfgrass guttation. *Environmental Toxicology and Chemistry* 34, 127–132. <https://doi.org/10.1002/etc.2768>
- Lavin, K.S., Hageman, K.J., 2012. Selective pressurised liquid extraction of halogenated pesticides and polychlorinated biphenyls from pine needles. *Journal of Chromatography A* 1258, 30–36. <https://doi.org/10.1016/j.chroma.2012.08.042>
- Lazo, P., Stafilov, T., Qarri, F., Allajbeu, S., Bekteshi, L., Frontasyeva, M., Harmens, H., 2019. Spatial distribution and temporal trend of airborne trace metal deposition in Albania studied by moss biomonitoring. *Ecological Indicators* 101, 1007–1017. <https://doi.org/10.1016/j.ecolind.2018.11.053>
- Lee, E.G., Magrm, R., Kusti, M., Kashon, M.L., Guffey, S., Costas, M.M., Boykin, C.J., Harper, M., 2017. Comparison between active (pumped) and passive (diffusive) sampling methods for formaldehyde in pathology and histology laboratories. *J Occup Environ Hyg* 14, 31–39. <https://doi.org/10.1080/15459624.2016.1211284>
- Lee, J., Jeong, J.-H., Park, S., Lee, K.-G., 2018. Monitoring and risk assessment of polycyclic aromatic hydrocarbons (PAHs) in processed foods and their raw materials. *Food Control* 92, 286–292. <https://doi.org/10.1016/j.foodcont.2018.05.012>
- Lee, J.E., Oh, H.B., Im, H., Han, S.B., Kim, K.H., 2020. Multiresidue analysis of 85 persistent organic pollutants in small human serum samples by modified QuEChERS preparation with different ionization sources in mass spectrometry. *Journal of Chromatography A* 1623, 461170. <https://doi.org/10.1016/j.chroma.2020.461170>
- Lee, K.K., Miller, M.R., Shah, A.S.V., 2018. Air Pollution and Stroke. *J Stroke* 20, 2–11. <https://doi.org/10.5853/jos.2017.02894>
- Lee, Y.-J., Rahman, Md.M., Abd El-Aty, A.M., Choi, J.-H., Chung, H.S., Kim, S.-W., Abdel-Aty, A.M., Shin, H.-C., Shim, J.-H., 2016. Detection of three herbicide, and one metabolite, residues in brown rice and rice straw using various versions of the QuEChERS method and liquid chromatography-tandem mass spectrometry. *Food Chemistry* 210, 442–450. <https://doi.org/10.1016/j.foodchem.2016.05.005>
- Lee, Y.-M., Jacobs Jr., D.R., Lee, D.-H., 2018. Persistent Organic Pollutants and Type 2 Diabetes: A Critical Review of Review Articles. *Front Endocrinol (Lausanne)* 9. <https://doi.org/10.3389/fendo.2018.00712>
- Lee, Y.-M., Kim, K.-S., Kim, S.-A., Hong, N.-S., Lee, S.-J., Lee, D.-H., 2014. Prospective associations between persistent organic pollutants and metabolic syndrome: A nested case-control study. *Science of The Total Environment* 496, 219–225. <https://doi.org/10.1016/j.scitotenv.2014.07.039>

- Leech, C., Tighe, M.K., Pereg, L., Winter, G., McMillan, M., Esmaeili, A., Wilson, S.C., 2020. Bioaccessibility constrains the co-composting bioremediation of field aged PAH contaminated soils. *International Biodeterioration & Biodegradation* 149, 104922. <https://doi.org/10.1016/j.ibiod.2020.104922>
- Lehotay, S.J., Son, K.A., Kwon, H., Koesukwiwat, U., Fu, W., Mastovska, K., Hoh, E., Leepipatpiboon, N., 2010. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *Journal of Chromatography A* 1217, 2548–2560. <https://doi.org/10.1016/j.chroma.2010.01.044>
- Lelieveld, J., Haines, A., Pozzer, A., 2018. Age-dependent health risk from ambient air pollution: a modelling and data analysis of childhood mortality in middle-income and low-income countries. *The Lancet Planetary Health* 2, e292–e300. [https://doi.org/10.1016/S2542-5196\(18\)30147-5](https://doi.org/10.1016/S2542-5196(18)30147-5)
- Lentola, A., David, A., Abdul-Sada, A., Tapparo, A., Goulson, D., Hill, E.M., 2017. Ornamental plants on sale to the public are a significant source of pesticide residues with implications for the health of pollinating insects. *Environmental Pollution* 228, 297–304. <https://doi.org/10.1016/j.envpol.2017.03.084>
- Les pesticides dans le bassin de la Seine | PIREN-Seine [WWW Document], n.d. URL <https://www.piren-seine.fr/fr/fascicules/les-pesticides-dans-le-bassin-de-la-seine> (accessed 10.5.20).
- Li, W., Zhang, Z., Zhang, R., Jiao, H., Sun, A., Shi, X., Chen, J., 2020. Effective removal matrix interferences by a modified QuEChERS based on the molecularly imprinted polymers for determination of 84 polychlorinated biphenyls and organochlorine pesticides in shellfish samples. *Journal of Hazardous Materials* 384, 121241. <https://doi.org/10.1016/j.jhazmat.2019.121241>
- Li, X., Dong, S., Wang, P., Su, X., Fu, J., 2019. Polychlorinated biphenyls are still alarming persistent organic pollutants in marine-origin animal feed (fishmeal). *Chemosphere* 233, 355–362. <https://doi.org/10.1016/j.chemosphere.2019.05.250>
- Li, Y., Fang, R., Liu, Z., Jiang, L., Zhang, J., Li, H., Liu, C., Li, F., 2021. The association between toxic pesticide environmental exposure and Alzheimer's disease: A scientometric and visualization analysis. *Chemosphere* 263, 128238. <https://doi.org/10.1016/j.chemosphere.2020.128238>
- Li, Y., Lohmann, R., Zou, X., Wang, C., Zhang, L., 2020. Air-water exchange and distribution pattern of organochlorine pesticides in the atmosphere and surface water of the open Pacific ocean. *Environmental Pollution* 265, 114956. <https://doi.org/10.1016/j.envpol.2020.114956>
- Liang, Y., Liu, D., Zhan, J., Luo, M., Han, J., Wang, P., Zhou, Z., 2020. New insight into the mechanism of POP-induced obesity: Evidence from DDE-altered microbiota. *Chemosphere* 244, 125123. <https://doi.org/10.1016/j.chemosphere.2019.125123>
- Liao, Z., Xie, J., Fang, X., Wang, Y., Zhang, Y., Xu, X., Fan, S., 2020. Modulation of synoptic circulation to dry season PM_{2.5} pollution over the Pearl River Delta region: An investigation based on self-organizing maps. *Atmospheric Environment* 230, 117482. <https://doi.org/10.1016/j.atmosenv.2020.117482>
- Lim, C.C., Hayes, R.B., Ahn, J., Shao, Y., Silverman, D.T., Jones, R.R., Garcia, C., Thurston, G.D., 2018. Association between long-term exposure to ambient air pollution and diabetes mortality in the US. *Environ Res* 165, 330–336. <https://doi.org/10.1016/j.envres.2018.04.011>
- Lind, P.M., Penell, J., Salihovic, S., van Bavel, B., Lind, L., 2014. Circulating levels of p,p'-DDE are related to prevalent hypertension in the elderly. *Environmental Research* 129, 27–31. <https://doi.org/10.1016/j.envres.2013.12.003>
- Lind, P.M., Salihovic, S., Stubleski, J., Kärrman, A., Lind, L., 2019. Association of Exposure to Persistent Organic Pollutants With Mortality Risk. *JAMA Netw Open* 2. <https://doi.org/10.1001/jamanetworkopen.2019.3070>
- Liu, H.-Y., Lin, S.-L., Fuh, M.-R., 2016. Determination of chloramphenicol, thiamphenicol and florfenicol in milk and honey using modified QuEChERS extraction coupled with polymeric monolith-based capillary liquid chromatography tandem mass spectrometry. *Talanta* 150, 233–239. <https://doi.org/10.1016/j.talanta.2015.12.045>
- Loos, G., Van Schepdael, A., Cabooter, D., 2016. Quantitative mass spectrometry methods for pharmaceutical analysis. *Philos Trans A Math Phys Eng Sci* 374. <https://doi.org/10.1098/rsta.2015.0366>
- Loos, M., Krauss, M., Fenner, K., 2012. Pesticide nonextractable residue formation in soil: insights from inverse modeling of degradation time series. *Environmental Science & Technology* 46, 9830–9837. <https://doi.org/10.1021/es300505r>
- Louzon, M., Zahn, S., Capelli, N., Massemin, S., Coeurdassier, M., Pauget, B., Gimbert, F., de Vaulfleur, A., 2020. Impact of ageing and soil contaminants on telomere length in the land snail. *Ecotoxicology and Environmental Safety* 201, 110766. <https://doi.org/10.1016/j.ecoenv.2020.110766>
- Louzon, M., Pauget, B., Pelfrène, A., Gimbert, F., de Vaulfleur, A., 2020. Combining human and snail indicators for an integrative risk assessment of metal(loid)-contaminated soils. *Journal of Hazardous Materials* 124182. <https://doi.org/10.1016/j.jhazmat.2020.124182>
- Louppis, A.P., Karabagias, I.K., Kontakos, S., Kontominas, M.G., Papastefanou, C., 2017. Botanical discrimination of Greek unifloral honeys based on mineral content in combination with physicochemical parameter analysis, using a validated chemometric approach. *Microchemical Journal* 135, 180–189. <https://doi.org/10.1016/j.microc.2017.09.004>
- Lucci, P., Pacetti, D., Núñez, O., Frega, N.G., 2012. Current Trends in Sample Treatment Techniques for Environmental and Food Analysis. *Chromatography - The Most Versatile Method of Chemical Analysis*. <https://doi.org/10.5772/47736>
- Luo, Y., Sun, J., Wang, P., Li, Y., Li, H., Xiao, K., Yang, R., Zhang, Q., Jiang, G., 2020. Age dependence accumulation of organochlorine pesticides and PAHs in needles with different forest types, southeast Tibetan Plateau. *Science of The Total Environment* 716, 137176. <https://doi.org/10.1016/j.scitotenv.2020.137176>
- Lushchak, V.I., Matviishyn, T.M., Husak, V.V., Storey, J.M., Storey, K.B., 2018. Pesticide toxicity: a mechanistic approach. *EXCLI J* 17, 1101–1136. <https://doi.org/10.17179/excli2018-1710>
- Ly, T.-K., Ho, T.-D., Behra, P., Nhu-Trang, T.-T., 2020. Determination of 400 pesticide residues in green tea leaves by UPLC-MS/MS and GC-MS/MS combined with QuEChERS extraction and mixed-mode SPE clean-up method. *Food Chemistry* 326, 126928. <https://doi.org/10.1016/j.foodchem.2020.126928>
- Macedo-Miranda, G., Avila-Pérez, P., Gil-Vargas, P., Zarazúa, G., Sánchez-Meza, J.C., Zepeda-Gómez, C., Tejeda, S., 2016. Accumulation of heavy metals in mosses: a biomonitoring study. *Springerplus* 5. <https://doi.org/10.1186/s40064-016-2524-7>
- Madadzada, A.I., Badawy, W.M., Hajiyeve, S.R., Veliyeva, Z.T., Hajiyeve, O.B., Shvetsova, M.S., Frontasyeva, M.V., 2019. Assessment of atmospheric deposition of major and trace elements using neutron activation analysis and GIS technology: Baku - Azerbaijan. *Microchemical Journal* 147, 605–614. <https://doi.org/10.1016/j.microc.2019.03.061>
- Maddah, D., Ghach, W., Farraj, N.A., Yehya, M., Khatib, J.A., Alami, N.H., 2019. The first community-based intervention to promote safe pesticide use by developing knowledge, attitudes, and practices among Lebanese farmers. *Human and Ecological Risk Assessment: An International Journal* 0, 1–12. <https://doi.org/10.1080/10807039.2019.1688639>
- Madeira, P.J.A., Florêncio, M.H., 2012. Applications of Tandem Mass Spectrometry: From Structural Analysis to Fundamental Studies. *Tandem Mass Spectrometry - Applications and Principles*. <https://doi.org/10.5772/31736>

- Madej, K., Kalenik, T.K., Piekoszewski, W., 2018. Sample preparation and determination of pesticides in fat-containing foods. *Food Chemistry* 269, 527–541. <https://doi.org/10.1016/j.foodchem.2018.07.007>
- Mahmood, I., Imadi, S., Shazadi, K., Gul, A., Hakeem, K., 2015. Effects of Pesticides on Environment. https://doi.org/10.1007/978-3-319-27455-3_13
- Makoś, P., Fernandes, A., Przyjazny, A., Boczkaj, G., 2018. Sample preparation procedure using extraction and derivatization of carboxylic acids from aqueous samples by means of deep eutectic solvents for gas chromatographic-mass spectrometric analysis. *Journal of Chromatography A* 1555, 10–19. <https://doi.org/10.1016/j.chroma.2018.04.054>
- Malisch, R., Kotz, A., 2014. Dioxins and PCBs in feed and food — Review from European perspective. *Science of The Total Environment* 491–492, 2–10. <https://doi.org/10.1016/j.scitotenv.2014.03.022>
- Mandal, S.C., Mandal, V., Das, A.K., 2015. Chapter 6 - Classification of Extraction Methods, in: Mandal, S.C., Mandal, V., Das, A.K. (Eds.), *Essentials of Botanical Extraction*. Academic Press, Boston, pp. 83–136. <https://doi.org/10.1016/B978-0-12-802325-9.00006-9>
- Manesiotis, P., Fitzhenry, L., Theodoridis, G., Jandera, P., 2012. 4.20 - Applications of SPE-MIP in the Field of Food Analysis, in: Pawliszyn, J. (Ed.), *Comprehensive Sampling and Sample Preparation*. Academic Press, Oxford, pp. 457–471. <https://doi.org/10.1016/B978-0-12-381373-2.00144-7>
- Manisalidis, I., Stavropoulou, E., Stavropoulos, A., Bezirtzoglou, E., 2020. Environmental and Health Impacts of Air Pollution: A Review. *Front. Public Health* 8. <https://doi.org/10.3389/fpubh.2020.00014>
- Maragou, N., Pavlidis, G., Karasali, H., Hatjina, F., 2017. Major and minor element levels in Greek apicultural products. *Global Nest Journal* 19, 423–429.
- Marcelino, A.F., Wachtel, C.C., Ghisi, N. de C., 2019. Are Our Farm Workers in Danger? Genetic Damage in Farmers Exposed to Pesticides. *International Journal of Environmental Research and Public Health* 16, 358. <https://doi.org/10.3390/ijerph16030358>
- Martinefski, M., Feizi, N., Lunar, M.L., Rubio, S., 2019. Supramolecular solvent-based high-throughput sample treatment platform for the biomonitoring of PAH metabolites in urine by liquid chromatography-tandem mass spectrometry. *Chemosphere* 237, 124525. <https://doi.org/10.1016/j.chemosphere.2019.124525>
- Martinello, M., Borin, A., Stella, R., Bovo, D., Biancotto, G., Gallina, A., Mutinelli, F., 2017. Development and validation of a QuEChERS method coupled to liquid chromatography and high resolution mass spectrometry to determine pyrrolizidine and tropane alkaloids in honey. *Food Chemistry* 234, 295–302. <https://doi.org/10.1016/j.foodchem.2017.04.186>
- Massimi, L., Castellani, F., Protano, C., Conti, M.E., Antonucci, A., Frezzini, M.A., Galletti, M., Mele, G., Pileri, A., Ristorini, M., Vitali, M., Canepari, S., 2021. Lichen transplants for high spatial resolution biomonitoring of Persistent Organic Pollutants (POPs) in a multi-source polluted area of Central Italy. *Ecological Indicators* 120, 106921. <https://doi.org/10.1016/j.ecolind.2020.106921>
- Massimi, L., Conti, M.E., Mele, G., Ristorini, M., Astolfi, M.L., Canepari, S., 2019. Lichen transplants as indicators of atmospheric element concentrations: a high spatial resolution comparison with PM10 samples in a polluted area (Central Italy). *Ecological Indicators* 101, 759–769. <https://doi.org/10.1016/j.ecolind.2018.12.051>
- Massoud, R., Shihadeh, Alan.L., Roumié, M., Youness, M., Gerard, J., Saliba, N., Zaarour, R., Abboud, M., Farah, W., Saliba, N.A., 2011. Intraurban variability of PM10 and PM2.5 in an Eastern Mediterranean city. *Atmospheric Research* 101, 893–901. <https://doi.org/10.1016/j.atmosres.2011.05.019>
- Matin, G., Kargar, N., Buyukisik, H.B., 2016. Bio-monitoring of cadmium, lead, arsenic and mercury in industrial districts of Izmir, Turkey by using honey bees, propolis and pine tree leaves. *Ecological Engineering* 90, 331–335. <https://doi.org/10.1016/j.ecoleng.2016.01.035>
- McLagan, D.S., Mitchell, C.P.J., Steffen, A., Hung, H., Shin, C., Stupple, G.W., Olson, M.L., Luke, W.T., Kelley, P., Howard, D., Edwards, G.C., Nelson, P.F., Xiao, H., Sheu, G.-R., Dreyer, A., Huang, H., Abdul Hussain, B., Lei, Y.D., Tavshunsky, I., Wania, F., 2018. Global evaluation and calibration of a passive air sampler for gaseous mercury. *Atmospheric Chemistry and Physics* 18, 5905–5919. <https://doi.org/10.5194/acp-18-5905-2018>
- Medeiros, A.S., Silva, D.B., Santos, A.O., Castro, S.S.L., Oliveira, T.M.B.F., 2020. Voltammetric sensing of E,E-dienestrol in fish tissue by combining a cathodically pretreated boron-doped diamond electrode and QuEChERS extraction method. *Microchemical Journal* 155, 104718. <https://doi.org/10.1016/j.microc.2020.104718>
- Mee Kin, C., Guan Huat, T., 2010. Headspace solid-phase microextraction for the evaluation of pesticide residue contents in cucumber and strawberry after washing treatment. *Food Chemistry* 123, 760–764. <https://doi.org/10.1016/j.foodchem.2010.05.038>
- Melody, S.M., Johnston, F.H., 2015. Coal mine fires and human health: What do we know? *International Journal of Coal Geology* 152, 1–14. <https://doi.org/10.1016/j.coal.2015.11.001>
- Melymuk, L., Bohlin-Nizzetto, P., Prokeš, R., Kukučka, P., Příbylová, P., Vojta, Š., Kohoutek, J., Lammel, G., Klánová, J., 2017. Uncertainties in monitoring of SVOCs in air caused by within-sampler degradation during active and passive air sampling. *Atmospheric Environment* 167, 553–565. <https://doi.org/10.1016/j.atmosenv.2017.08.038>
- Merhaby, D., Ouddane, B., Net, S., Halwani, J., 2020. Assessment of persistent organic pollutants in surface sediments along Lebanese coastal zone. *Marine Pollution Bulletin* 153, 110947. <https://doi.org/10.1016/j.marpolbul.2020.110947>
- Merhaby, D., Rabodonirina, S., Net, S., Ouddane, B., Halwani, J., 2019. Overview of sediments pollution by PAHs and PCBs in mediterranean basin: Transport, fate, occurrence, and distribution. *Marine Pollution Bulletin* 149, 110646. <https://doi.org/10.1016/j.marpolbul.2019.110646>
- Mittal, R.D., 2015. Tandem Mass Spectroscopy in Diagnosis and Clinical Research. *Indian J Clin Biochem* 30, 121–123. <https://doi.org/10.1007/s12291-015-0498-9>
- Mleiki, A., Zaldibar, B., Izagirre, U., El Menif, N.T., Marigómez, I., 2018. Effects of dietary Pb and Cd and their combination on lysosomal and tissue-level biomarkers and histopathology in digestive gland of the land snail, *Cantareus apertus* (Born, 1778). *Ecotoxicology and Environmental Safety* 156, 301–310. <https://doi.org/10.1016/j.ecoenv.2018.02.079>
- Mokalled, T., Adjizian Gérard, J., Abboud, M., Liaud, C., Nassreddine, R., Le Calvé, S., 2019. An assessment of indoor air quality in the maintenance room at Beirut-Rafic Hariri International Airport. *Atmospheric Pollution Research* 10, 701–711. <https://doi.org/10.1016/j.apr.2018.11.008>
- Mokalled, T., Le Calvé, S., Badaro-Saliba, N., Abboud, M., Zaarour, R., Farah, W., Adjizian-Gérard, J., 2018. Identifying the impact of Beirut Airport's activities on local air quality - Part I: Emissions inventory of NO2 and VOCs. *Atmospheric Environment* 187, 435–444. <https://doi.org/10.1016/j.atmosenv.2018.04.036>
- Moreno-González, D., Cutillas, V., Hernando, M.D., Alcántara-Durán, J., García-Reyes, J.F., Molina-Díaz, A., 2020. Quantitative determination of pesticide residues in specific parts of bee specimens by nanoflow liquid chromatography high resolution mass spectrometry. *Science of The Total Environment* 715, 137005. <https://doi.org/10.1016/j.scitotenv.2020.137005>

- Moreno-González, D., Huertas-Pérez, J.F., García-Campaña, A.M., Gámiz-Gracia, L., 2014. Determination of carbamates in edible vegetable oils by ultra-high performance liquid chromatography–tandem mass spectrometry using a new clean-up based on zirconia for QuEChERS methodology. *Talanta* 128, 299–304. <https://doi.org/10.1016/j.talanta.2014.04.045>
- Mostafalou, S., Abdollahi, M., 2018. The link of organophosphorus pesticides with neurodegenerative and neurodevelopmental diseases based on evidence and mechanisms. *Toxicology* 409, 44–52. <https://doi.org/10.1016/j.tox.2018.07.014>
- Mu, P., Xu, N., Chai, T., Jia, Q., Yin, Z., Yang, S., Qian, Y., Qiu, J., 2016. Simultaneous determination of 14 antiviral drugs and relevant metabolites in chicken muscle by UPLC–MS/MS after QuEChERS preparation. *Journal of Chromatography B* 1023–1024, 17–23. <https://doi.org/10.1016/j.jchromb.2016.04.036>
- Muhammad, N., Subhani, Q., Wang, F., Guo, D., Zhao, Q., Wu, S., Zhu, Y., 2017. Application of a simple column-switching ion chromatography technique for removal of matrix interferences and sensitive fluorescence determination of acidic compounds (pharmaceutical drugs) in complex samples. *Journal of Chromatography A* 1515, 69–80. <https://doi.org/10.1016/j.chroma.2017.07.007>
- Mukhopadhyay, S., Dutta, R., Das, P., 2020. A critical review on plant biomonitors for determination of polycyclic aromatic hydrocarbons (PAHs) in air through solvent extraction techniques. *Chemosphere* 251, 126441. <https://doi.org/10.1016/j.chemosphere.2020.126441>
- Musarurwa, H., Tavengwa, N.T., 2020. Deep eutectic solvent-based dispersive liquid-liquid micro-extraction of pesticides in food samples. *Food Chemistry* 127943. <https://doi.org/10.1016/j.foodchem.2020.127943>
- Musarurwa, H., Chimuka, L., Pakade, V.E., Tavengwa, N.T., 2019. Recent developments and applications of QuEChERS based techniques on food samples during pesticide analysis. *Journal of Food Composition and Analysis* 84, 103314. <https://doi.org/10.1016/j.jfca.2019.103314>
- Nadal, M., Marquès, M., Mari, M., Domingo, J.L., 2015. Climate change and environmental concentrations of POPs: A review. *Environmental Research* 143, 177–185. <https://doi.org/10.1016/j.envres.2015.10.012>
- Nardelli, V., D'Amico, V., Della Rovere, I., Casamassima, F., Marchesiello, W.M.V., Nardiello, D., Quinto, M., 2020. Box Behn ken design-based optimized extraction of non-dioxin-like PCBs for GC-ECD and GC-MS analyses in milk samples. *Emerging Contaminants* 6, 303–311. <https://doi.org/10.1016/j.emcon.2020.08.002>
- Narenderan, S.T., Meyyanathan, S.N., Karri, V.V.S.R., Babu, B., Chintamaneni, P., 2019. Multivariate response surface methodology assisted modified QuEChERS extraction method for the evaluation of organophosphate pesticides in fruits and vegetables cultivated in Nilgiris, South India. *Food Chemistry* 300, 125188. <https://doi.org/10.1016/j.foodchem.2019.125188>
- Negri, I., Mavris, C., Di Prisco, G., Caprio, E., Pellecchia, M., 2015. Honey Bees (*Apis mellifera*, L.) as Active Samplers of Airborne Particulate Matter. *PLoS One* 10. <https://doi.org/10.1371/journal.pone.0132491>
- Newton, S., Sellström, U., Harrad, S., Yu, G., de Wit, C.A., 2016. Comparisons of indoor active and passive air sampling methods for emerging and legacy halogenated flame retardants in Beijing, China offices. *Emerging Contaminants* 2, 80–88. <https://doi.org/10.1016/j.emcon.2016.02.001>
- Nica, D., Borden, D.-M., Borožan, A., Gergen, I., Bura, M., Banatean-Dunea, I., 2013. Use of Land Snails (Pulmonata) for Monitoring Copper Pollution in Terrestrial Ecosystems.
- Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L., 2016. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. *Front Public Health* 4. <https://doi.org/10.3389/fpubh.2016.00148>
- Niell, S., Jesús, F., Pérez, N., Pérez, C., Pareja, L., Abbate, S., Carrasco-Letelier, L., Díaz, S., Mendoza, Y., Cesio, V., Heinzen, H., 2017. Neonicotinoids transference from the field to the hive by honey bees: Towards a pesticide residues biomonitor. *Science of The Total Environment* 581–582, 25–31. <https://doi.org/10.1016/j.scitotenv.2017.01.011>
- Noth, E.M., Katharine Hammond, S., Biging, G.S., Tager, I.B., 2013. Mapping and modeling airborne urban phenanthrene distribution using vegetation biomonitoring. *Atmospheric Environment* 77, 518–524. <https://doi.org/10.1016/j.atmosenv.2013.05.056>
- Nouira, T., Risso, C., Chouba, L., Budzinski, H., Boussetta, H., 2013. Polychlorinated biphenyls (PCBs) and Polybrominated Diphenyl Ethers (PBDEs) in surface sediments from Monastir Bay (Tunisia, Central Mediterranean): Occurrence, distribution and seasonal variations. *Chemosphere* 93, 487–493. <https://doi.org/10.1016/j.chemosphere.2013.06.017>
- Odabasi, M., Ozgunerge Falay, E., Tuna, G., Altioğ, H., Kara, M., Dumanoglu, Y., Bayram, A., Tolunay, D., Elbir, T., 2015. Biomonitoring the Spatial and Historical Variations of Persistent Organic Pollutants (POPs) in an Industrial Region. *Environ. Sci. Technol.* 49, 2105–2114. <https://doi.org/10.1021/es506316t>
- Oellig, C., 2016. Acetonitrile extraction and dual-layer solid phase extraction clean-up for pesticide residue analysis in propolis. *Journal of Chromatography A* 1445, 19–26. <https://doi.org/10.1016/j.chroma.2016.03.082>
- Ogunkunle, C.O., Ziyath, A.M., Rufai, S.S., Fatoba, P.O., 2016. Surrogate approach to determine heavy metal loads in a moss species – *Barbula lambaranensis*. *Journal of King Saud University - Science* 28, 193–197. <https://doi.org/10.1016/j.jksus.2015.11.002>
- Oishi, Y., 2018. Comparison of moss and pine needles as bioindicators of transboundary polycyclic aromatic hydrocarbon pollution in central Japan. *Environmental Pollution* 234, 330–338. <https://doi.org/10.1016/j.envpol.2017.11.035>
- Okeme, J.O., Saini, A., Yang, C., Zhu, J., Smedes, F., Klánová, J., Diamond, M.L., 2016. Calibration of polydimethylsiloxane and XAD-Pocket passive air samplers (PAS) for measuring gas- and particle-phase SVOCs. *Atmospheric Environment* 143, 202–208. <https://doi.org/10.1016/j.atmosenv.2016.08.023>
- Olatunji, O.S., Fatoki, O.S., Opeolu, B.O., Ximba, B.J., 2014. Determination of polycyclic aromatic hydrocarbons [PAHs] in processed meat products using gas chromatography – Flame ionization detector. *Food Chemistry* 156, 296–300. <https://doi.org/10.1016/j.foodchem.2014.01.120>
- Oliveira, César, Martins, N., Tavares, J., Pio, C., Cerqueira, M., Matos, M., Silva, H., Oliveira, Cristina, Camões, F., 2011. Size distribution of polycyclic aromatic hydrocarbons in a roadway tunnel in Lisbon, Portugal. *Chemosphere* 83, 1588–1596. <https://doi.org/10.1016/j.chemosphere.2011.01.011>
- Omwoma, S., Mbithi, B.M., Pandelova, M., Ssebugere, P., Lalah, J.O., Wang, Y., Bi, Y., Henkelmann, B., Schramm, K.-W., 2019. Comparative exposomics of persistent organic pollutants (PCBs, OCPs, MCCPs and SCCPs) and polycyclic aromatic hydrocarbons (PAHs) in Lake Victoria (Africa) and Three Gorges Reservoir (China). *Science of The Total Environment* 695, 133789. <https://doi.org/10.1016/j.scitotenv.2019.133789>
- Ong, C.B., Ng, L.Y., Mohammad, A.W., 2018. A review of ZnO nanoparticles as solar photocatalysts: Synthesis, mechanisms and applications. *Renewable and Sustainable Energy Reviews* 81, 536–551. <https://doi.org/10.1016/j.rser.2017.08.020>
- Orisakwe, O.E., Igweze, Z.N., Okolo, K.O., Udowelle, N.A., 2015. Human health hazards of poly aromatic hydrocarbons in Nigerian smokeless tobacco. *Toxicology Reports* 2, 1019–1023. <https://doi.org/10.1016/j.toxrep.2015.07.011>
- Ostiguy, N., Drummond, F.A., Aronstein, K., Eitzer, B., Ellis, J.D., Spivak, M., Sheppard, W.S., 2019. Honey Bee Exposure to Pesticides: A Four-Year Nationwide Study. *Insects* 10. <https://doi.org/10.3390/insects10010013>

- Osyczka, P., Boroń, P., Lenart-Boroń, A., Rola, K., 2018. Modifications in the structure of the lichen *Cladonia thallus* in the aftermath of habitat contamination and implications for its heavy-metal accumulation capacity. *Environ Sci Pollut Res* 25, 1950–1961. <https://doi.org/10.1007/s11356-017-0639-1>
- Pacitto, A., Stabile, L., Scungio, M., Rizza, V., Buonanno, G., 2018. Characterization of airborne particles emitted by an electrically heated tobacco smoking system. *Environmental Pollution* 240, 248–254. <https://doi.org/10.1016/j.envpol.2018.04.137>
- Panio, A., Fabbri Corsarini, S., Bruno, A., Lasagni, M., Labra, M., Saliu, F., 2020. Determination of phthalates in fish fillets by liquid chromatography tandem mass spectrometry (LC-MS/MS): A comparison of direct immersion solid phase microextraction (SPME) versus ultrasonic assisted solvent extraction (UASE). *Chemosphere* 255, 127034. <https://doi.org/10.1016/j.chemosphere.2020.127034>
- Panuwet, P., Hunter, R.E., D'Souza, P.E., Chen, X., Radford, S.A., Cohen, J.R., Marder, M.E., Kartavenka, K., Ryan, P.B., Barr, D.B., 2016. Biological Matrix Effects in Quantitative Tandem Mass Spectrometry-Based Analytical Methods: Advancing Biomonitoring. *Crit Rev Anal Chem* 46, 93–105. <https://doi.org/10.1080/10408347.2014.980775>
- Pariatamby, A., Kee, Y.L., 2016. Persistent Organic Pollutants Management and Remediation. *Procedia Environmental Sciences* 31, 842–848. <https://doi.org/10.1016/j.proenv.2016.02.093>
- Park, M.G., Blitzer, E.J., Gibbs, J., Losey, J.E., Danforth, B.N., 2015. Negative effects of pesticides on wild bee communities can be buffered by landscape context. *Proc Biol Sci* 282. <https://doi.org/10.1098/rspb.2015.0299>
- Parmar, T.K., Rawtani, D., Agrawal, Y.K., 2016. Bioindicators: the natural indicator of environmental pollution. *Frontiers in Life Science* 9, 110–118. <https://doi.org/10.1080/21553769.2016.1162753>
- Parrilla Vázquez, P., Ferrer, C., Martínez Bueno, M.J., Fernández-Alba, A.R., 2019. Pesticide residues in spices and herbs: Sample preparation methods and determination by chromatographic techniques. *TrAC Trends in Analytical Chemistry* 115, 13–22. <https://doi.org/10.1016/j.trac.2019.03.022>
- Peters, C.E., Parent, M.-É., Harris, S.A., Bogaert, L., Latifovic, L., Kachuri, L., Villeneuve, P.J., 2018. Occupational Exposure to Diesel and Gasoline Engine Exhausts and the Risk of Kidney Cancer in Canadian Men. *Ann Work Expo Health* 62, 978–989. <https://doi.org/10.1093/annweh/wxy059>
- Pies, C., Hoffmann, B., Petrowsky, J., Yang, Y., Ternes, T.A., Hofmann, T., 2008. Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in river bank soils. *Chemosphere* 72, 1594–1601. <https://doi.org/10.1016/j.chemosphere.2008.04.021>
- Pineda-Martínez, L.F., Carbajal, N., Campos-Ramos, A., Aragón-Piña, A., García, A.R., 2014. Dispersion of atmospheric coarse particulate matter in the San Luis Potosí, Mexico, urban area. *Atmósfera* 27, 5–19. [https://doi.org/10.1016/S0187-6236\(14\)71097-5](https://doi.org/10.1016/S0187-6236(14)71097-5)
- Piri-Moghadam, H., Ahmadi, F., Pawliszyn, J., 2016. A critical review of solid phase microextraction for analysis of water samples. *TrAC Trends in Analytical Chemistry, On-site and In-vivo Instrumentation and Applications* 85, 133–143. <https://doi.org/10.1016/j.trac.2016.05.029>
- Proum, S., Santos, J.H., Lim, L.H., Marshall, D.J., 2016. Metal accumulation in the tissues and shells of *Indothais gradata* snails inhabiting soft and hard substrata in an acidified tropical estuary (Brunei, South East Asia). *Regional Studies in Marine Science* 8, 487–497. <https://doi.org/10.1016/j.rsma.2016.03.010>
- Qiu, Y.-W., Zeng, E.Y., Qiu, H., Yu, K., Cai, S., 2017. Bioconcentration of polybrominated diphenyl ethers and organochlorine pesticides in algae is an important contaminant route to higher trophic levels. *Science of The Total Environment* 579, 1885–1893. <https://doi.org/10.1016/j.scitotenv.2016.11.192>
- Raaschou-Nielsen, O., Beelen, R., Wang, M., Hoek, G., Andersen, Z.J., Hoffmann, B., Stafoggia, M., Samoli, E., Weinmayr, G., Dimakopoulou, K., Nieuwenhuijsen, M., Xun, W.W., Fischer, P., Eriksen, K.T., Sørensen, M., Tjønneland, A., Ricceri, F., de Hoogh, K., Key, T., Eeftens, M., Peeters, P.H., Bueno-de-Mesquita, H.B., Meliefste, K., Oftedal, B., Schwarze, P.E., Nafstad, P., Galassi, C., Migliore, E., Ranzi, A., Cesaroni, G., Badaloni, C., Forastiere, F., Penell, J., De Faire, U., Korek, M., Pedersen, N., Östenson, C.-G., Pershagen, G., Fratiglioni, L., Concin, H., Nagel, G., Jaensch, A., Ineichen, A., Naccarati, A., Katsoulis, M., Trichopoulou, A., Keuken, M., Jedynska, A., Kooter, I.M., Kukkonen, J., Brunekreef, B., Sokhi, R.S., Katsouyanni, K., Vineis, P., 2016. Particulate matter air pollution components and risk for lung cancer. *Environment International* 87, 66–73. <https://doi.org/10.1016/j.envint.2015.11.007>
- Rahman, Md.M., Lee, H.S., Abd El-Aty, A.M., Kabir, Md.H., Chung, H.S., Park, J.-H., Kim, M.-R., Kim, J., Shin, H.-C., Shin, S.S., Shim, J.-H., 2018. Determination of endrin and δ -keto endrin in five food products of animal origin using GC- μ ECD: A modified QuEChERS approach to traditional detection. *Food Chemistry* 263, 59–66. <https://doi.org/10.1016/j.foodchem.2018.04.099>
- Raina-Fulton, R., Xie, Z., 2017. Sample Preparation Methods for Pesticide Analysis in Food Commodities, Biological and Environment Matrices. *Ideas and Applications Toward Sample Preparation for Food and Beverage Analysis*. <https://doi.org/10.5772/intechopen.69791>
- Rajski, Ł., Lozano, A., Uclés, A., Ferrer, C., Fernández-Alba, A.R., 2013. Determination of pesticide residues in high oil vegetal commodities by using various multi-residue methods and clean-ups followed by liquid chromatography tandem mass spectrometry. *Journal of Chromatography A* 1304, 109–120. <https://doi.org/10.1016/j.chroma.2013.06.070>
- Ramli, N.H., Yusup, S., Quitain, A.T., Johari, K., Kueh, B.W.B., 2019. Optimization of saponin extracts using microwave-assisted extraction as a sustainable biopesticide to reduce *Pomacea canaliculata* population in paddy cultivation. *Sustainable Chemistry and Pharmacy* 11, 23–35. <https://doi.org/10.1016/j.scp.2018.12.002>
- Randoll, R., Wondrak, W., Schletz, A., 2014. Dielectric strength and thermal performance of PCB-embedded power electronics. *Microelectronics Reliability, SI: ESREF 2014* 54, 1872–1876. <https://doi.org/10.1016/j.microrel.2014.07.139>
- Rasheed, T., Bilal, M., Nabeel, F., Adeel, M., Iqbal, H.M.N., 2019. Environmentally-related contaminants of high concern: Potential sources and analytical modalities for detection, quantification, and treatment. *Environment International* 122, 52–66. <https://doi.org/10.1016/j.envint.2018.11.038>
- Ratola, N., Alves, A., Santos, L., Lacorte, S., 2011a. Pine needles as passive bio-samplers to determine polybrominated diphenyl ethers. *Chemosphere* 85, 247–252. <https://doi.org/10.1016/j.chemosphere.2011.06.005>
- Ratola, N., Amigo, J.M., Oliveira, M.S.N., Araújo, R., Silva, J.A., Alves, A., 2011b. Differences between *Pinus pinea* and *Pinus pinaster* as bioindicators of polycyclic aromatic hydrocarbons. *Environmental and Experimental Botany* 72, 339–347. <https://doi.org/10.1016/j.envexpbot.2011.04.012>
- Ratola, N., Homem, V., Silva, J.A., Araújo, R., Amigo, J.M., Santos, L., Alves, A., 2014. Biomonitoring of pesticides by pine needles — Chemical scoring, risk of exposure, levels and trends. *Science of The Total Environment* 476–477, 114–124. <https://doi.org/10.1016/j.scitotenv.2014.01.003>
- Ravindra, K., Sokhi, R., Van Grieken, R., 2008. Atmospheric polycyclic aromatic hydrocarbons: Source attribution, emission factors and regulation. *Atmospheric Environment* 42, 2895–2921. <https://doi.org/10.1016/j.atmosenv.2007.12.010>

- Reddy, A.V.B., Moniruzzaman, M., Aminabhavi, T.M., 2019. Polychlorinated biphenyls (PCBs) in the environment: Recent updates on sampling, pretreatment, cleanup technologies and their analysis. *Chemical Engineering Journal* 358, 1186–1207. <https://doi.org/10.1016/j.cej.2018.09.205>
- Rockwood, A.L., Kushnir, M.M., Clarke, N.J., 2018. 2 - Mass Spectrometry, in: Rifai, N., Horvath, A.R., Wittwer, C.T. (Eds.), *Principles and Applications of Clinical Mass Spectrometry*. Elsevier, pp. 33–65. <https://doi.org/10.1016/B978-0-12-816063-3.00002-5>
- Rodrigues, A.A.Z., Neves, A.A., Queiroz, M.E.L.R. de, Oliveira, A.F. de, Prates, L.H.F., Morais, E.H. da C., 2018. Optimization and validation of the salting-out assisted liquid-liquid extraction method and analysis by gas chromatography to determine pesticides in water. *Eclética Química* 43, 11–21.
- Rodríguez López, D., Ahumada, D.A., Díaz, A.C., Guerrero, J.A., 2014. Evaluation of pesticide residues in honey from different geographic regions of Colombia. *Food Control* 37, 33–40. <https://doi.org/10.1016/j.foodcont.2013.09.011>
- Rola, K., Lenart-Boroń, A., Boroń, P., Osyczka, P., 2021. Heavy-metal pollution induces changes in the genetic composition and anatomical properties of photobionts in pioneer lichens colonising post-industrial habitats. *Science of The Total Environment* 750, 141439. <https://doi.org/10.1016/j.scitotenv.2020.141439>
- Rola, K., Osyczka, P., 2018. Cryptogamic communities as a useful bioindication tool for estimating the degree of soil pollution with heavy metals. *Ecological Indicators* 88, 454–464. <https://doi.org/10.1016/j.ecolind.2018.01.013>
- Roll, I.B., Halden, R.U., 2016. Critical Review of Factors Governing Data Quality of Integrative Samplers Employed in Environmental Water Monitoring. *Water Res* 94, 200–207. <https://doi.org/10.1016/j.watres.2016.02.048>
- Roumié, M., Saliba, N., Nsouli, B., Younes, M., Noun, M., Massoud, R., 2011. PIXE identification of fine and coarse particles of aerosol samples and their distribution across Beirut. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, Proceedings of the 10th European Conference on Accelerators in Applied Research and Technology (ECAART10)* 269, 3106–3110. <https://doi.org/10.1016/j.nimb.2011.04.081>
- Ruiz del Castillo, M.L., Rodríguez-Valenciano, M., Flores, G., Blanch, G.P., 2019. New method based on Solid Phase Microextraction and Multidimensional gas chromatography-mass spectrometry to determine pesticides in strawberry jam. *LWT* 99, 283–290. <https://doi.org/10.1016/j.lwt.2018.09.063>
- Ruiz-Suárez, N., Boada, L.D., Henríquez-Hernández, L.A., González-Moreo, F., Suárez-Pérez, A., Camacho, M., Zumbado, M., Almeida-González, M., del Mar Travieso-Aja, M., Luzardo, O.P., 2015. Continued implication of the banned pesticides carbofuran and aldicarb in the poisoning of domestic and wild animals of the Canary Islands (Spain). *Science of The Total Environment* 505, 1093–1099. <https://doi.org/10.1016/j.scitotenv.2014.10.093>
- Rusin, M., Dziubanek, G., Marchwińska-Wyrwał, E., Ćwieląg-Drabek, M., Razzaghi, M., Piekut, A., 2019. PCDDs, PCDFs and PCBs in locally produced foods as health risk factors in Silesia Province, Poland. *Ecotoxicology and Environmental Safety* 172, 128–135. <https://doi.org/10.1016/j.ecoenv.2019.01.052>
- Sabarwal, A., Kumar, K., Singh, R.P., 2018. Hazardous effects of chemical pesticides on human health—Cancer and other associated disorders. *Environmental Toxicology and Pharmacology* 63, 103–114. <https://doi.org/10.1016/j.etap.2018.08.018>
- Saburouh, N., Jabbari, A., Parastar, H., 2020. An innovative chemometric approach for simultaneous determination of polycyclic aromatic hydrocarbons in oil-contaminated waters based on dispersive micro-solid phase extraction followed by gas chromatography. *Microchemical Journal* 159, 105407. <https://doi.org/10.1016/j.microc.2020.105407>
- Sadowska, M., Gogolewska, H., Pawelec, N., Sentkowska, A., Krasnodęska-Ostręga, B., 2019. Comparison of the contents of selected elements and pesticides in honey bees with regard to their habitat. *Environ Sci Pollut Res Int* 26, 371–380. <https://doi.org/10.1007/s11356-018-3612-8>
- Sajid, M., Nazal, M.K., Ihsanullah, I., 2020. Novel materials for dispersive (micro) solid-phase extraction of polycyclic aromatic hydrocarbons in environmental water samples: A review. *Analytica Chimica Acta* S0003267020308023. <https://doi.org/10.1016/j.aca.2020.07.064>
- Sakin, A.E., Esen, F., Tasdemir, Y., 2017. Effects of sampling interval on the passive air sampling of atmospheric PCBs levels. *Journal of Environmental Science and Health, Part A* 52, 673–679. <https://doi.org/10.1080/10934529.2017.1297148>
- Salameh, P.R., Baldi, I., Brochard, P., Saleh, B.A., 2004. Pesticides in Lebanon: a knowledge, attitude, and practice study. *Environmental Research* 94, 1–6. [https://doi.org/10.1016/S0013-9351\(03\)00092-6](https://doi.org/10.1016/S0013-9351(03)00092-6)
- Salemi, A., Khaleghifard, N., Mirikaram, N., 2019. Optimization and comparison of membrane-protected micro-solid-phase extraction coupled with dispersive liquid-liquid microextraction for organochlorine pesticides using three different sorbents. *Microchemical Journal* 144, 215–220. <https://doi.org/10.1016/j.microc.2018.09.011>
- Salo, H., Bučko, M.S., Vaahotvuo, E., Limo, J., Mäkinen, J., Pesonen, L.J., 2012. Biomonitoring of air pollution in SW Finland by magnetic and chemical measurements of moss bags and lichens. *Journal of Geochemical Exploration* 115, 69–81. <https://doi.org/10.1016/j.gexplo.2012.02.009>
- Sanchez-Bayo, F., Goka, K., 2014. Pesticide Residues and Bees – A Risk Assessment. *PLOS ONE* 9, e94482. <https://doi.org/10.1371/journal.pone.0094482>
- Santibáñez-Andrade, M., Quezada-Maldonado, E.M., Osornio-Vargas, Á., Sánchez-Pérez, Y., García-Cuellar, C.M., 2017. Air pollution and genomic instability: The role of particulate matter in lung carcinogenesis. *Environmental Pollution* 229, 412–422. <https://doi.org/10.1016/j.envpol.2017.06.019>
- Sari, M.F., Esen, F., Tasdemir, Y., 2021. Levels of polychlorinated biphenyls (PCBs) in honeybees and bee products and their evaluation with ambient air concentrations. *Atmospheric Environment* 244, 117903. <https://doi.org/10.1016/j.atmosenv.2020.117903>
- Saraji, M., Jafari, M.T., Mossaddegh, M., 2016. Carbon nanotubes@silicon dioxide nanohybrids coating for solid-phase microextraction of organophosphorus pesticides followed by gas chromatography–corona discharge ion mobility spectrometric detection. *Journal of Chromatography A* 1429, 30–39. <https://doi.org/10.1016/j.chroma.2015.12.008>
- Sari, M.F., Esen, F., Tasdemir, Y., 2021. Levels of polychlorinated biphenyls (PCBs) in honeybees and bee products and their evaluation with ambient air concentrations. *Atmospheric Environment* 244, 117903. <https://doi.org/10.1016/j.atmosenv.2020.117903>
- Scaramozzino, P., Battisti, S., Desiato, R., Tamba, M., Fedrizzi, G., Ubaldi, A., Neri, B., Abete, M.C., Ru, G., 2019. Application of a risk-based standardized animal biomonitoring approach to contaminated sites. *Environ Monit Assess* 191. <https://doi.org/10.1007/s10661-019-7653-3>
- Schikowski, T., Altuğ, H., 2020. The role of air pollution in cognitive impairment and decline. *Neurochemistry International* 136, 104708. <https://doi.org/10.1016/j.neuint.2020.104708>
- Scopus preview - Scopus - Document details [WWW Document], n.d. <https://doi.org/10.1016/j.envpol.2007.09.004>
- Sekhotha, M.M., Monyeki, K.D., Sibuyi, M.E., 2016. Exposure to Agrochemicals and Cardiovascular Disease: A Review. *Int J Environ Res Public Health* 13. <https://doi.org/10.3390/ijerph13020229>

- Sgargi, D., Adam, B., Budnik, L.T., Dinelli, G., Moldovan, H.R., Perry, M.J., Scheepers, P.T.J., Schlünssen, V., Teixeira, J.P., Mandrioli, D., Belpoggi, F., 2020. Protocol for a systematic review and meta-analysis of human exposure to pesticide residues in honey and other bees' products. *Environmental Research* 186, 109470. <https://doi.org/10.1016/j.envres.2020.109470>
- Shaltout, A.A., Hassan, S.K., Karydas, A.G., Zaki, Z.I., Mostafa, N.Y., Kregsamer, P., Wobrauschek, P., Strelci, C., 2018. Comparative elemental analysis of fine particulate matter (PM 2.5) from industrial and residential areas in Greater Cairo-Egypt by means of a multi-secondary target energy dispersive X-ray fluorescence spectrometer. *Spectrochimica Acta Part B: Atomic Spectroscopy* 145, 29–35. <https://doi.org/10.1016/j.sab.2018.04.003>
- Shamsipur, M., Yazdanfar, N., Ghambarian, M., 2016. Combination of solid-phase extraction with dispersive liquid-liquid microextraction followed by GC-MS for determination of pesticide residues from water, milk, honey and fruit juice. *Food Chemistry* 204, 289–297. <https://doi.org/10.1016/j.foodchem.2016.02.090>
- Shendy, A.H., Al-Ghobashy, M.A., Gad Alla, S.A., Lotfy, H.M., 2016. Development and validation of a modified QuEChERS protocol coupled to LC-MS/MS for simultaneous determination of multi-class antibiotic residues in honey. *Food Chemistry* 190, 982–989. <https://doi.org/10.1016/j.foodchem.2015.06.048>
- Shihadeh, A., Azar, S., Antonios, C., Haddad, A., 2004. Towards a topographical model of narghile water-pipe café smoking: a pilot study in a high socioeconomic status neighborhood of Beirut, Lebanon. *Pharmacology Biochemistry and Behavior* 79, 75–82. <https://doi.org/10.1016/j.pbb.2004.06.005>
- Shmuel, S., White, A.J., Sandler, D.P., 2017. Residential exposure to vehicular traffic-related air pollution during childhood and breast cancer risk. *Environmental Research* 159, 257–263. <https://doi.org/10.1016/j.envres.2017.08.015>
- Silva, A.S., Araújo, S.B., Souza, D.C., Silva, F.A.S. e, 2012. Study of the Cu, Mn, Pb and Zn dynamics in soil, plants and bee pollen from the region of Teresina (PI), Brazil. *Anais da Academia Brasileira de Ciências* 84, 881–889. <https://doi.org/10.1590/S0001-37652012005000065>
- Silva, B., Gonzaga, L.V., Fett, R., Oliveira Costa, A.C., 2019. Improved strategy based on QuEChERS method followed by HPLC/DAD for the quantification of phenolic compounds from Mimosa scabrella Benth honeydew honeys. *LWT* 116, 108471. <https://doi.org/10.1016/j.lwt.2019.108471>
- Silva, J.A., Ratola, N., Ramos, S., Homem, V., Santos, L., Alves, A., 2015. An analytical multi-residue approach for the determination of semi-volatile organic pollutants in pine needles. *Analytica Chimica Acta* 858, 24–31. <https://doi.org/10.1016/j.aca.2014.12.042>
- Skorbiłowicz, E., Skorbiłowicz, M., Cieśluk, I., 2018. Bees as Bioindicators of Environmental Pollution with Metals in an Urban Area. *Journal of Ecological Engineering* 19, 229–234. <https://doi.org/10.12911/22998993/85738>
- Slezakova, K., Castro, D., Delerue-Matos, C., Alvim-Ferraz, M. da C., Morais, S., Pereira, M. do C., 2013. Impact of vehicular traffic emissions on particulate-bound PAHs: Levels and associated health risks. *Atmospheric Research* 127, 141–147. <https://doi.org/10.1016/j.atmosres.2012.06.009>
- Soares, K.L., Cerqueira, M.B.R., Caldas, S.S., Primel, E.G., 2017. Evaluation of alternative environmentally friendly matrix solid phase dispersion solid supports for the simultaneous extraction of 15 pesticides of different chemical classes from drinking water treatment sludge. *Chemosphere* 182, 547–554. <https://doi.org/10.1016/j.chemosphere.2017.05.062>
- Souza Tette, P.A., Rocha Guidi, L., de Abreu Glória, M.B., Fernandes, C., 2016. Pesticides in honey: A review on chromatographic analytical methods. *Talanta* 149, 124–141. <https://doi.org/10.1016/j.talanta.2015.11.045>
- Song, S., Xue, J., Lu, Y., Zhang, H., Wang, C., Cao, X., Li, Q., 2018. Are unintentionally produced polychlorinated biphenyls the main source of polychlorinated biphenyl occurrence in soils? *Environmental Pollution* 243, 492–500. <https://doi.org/10.1016/j.envpol.2018.09.027>
- Srivastava, K., Bhattacharya, P., 2016. Lichen as a Bio-Indicator Tool for Assessment of Climate and Air Pollution Vulnerability: Review. *International Research Journal of Environment Sciences* 4, 2319–1414.
- Srogi, K., 2007. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. *Environ Chem Lett* 5, 169–195. <https://doi.org/10.1007/s10311-007-0095-0>
- Stader, C., Deventer, M.J., Klemm, O., Achten, C., 2016. Turbulent transport of particle-bound PAHs: A feasibility study combining GC-APLI-MS and eddy covariance. *Atmospheric Pollution Research* 7, 603–610. <https://doi.org/10.1016/j.apr.2016.02.005>
- Stocka, J., Tankiewicz, M., Biziuk, M., Namieśnik, J., 2011. Green Aspects of Techniques for the Determination of Currently Used Pesticides in Environmental Samples. *Int J Mol Sci* 12, 7785–7805. <https://doi.org/10.3390/ijms12117785>
- Sturba, L., Liberatori, G., Vannuccini, M.L., Ancora, S., Corsi, I., 2018. Uptake and biological responses in land snail Cornu aspersum exposed to vaporized CdCl₂. *Ecotoxicology and Environmental Safety* 148, 377–383. <https://doi.org/10.1016/j.ecoenv.2017.10.050>
- Suganthi, A., Bhuvaneswari, K., Ramya, M., 2018. Determination of neonicotinoid insecticide residues in sugarcane juice using LCMSMS. *Food Chemistry* 241, 275–280. <https://doi.org/10.1016/j.foodchem.2017.08.098>
- Suman, S., Sinha, A., Tarafdar, A., 2016. Polycyclic aromatic hydrocarbons (PAHs) concentration levels, pattern, source identification and soil toxicity assessment in urban traffic soil of Dhanbad, India. *Science of The Total Environment* 545–546, 353–360. <https://doi.org/10.1016/j.scitotenv.2015.12.061>
- Sun, C., Tan, H., Zhang, Y., Zhang, H., 2016. Phenolics and abscisic acid identified in acacia honey comparing different SPE cartridges coupled with HPLC-PDA. *Journal of Food Composition and Analysis* 53, 91–101. <https://doi.org/10.1016/j.jfca.2016.08.006>
- Sun, Y., Wu, S., 2020. Analysis of PAHs in oily systems using modified QuEChERS with EMR-Lipid T clean-up followed by GC-QqQ-MS. *Food Control* 10.
- Szulejko, J.E., Kim, K.-H., Brown, R.J.C., Bae, M.-S., 2014. Review of progress in solvent-extraction techniques for the determination of polyaromatic hydrocarbons as airborne pollutants. *TrAC Trends in Analytical Chemistry* 61, 40–48. <https://doi.org/10.1016/j.trac.2014.07.001>
- Tan, J., Lu, X., Fu, L., Yang, G., Chen, J., 2019. Quantification of Cl-PAHs and their parent compounds in fish by improved ASE method and stable isotope dilution GC-MS. *Ecotoxicology and Environmental Safety* 186, 109775. <https://doi.org/10.1016/j.ecoenv.2019.109775>
- Tang, D., Liu, X., He, H., Cui, Z., Gan, H., Xia, Z., 2020. Distribution, sources and ecological risks of organochlorine compounds (DDTs, HCHs and PCBs) in surface sediments from the Pearl River Estuary, China. *Marine Pollution Bulletin* 152, 110942. <https://doi.org/10.1016/j.marpolbul.2020.110942>
- Tang, J., Ma, S., Liu, R., Yue, C., Li, G., Yu, Y., Yang, Y., An, T., 2020. The pollution profiles and human exposure risks of chlorinated and brominated PAHs in indoor dusts from e-waste dismantling workshops: Comparison of GC-MS, GC-MS/MS and GC × GC-MS/MS determination methods. *Journal of Hazardous Materials* 394, 122573. <https://doi.org/10.1016/j.jhazmat.2020.122573>
- Tang, R., Luo, J., Yang, P., She, J., Chen, Y., Gong, Y., Zhou, J., 2014. Trace metals of needles and litter in timberline forests in the Eastern of Tibetan Plateau, China. *Ecological Indicators* 45, 669–676. <https://doi.org/10.1016/j.ecolind.2014.06.003>

- Tang, X., Hashmi, M.Z., Zeng, B., Yang, J., Shen, C., 2015. Application of iron-activated persulfate oxidation for the degradation of PCBs in soil. *Chemical Engineering Journal* 279, 673–680. <https://doi.org/10.1016/j.cej.2015.05.059>
- Tang-Péronard, J.L., Heitmann, B.L., Jensen, T.K., Vinggaard, A.M., Madsbad, S., Steuerwald, U., Grandjean, P., Weihe, P., Nielsen, F., Andersen, H.R., 2015. Prenatal exposure to persistent organochlorine pollutants is associated with high insulin levels in 5-year-old girls. *Environmental Research* 142, 407–413. <https://doi.org/10.1016/j.envres.2015.07.009>
- Tartu, S., Angelier, F., Wingfield, J.C., Bustamante, P., Labadie, P., Budzinski, H., Weimerskirch, H., Bustnes, J.O., Chastel, O., 2015. Corticosterone, prolactin and egg neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird. *Science of The Total Environment* 505, 180–188. <https://doi.org/10.1016/j.scitotenv.2014.10.008>
- Tavalieri, Y.E., Galoppo, G.H., Canesini, G., Luque, E.H., Muñoz-de-Toro, M.M., 2020. Effects of agricultural pesticides on the reproductive system of aquatic wildlife species, with crocodilians as sentinel species. *Molecular and Cellular Endocrinology* 110918. <https://doi.org/10.1016/j.mce.2020.110918>
- Tette, P.A.S., da Silva Oliveira, F.A., Pereira, E.N.C., Silva, G., de Abreu Glória, M.B., Fernandes, C., 2016. Multiclass method for pesticides quantification in honey by means of modified QuEChERS and UHPLC-MS/MS. *Food Chemistry* 211, 130–139. <https://doi.org/10.1016/j.foodchem.2016.05.036>
- Tian, F., Qiao, C., Luo, J., Guo, L., Pang, T., Pang, R., Li, J., Wang, C., Wang, R., Xie, H., 2020. Development of a fast multi-residue method for the determination of succinate dehydrogenase inhibitor fungicides in cereals, vegetables and fruits by modified QuEChERS and UHPLC-MS/MS. *Journal of Chromatography B* 1152, 122261. <https://doi.org/10.1016/j.jchromb.2020.122261>
- Tibbetts, J.H., 2015. Managing Marine Plastic Pollution: Policy Initiatives to Address Wayward Waste. *Environ Health Perspect* 123, A90–A93. <https://doi.org/10.1289/ehp.123-A90>
- Tiwari, G., Tiwari, R., 2010. Bioanalytical method validation: An updated review. *Pharm Methods* 1, 25–38. <https://doi.org/10.4103/2229-4708.72226>
- Tobiszewski, M., Namieśnik, J., 2012. PAH diagnostic ratios for the identification of pollution emission sources. *Environmental Pollution* 162, 110–119. <https://doi.org/10.1016/j.envpol.2011.10.025>
- Tuduri, L., Millet, M., Briand, O., Montury, M., 2012. Passive air sampling of semi-volatile organic compounds. *TrAC Trends in Analytical Chemistry* 31, 38–49. <https://doi.org/10.1016/j.trac.2011.08.007>
- Turkylmaz, A., Sevik, H., Cetin, M., 2018. The use of perennial needles as biomonitors for recently accumulated heavy metals. *Landscape Ecol Eng* 14, 115–120. <https://doi.org/10.1007/s11355-017-0335-9>
- Tuzimski, T., Rejczak, T., 2016. Application of HPLC-DAD after SPE/QuEChERS with ZrO₂-based sorbent in d-SPE clean-up step for pesticide analysis in edible oils. *Food Chemistry* 190, 71–79. <https://doi.org/10.1016/j.foodchem.2015.05.072>
- Tuzimski, T., Rejczak, T., 2014. Determination of Pesticides in Sunflower Seeds by High-Performance Liquid Chromatography Coupled with a Diode Array Detector. *J AOAC Int* 97, 1012–1020. <https://doi.org/10.5740/jaoacint.SGETuzimski>
- Vaccher, V., Ingenbleek, L., Adegboye, A., Hossou, S.E., Koné, A.Z., Oyedele, A.D., Kisito, C.S.K.J., Dembélé, Y.K., Hu, R., Abdel Malak, I., Cariou, R., Vénisseau, A., Veyrand, B., Marchand, P., Eyangoh, S., Verger, P., Dervilly-Pinel, G., Leblanc, J.-C., Le Bizet, B., 2020. Levels of persistent organic pollutants (POPs) in foods from the first regional Sub-Saharan Africa Total Diet Study. *Environment International* 135, 105413. <https://doi.org/10.1016/j.envint.2019.105413>
- Van der Wat, L., Forbes, P.B.C., 2015. Lichens as biomonitors for organic air pollutants. *TrAC Trends in Analytical Chemistry* 64, 165–172. <https://doi.org/10.1016/j.trac.2014.09.006>
- Vandeyck, T., Keramidis, K., Kitous, A., Spadaro, J.V., Van Dingenen, R., Holland, M., Saveyn, B., 2018. Air quality co-benefits for human health and agriculture counterbalance costs to meet Paris Agreement pledges. *Nat Commun* 9. <https://doi.org/10.1038/s41467-018-06885-9>
- Vannini, A., Guarnieri, M., Paoli, L., Sorbo, S., Basile, A., Loppi, S., 2016. Bioaccumulation, physiological and ultrastructural effects of glyphosate in the lichen *Xanthoria parietina* (L.) Th. Fr. *Chemosphere* 164, 233–240. <https://doi.org/10.1016/j.chemosphere.2016.08.058>
- Varga, T., Sajtos, Z., Gajdos, Z., Jull, A.J.T., Molnár, M., Baranyai, E., 2020. Honey as an indicator of long-term environmental changes: MP-AES analysis coupled with 14C-based age determination of Hungarian honey samples. *Science of The Total Environment* 736, 139686. <https://doi.org/10.1016/j.scitotenv.2020.139686>
- Villalba, A., Maggi, M., Ondarza, P.M., Szawarski, N., Miglioranza, K.S.B., 2020a. Influence of land use on chlorpyrifos and persistent organic pollutant levels in honey bees, bee bread and honey: Beehive exposure assessment. *Science of The Total Environment* 713, 136554. <https://doi.org/10.1016/j.scitotenv.2020.136554>
- Villalba, A., Maggi, M., Ondarza, P.M., Szawarski, N., Miglioranza, K.S.B., 2020b. Influence of land use on chlorpyrifos and persistent organic pollutant levels in honey bees, bee bread and honey: Beehive exposure assessment. *Science of The Total Environment* 713, 136554. <https://doi.org/10.1016/j.scitotenv.2020.136554>
- Visha, A., Gandhi, N., Bhavsar, S.P., Arhonditsis, G.B., 2018. A Bayesian assessment of polychlorinated biphenyl contamination of fish communities in the Laurentian Great Lakes. *Chemosphere* 210, 1193–1206. <https://doi.org/10.1016/j.chemosphere.2018.07.070>
- Vitali, M., Antonucci, A., Owczarek, M., Guidotti, M., Astolfi, M.L., Manigrasso, M., Avino, P., Bhattacharya, B., Protano, C., 2019. Air quality assessment in different environmental scenarios by the determination of typical heavy metals and Persistent Organic Pollutants in native lichen *Xanthoria parietina*. *Environmental Pollution* 254, 113013. <https://doi.org/10.1016/j.envpol.2019.113013>
- Vizcaino, E., Grimalt, J.O., Fernández-Somoano, A., Tardon, A., 2014. Transport of persistent organic pollutants across the human placenta. *Environment International* 65, 107–115. <https://doi.org/10.1016/j.envint.2014.01.004>
- Vukavić, T., Vojinović Miloradov, M., Mihajlović, I., Ristivojević, A., 2013. Human milk POPs and neonatal risk trend from 1982 to 2009 in the same geographic region in Serbia. *Environment International* 54, 45–49. <https://doi.org/10.1016/j.envint.2013.01.008>
- Wahlang, B., Hardesty, J.E., Jin, J., Falkner, K.C., Cave, M.C., 2019. Polychlorinated biphenyls and nonalcoholic fatty liver disease. *Current Opinion in Toxicology* 14, 21–28. <https://doi.org/10.1016/j.cotox.2019.06.001>
- Waked, A., Afif, C., Seigneur, C., 2012a. An atmospheric emission inventory of anthropogenic and biogenic sources for Lebanon. *Atmospheric Environment* 50, 88–96. <https://doi.org/10.1016/j.atmosenv.2011.12.058>
- Waked, A., Seigneur, C., Couvidat, F., Kim, Y., Sartelet, K., Afif, C., Borbon, A., Formenti, P., Sauvage, S., 2012b. Modeling air pollution in Lebanon: evaluation at a suburban site in Beirut. *Atmospheric Chemistry and Physics Discussions* 12. <https://doi.org/10.5194/acpd-12-29571-2012>
- Wang, J., Xu, J., Ji, X., Wu, H., Yang, H., Zhang, H., Zhang, X., Li, Z., Ni, X., Qian, M., 2020. Determination of veterinary drug/pesticide residues in livestock and poultry excrement using selective accelerated solvent extraction and magnetic material purification combined with ultra-high-performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A* 1617, 460808. <https://doi.org/10.1016/j.chroma.2019.460808>

- Wang, L., Wang, X., Zhou, J.-B., Zhao, R.-S., 2016. Carbon nanotube sponges as a solid-phase extraction adsorbent for the enrichment and determination of polychlorinated biphenyls at trace levels in environmental water samples. *Talanta* 160, 79–85. <https://doi.org/10.1016/j.talanta.2016.07.005>
- Wang, S., Li, M., Li, Xiuqin, Li, Xianjiang, Li, Xiaomin, Li, S., Zhang, Q., Li, H., 2020. A functionalized carbon nanotube nanohybrids-based QuEChERS method for detection of pesticide residues in vegetables and fruits. *Journal of Chromatography A* 1631, 461526. <https://doi.org/10.1016/j.chroma.2020.461526>
- Wang, S., Qi, P., Di, S., Wang, J., Wu, S., Wang, Xiangyun, Wang, Z., Wang, Q., Wang, Xinquan, Zhao, C., Li, Q., 2019. Significant role of supercritical fluid chromatography - mass spectrometry in improving the matrix effect and analytical efficiency during multi-pesticides residue analysis of complex chrysanthemum samples. *Analytica Chimica Acta* 1074, 108–116. <https://doi.org/10.1016/j.aca.2019.04.063>
- Wang, X., Jia, R., Song, Y., Wang, M., Zhao, Q., Sun, S., 2019. Determination of pesticides and their degradation products in water samples by solid-phase extraction coupled with liquid chromatography-mass spectrometry. *Microchemical Journal* 149, 104013. <https://doi.org/10.1016/j.microc.2019.104013>
- Wei, C., Bandowe, B.A.M., Han, Y., Cao, J., Zhan, C., Wilcke, W., 2015. Polycyclic aromatic hydrocarbons (PAHs) and their derivatives (alkyl-PAHs, oxygenated-PAHs, nitrated-PAHs and azaarenes) in urban road dusts from Xi'an, Central China. *Chemosphere* 134, 512–520. <https://doi.org/10.1016/j.chemosphere.2014.11.052>
- WHO | Ambient air pollution: Health impacts. <http://www.who.int/airpollution/ambient/health-impacts/en/>
- WHO | Persistent organic pollutants (POPs). https://www.who.int/foodsafety/areas_work/chemical-risks/pops/en/
- WHO | World Health Statistics 2019: Monitoring health for the SDGs. http://www.who.int/gho/publications/world_health_statistics/2019/en/
- Wilm, M., 2011. Principles of Electrospray Ionization. *Molecular & Cellular Proteomics* 10. <https://doi.org/10.1074/mcp.M111.009407>
- Wolf, K., Bongaerts, B.W.C., Schneider, Alexandra, Huth, C., Meisinger, C., Peters, A., Schneider, Andrea, Wittsiepe, J., Schramm, K.-W., Greiser, K.H., Hartwig, S., Kluttig, A., Rathmann, W., 2019. Persistent organic pollutants and the incidence of type 2 diabetes in the CARLA and KORA cohort studies. *Environment International* 129, 221–228. <https://doi.org/10.1016/j.envint.2019.05.030>
- Wood, T.J., Goulson, D., 2017. The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. *Environ Sci Pollut Res Int* 24, 17285–17325. <https://doi.org/10.1007/s11356-017-9240-x>
- Wu, J., Hu, J., Wang, S., Jin, JingXi, Wang, R., Wang, Y., Jin, Jun, 2018. Levels, sources, and potential human health risks of PCNs, PCDD/Fs, and PCBs in an industrial area of Shandong Province, China. *Chemosphere* 199, 382–389. <https://doi.org/10.1016/j.chemosphere.2018.02.039>
- Wu, J.-P., Chen, X.-Y., Wu, S.-K., Tao, L., She, Y.-Z., Luo, X.-J., Mai, B.-X., 2019. Polychlorinated biphenyls in apple snails from an abandoned e-waste recycling site, 2010–2016: A temporal snapshot after the regulatory efforts and the bioaccumulation characteristics. *Science of The Total Environment* 650, 779–785. <https://doi.org/10.1016/j.scitotenv.2018.09.074>
- Wu, M., Wang, L., Zeng, B., Zhao, F., 2016. Ionic liquid polymer functionalized carbon nanotubes-doped poly(3,4-ethylenedioxythiophene) for highly-efficient solid-phase microextraction of carbamate pesticides. *Journal of Chromatography A* 1444, 42–49. <https://doi.org/10.1016/j.chroma.2016.03.074>
- Wurita, A., Hasegawa, K., Nozawa, H., Yamagishi, I., Minakata, K., Watanabe, K., Suzuki, O., 2020. Postmortem distribution/redistribution of bufonin in body fluids and solid tissues in an autopsy case using liquid chromatography-tandem mass spectrometry with QuEChERS extraction method. *Forensic Science International* 314, 110376. <https://doi.org/10.1016/j.forsciint.2020.110376>
- Xu, Q., Zhu, X., Henkelmann, B., Schramm, K.-W., Chen, J., Ni, Y., Wang, W., Pfister, G., Mu, J., Qin, S., Li, Y., 2013. Simultaneous monitoring of PCB profiles in the urban air of Dalian, China with active and passive samplings. *Journal of Environmental Sciences* 25, 133–143. [https://doi.org/10.1016/S1001-0742\(12\)60030-8](https://doi.org/10.1016/S1001-0742(12)60030-8)
- Xu, X., Liu, J., Huang, C., Lu, F., Chiung, Y.M., Huo, X., 2015. Association of polycyclic aromatic hydrocarbons (PAHs) and lead co-exposure with child physical growth and development in an e-waste recycling town. *Chemosphere* 139, 295–302. <https://doi.org/10.1016/j.chemosphere.2015.05.080>
- Xun, E., Zhang, Y., Zhao, J., Guo, J., 2018. Heavy metals in nectar modify behaviors of pollinators and nectar robbers: Consequences for plant fitness. *Environmental Pollution* 242, 1166–1175. <https://doi.org/10.1016/j.envpol.2018.07.128>
- Yadav, I.C., Devi, N.L., Syed, J.H., Cheng, Z., Li, J., Zhang, G., Jones, K.C., 2015. Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: A comprehensive review of India. *Science of The Total Environment* 511, 123–137. <https://doi.org/10.1016/j.scitotenv.2014.12.041>
- Yao, T., He, C., Zhang, P., Gao, H., Zhou, C., 2013. Distribution and Sources of Polychlorinated Biphenyls (PCBs) and Organochlorine Pesticides (OCPs) in Surface Waters of Jinzhou Bay in China. *Procedia Environmental Sciences* 18, 317–322. <https://doi.org/10.1016/j.proenv.2013.04.041>
- Ye, M., Beach, J., Martin, J.W., Senthilselvan, A., 2013. Occupational Pesticide Exposures and Respiratory Health. *Int J Environ Res Public Health* 10, 6442–6471. <https://doi.org/10.3390/ijerph10126442>
- Yetim, N.K., Hasanoğlu Özkan, E., Özcan, C., Sarı, N., 2020. Preparation of AChE immobilized microspheres containing thiophene and furan for the determination of pesticides by the HPLC-DAD method. *Journal of Molecular Structure* 1222, 128931. <https://doi.org/10.1016/j.molstruc.2020.128931>
- Yin, H., Cai, Y., Duan, H., Gao, J., Fan, C., 2014. Use of DGT and conventional methods to predict sediment metal bioavailability to a field inhabitant freshwater snail (*Bellamya aeruginosa*) from Chinese eutrophic lakes. *Journal of Hazardous Materials* 264, 184–194. <https://doi.org/10.1016/j.jhazmat.2013.11.030>
- Yin, L., Hu, Q., Mondal, S., Xu, J., Ouyang, G., 2019. Peanut shell-derived biochar materials for effective solid-phase microextraction of polycyclic aromatic hydrocarbons in environmental waters. *Talanta* 202, 90–95. <https://doi.org/10.1016/j.talanta.2019.04.020>
- Yin, P., Brauer, M., Cohen, A.J., Wang, H., Li, J., Burnett, R.T., Stanaway, J.D., Causey, K., Larson, S., Godwin, W., Frostad, J., Marks, A., Wang, L., Zhou, M., Murray, C.J.L., 2020. The effect of air pollution on deaths, disease burden, and life expectancy across China and its provinces, 1990–2017: an analysis for the Global Burden of Disease Study 2017. *The Lancet Planetary Health* 4, e386–e398. [https://doi.org/10.1016/S2542-5196\(20\)30161-3](https://doi.org/10.1016/S2542-5196(20)30161-3)
- Youssef, L., Younes, G., Kouzayha, A., Jaber, F., 2015. Occurrence and levels of pesticides in South Lebanon water. *Chemical Speciation & Bioavailability* 27, 62–70. <https://doi.org/10.1080/09542299.2015.1023092>
- Yu, S., Zhu, B., Lv, F., Li, S., Huang, W., 2012. Rapid analysis of cyclamate in foods and beverages by gas chromatography-electron capture detector (GC-ECD). *Food Chemistry* 134, 2424–2429. <https://doi.org/10.1016/j.foodchem.2012.04.028>

- Yuan, C., Liu, L., Ye, J., Ren, G., Zhuo, D., Qi, X., 2017. Assessing the effects of rural livelihood transition on non-point source pollution: a coupled ABM-IECM model. *Environ Sci Pollut Res* 24, 12899–12917. <https://doi.org/10.1007/s11356-017-8812-0>
- Yue, M.-E., Yu, K., Lin, Q., Xu, J., Jiang, T.-F., 2019. Determination of N-acylhomoserine lactones from spoilage bacteria and aquatic product by integrative coupling method of solvent-terminated dispersive liquid-liquid microextraction and micellar electrokinetic capillary chromatography. *Microchemical Journal* 146, 1263–1268. <https://doi.org/10.1016/j.microc.2019.02.062>
- Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D., Sylvestre, S., 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Organic Geochemistry* 33, 489–515. [https://doi.org/10.1016/S0146-6380\(02\)00002-5](https://doi.org/10.1016/S0146-6380(02)00002-5)
- Zahiri, E., Khandaghi, J., Farajzadeh, M.A., Afshar Mogaddam, M.R., 2020. Combination of dispersive solid phase extraction with solidification organic drop-dispersive liquid-liquid microextraction based on deep eutectic solvent for extraction of organophosphorous pesticides from edible oil samples. *Journal of Chromatography A* 1627, 461390. <https://doi.org/10.1016/j.chroma.2020.461390>
- Zeng, S., Gan, N., Weideman-Mera, R., Cao, Y., Li, T., Sang, W., 2013. Enrichment of polychlorinated biphenyl 28 from aqueous solutions using Fe₃O₄ grafted graphene oxide. *Chemical Engineering Journal* 218, 108–115. <https://doi.org/10.1016/j.cej.2012.12.030>
- Zhang, N., Su, L., Man, S., Lei, X., Huang, T., Zhu, C., Zhang, L., Wu, X., 2019. Task-specific solid-phase microextraction based on ionic liquid/polyhedral oligomeric silsesquioxane hybrid coating for sensitive analysis of polycyclic aromatic hydrocarbons by gas chromatography-mass spectrometry. *Journal of Chromatography A* 1598, 49–57. <https://doi.org/10.1016/j.chroma.2019.03.062>
- Zhang, Q.-H., Zhou, L.-D., Chen, H., Wang, C.-Z., Xia, Z.-N., Yuan, C.-S., 2016. Solid-phase microextraction technology for in vitro and in vivo metabolite analysis. *TrAC Trends in Analytical Chemistry* 80, 57–65. <https://doi.org/10.1016/j.trac.2016.02.017>
- Zhang, S., Yang, Q., Yang, X., Wang, W., Li, Z., Zhang, L., Wang, C., Wang, Z., 2017. A zeolitic imidazolate framework based nanoporous carbon as a novel fiber coating for solid-phase microextraction of pyrethroid pesticides. *Talanta* 166, 46–53. <https://doi.org/10.1016/j.talanta.2017.01.042>
- Zhang, W., Zhang, S., Wan, C., Yue, D., Ye, Y., Wang, X., 2008. Source diagnostics of polycyclic aromatic hydrocarbons in urban road runoff, dust, rain and canopy throughfall. *Environmental Pollution* 153, 594–601. <https://doi.org/10.1016/j.envpol.2007.09.004>
- Zhang, Y., Zhang, X., Jiao, B., 2014. Determination of ten pyrethroids in various fruit juices: Comparison of dispersive liquid-liquid microextraction sample preparation and QuEChERS method combined with dispersive liquid-liquid microextraction. *Food Chemistry* 159, 367–373. <https://doi.org/10.1016/j.foodchem.2014.03.028>
- Zhao, L., Szakas, T., Churley, M., Lucas, D., 2019. Multi-class multi-residue analysis of pesticides in edible oils by gas chromatography-tandem mass spectrometry using liquid-liquid extraction and enhanced matrix removal lipid cartridge cleanup. *Journal of Chromatography A* 1584, 1–12. <https://doi.org/10.1016/j.chroma.2018.11.022>
- Zhao, T., Guan, X., Tang, W., Ma, Y., Zhang, H., 2015. Preparation of temperature sensitive molecularly imprinted polymer for solid-phase microextraction coatings on stainless steel fiber to measure ofloxacin. *Analytica Chimica Acta* 853, 668–675. <https://doi.org/10.1016/j.aca.2014.10.019>
- Zheng, H.-B., Ding, J., Zheng, S.-J., Zhu, G.-T., Yuan, B.-F., Feng, Y.-Q., 2016. Facile synthesis of magnetic carbon nitride nanosheets and its application in magnetic solid phase extraction for polycyclic aromatic hydrocarbons in edible oil samples. *Talanta* 148, 46–53. <https://doi.org/10.1016/j.talanta.2015.10.059>
- Zheng, W., Park, J.-A., Abd El-Aty, A.M., Kim, S.-K., Cho, S.-H., Choi, J., Yi, H., Cho, S.-M., Ramadan, A., Jeong, J.H., Shim, J.-H., Shin, H.-C., 2018. Development and validation of modified QuEChERS method coupled with LC-MS/MS for simultaneous determination of cymiazole, fipronil, coumaphos, fluvalinate, amitraz, and its metabolite in various types of honey and royal jelly. *Journal of Chromatography B* 1072, 60–69. <https://doi.org/10.1016/j.jchromb.2017.11.011>
- Zhou, Q.-X., Gao, Y.-Y., 2014. Combination of ionic liquid dispersive liquid-phase microextraction and high performance liquid chromatography for the determination of triazine herbicides in water samples. *Chinese Chemical Letters* 25, 745–748. <https://doi.org/10.1016/j.cclet.2014.01.026>
- Zhou, X., Wu, B., Jin, Q., 2018. Analysis of User Network and Correlation for Community Discovery Based on Topic-Aware Similarity and Behavioral Influence. *IEEE Transactions on Human-Machine Systems* 48, 559–571. <https://doi.org/10.1109/THMS.2017.2725341>
- Zhu, J., Wang, L., Xiao, Z., Niu, Y., 2018. Characterization of the key aroma compounds in mulberry fruits by application of gas chromatography-olfactometry (GC-O), odor activity value (OAV), gas chromatography-mass spectrometry (GC-MS) and flame photometric detection (FPD). *Food Chemistry* 245, 775–785. <https://doi.org/10.1016/j.foodchem.2017.11.112>
- Zhu, Z., Zhang, Y., Wang, J., Li, X., Wang, W., Huang, Z., 2019. Sugaring-out assisted liquid-liquid extraction coupled with high performance liquid chromatography-electrochemical detection for the determination of 17 phenolic compounds in honey. *Journal of Chromatography A* 1601, 104–114. <https://doi.org/10.1016/j.chroma.2019.06.023>
- Zięba, K., Szostak, E., Czekońska, K., Miśkowiec, P., Moos-Matysik, A., Nyczyk-Malinowska, A., Szentgyörgyi, H., 2020. Usefulness of bee bread and capped brood for the assessment of monocyclic aromatic hydrocarbon levels in the environment. *Environmental Pollution* 265, 114882. <https://doi.org/10.1016/j.envpol.2020.114882>
- Zioga, E., Kelly, R., White, B., Stout, J.C., 2020. Plant protection product residues in plant pollen and nectar: A review of current knowledge. *Environmental Research* 189, 109873. <https://doi.org/10.1016/j.envres.2020.109873>
- Zuhara, S., Isaifan*, R., 2018. The Impact of Criteria Air Pollutants on Soil and Water: A Review. *Journal of Environmental Science and Pollution Research* 278–284. <https://doi.org/10.30799/jespr.133.18040205>

Chapitre II : Matériels et méthodes & résultats

I. Snail as sentinel organism for monitoring the environmental pollution; a review

Résumé

Cette revue scientifique publiée dans le journal « *Ecological Indicators* » présente un apport bibliographique sur l'usage des escargots en tant que biomoniteur de la pollution de l'air, les polluants couramment détectés ainsi que les méthodes permettant l'extraction et l'analyse de ces polluants.

Les animaux, comme les escargots, utilisés comme biomoniteurs de la pollution environnementale, présentent de multiples mécanismes physiologiques pour contrer les effets des toxines dans l'environnement en raison de leur sensibilité à divers contaminants. Ces espèces occupent une place importante dans l'interface sol - air - végétation du milieu et se trouvent en contact avec de nombreuses sources de pollution (sol, atmosphère, végétaux) à travers les voies digestive, respiratoire et / ou cutanée.

L'intérêt de cette revue était d'explorer la possibilité d'utiliser différents types d'escargots terrestres (*Helix aspersa*, *Cepaea nemoralis*, *Theba pisana* et *Eobania vermiculata*), en raison de leur échantillonnage facile, leur large distribution et leur capacité à accumuler divers contaminants dans leurs tissus. Ces matrices sont étudiées comme biomoniteurs potentielles et idéales pour évaluer la pollution de l'air et de détecter la concentration des métaux lourds, des pesticides, des composés organochlorés (PCBs et OCPs), des diphényléthers polybromés, des HAPs ainsi que le glufosinate et glyphosate persistants en utilisant différentes techniques d'extraction, notamment l'extraction soxhlet, l'ASE, la SPE, la MAE et l'extraction d'eau chaude sous pression.

L'étude de l'impact des métaux et autres polluants sur la physiologie des organismes contribue au développement de nombreuses études toxiques qui peuvent être utilisées comme outil d'évaluation environnementale. Leur utilisation dans les essais biologiques de toxicité est un processus efficace car les escargots sont faciles à adapter et à manipuler en laboratoire et ils peuvent être traités avec les quantités souhaitées de contaminants sur des régimes spécifiques et réagir rapidement à la contamination par les polluants.

Pour toutes ces raisons, l'utilisation d'escargots comme matrice est fortement recommandée afin de surveiller une large classe de polluants environnementaux qui pourraient être présents dans l'air.

Highlights

- Studies regarding the use of snails as biomonitors for environmental pollution were reviewed and discussed.
- Snails were shown to be efficient biomonitor species for environmental pollution by organic pollutants and heavy metals.
- The ability of snails to bioaccumulate heavy metals and organic pollutants in their different organs are reviewed.

ABSTRACT

Environmental pollution, one of the most serious problems facing human health, ecosystems and biodiversity, is defined as the contamination of the physical and biological components of the atmosphere system which has harmful consequences for normal environmental processes. Animals, such as snails used as environmental pollution biomonitors, show multiple physiological mechanisms to counteract the effects of toxins in the environment due to their sensitivity to various contaminants and their ability to accumulate them through their tissues. The objective of this review is to explore the possibility of using different types of snails as potential and ideal monitoring matrices to assess air pollution and to detect heavy metal and POPs concentration by different extraction techniques including Soxhlet extraction, Accelerated Solvent Extraction, Solid Phase Extraction, Microwave-Assisted Extraction, Pressurized Hot Water extraction and Microwave Acid Digestion.

Keywords: Air pollution; Bioindicator; Snails; Metallic trace elements; Organic pollutants.

1. Introduction

The World Health Organization (WHO) remains strictly bound by the principles set out in the constitution preface that described health as “a complete state of physical, mental and social well-being and not just the absence of disease or infirmity” (Ortmann et al., 2016). Several factors, such as demographic, socio-economic, environmental geographic, climatic and meteorological factors, affect directly or indirectly the health of individuals and populations. In fact, the contribution of all these factors is of paramount importance on health sector (Ebi et al., 2017).

Various human activities, industrial and other anthropogenic waste, concentrated around the world, exceed the critical levels set by the European Union and other environmental governments leading to serious problems (Guerreiro et al., 2014). Indeed, air pollution's exposure is characterized by a person's exposure to pollutant concentration over a time period that depends on the concentrations of air pollutants present at the sites through which the person moves as well as the time spent at each site causing respiratory, cardiovascular and cancer diseases problems at excessive levels (Reames and Bravo, 2019, Lee et al., 2014, Le Tertre et al., 2002, Stieb et al., 2002). The existence of a pollutant in the soil is therefore not a direct risk, but the danger occurs as soon as this pollutant can be activated and acts on the atmosphere or on humans (Tchounwou et al., 2012). By definition, a complete risk model, consisting of five components (sources, transport, exposure, dose and effect), is used as a method of adjusting the concentration of a toxic pollutant to estimate the variance in health risk (Laumbach et al., 2015, Muralikrishna and Manickam, 2017).

Therefore, monitoring and predicting occurring changes in the environment as well as the effects of air pollution is critical. Such monitoring stations evaluate long-term environmental changes but are complicated to implement and maintain. Thus, biomonitoring can provide alternative method for assessing air pollution (Marshall et al., 2019). In recent years, the expansion of passive samplers has been one of the significant developments in air sampling technology. Palmes and Gunnison first applied air sampling to the safety and health field in 1973 (Palmes and Gunnison, 1973). As the applications of this technology have developed and changed recently, the number and different kinds of

passive samplers are increasing rapidly and become a crucial element in the range of air sampling equipment (Amato et al., 2018, Osytek et al., 2008).

Among the very well-known passive samplers, figure the biomonitors. In fact, a biomonitor, described as a species of animals, plants or fungi providing information on the quality of the environment in which it resides, is usually characterized by its sedentary lifestyle, wide distribution and ease of identification and collection (Parmar et al., 2016, Kovalchuk and Kovalchuk, 2008). Invertebrate species, such as snails are recognized as appropriate biological indicators due to their potential accumulation of persistent organic pollutants (POPs) and metallic trace elements and are also used as a research species to evaluate the effect of these pollutants on their development (Cossi et al., 2018, Krupnova et al., 2018, Hodgkinson and Jackson, 2005) (Fig. 1).

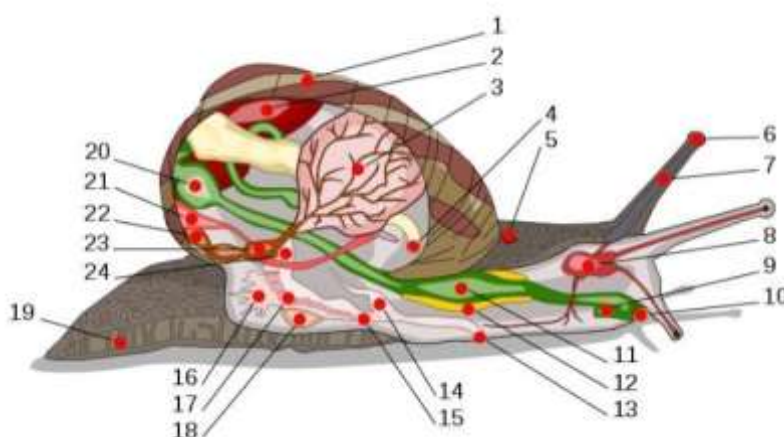


Fig. 1. Sectional diagram of the anatomy of a snail (Winkelmann A, 2007). 1: Shell. 2: Liver. 3: Lung. 4: Anus. 5: Respiratory pore. 6: Eye. 7: Tentacle. 8: Brain. 9: Salivary duct. 10: Mouth. 11: Panse. 12: Salivary gland. 13: Genital opening. 14: Penis. 15: Vagina. 16: mucous gland. 17: Oviduct. 18: Bag of darts. 19: Foot. 20: Stomach. 21: Kidney. 22: Coat. 23: Heart. 24: vas deferens.

For instance, terrestrial snails (*Helix aspersa*, *Cepaea nemoralis*, *Theba pisana* and *Eobania vermiculata*) are excellent biomonitors of environmental contamination due to their easy sampling, wide distribution, high tolerance to stress and their ability to accumulate diverse contaminants in the air, soil and flora (Soltani et al., 2013, Itziou and Dimitriadis, 2011, De Vaufleury and Pihan, 2000).

The purpose of this study is to assess the feasibility to use naturally distributed snails to control the concentration of contaminants throughout the ecosystem. The theory is that snails feeding on species that are directly exposed to dry and wet pollutant deposition and

inhaling major urban air pollutants can be an important biomonitor sentinel for determining pollutants and heavy metals' spatial distribution and bioavailability. This review sheds light on some parameters including production of nitric oxide, phenol oxidase, and lysozymes that are associated with the immune response that is resulting from the interaction between the snail and the particles and organisms. The quality of the growth of some snails gives indices of degree of soil pollution by pesticides or some metal trace elements. It allows for example the evaluation of the bioassimilable chromium content of a soil for example or of organophosphorus pesticides or even to study the bioaccumulation of heavy metals.

2. Snails as atmospheric pollution biomonitoring tools

Representative species of the environment must be studied to assess the effects of pollutants in a matrix. In fact, a biomonitor can be identified as an organism or group of organisms that are important for qualitative and quantitative environmental pollution determination (Fränzle, 2006). Many studies have shown that gastropod mollusks including terrestrial snails are pollution bioindicators, including metals, pesticides, PAHs, PCBs and PBDEs (Beeby and Richmond, 2002, Fu et al., 2011, De Vaufleury and Pihan, 2000, Silva et al., 2019). Such species occupy an important place in the soil-air-vegetation interface of the environment and incorporate many pollution sources (soil, atmosphere, plants) through the digestive, respiratory and/or cutaneous tracts. Multiple species of snails have been recorded, for example *Papillifera papillaris* (Emilia et al., 2016), *Indothais gradata* (Proum et al., 2016), *Pomacea canaliculata* (Ramli et al., 2019, Dummee et al., 2012), *Cantareus apertus* (Mleiki et al., 2016), *Helix aspersa* (Viard et al., 2004, Abdel-Halim et al., 2013), *Eobania vermiculata* (El-Shenawy et al., 2012, Itziou and Dimitriadis, 2011, Itziou et al., 2011), *Cepaea nemoralis* (Boshoff et al., 2015), *Bellamya aeruginosa* (Yin et al., 2014), *Theodoxus niloticus* (Abdel Gawad, 2018) and *Achatina fulica* (Cho et al., 2019). Such snails colonize most environments and frequent the periphery of agricultural and forest areas. In contrast, several species have shown their ability to resist and accumulate contaminants (Notten et al., 2005, Radwan et al., 2010, Scheifler et al., 2002). For this reason, snails can be useful biomonitoring organisms commonly used as effective indicators in recent studies to determine the effects of pollutant mixtures on environments in space and time, based on the species collected (Cœurdassier et al., 2002, Druart et al., 2011, Gimbert et al., 2006).

2.1. Characteristics of snails

Snails have long been used to study pollutant accumulation. The assessment of the accumulation of contaminants such as metals is dependent on the assessment of internal concentrations after a specified exposure period and authorize the possibility to assess the accumulation capacity of snails, their bioavailability and the intensity of the transfer of contaminants from the environment (food and / or soil) (Gimbert et al., 2006). Invertebrate species accumulate pollutants as a consequence of absorption-assimilation, storage, transmission and excretion processes (Regoli et al., 2006). Once pollutants are absorbed, terrestrial snails have a non-regulation strategy that is influenced by calcium metabolism (Notten et al., 2005), and the need to prevent excessive water losses (Dallinger et al., 2001). In fact, there is two great parts to be considered: the foot and the viscera. The viscera refer to the shell's organs and includes the kidney, hepatopancreas, heart and part of the genital system that also extends into the foot. The foot essentially comprises the anterior part of the digestive tract and the nervous system. There is a dose-dependent increase in the concentration of pollutants in both organs analyzed (hepatopancreas and kidney) and their accumulation is related to their organism's bioavailability and environmental concentrations (Cœurdassier et al., 2002). Due to the open circulatory system of snails, there is no distinct difference between the blood and the lymph. As a consequence, the circulatory fluid is generally known to as a haemolymph, instead of blood. The organic and inorganic composition of the haemolymph is variable, and factors such as temperature, photoperiod, activity, hibernation, hydration and feeding, affect the composition of the haemolymph. However, shell size and age, influences the haemolymph pollutants accumulation (Pagano et al., 2017). Moreover, Itziou et al., in 2011 showed the use of land snails as biomarkers for early detection of organic pollutants in the environment, since significant alterations have been reported in haemolymph and digestive gland of snails exposed to organic contaminants (Itziou et al., 2011).

For instance, a high accumulation capacity of heavy metals, particularly of Cu, Zn, Cd and Pb, was shown in gastropod mollusks in 2004, as indicated by Viard et al. (Viard et al., 2004). Additionally, particularly in the Mediterranean and oceanic regions, snails are widespread, and they colonize anthropogenic environments and are also found in woods, rocks and areas of agriculture. They mainly feed on plants, lichens and fungi and adapt to

the plants that colonize the ecosystem (Chevalier et al., 2001, Guiller et al., 2012). Therefore, the soil is also a part of the diet of snails and can affect their growth, so a low calcium soil could be a restricting factor in the development of snails (Dallinger et al., 2001). In addition, snails exposed to terrestrial environment, are subjects to frequent and seasonal fluctuations in temperature. In order to resist to these fluctuations such as heat and aridity over long periods of estivation, snails are able to form an epiphragm by their dried mucous. Such snails normally resume activities in the early spring when temperatures are around 12–14 °C (Arad and Heller, 2009, Eugene Havel et al., 2014, Gaitán-Espitia et al., 2013).

In common with other invertebrates, mollusks are known by their innate immunity composed of both cellular and humoral elements consider as the first line of defense that reflects the immune response against foreign particles and organisms by the phagocytic cells, anatomic barriers and physiological components. Nitric oxide, phenyloxidase system, lysozyme activity and lectins are the humoral components, while the circulating phagocytic hemocytes are the cellular components of snail immunity. Small invaders are eliminated by phagocytic hemocytes, while large invaders are eliminated by encapsulation. The pathogens and foreign invaders are then hemolyzed by the action of certain toxic enzymes that catalyze oxidative burst reactions (Wang et al., 2018).

2.2. Snails, matrix used for biomonitoring

Owing to their wide distribution, snails can be used to evaluate soil contaminant bioavailability by measuring soil-snail or soil-plant-snail transmission and to analyze the bioavailability of environmental pollutants (soil, plants air) by measuring their concentration in caged snails during a specified time period. Snails may reflect the quality of their atmosphere by accumulating some toxic pollutants present in their biotope in their twist or visceral mass and shell. Their mucus protects them from external threats and from the infections of bacteria and fungi (De Vaufleury and Gimbert, 2009).

Ecotoxicology is important for assessing the value of ecosystems and biomes: it is particularly interesting in interactions between environmental chemicals and biotope, covering several areas including bioindicator development. The snails can be easily collected, raised, identified and found nearly anywhere. In addition, snails are in contact

with different pollutants that they consume transcutaneous, digestively and respiratory from water, plants and contact with the ground soil (De Vaufleury and Pihan, 2000).

The existence of pollutants has shown that snails are more likely to accumulate in the viscera than in the foot, which indicates the appropriateness of the viscera to expose the pollutant bioavailability in the ecosystem and illustrating the utility of separately analyzing foot and viscera (De Vaufleury and Pihan, 2000). Bioaccumulation of pollutant in snails depends on the duration of exposure, which implies that wild snails are highly contaminated due to their lifetime exposure to environmental pollutants. The exposure period of several weeks reflects the harmful effects and demonstrates that bioaccumulation is modulated by pollution type compared to short exposures that may be sufficient to detect bioaccumulation (Eeva et al., 2010, Nica et al., 2013).

In fact, snails can accumulate in their shell toxic minerals and metals (Beeby and Richmond, 2011). Studying toxic accumulation in snails is allowed by modifying two parameters:

- Biological parameters expressed by the organ growth and weight
- Chemical parameters presented through the phase of bioaccumulation (Abdel-Halim et al., 2013, Gimbert et al., 2008).

Several snails are herbivorous with lungs that provide information on air, soil and flora quality. They absorb air through breathing to measure the atmospheric pollution and have the capacity to accumulate large quantities of pollutants in their body without any hurtful effects on their life cycle (De Vaufleury and Gimbert, 2009). Furthermore, snails have the particularity of concentrating the chemical substances present in the soil, the air and the plants of their environment in their tissues and can be used both as test organisms for the study the toxicity of metals and as bioindicators of terrestrial pollution (Druart et al., 2012, Kramarz et al., 2009). The pollution state of a soil observed by the analysis of what is accumulated in the gastropod organism, could be estimated as well as the amount of pollutants and their evaluation, such as pesticides likely to disperse in nature and contaminate living things (Scheifler et al., 2003, Gimbert et al., 2006).

2.3. Methods of pollutants extraction and analysis

Various snail species have been studied, such as *Achatina fulica* (Cho et al., 2019), *Indothais gradata* (Proum et al., 2016), *Helix aspersa* (Abdel-Halim et al., 2013, Viard et al., 2004), *Papillifera papillaris* (Emilia et al., 2016), *Eobania vermiculata* (El-Shenawy et al., 2012, Itziou and Dimitriadis, 2011), *Cantareus apertus* (Mleiki et al., 2016), and *Pomacea canaliculata* (Ramli et al., 2019, Wu et al., 2019, Dummee et al., 2012). Among these species, *Helix aspersa* was used to investigate fungicides (tebuconazole, folpet, pyraclostrobin and cymoxanil) and herbicides (glufosinate and glyphosate) contamination using fluorescence detection GC–MS and HPLC respectively (Druart et al., 2011) while analysis of polycyclic aromatic compounds was done using liquid chromatography coupled to a fluorimetric detector (Sverdrup et al., 2006).

Table 1. summarizes some snail matrices commonly used in studies on biomonitoring, the aim of the study and the target analytes and their extraction methods and analysis.

Table 1. Summary of the use of snails as biomonitors.

Matrix	Aim of the study	Analytes	Extraction and clean up	Analytical method	Ref.
<i>Helix aspersa</i>	Effects and uptake of polycyclic aromatic compounds <i>Helix aspersa</i>	Polycyclic aromatic compounds	Accelerated solvent extraction	Liquid chromatography coupled to a fluorimetric detector	(Sverdrup et al., 2006)
<i>Buccinum spp.</i> and <i>N. lyrata</i>	Analysis of pyrene metabolites in marine snails	Polycyclic aromatic hydrocarbons: pyrene	Solid phase extraction	Liquid chromatography using fluorescence and mass spectrometry detection	(Beach et al., 2009)
<i>Achatina fulica</i>	Optimization and characterization of polysaccharides extraction <i>Achatina fulica</i> using	Polysaccharides	Pressurized hot water extraction (PHWE)	-High-performance liquid chromatography (HPLC) -Gel permeation chromatography (GPC)	(Cho et al., 2019)

<i>Pomacea canaliculata</i>	Optimization of saponin extracts using microwave-assisted extraction as a sustainable biopesticide to reduce <i>Pomacea canaliculata</i> population in paddy cultivation	Saponin	Microwave-assisted extraction	-HPLC-UV -UV/Vis spectrophotometric	(Ramli et al., 2019)
<i>Ampullariidae</i>	Spatial distribution of polychlorinated biphenyls and polybrominated biphenyl ethers in China	-Polychlorinated biphenyls (PCBs) -Polybrominated biphenyl ethers (PBDEs)	Isotope dilution methods	Gas chromatograph coupled with high-resolution mass spectrometer	(Fu et al., 2011)
<i>Helix aspersa</i>	Measure the exposure, the transfer and the effects of pesticides on a non-target soil invertebrate, the land snail <i>Helix aspersa</i>	-Herbicides (glyphosate and glufosinate) -Fungicides (cymoxanil, folpet, tebuconazole and pyraclostrobin)	Liquid-liquid extraction	-Herbicides : HPLC -Fongicides : GC-MS	(Druart et al., 2011)
<i>Cepaea nemoralis</i>	Trace metal transfer in a soil-plant-snail microcosm field experiment and biomarker responses in snails	As, Ni, Pb and Zn	Digestion with a mixed solution of nitric acid and hydrochloric acid	Inductively Coupled Plasma Mass Spectrometer (ICP-MS)	(Boshoff et al., 2015)
<i>Hexaplex trunculus</i>	Levels of polychlorinated biphenyls in <i>Hexaplex trunculus</i>	Polychlorinated biphenyls	Soxhlet	Thermo Trace GC connected with a Thermo PolarisQ MS operated in electron impact ionization	(Storelli et al., 2014)
<i>Theodoxus niloticus</i>	Acute toxicity of some heavy metals to the snail, <i>Theodoxus niloticus</i>	Zn, Fe and Pb	Digestion with acidified water samples, HNO ₃ and perchloric acid	Atomic Absorption Reader	(Abdel Gawad, 2018)
<i>Cipangopaludina chinensis</i>	Effect of periphyton community structure on heavy metal accumulation <i>Cipangopaludina chinensis</i>	V, Cr, Co, Ni, Cu, Cd, and Pb	Microwave-Assisted Acid Digestion System	ICP-MS	(Cui et al., 2012)

<i>Pomacea canaliculata</i>	Bioaccumulation of heavy metals in water, sediments, aquatic plant and histopathological effects on the golden apple snail	Cu, Mn, Fe, Zn, Pb and Cd	Digestion with a mixed solution of nitric acid-perchloric acid and concentrated hydrochloric acid	Flame atomic absorption spectrophotometer	(Dumme et al., 2012)
<i>Eobania vermiculata</i>	Using the enzymatic and non-enzymatic antioxidant defense system of <i>Eobania vermiculata</i> as biomarkers of terrestrial heavy metal pollution	Cd, Cu, Fe, Ca, Pb and Zn	Digestion with concentrated nitric acid in a microwave digester using microwave digestion program	Inductively coupled plasma optical emission spectrometry (ICPOES)	(El-Shenawy et al., 2012)
<i>Papillifera papillaris</i>	<i>Papillifera papillaris</i> , as indicator of metal deposition and bioavailability in urban environments	Al, Cd, Cr, Ni, Pb, Hg, Cu, Fe, Mn and Zn	Digestion in Teflon containers with HNO ₃ and H ₂ O ₂	-Atomic absorption spectrometry with graphite furnace for Al, Cd, Cr, Ni and Pb. -Inductively coupled plasma emission spectrometry for Cu, Fe, Mn and Zn.	(Emilia et al., 2016)
<i>Cepaea nemoralis</i>	Associations between shell strength, shell morphology and heavy metals in <i>Cepaea nemoralis</i>	Ca, Cd, Cr, Pb and Zn	Digestion in nitric acid using the microwave digestion procedure	Inductively coupled plasma-atomic emission spectrophotometry (ICP-AES)	(Jordaens et al., 2006)
<i>Theba pisana</i>	Biomarkers of oxidative stress in <i>Theba pisana</i> for assessing ecotoxicological effects of urban metal pollution	Zn, Cu, Pb and Cd	Digestion in concentrated nitric acid and deionized water	Atomic absorption spectrophotometry (AAS)	(Radwan et al., 2010)
<i>Bellamya aeruginosa</i>	Use of DGT and conventional methods to predict sediment metal bioavailability to a field inhabitant freshwater snail	Cr, Ni, Cu, Zn, Cd and Pb	Digestion with concentrated ultrapure nitric acid in a microwave digestion system	ICP-MS	(Yin et al., 2014)

<i>Cantareus apertus</i>	Bioaccumulation and tissue distribution of Pb and Cd and growth effects in <i>Cantareus apertus</i> after dietary exposure to the metals alone and in combination	Pb and Cd	Digestion with cadmium chloride (CdCl ₂) and lead nitrate (PbNO ₃)	Inductively coupled plasma mass spectrometry (ICP-MS)	(Mleiki et al., 2016)
<i>Nassarius reticulatus</i>	Biomonitoring of metal contamination in <i>Nassarius reticulatus</i>	Cu, Zn, Cd, Hg and Pb	Digestion with HNO ₃ and H ₂ O ₂	Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)	(Santos et al., 2009)
<i>Ulva thalli</i>	Freshwater Ulva as a bioaccumulator of selected heavy metals and alkaline earth metals	Cd, Ni, Pb, Ca and Mg	Digestion with a mixture of HNO ₃ and H ₂ O ₂	Inductively coupled plasma Emission spectrometer	(Rybak et al., 2012)

As shown in Table 1, snails are widely used as environmental detectors for heavy metals, glufosinate, glyphosate, PAHs, PBDEs, PCBs and pesticides including OCPs. Several methods for extraction of organic pollutants and heavy metals from snails have been published in the literature (Wu et al., 2019). For instance, organic pollutants were mainly extracted by Accelerated Solvent Extraction (ASE) (Sverdrup et al., 2006), Solid Phase Extraction (SPE) (Beach et al., 2009), Pressurized Hot Water Extraction (PHWE) (Cho et al., 2019) and Microwave-Assisted Extraction (MAE) (Ramli et al., 2019). However, the extraction of heavy metals was especially done by digestion with a mixture of nitric acid (HNO₃), deionized water (H₂O₂), perchloric acid (HClO₄), chloride (CdCl₂), lead nitrate (PbNO₃) using microwave-assisted acid digestion technology (Abdel Gawad, 2018, Dummee et al., 2012, Emilia et al., 2016). However, a purification step may be required by simple centrifugation or filtration, SPE or chromatography technique.

Furthermore, there are many separation and detection techniques available for the study of emerging pollutants from such species such as liquid chromatography (LC) (Cho et al., 2019, Ramli et al., 2019, Druart et al., 2011, Beach et al., 2009, Sverdrup et al., 2006), Gas chromatography (GC) (Fu et al., 2011), Atomic Absorption Spectrophotometry (AAS) (Abdel Gawad, 2018, Emilia et al., 2016, Radwan et al., 2010) and Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Emilia et al., 2016, Boshoff et al., 2015, Cui et al.,

2012). Moreover, it was shown that LC was the main process of analyzing POPs in combination with a fluorometric detector to analyze polycyclic aromatic hydrocarbons (Sverdrup et al., 2006, Beach et al., 2009) and to mass spectrometry to analyze polychlorinated biphenyls (Storelli et al., 2014). However, GC-MS could also be used for the assessment of several organic pollutants in snails (Druart et al., 2011). On the other side, the AAS, ICP-AES and ICP-MS are the most widely used methods for the assessment of heavy metals in environmental samples (Sastre et al., 2002).

In addition, it has been confirmed that the apple snail "*Pomacea canaliculata*" is identified as bioindicators for many environmental pollutants, indicating both the level and the profile of pollutants, as well as for persistent organic pollutants (Harmon and Wiley, 2010, Fu et al., 2011) as for metals and organometallic compounds (Giraud-Billoud et al., 2018, Cueto et al., 2013, Campoy-Diaz et al., 2018). Their ecology and biology can be classified as having the main properties of an ideal indicator species to evaluate the pollution of the environment, such as the capacity for bioaccumulation, short lifetime, limited movement range, wide distribution and ease of collection compared to other animals like fish or birds (Tanabe and Subramanian, 2006, Gerlach et al., 2013).

3. Presence and detection of pollutants in snails

3.1. Snail pollution by metallic trace elements (MTE)

Metal trace elements (MTEs) are part of trace elements family and constitute only 0.6% of the total elements. Their denomination is due to their low concentration, which usually does not exceed 1000 mg kg^{-1} naturally in soils (Crémazy et al., 2019, Emilia et al., 2016). Among these MTEs, zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb) and chromium (Cr) are classified in the metal class while arsenic (As) and antimony (Sb) are among the metalloid group. Due to their harmful effects on the environment and their classification as carcinogenic or dangerous to human health, their presence in the environment and soil is a significant source of concern. However, three of these metals (mercury (Hg), cadmium (Cd) and lead (Pb)) are listed as priority dangerous substances in Decision 2455/2001/EC of the European Council (Cheng and Yap, 2015, Emilia et al., 2016).

Snails are well known for the bioaccumulation of these pollutants (Viard et al., 2004, Cœurdassier et al., 2002). In fact, it has been observed that about 68% of the

cadmium (Cd), 90% of the copper (Cu), 43% of the lead (Pb), and 60% of the zinc (Zn) ingested were accumulated in *Helix aspersa* snails' soft tissue, shells, and feces that were analyzed by flame atomic absorption spectrometry after mineralization and extraction using concentrated HNO₃. Snails seem to be more significant pathways for transport along the Cu and Cd food chains than Zn and Pb and are not able of depositing large amounts of metals into their shells. Resistance to the appearance of effects associated with the accumulation of metals by the snails may cause their predators to be contaminated (Laskowski and Hopkin, 1996). However, snails have many predators, including vertebrates, such as birds, small mammals, reptiles and invertebrates, such as carabids (Liew and Schilthuizen, 2014). Staikou and Lazaridou emphasize the role of snails in the transfer of material and energy from producers to higher trophic levels (Staikou and Lazaridou, 2013), suggesting their potential involvement in the transfer of metallic pollutants along trophic chains (Dar et al., 2019, Hispard et al., 2008). Human consumption of the *Helix aspersa* snail is also small (15 tons of canned food in France in 2008) relative to the Bourgogne *Helix pomatia* snail (876 tons of canned food in France in 2008) (Druart et al., 2011).

In addition, mosses absorb metals in deposition of wet and dry atmosphere via passive cation exchange mechanisms and the detection of airborne particles (Bargagli, 2016), while metal absorption may occur via various processes in snails, like inhalation, contact with the surfaces of the walls and ingestion of lichens, herbs, algae, soil particles and mosses. Metabolism and detoxification processes of cadmium (Cd), zinc (Zn), chromium (Cr), mercury (Hg), copper (Cu), and lead (Pb) are conducted in digestive gland, and their bioaccumulation is mainly due to the compartmentalization in the granules inside the cells or vesicles in the digestive gland or other tissues like foot (Boshoff et al., 2013, Regoli et al., 2006). Soft snail tissues purged of their intestine content have lower metals levels and their composition may provide an accurate image of emissions from environmental metals. For example, lead (Pb) concentrations in Italian urban areas can be detected in very high concentrations in street dust suspended by a vehicle, and even in low concentrations that have relatively small impact on the chains of foods (Barca et al., 2014).

Moreover, the adsorption and precipitation of trace metals increase under alkaline soils. A higher percentage of metals in their ionic state with a lower pH level. In order to succeed

in binding to the exchange sites, such metal ions should interact with specific cations including magnesium (Mg^{2+}), calcium (Ca^{2+}), iron (Fe^{2+}), aluminum (Al^{3+}) and hydrogen (H^+) (Bakircioglu et al., 2011). Metals can thus be more attainable for plant absorption at lower pH levels (Bakircioglu et al., 2011). In fact, in their study, Pauget et al. in 2012, demonstrated an important correlation between *Cantareus aspersus* metal accumulation and soil physicochemical properties such as cation exchange capacity and pH (Pauget et al., 2012). Nonetheless, based on the maximum residue limit (MLR), it was not reasonable to deduce with confidence that physicochemical properties of the soil and metals contributed to the cumulation of metal in the digestive gland, although the physicochemical characteristics of the soil might have indirectly affected the metal's accumulation in snails (*Cepaea nemoralis*) and determined the accumulation of metals in plants (*Urtica dioica*) as well as snails by their digestive exposure (Gimbert et al., 2006, Cœurdassier et al., 2002). Moreover, Boshoff et al., in 2013 and 2014 and Notten et al., in 2006, have reported differing levels of cadmium (Cd) such as 60–150 mg kg⁻¹, 33.93–148.40 mg kg⁻¹ and 94 mg kg⁻¹ in several studies in *Cepaea nemoralis*, respectively (Boshoff et al., 2015, Boshoff et al., 2013, Notten et al., 2006). The absorption of cadmium (Cd) as a non-essential element is not controlled at an accurate level or is less efficiently regulated than fundamental elements (Tchounwou et al., 2012). Higher concentrations are detected in the *helix aspersa* snail that fed a cadmium-rich diet (Cd) (Scheifler et al., 2002) by binding to metallothionein-like proteins present in the digestive gland that accumulate metal at higher levels without severe effects (Nica et al., 2013, Gimbert et al., 2006, Manzl et al., 2004). For snail physiological functioning, copper (Cu) and zinc (Zn) are required and their absorption is controlled till it exceeds the threshold rate (Nica et al., 2012). Specifically, snails require large amounts of copper as an element of hemocyanin that is converted and absorbed after accumulation (Manzl et al., 2004). Snails can maintain zinc in tissues for essential functions at low levels and can affect the feeding and development at high concentrations (Swaileh and Ezzughayyar, 2001).

Moreover, snails living in areas highly contaminated with metals also exhibit physiological and morphological modifications due to higher energy costs correlated with process of excretion and detoxification (Radwan et al., 2010, Regoli et al., 2006). As the strength of the shell increases slightly, there was no significant change in the thickness or strength of the shell that can be correlated with the site and time interaction even when

exposed to contaminated soil. Similarly, there are no detected effects of metal toxicity such as lead (Pb) and cadmium (Cd) on the weight or size of the snail shell (Mourier et al., 2011, Jordaens et al., 2006). Although adult snails have a completely formed shell, the morphology of wild population shells may change (De Vaufleury and Pihan, 2000).

Furthermore, snails have mechanisms for metabolizing, exporting storing and excreting metals, and these mechanisms are triggered by the acclimatization process, such as transfer to new environment, physiological changes resulting from experimental stressors or response to stress due to the current microcosm conditions (Bighiu et al., 2017). Cumulative metals will be stable after a specified exposure time and therefore cannot interact with biochemical reactions (Tchounwou et al., 2012, Rainbow, 2007).

Some studies have shown that high doses and duration of metal exposure cause more serious effects, such as kidney failure caused by the combination of inorganic arsenic and cadmium, compared to low doses and individual exposure to various elements (Wang and Fowler, 2008, Nordberg et al., 2005).

In general, digestion with HNO₃ and H₂O₂ mixture (Abdel Gawad, 2018, Emilia et al., 2016, Dummee et al., 2012, Rybak et al., 2012, Santos et al., 2009) followed by the inductively coupled plasma mass spectrometer (ICP-MS) (Mleiki et al., 2016, Yin et al., 2014, Rybak et al., 2012, Cui et al., 2012, Santos et al., 2009) was the main multi-residue extraction and analytical procedure of heavy metals from the different snail species used.

3.2. Snail pollution by organic pollutants

The high use of pesticides as a result of the increasing world population growth and plant protection policies as well, led to an increase of the invertebrates' contamination by these pollutants. They are scarcely used separately in agriculture and can be used in association during crop production at specific times (Ngowi et al., 2007). Due to their usage and application, the presence of pesticides overlaps in space and time (Smiley et al., 2014). Snails are widely used in ecotoxicological studies to investigate pesticide exposure effects that observed in response to each of the individual and combined pesticides exposures (Hock and Poulin, 2012, Mora et al., 2011). The effects of pesticides and their accumulation via different routes on snails that affecting their response, are influenced by several factors, such as, the sensitivity of species to pesticide (Lushchak et al., 2018), the

chemical mode of action (Staley et al., 2015) and their exposure period (Damalas and Eleftherohorinos, 2011). Exposure can have direct effects on biological organization at all levels, while toxicant mode of action mainly defines the class of organisms are affected. Several characteristics of snails such as, fertility (Coutellec et al., 2008), survival (Qiu et al., 2011), and movement (Perez et al., 2009) can be quantified to estimate the fitness of snails which decrease in response to stress associated with the environment (Coutellec et al., 2008), including pesticides contamination (Coutellec and Lagadic, 2006) and physicochemical parameters (Elias and Bernot, 2017). In addition, the effects of pesticides on snails can either directly affect their egestion and movement or indirectly affect food chains and predator interactions. Lower snail egestion levels once exposed to pesticides may limit the availability of carbon and nitrogen that affect biomass algae and nutrient flows (Fink and Elert, 2006). In addition to the pesticide mode of action, it is necessary to consider the sensitivity of organisms to pesticides in combination with different exposures periods to comprehend the potential adverse environmental impacts of co-occurring pesticides on biodiversity. The ecology and biology of snails, such as large distribution, bioaccumulation capacity and ease of sampling reflect most of the essential characteristics of an ideal bioindicator (Hall et al., 2009), and these advantages make the snail field an effective bioindicator for toxins and other natural substances such as *Pomacea canaliculata* (Martín et al., 2019, Koch et al., 2013, Fu et al., 2011).

It is also noted that snails are involved in multiple food chains: carabids, amphibians, mammals, birds, other gastropods and humans. Consequently, the contamination of snails by pesticides can be transferred for consumers leading to a risk of secondary poisoning. The pesticide concentrations determined in snails exposed exceed the MRLs (Maximum Residue Limit) set for a type of animal food (Glyphosate: Animal product MRL = 0.1 mg kg⁻¹, Snail MRL 6 mg kg⁻¹) (Druart et al., 2011).

However, the effects of pesticides on the snails are limited to few studies. Among the available data, it appears that carbamates and organophosphorus insecticides cause low mortality in snails feeding for 10 days with contaminated food (Schuytema et al., 1994). The insecticide pentachlorophenol is not fatal for snails at concentrations of 1000 mg kg⁻¹ in food (De Vaufleury, 2000). Moreover, Coeurdassier et al., in 2002 studied the effects on survival, growth, and acetylcholinesterase (AChE) of an organophosphorus insecticide, dimethoate, and demonstrated growth inhibition in relation to its

accumulation. These authors also observed that snails are more sensitive to pesticides contained in the soil than in food (Cœurdassier et al., 2002).

4. Conclusion

Snails used as sensitive environmental pollution indicators has taken considerable importance and been applied for incorporate the pollution signal over a period of time or an area. Their use in toxicity bioassays is an effective process as snails are easy to adapt and manipulate in the laboratory and it can be treated with the desired amounts of contaminants on specific diets and react quickly to pollutant contamination in the sublethal dosage range. Such organisms are also chosen as sentinels due to their limited toxic response or little ability to control their tissue levels. Studying the impact of metals and other pollutants on organism physiology contributes to the development of many toxic studies which can be used as an environmental evaluation tool.

For all these reasons, the use of snails as matrix is highly recommended in order to monitor a wide class of environmental pollutants that might be present in the environment.

References

- Abdel Gawad, S.S., 2018. Acute toxicity of some heavy metals to the fresh water snail, *Theodoxus niloticus* (Reeve, 1856). *The Egyptian Journal of Aquatic Research* 44, 83–87. <https://doi.org/10.1016/j.ejar.2018.06.004>
- Abdel-Halim, K.Y., Abo El-Saad, A.M., Talha, M.M., Hussein, A.A., Bakry, N.M., 2013. Oxidative stress on land snail *Helix aspersa* as a sentinel organism for ecotoxicological effects of urban pollution with heavy metals. *Chemosphere* 93, 1131–1138. <https://doi.org/10.1016/j.chemosphere.2013.06.042>
- Amato, E.D., Covaci, A., Town, R.M., Hereijgers, J., Bellekens, B., Giacometti, V., Breugelmans, T., Weyn, M., Dardenne, F., Bervoets, L., Blust, R., 2018. A novel active-passive sampling approach for measuring time-averaged concentrations of pollutants in water. *Chemosphere* 209, 363–372. <https://doi.org/10.1016/j.chemosphere.2018.06.079>
- Arad, Z., HELLER, J., 2009. Resistance to desiccation and distribution patterns in the land snail *Sphincterochila*. *Journal of Zoology* 218, 353–364. <https://doi.org/10.1111/j.1469-7998.1989.tb02549.x>
- Bakircioglu, D., Kurtulus, Y.B., Ucar, G., 2011. Determination of some traces metal levels in cheese samples packaged in plastic and tin containers by ICP-OES after dry, wet and microwave digestion. *Food and Chemical Toxicology* 49, 202–207. <https://doi.org/10.1016/j.fct.2010.10.017>
- Barca, D., Comite, V., Belfiore, C., Bonazza, A., La Russa, M., Ruffolo, S., Crisci, G., Pezzino, A., Sabbioni, C., 2014. Impact of air pollution in deterioration of carbonate building materials in Italian urban environments. *Applied Geochemistry* 48, 122–131. <https://doi.org/10.1016/j.apgeochem.2014.07.002>
- Bargagli, R., 2016. Moss and lichen biomonitoring of atmospheric mercury: A review. *Science of The Total Environment* 572, 216–231. <https://doi.org/10.1016/j.scitotenv.2016.07.202>
- Beach, D.G., Quilliam, M.A., Hellou, J., 2009. Analysis of pyrene metabolites in marine snails by liquid chromatography using fluorescence and mass spectrometry detection. *Journal of Chromatography B* 877, 2142–2152. <https://doi.org/10.1016/j.jchromb.2009.06.006>
- Beeby, A., Richmond, L., 2002. Evaluating *Helix aspersa* as a sentinel for mapping metal pollution. *Ecological Indicators* 1, 261–270. [https://doi.org/10.1016/S1470-160X\(02\)00022-5](https://doi.org/10.1016/S1470-160X(02)00022-5)
- Beeby, A., Richmond, L., 2011. Sources of variation in the assimilation of lead by a common gastropod sentinel *Cantareus aspersus*. *Science of The Total Environment* 409, 5499–5504. <https://doi.org/10.1016/j.scitotenv.2011.09.015>
- Bighiu, M.A., Watermann, B., Guo, X., Almroth, B.C., Eriksson-Wiklund, A.-K., 2017. Mortality and histopathological effects in harbour-transplanted snails with different exposure histories. *Aquatic Toxicology* 190, 11–20. <https://doi.org/10.1016/j.aquatox.2017.06.018>
- Boshoff, M., Jordaens, K., Backeljau, T., Lettens, S., Tack, F., Vandecasteele, B., De Jonge, M., Bervoets, L., 2013. Organ- and species-specific accumulation of metals in two land snail species (Gastropoda, Pulmonata). *Science of The Total Environment* 449, 470–481. <https://doi.org/10.1016/j.scitotenv.2013.02.003>

- Boshoff, M., Jordaens, K., Baguet, S., Bervoets, L., 2015. Trace metal transfer in a soil–plant–snail microcosm field experiment and biomarker responses in snails. *Ecological Indicators* 48, 636–648. <https://doi.org/10.1016/j.ecolind.2014.08.037>
- Campoy-Diaz, A.D., Arribère, M.A., Guevara, S.R., Vega, I.A., 2018. Bioindication of mercury, arsenic and uranium in the apple snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae): Bioconcentration and depuration in tissues and symbiotic corpuscles. *Chemosphere* 196, 196–205. <https://doi.org/10.1016/j.chemosphere.2017.12.145>
- Cheng, W.H., Yap, C.K., 2015. Potential human health risks from toxic metals via mangrove snail consumption and their ecological risk assessments in the habitat sediment from Peninsular Malaysia. *Chemosphere* 135, 156–165. <https://doi.org/10.1016/j.chemosphere.2015.04.013>
- Chevalier, L., Desbuquois, C., Le Lannic, J., Charrier, M., 2001. Poaceae in the natural diet of the snail *Helix aspersa* Müller (Gastropoda, Pulmonata). *Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie* 324, 979–987. [https://doi.org/10.1016/S0764-4469\(01\)01382-8](https://doi.org/10.1016/S0764-4469(01)01382-8)
- Cho, Y.J., Getachew, A.T., Saravana, P.S., Chun, B.S., 2019. Optimization and characterization of polysaccharides extraction from Giant African snail (*Achatina fulica*) using pressurized hot water extraction (PHWE). *Bioactive Carbohydrates and Dietary Fibre* 18, 100179. <https://doi.org/10.1016/j.bcdf.2019.100179>
- Cœurdassier, M., De Vaufléury, A., Lovy, C., Badot, P.-M., 2002. Is the cadmium uptake from soil important in bioaccumulation and toxic effects for snails? *Ecotoxicology and Environmental Safety* 53, 425–431. [https://doi.org/10.1016/S0147-6513\(02\)00004-0](https://doi.org/10.1016/S0147-6513(02)00004-0)
- Cossi, P.F., Herbert, L.T., Yusseppone, M.S., Pérez, A.F., Kristoff, G., 2018. Environmental concentrations of azinphos-methyl cause different toxic effects without affecting the main target (cholinesterases) in the freshwater gastropod *Biomphalaria straminea*. *Ecotoxicology and Environmental Safety* 162, 287–295. <https://doi.org/10.1016/j.ecoenv.2018.06.091>
- Coutellec, M.-A., Delous, G., Cravedi, J.-P., Lagadic, L., 2008. Effects of the mixture of diquat and a nonylphenol polyethoxylate adjuvant on fecundity and progeny early performances of the pond snail *Lymnaea stagnalis* in laboratory bioassays and microcosms. *Chemosphere* 73, 326–336. <https://doi.org/10.1016/j.chemosphere.2008.05.068>
- Coutellec, M.-A., Lagadic, L., 2006. Effects of Self-Fertilization, Environmental Stress and Exposure to Xenobiotics on Fitness-Related Traits of the Freshwater Snail *Lymnaea stagnalis*. *Ecotoxicology (London, England)* 15, 199–213. <https://doi.org/10.1007/s10646-005-0049-x>
- Crémazy, A., Brix, K.V., Wood, C.M., 2019. Using the Biotic Ligand Model framework to investigate binary metal interactions on the uptake of Ag, Cd, Cu, Ni, Pb and Zn in the freshwater snail *Lymnaea stagnalis*. *Science of The Total Environment* 647, 1611–1625. <https://doi.org/10.1016/j.scitotenv.2018.07.455>
- Cueto, J.A., Vega, I.A., Castro-Vazquez, A., 2013. Multicellular spheroid formation and evolutionary conserved behaviors of apple snail hemocytes in culture. *Fish & Shellfish Immunology* 34, 443–453. <https://doi.org/10.1016/j.fsi.2012.11.035>
- Cui, J., Shan, B., Tang, W., 2012. Effect of periphyton community structure on heavy metal accumulation in mystery snail (*Cipangopaludina chinensis*): A case study of the Bai River, China. *Journal of Environmental Sciences* 24, 1723–1730. [https://doi.org/10.1016/S1001-0742\(11\)60976-5](https://doi.org/10.1016/S1001-0742(11)60976-5)
- Dallinger, R., Berger, B., Triebkorn-Köhler, R., Köhler, H., 2001. Soil biology and ecotoxicology., in: Barker, G.M. (Ed.), *The Biology of Terrestrial Molluscs*. CABI, Wallingford, pp. 489–525. <https://doi.org/10.1079/9780851993188.0489>
- Damalas, C.A., Eleftherohorinos, I.G., 2011. Pesticide Exposure, Safety Issues, and Risk Assessment Indicators. *Int J Environ Res Public Health* 8, 1402–1419. <https://doi.org/10.3390/ijerph8051402>
- Dar, M.I., Green, I.D., Khan, F.A., 2019. Trace metal contamination: Transfer and fate in food chains of terrestrial invertebrates. *Food Webs* 20, e00116. <https://doi.org/10.1016/j.fooweb.2019.e00116>
- De Vaufléury, A., 2015. Landsnail for Ecotoxicological Assessment of Chemicals and Soil Contamination – Ecotoxicological Assessment of Chemicals and Contaminated Soils Using the Terrestrial Snail, *Helix aspersa*, at Various Stage of Its Life Cycle: A Review, in: Armon, R.H., Hänninen, O. (Eds.), *Environmental Indicators*. Springer Netherlands, Dordrecht, pp. 365–391. https://doi.org/10.1007/978-94-017-9499-2_23
- De Vaufléury, A., 2000. Standardized Growth Toxicity Testing (Cu, Zn, Pb, and Pentachlorophenol) with *Helix aspersa*. *Ecotoxicology and Environmental Safety* 46, 41–50. <https://doi.org/10.1006/eesa.1999.1872>
- De Vaufléury, A., Gimbert, F., 2009. Obtention du cycle de vie complet d'*Helix aspersa* Born de sites tunisiens en conditions contrôlées. Influence de la photopériode. *Comptes Rendus Biologies* 332, 795–805. <https://doi.org/10.1016/j.crvi.2009.04.004>
- De Vaufléury, A., Pihan, F., 2000. Growing snails used as sentinels to evaluate terrestrial environment contamination by trace elements. *Chemosphere* 40, 275–284. [https://doi.org/10.1016/S0045-6535\(99\)00246-5](https://doi.org/10.1016/S0045-6535(99)00246-5)
- Druart, C., Millet, M., Scheifler, R., Delhomme, O., Raepel, C., De Vaufléury, A., 2011. Snails as indicators of pesticide drift, deposit, transfer and effects in the vineyard. *Science of The Total Environment* 409, 4280–4288. <https://doi.org/10.1016/j.scitotenv.2011.07.006>
- Druart, C., Scheifler, R., Millet, M., De Vaufléury, A., 2012. Landsnail eggs bioassays: A new tool to assess embryotoxicity of contaminants in the solid, liquid or gaseous phase of soil. *Applied Soil Ecology* 53, 56–64. <https://doi.org/10.1016/j.apsoil.2011.11.006>
- Dumme, V., Kruatrachue, M., Trinachartvanit, W., Tanhan, P., Pokethitiyook, P., Damrongphol, P., 2012. Bioaccumulation of heavy metals in water, sediments, aquatic plant and histopathological effects on the golden apple snail in Beung Boraphet reservoir, Thailand. *Ecotoxicology and Environmental Safety* 86, 204–212. <https://doi.org/10.1016/j.ecoenv.2012.09.018>
- Ebi, K.L., Hess, J.J., Watkiss, P., 2017. Health Risks and Costs of Climate Variability and Change, in: Mock, C.N., Nugent, R., Kobusingye, O., Smith, K.R. (Eds.), *Injury Prevention and Environmental Health*. The International Bank for Reconstruction and Development / The World Bank, Washington (DC).
- Eeva, T., Rainio, K., Suominen, O., 2010. Effects of pollution on land snail abundance, size and diversity as resources for pied flycatcher, *Ficedula hypoleuca*. *The Science of the total environment* 408, 4165–9. <https://doi.org/10.1016/j.scitotenv.2010.05.028>
- Elias, D., Bernot, M.J., 2017. Effects of Individual and Combined Pesticide Commercial Formulations Exposure to Egestion and Movement of Common Freshwater Snails, *Physa acuta* and *Helisoma anceps*. *amid* 178, 97–111. <https://doi.org/10.1674/0003-0031-178.1.97>
- El-Shenawy, N.S., Mohammadden, A., Al-Fahmie, Z.H., 2012. Using the enzymatic and non-enzymatic antioxidant defense system of the land snail *Eobania vermiculata* as biomarkers of terrestrial heavy metal pollution. *Ecotoxicology and Environmental Safety* 84, 347–354. <https://doi.org/10.1016/j.ecoenv.2012.08.014>
- Emilia, R., Debora, B., Stefania, A., Nicola, B., Roberto, B., 2016. *Papillifera papillaris* (O.F. Müller), a small snail living on stones and monuments, as indicator of metal deposition and bioavailability in urban environments. *Ecological Indicators* 69, 360–367. <https://doi.org/10.1016/j.ecolind.2016.04.024>
- Eugene Havel, J., Bruckerhoff, L., Funkhouser, M., Gemberling, A., 2014. Resistance to desiccation in aquatic invasive snails and implications for their overland dispersal. *Hydrobiologia* 741, 1–12. <https://doi.org/10.1007/s10750-014-1839-z>

- Fink, P., Elert, E., 2006. Physiological responses to stoichiometric constraints: Nutrient limitation and compensatory feeding in a freshwater snail. *Oikos* 115, 484–494. <https://doi.org/10.1111/j.2006.0030-1299.14951.x>
- Fränzle, O., 2006. Complex bioindication and environmental stress assessment. *Ecological Indicators* 6, 114–136. <https://doi.org/10.1016/j.ecolind.2005.08.015>
- Fu, J., Wang, Y., Zhang, A., Zhang, Q., Zhao, Z., Wang, T., Jiang, G., 2011. Spatial distribution of polychlorinated biphenyls (PCBs) and polybrominated biphenyl ethers (PBDEs) in an e-waste dismantling region in Southeast China: Use of apple snail (*Ampullariidae*) as a bioindicator. *Chemosphere* 82, 648–655. <https://doi.org/10.1016/j.chemosphere.2010.11.014>
- Gaitán-Espitia, J.D., Arias, M., Lardies, M., Nespolo, R., 2013. Variation in Thermal Sensitivity and Thermal Tolerances in an Invasive Species across a Climatic Gradient: Lessons from the Land Snail *Cornu aspersum*. *PloS one* 8, e70662. <https://doi.org/10.1371/journal.pone.0070662>
- Gerlach, J., Samways, M., Pryke, J., 2013. Terrestrial invertebrates as bioindicators: An overview of available taxonomic groups. *Journal of Insect Conservation* 17. <https://doi.org/10.1007/s10841-013-9565-9>
- Gimbert, F., De Vaufléury, A., Douay, F., Coeurdassier, M., Scheifler, R., Badot, P.-M., 2008. Long-term responses of snails exposed to cadmium-contaminated soils in a partial life-cycle experiment. *Ecotoxicology and Environmental Safety* 70, 138–146. <https://doi.org/10.1016/j.ecoenv.2007.05.014>
- Gimbert, F., De Vaufléury, A., Douay, F., Scheifler, R., Coeurdassier, M., Badot, P.-M., 2006. Modelling chronic exposure to contaminated soil: A toxicokinetic approach with the terrestrial snail *Helix aspersa*. *Environment International* 32, 866–875. <https://doi.org/10.1016/j.envint.2006.05.006>
- Giraud-Billoud, M., Castro-Vazquez, A., Campoy-Diaz, A.D., Giuffrida, P.M., Vega, I.A., 2018. Tolerance to hypometabolism and arousal induced by hibernation in the apple snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 224, 129–137. <https://doi.org/10.1016/j.cbpb.2017.12.015>
- Guerreiro, C.B.B., Foltescu, V., de Leeuw, F., 2014. Air quality status and trends in Europe. *Atmospheric Environment* 98, 376–384. <https://doi.org/10.1016/j.atmosenv.2014.09.017>
- Guiller, A., Martin, M.-C., Hiraux, C., Madec, L., 2012. Tracing the Invasion of the Mediterranean Land Snail *Cornu aspersum aspersum* Becoming an Agricultural and Garden Pest in Areas Recently Introduced. *PLoS One* 7. <https://doi.org/10.1371/journal.pone.0049674>
- Hall, C., Rhind, S., Wilson, M., 2009. The potential for use of gastropod molluscs as bioindicators of endocrine disrupting compounds in the terrestrial environment. *Journal of environmental monitoring: JEM* 11, 491–7. <https://doi.org/10.1039/b804320e>
- Harmon, S., Wiley, F., 2010. Effects of Pollution on Freshwater Organisms. *Water Environment Research* 82, 1945–2000. <https://doi.org/10.2175/106143010X12756668802139>
- Hispard, F., De Vaufléury, A., Cosson, R.P., Devaux, S., Scheifler, R., Coeurdassier, M., Gimbert, F., Martin, H., Richert, L., Berthelot, A., Badot, P.-M., 2008. Comparison of transfer and effects of Cd on rats exposed in a short experimental snail–rat food chain or to CdCl₂ dosed food. *Environment International* 34, 381–389. <https://doi.org/10.1016/j.envint.2007.09.003>
- Hock, S.D., Poulin, R., 2012. Exposure of the snail *Potamopyrgus antipodarum* to herbicide boosts output and survival of parasite infective stages. *International Journal for Parasitology: Parasites and Wildlife* 1, 13–18. <https://doi.org/10.1016/j.ijppaw.2012.10.002>
- Hodkinson, I.D., Jackson, J.K., 2005. Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. *Environ Manage* 35, 649–666. <https://doi.org/10.1007/s00267-004-0211-x>
- Itziou, A., Dimitriadis, V.K., 2011. Introduction of the land snail *Eobania vermiculata* as a bioindicator organism of terrestrial pollution using a battery of biomarkers. *Sci. Total Environ.* 409, 1181–1192. <https://doi.org/10.1016/j.scitotenv.2010.12.009>
- Itziou, A., Kaloyianni, M., Dimitriadis, V.K., 2011. Effects of organic contaminants in reactive oxygen species, protein carbonylation and DNA damage on digestive gland and haemolymph of land snails. *Chemosphere* 85, 1101–1107. <https://doi.org/10.1016/j.chemosphere.2011.07.043>
- Jordaens, K., De Wolf, H., Vandecasteele, B., Blust, R., Backeljau, T., 2006. Associations between shell strength, shell morphology and heavy metals in the land snail *Cepaea nemoralis* (Gastropoda, Helicidae). *Science of The Total Environment* 363, 285–293. <https://doi.org/10.1016/j.scitotenv.2005.12.002>
- Koch, E., Altamirano, J., Covaci, A., Lana, B., Ciocco, N., 2013. Should apple snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae) be used as bioindicator for BDE-209? *Environmental science and pollution research international* 21. <https://doi.org/10.1007/s11356-013-2109-8>
- Kovalchuk, I., Kovalchuk, O., 2008. Transgenic Plants as Sensors of Environmental Pollution Genotoxicity. *Sensors (Basel)* 8, 1539–1558.
- Kramarz, P., De Vaufléury, A., Gimbert, F., Cortet, J., Tabone, E., Andersen, M.N., Krogh, P.H., 2009. Effects of Bt-maize material on the life cycle of the land snail *Cantareus aspersus*. *Applied Soil Ecology* 42, 236–242. <https://doi.org/10.1016/j.apsoil.2009.04.007>
- Krupnova, T.G., Mashkova, I.V., Kostyukova, A.M., Schelkanova, E.E., Gavrilkina, S.V., 2018. Gastropods as potential biomonitors of contamination caused by heavy metals in South Ural lakes, Russia. *Ecological Indicators* 95, 1001–1007. <https://doi.org/10.1016/j.ecolind.2017.12.005>
- Laskowski, R., Hopkin, S.P., 1996. Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*): Implications for predators. *Environmental Pollution* 91, 289–297. [https://doi.org/10.1016/0269-7491\(95\)00070-4](https://doi.org/10.1016/0269-7491(95)00070-4)
- Laumbach, R., Meng, Q., Kipen, H., 2015. What can individuals do to reduce personal health risks from air pollution? *J Thorac Dis* 7, 96–107. <https://doi.org/10.3978/j.issn.2072-1439.2014.12.21>
- Le Tertre, A., Medina, S., Samoli, E., Forsberg, B., Michelozzi, P., Boumghar, A., Vonk, J.M., Bellini, A., Atkinson, R., Ayres, J.G., Sunyer, J., Schwartz, J., Katsouyanni, K., 2002. Short-term effects of particulate air pollution on cardiovascular diseases in eight European cities. *J Epidemiol Community Health* 56, 773–779. <https://doi.org/10.1136/jech.56.10.773>
- Lee, B.-J., Kim, B., Lee, K., 2014. Air Pollution Exposure and Cardiovascular Disease. *Toxicol Res* 30, 71–75. <https://doi.org/10.5487/TR.2014.30.2.071>
- Liew, T.-S., Schilthuisen, M., 2014. Association between shell morphology of micro-land snails (genus *Plectostoma*) and their predator's predatory behaviour. *PeerJ* 2. <https://doi.org/10.7717/peerj.329>
- Lushchak, V.I., Matviishyn, T.M., Husak, V.V., Storey, J.M., Storey, K.B., 2018. Pesticide toxicity: a mechanistic approach. *EXCLI J* 17, 1101–1136. <https://doi.org/10.17179/excli2018-1710>
- Manzl, C., Krumschnabel, G., Schwarzbach, P.J., Dallinger, R., 2004. Acute toxicity of cadmium and copper in hepatopancreas cells from the Roman snail (*Helix pomatia*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 138, 45–52. <https://doi.org/10.1016/j.cca.2004.04.008>

- Marshall, D.J., Abdelhady, A.A., Wah, D.T.T., Mustapha, N., Gödeke, S.H., De Silva, L.C., Hall-Spencer, J.M., 2019. Biomonitoring acidification using marine gastropods. *Science of The Total Environment* 692, 833–843. <https://doi.org/10.1016/j.scitotenv.2019.07.041>
- Martín, P., Burela, S., Seuffert, M., Tamburi, N., Saveanu, L., 2019. Invasive Pomacea snails: actual and potential environmental impacts and their underlying mechanisms. *CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources* 14, 1–11. <https://doi.org/10.1079/pavsnr.201914042>
- Mleiki, A., Irizar, A., Zaldibar, B., El Menif, N.T., Marigómez, I., 2016. Bioaccumulation and tissue distribution of Pb and Cd and growth effects in the green garden snail, *Cantareus apertus* (Born, 1778), after dietary exposure to the metals alone and in combination. *Science of The Total Environment* 547, 148–156. <https://doi.org/10.1016/j.scitotenv.2015.12.162>
- Mora, M.A., Baxter, C., Sericano, J.L., Montoya, A.B., Gallardo, J.C., Rodríguez-Salazar, J.R., 2011. PBDEs, PCBs, and DDE in eggs and their impacts on aplomado falcons (*Falco femoralis*) from Chihuahua and Veracruz, Mexico. *Environmental Pollution* 159, 3433–3438. <https://doi.org/10.1016/j.envpol.2011.08.025>
- Mourier, B., Fritsch, C., Dhivert, E., Gimbert, F., Coeurdassier, M., Pauget, B., De Vaufléury, A. de, Scheifler, R., 2011. Chemical extractions and predicted free ion activities fail to estimate metal transfer from soil to field land snails. *Chemosphere* 85, 1057–1065. <https://doi.org/10.1016/j.chemosphere.2011.07.035>
- Muralikrishna, I.V., Manickam, V., 2017. Chapter Eight - Environmental Risk Assessment, in: Muralikrishna, I.V., Manickam, V. (Eds.), *Environmental Management*. Butterworth-Heinemann, pp. 135–152. <https://doi.org/10.1016/B978-0-12-811989-1.00008-7>
- Ngowi, A.V.F., Mbise, T.J., Ijani, A.S.M., London, L., Ajayi, O.C., 2007. Pesticides use by smallholder farmers in vegetable production in Northern Tanzania. *Crop Prot* 26, 1617–1624. <https://doi.org/10.1016/j.cropro.2007.01.008>
- Nica, D., Bordean, D.-M., Boroșan, A., Gergen, I., Bura, M., Banatean-Dunea, I., 2013. Use of Land Snails (Pulmonata) for Monitoring Copper Pollution in Terrestrial Ecosystems.
- Nica, D.V., Bura, M., Gergen, I., Harmanescu, M., Bordean, D.-M., 2012. Bioaccumulative and conchological assessment of heavy metal transfer in a soil-plant-snail food chain. *Chem Cent J* 6, 55. <https://doi.org/10.1186/1752-153X-6-55>
- Nordberg, G.F., Jin, T., Hong, F., Zhang, A., Buchet, J.P., Bernard, A., 2005. Biomarkers of cadmium and arsenic interactions. *Toxicol. Appl. Pharmacol.* 206, 191–197. <https://doi.org/10.1016/j.taap.2004.11.028>
- Notten, M.J.M., Oosthoek, A.J.P., Rozema, J., Aerts, R., 2005. Heavy metal concentrations in a soil–plant–snail food chain along a terrestrial soil pollution gradient. *Environmental Pollution* 138, 178–190. <https://doi.org/10.1016/j.envpol.2005.01.011>
- Notten, M.J.M., Oosthoek, A.J.P., Rozema, J., Aerts, R., 2006. The landsnail *Cepaea nemoralis* regulates internal Cd levels when fed on Cd-enriched stinging nettle (*Urtica dioica*) leaves at low, field-relevant concentrations. *Environmental Pollution* 139, 296–305. <https://doi.org/10.1016/j.envpol.2005.05.007>
- Ortmann, L.W., Barrett, D.H., Saenz, C., Bernheim, R.G., Dawson, A., Valentine, J.A., Reis, A., 2016. Public Health Ethics: Global Cases, Practice, and Context, in: H. Barrett, D., W. Ortmann, L., Dawson, A., Saenz, C., Reis, A., Bolan, G. (Eds.), *Public Health Ethics: Cases Spanning the Globe*. Springer, Cham (CH).
- Osytek, A., Biesaga, M., Pyrzynska, K., Szewczyńska, M., 2008. Quantification of some active compounds in air samples at pharmaceutical workplaces by HPLC. *Journal of Biochemical and Biophysical Methods* 70, 1283–1286. <https://doi.org/10.1016/j.jbbm.2007.10.003>
- Pagano, M., Porcino, C., Briglia, M., Fiorino, E., Vazzana, M., Silvestro, S., Faggio, C., 2017. The Influence of Exposure of Cadmium Chloride and Zinc Chloride on Haemolymph and Digestive Gland Cells from *Mytilus galloprovincialis*. *Int J Environ Res* 11, 207–216. <https://doi.org/10.1007/s41742-017-0020-8>
- Palmer, E.D., Gunnison, A.F., 1973. Personal Monitoring Device for Gaseous Contaminants. *American Industrial Hygiene Association Journal* 34, 78–81. <https://doi.org/10.1080/0002889738506810>
- Parmar, T., Rawtani, D., Agrawal, Y., 2016. Bioindicators: the natural indicator of environmental pollution. *Frontiers in Life Science* 9, 1–9. <https://doi.org/10.1080/21553769.2016.1162753>
- Pauget, B., Gimbert, F., Scheifler, R., Coeurdassier, M., De Vaufléury, A., 2012. Soil parameters are key factors to predict metal bioavailability to snails based on chemical extractant data. *Science of The Total Environment* 431, 413–425. <https://doi.org/10.1016/j.scitotenv.2012.05.048>
- Perez, K.O., Carlson, R.L., Shulman, M.J., Ellis, J.C., 2009. Why are intertidal snails rare in the subtidal? Predation, growth and the vertical distribution of *Littorina littorea* (L.) in the Gulf of Maine. *Journal of Experimental Marine Biology and Ecology* 369, 79–86. <https://doi.org/10.1016/j.jembe.2008.09.019>
- Proum, S., Santos, J.H., Lim, L.H., Marshall, D.J., 2016. Metal accumulation in the tissues and shells of *Indothais gradata* snails inhabiting soft and hard substrata in an acidified tropical estuary (Brunei, South East Asia). *Regional Studies in Marine Science* 8, 487–497. <https://doi.org/10.1016/j.rsma.2016.03.010>
- Qiu, J.-W., Chan, M., Kwong, K., Sun, J., 2011. Consumption, survival and growth in the invasive freshwater snail *Pomacea canaliculata*: Does food freshness matter? *Journal of Molluscan Studies* 77. <https://doi.org/10.1093/mollus/eyr005>
- Radwan, M.A., El-Gendy, K.S., Gad, A.F., 2010. Biomarkers of oxidative stress in the land snail, *Theba pisana* for assessing ecotoxicological effects of urban metal pollution. *Chemosphere* 79, 40–46. <https://doi.org/10.1016/j.chemosphere.2010.01.056>
- Rainbow, P.S., 2007. Trace metal bioaccumulation: Models, metabolic availability and toxicity. *Environment International* 33, 576–582. <https://doi.org/10.1016/j.envint.2006.05.007>
- Ramli, N.H., Yusup, S., Quitain, A.T., Johari, K., Kueh, B.W.B., 2019. Optimization of saponin extracts using microwave-assisted extraction as a sustainable biopesticide to reduce *Pomacea canaliculata* population in paddy cultivation. *Sustainable Chemistry and Pharmacy* 11, 23–35. <https://doi.org/10.1016/j.scp.2018.12.002>
- Reames, T.G., Bravo, M.A., 2019. People, place and pollution: Investigating relationships between air quality perceptions, health concerns, exposure, and individual- and area-level characteristics. *Environment International* 122, 244–255. <https://doi.org/10.1016/j.envint.2018.11.013>
- Regoli, F., Gorbi, S., Fattorini, D., Tedesco, S., Notti, A., Machella, N., Bocchetti, R., Benedetti, M., Piva, F., 2006. Use of the land snail *Helix aspersa* as sentinel organism for monitoring ecotoxicologic effects of urban pollution: an integrated approach. *Environ. Health Perspect.* 114, 63–69. <https://doi.org/10.1289/ehp.8397>
- Rybak, A., Messyas, B., Łęska, B., 2012. Freshwater *Ulva* (Chlorophyta) as a bioaccumulator of selected heavy metals (Cd, Ni and Pb) and alkaline earth metals (Ca and Mg). *Chemosphere* 89, 1066–1076. <https://doi.org/10.1016/j.chemosphere.2012.05.071>
- Santos, M.C., Wagner, M., Wu, B., Scheider, J., Oehlmann, J., Cadore, S., Becker, J.S., 2009. Biomonitoring of metal contamination in a marine prosobranch snail (*Nassarius reticulatus*) by imaging laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). *Talanta* 80, 428–433. <https://doi.org/10.1016/j.talanta.2009.06.080>

- Sastre, J., Sahuquillo, A., Vidal, M., Rauret, G., 2002. Determination of Cd, Cu, Pb and Zn in environmental samples: Microwave-assisted total digestion versus aqua regia and nitric acid extraction. *Analytica Chimica Acta* 462, 59–72. [https://doi.org/10.1016/S0003-2670\(02\)00307-0](https://doi.org/10.1016/S0003-2670(02)00307-0)
- Scheifler, R., Braham, M.B., De Vaufléury, A., Carnus, J.-M., Badot, P.-M., 2003. A field method using microcosms to evaluate transfer of Cd, Cu, Ni, Pb and Zn from sewage sludge amended forest soils to *Helix aspersa* snails. *Environmental Pollution* 122, 343–350. [https://doi.org/10.1016/S0269-7491\(02\)00333-0](https://doi.org/10.1016/S0269-7491(02)00333-0)
- Scheifler, R., De Vaufléury, A., Badot, P.-M., 2002. Transfer of Cadmium from Plant Leaves and Vegetable Flour to the Snail *Helix aspersa*: Bioaccumulation and Effects. *Ecotoxicology and Environmental Safety* 53, 148–153. <https://doi.org/10.1006/eesa.2002.2216>
- Schuytema, G.S., Nebeker, A.V., Griffis, W.L., 1994. Effects of dietary exposure to forest pesticides on the brown garden snail *Helix aspersa* Müller. *Arch. Environ. Contam. Toxicol.* 26, 23–28. <https://doi.org/10.1007/BF00212789>
- Silva, C.O., Novais, S.C., Alves, L., Soares, A.M.V.M., Barata, C., Lemos, M.F.L., 2019. Linking cholinesterase inhibition with behavioural changes in the sea snail *Gibbula umbilicalis*: Effects of the organophosphate pesticide chlorpyrifos. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 108570. <https://doi.org/10.1016/j.cbpc.2019.108570>
- Smiley, P.C., King, K.W., Fausey, N.R., 2014. Annual and seasonal differences in pesticide mixtures within channelized agricultural headwater streams in central Ohio. *Agriculture, Ecosystems & Environment* 193, 83–95. <https://doi.org/10.1016/j.agee.2014.04.022>
- Soltani, N., 2013. LARBA R. & SOLTANI N., 2013. Diversity of the terrestrial gastropods in the Northeast Algeria: spatial and temporal distribution. *European Journal of Experimental Biology*, 3(4): 209-215. www.pelagiaresearchlibrary.com ISSN: 2248 -9215 CODEN (USA): EJEBAU. *European Journal of Experimental Biology* 3, 209–215.
- Staïkou, A., Lazaridou, M., 2013. Effect of crowding on growth and mortality in the edible snail *Helix lucorum* (Gastropoda: Pulmonata) in Greece. *Israel Journal of Zoology* 36, 1–9. <https://doi.org/10.1080/00212210.1989.10688620>
- Staley, Z.R., Harwood, V.J., Rohr, J.R., 2015. A Synthesis of the Effects of Pesticides on Microbial Persistence in Aquatic Ecosystems. *Crit Rev Toxicol* 45, 813–836. <https://doi.org/10.3109/10408444.2015.1065471>
- Stieb, D.M., Judek, S., Burnett, R.T., 2002. Meta-analysis of time-series studies of air pollution and mortality: effects of gases and particles and the influence of cause of death, age, and season. *J Air Waste Manag Assoc* 52, 470–484.
- Storelli, M.M., Dambrosio, A., Storelli, A., Barone, G., Ioanna, F., Normanno, G., 2014. Levels of polychlorinated biphenyls (PCBs) in marine gastropod *Hexaplex trunculus*: Compliance with European Union legislation. *Journal of Food Composition and Analysis* 36, 35–39. <https://doi.org/10.1016/j.jfca.2014.06.008>
- Sverdrup, L.E., De Vaufléury, A., Hartnik, T., Hagen, S.B., Loibner, A.P., Jensen, J., 2006. EFFECTS AND UPTAKE OF POLYCYCLIC AROMATIC COMPOUNDS IN SNAILS (*HELIX ASPERSA*). *Environmental Toxicology and Chemistry* 25, 1941. <https://doi.org/10.1897/05-620R1.1>
- Swaileh, K.M., Ezzughayyar, A., 2001. Dose-Dependent Effects of Dietary Pb and Zn on Feeding and Growth Rates of the Landsnail *Helix engaddensis*. *Ecotoxicology and Environmental Safety* 50, 9–14. <https://doi.org/10.1006/eesa.2001.2051>
- Tanabe, S., Subramanian, A., 2006. Bioindicators of POPs: monitoring in developing countries. Kyoto University Press; Trans Pacific Press, Kyoto, Japan : Melbourne.
- Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy Metals Toxicity and the Environment. *EXS* 101, 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6
- Viard, B., Pihan, F., Promeyrat, S., Pihan, J.-C., 2004. Integrated assessment of heavy metal (Pb, Zn, Cd) highway pollution: bioaccumulation in soil, Gramineae and land snails. *Chemosphere* 55, 1349–1359. <https://doi.org/10.1016/j.chemosphere.2004.01.003>
- Wang, G., Fowler, B.A., 2008. Roles of biomarkers in evaluating interactions among mixtures of lead, cadmium and arsenic. *Toxicol. Appl. Pharmacol.* 233, 92–99. <https://doi.org/10.1016/j.taap.2008.01.017>
- Wang, W., Song, X., Wang, L., Song, L., 2018. Pathogen-Derived Carbohydrate Recognition in Molluscs Immune Defense. *International Journal of Molecular Sciences* 19, 721. <https://doi.org/10.3390/ijms19030721>
- Winkelmann, A., 2007. Anatomical dissection as a teaching method in medical school: a review of the evidence. *Medical Education* 41, 15–22. <https://doi.org/10.1111/j.1365-2929.2006.02625.x>
- Wu, J.-P., Chen, X.-Y., Wu, S.-K., Tao, L., She, Y.-Z., Luo, X.-J., Mai, B.-X., 2019. Polychlorinated biphenyls in apple snails from an abandoned e-waste recycling site, 2010–2016: A temporal snapshot after the regulatory efforts and the bioaccumulation characteristics. *Science of The Total Environment* 650, 779–785. <https://doi.org/10.1016/j.scitotenv.2018.09.074>
- Yin, H., Cai, Y., Duan, H., Gao, J., Fan, C., 2014. Use of DGT and conventional methods to predict sediment metal bioavailability to a field inhabitant freshwater snail (*Bellamya aeruginosa*) from Chinese eutrophic lakes. *Journal of Hazardous Materials* 264, 184–194. <https://doi.org/10.1016/j.jhazmat.2013.11.030>

II. A multiresidue method for the analysis of pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in snails used as environmental biomonitors

Résumé

Cette étude publiée dans « *Journal of Chromatography A* », contribue au développement d'une méthode multi-résidus pour l'analyse des pesticides, des HAPs et des PCBs à partir des escargots.

Une stratégie d'extraction a été mise au point pour le criblage simultané de 120 pesticides, 16 HAPs et 22 PCBs à partir de l'escargot terrestre *Helix aspersa*. La méthode d'extraction optimisée était basée sur le QuEChERS utilisant de l'ACN, suivi d'un nettoyage par extraction en phase solide dispersive à l'aide de PSA et de sorbants l'octadécylsilane (C₁₈). Les extraits obtenus ont été analysés par LC-MS/MS et GC-MS/MS. La GC-MS/MS a été précédée d'une étape de préconcentration par SPME avec des fibres de polyacrylate pour l'analyse des pesticides volatiles et de polydiméthylsiloxane pour l'analyse des HAPs et des PCBs.

La validation montre que la méthode analytique utilisée est fiable, précise, reproductible et robuste avec un (R^2) obtenu supérieur à 0.99 pour la plupart des composés cibles. Cette validation a révélé un bon taux de récupération compris entre 60 et 110%, avec des LOD et des LOQ inférieures à 20 ng g⁻¹ pour les composés analysés.

La comparaison de la méthode basée sur QuEChERS avec l'ASE suivie d'une SPE a montré une meilleure efficacité, sensibilité et précision pour la première méthode. La validation de la méthode a donné des résultats positifs, indiquant de bonnes performances en termes de linéarité, d'exactitude et de précision. Enfin, l'ACN en présentant de meilleurs taux de récupération que l'AE a prouvé une plus grande efficacité.

En conclusion, cette méthode d'extraction suivie d'une analyse chromatographique est un outil fiable pour l'analyse d'une large gamme de composés dans les escargots. Cette méthode a été testée pour montrer son efficacité sur trois échantillons de *Helix aspersa* prélevés dans la région d'Akkar, située au nord du Liban, dans lesquels des résidus de pesticides et de HAPs ont été détectés.

Highlights

- QUECHERS-SPME for the quantification of pesticides, PAHs and PCBs in snails' samples.
- Comparison of ASE and QUECHERS for snails' analysis.
- Comparison of acetonitrile and ethyl acetate as solvent extraction for QuEChERS extraction.
- Application on the use of snails as environmental biomonitors.

Abstract

This paper reports an optimized multiresidue extraction strategy based on the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction procedure and on solid-phase microextraction (SPME) for the simultaneous screening of 120 pesticides, 16 polycyclic aromatic hydrocarbons, and 22 polychlorinated biphenyls from the terrestrial snail *Helix aspersa*. The optimized extraction method was based on QuEChERS using acetonitrile, followed by dispersive-Solid-phase extraction clean-up using primary secondary amine and octadecyl (C₁₈) sorbents. The obtained extracts were analyzed by liquid chromatography coupled with tandem mass spectrometry and gas chromatography coupled with tandem mass spectrometry. This latest technique was preceded by a pre-concentration step using SPME with appropriate fibers. Afterwards, the method was validated for its linearity, sensitivity, recovery, and precision. Results showed high sensitivity, accuracy, and precision, with limits of detection and quantification lower than 20 ng g⁻¹ for most considered pollutants. Both inter and intra-day analyses revealed low relative standard deviation (%), which was lower than 20% for most targeted compounds. Moreover, the obtained regression coefficient (R²) was higher than 0.98 and the recoveries were higher than 60% for the majority of the assessed pollutants.

Keywords: Snails; *Helix aspersa*; Quechers; SPME; Environmental biomonitoring.

1. Introduction

Recent excessive polluting factors due to increased industrial activities, excessive agricultural practices (such as the use of pesticides and fertilizers), and intensive population growth and tobacco smoking need to be monitored to protect the ecosystem from their negative effects [1-4]. Environmental biomonitoring or biological environmental monitoring is generally defined as “the systematic use of living organisms or their responses to determine the state or changes in the environment” [5]. These samplers, known as biomonitors, are living organisms naturally present in the environment and characterized by a high ability to accumulate pollutants in their tissues [6]. These species should be accumulative and characterized by different criteria such as specificity, accumulation ratio, occurrence, time-integrative behavior, and biodiversity [7-9].

Among these species, mollusks are well known as filtering organisms, which have been successfully used in persistent organic pollutant (POP) monitoring programs due to their high bioaccumulation capacity, fixed location, and high population density [10, 11]. The use of snails as sentinel indicators is efficient due to their wide distribution, easy sampling, and ability to accumulate various type of pollutants [10, 12, 13]. Furthermore, snails live at the soil–plant–air interface and then integrate different sources and paths of contamination, which make them prone to various types of pollutants that accumulate in their soft tissues and become incorporated in their shells [14-19].

Among different type of mollusks, the terrestrial gastropod *Helix aspersa* is known for its biomonitoring properties, easy adaptation and manipulation in the laboratory, and sensitivity to genotoxicity assays [20, 21]. *H. aspersa* can accumulate different classes of chemicals and serve as appropriate species for environmental biomonitoring for pesticides [22], trace metals [23-25], polycyclic aromatic compounds [26], industrial contamination [27], and urban pollution agents [28].

Therefore, the development of analytical tools for the quantification of traces of organic pollutants in such organisms seems efficient to establish a specific environmental control. Prior to chromatographic analysis, sample preparation is required to remove all types of interferences that may disturb the detection of the pollutants of concern, decrease the

separation efficiency, or shorten the chromatographic column life [10, 29]. Although snails are well known for their ability to accumulate pollutants, studies on the extraction of pesticides and POPs from these matrices remain limited.

Accelerated solvent extraction (ASE) is an example of an extraction technique that has been used for the assessment of organic pollutants [30]. However, ASE requires efficient clean-up to remove all interfering impurities that give rise to high labor costs. SPE and solid-phase microextraction (SPME) are two well-known extraction / purification / concentration methods used to obtain a pure extract that can be analyzed by chromatographic techniques [31, 32].

In 2003, a new method was developed by Anastassiades et al. to overcome all critical flaws and practical limitations of the extraction procedures. This method known as QuEChERS was first used for the analysis of multiresidue pesticides in food [33]. This technique consists of soft liquid–liquid extraction followed by an optional clean-up step and requires few minutes and 5–15 mL of extracting solvent [34]. Although the original method showed remarkable efficiency for hundreds of analytes, several adjustments were made to improve the method's performance and make it even more rugged and efficient for other difficult analytes in different complex matrices [35–37].

After extraction, organic pollutants are analyzed using commonly known analytical techniques, namely, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) and gas chromatography coupled with tandem mass spectrometry (GC–MS/MS) [38]. The choice of the separation technique depends mostly on the characteristics of the pollutants of interest [39, 40].

For all these reasons, this study aimed to develop a simple, fast, sensitive, and reliable analytical method for the trace analysis of a large number of environmental contaminants in the terrestrial snail *H. aspersa*. For this purpose, 120 pesticides, 16 polycyclic aromatic hydrocarbons (PAHs), and 22 polychlorinated biphenyls (PCBs) were selected to cover a wide number of organic environmental contaminants that can be monitored in such organisms. Two extraction protocols were compared to choose the method with a high extraction potential, allowing the best extraction recovery. The first protocol was based on ASE–SPE, and the second was based on QuEChERS. Both techniques were followed by

a pre-concentration step using SPME. Both liquid and gas chromatography, coupled with MS/MS, were used to analyze this wide range of pollutants. The chosen matrices have yet to be studied for contamination by all these pollutants. Furthermore, the two extraction protocols were never carried out on snails, enabling their use as potential biomonitor candidates.

2. Materials and methods

2.1. Materials and reagents

Pesticide analysis included 30 nonvolatile compounds analyzed by LC-MS/MS and 90 semi-volatile compounds including 21 organochlorine pesticides (OCPs) analyzed by GC-MS/MS. All pesticides, except OCPs, were purchased from Sigma-Aldrich (L'Isle d'Abeau, France) with purity higher than 97%.

For LC-MS/MS pesticide analysis, the 30 pesticides were as follows: pymetrozine, carbendazim, chloridazon, acetamiprid, nicosulfuron, thiacloprid, chlortoluron, carbetamide, terbutryn, spinosad A, isoproturon, diuron, metalaxyl-M, spinosad D, dimethenamid-p, penconazole, isoxadifen, tebuconazole, diflubenzuron, epoxiconazole, prothioconazole, propiconazole, chlorfenvinphos, triflusulfuron methyl, pendimethalin, cyazofamid, pyraclostrobine, diflufenican, flufenoxuron, and lufenuron. A stock solution of each of these standards at 1 g L^{-1} was prepared in acetonitrile.

For GC-MS/MS pesticide analysis, the pesticides (except OCPs) were as follows: clofentezine, dichlobenil, etridiazole, diphenylamine, trifluralin, chlorpropham, tebutam, clomazone, propyzamide, lindane, pyrimethanil, dimethenamid-P, dimethachlor, acetochlor, alachlor, fenpropidin, carbaryl, ethofumesate, malathion, fenpropimorph, metolachlor-S, chlorpyrifos, flurochloridone, cyprodinil, pendimethalin, tolyfluanid, metazachlor, penconazole, procymidone, captan, folpet, oxadiazon, buprofezine, kresoxim-methyl, bupirimate, flusilazole, myclobutanil, aclonifen, trifloxystrobin, bromoxynil octanoate, propiconazole, quinoxifen, lenacile, diclofop-methyl, chloridazon, diflufenicanil, fluazinam, tebuconazole, bifenthrin, dimoxystrobin, epoxiconazole, fenoxycarb, isoxaflutole, tebufenpyrad, bifenox, lambda cyhalothrin, fenarimol, pyraclostrobin, prochloraz, cypermethrin, boscalid, indoxacarb, difenoconazole,

deltamethrin, azoxystrobin, dimethomorph, spiromoxamine, and metamitron. A stock solution of each of these standards at 1 g L^{-1} was prepared in acetonitrile.

For OCP analysis, a mixture at 0.1 g L^{-1} of 21 OCPs, including α -HCH, γ -HCH, β -HCH, δ -HCH, heptachlor epoxide A, methoxychlor, o,p'-DDD, o,p'-DDT, p,p'-DDD, p,p'-DDT, α -endosulfan, o,p'-DDE, p,p'-DDE, aldrin, heptachlor, dieldrin, hexachlorobenzene, heptachlor epoxide B, *trans*-chlordane, and *cis*-chlordane, was purchased from Cluzeau Info Labo (St. Foy la Grande, France).

For PAH analysis, a mixture at 0.1 g L^{-1} of 16 PAHs (i.e., naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo(a)pyrene, indeno (1,2,3-cd) pyrene, benzo[*g,h,i*]perylene, and dibenzo[*a,h*]anthracene) was prepared from individual standards purchased from Sigma-Aldrich (L'Isle D'Abeau, France).

For PCB analysis, a mixture at 0.1 g L^{-1} of 22 PCBs (i.e., PCB 18, PCB 31, PCB 28, PCB 52, PCB 44, PCB 70, PCB 81, PCB 101, PCB 123, PCB 118, PCB 114, PCB 105, PCB 126, PCB 149, PCB 153, PCB 138, PCB 167, PCB 156, PCB 157, PCB 169, PCB 180, and PCB 189) was purchased from Cluzeau Info Labo (St. Foy la Grande, France).

All prepared solutions were stored at -18°C .

Four internal standards for LC-MS/MS were obtained from CDN isotopes (Quebec, Canada): carbendazim- d^4 (99.3%), diuron- d^6 (99.8%), pendimethalin- d^5 (99%), and nicosulfuron- d^6 (99%). A standard solution of each compound at 0.05 g L^{-1} in acetonitrile was prepared, and a mixture of each compound at 0.01 g L^{-1} in acetonitrile was prepared and stored at -18°C for alternative use as internal standard (IS) solution.

Internal standards for GC-MS/MS were trifluralin- d^{14} , 4-nitrophenol- d^4 , and naphthalene- d^8 (99%), and they were purchased from Sigma-Aldrich (L'Isle d'Abeau, France) and Cambridge Isotope Laboratories (Cluzeau Info Labo, France). A mixture of trifluralin- d^{14} and 4-nitrophenol- d^4 at 0.01 g L^{-1} in acetonitrile was used as IS solution for pesticide analysis except OCPs. A solution of naphthalene- d^8 at 0.01 g L^{-1} in acetonitrile was used as IS solution for OCP, PAH, and PCB analyses.

HPLC-grade acetonitrile, toluene (TOL) from Biosolve (Dieuze, France), methanol (MeOH), ethyl acetate (EA), “Fontainebleau” sand from Prolabo (France), and silica gel (Merck, Germany) were used. LC–MS-grade acetonitrile, LC–MS-grade water, formic acid, and HPLC-grade acetonitrile were purchased from Sigma–Aldrich (L'Isle D'Abeau, France). Ultrapure water used was purified by an Elga system (Antony, France).

Kits for QuEChERS sample preparations were purchased as ready to use from RESTEK, France. Buffered extraction kits (EN 1566 method) containing 4 g of MgSO₄, 1 g of NaCl, 1 g of trisodium citrate dihydrate, and 0.5 g of disodium hydrogencitrate sesquihydrate were used. For clean-up, sample clean-up kits (AOAC 2007 method) containing 1.2 g of MgSO₄, 400 mg of PSA, and 400 mg of C₁₈ were used.

SPE CHROMABOND® EASY cartridges consisting of a polar-modified polystyrene divinyl benzene copolymer were used as adsorbents (mean pore diameter 60 Å, surface area 623 m²/g, and mean particle size 91 µm) and purchased from Macherey–Nagel, France.

SPME polyacrylate (PA) fiber (65 µm) was purchased from Supelco-Sigma Aldrich (Saint Martin d'Hère France) and used for the extraction of semi-volatile pesticides except OCPs.

SPME polydimethylsiloxane (PDMS) fiber (100 µm) was purchased from Supelco- Sigma Aldrich (Saint Martin d'Hère France) and used for the extraction of OCPs, PCBs, and PAHs.

2.2. Sample collection

Certified *H. aspersa* blank snails were purchased from “Cap' Hélix Escargots, 2, Bréharadec, 29,770 GOULIEN, France.” The snails were transported frozen to the laboratory where they were kept at –18 °C until used.

For real sample analysis, three samples of *H. aspersa* were collected from three different lands in Akkar region situated in northern Lebanon (34°33'02"N 036°04'41"E). This region is known for its high population rate and agricultural production.

Samples were collected in propylene tubes and then transported frozen to the laboratory, where they were stored at –18 °C until analysis.

2.3. Extraction procedures

About 5 g of homogenized blank snails samples was weighed in 50 mL centrifuge tubes and fortified with 200 μ L of each mixture. Fortified samples were kept at 4 °C overnight, followed by the extraction procedure cited below. All extractions were conducted in triplicate.

2.3.1. ASE–SPE-based extraction

2.3.1.1. ASE

About 5 g of the fortified snails was extracted by ASE. For ASE, 33 mL cells were fitted at the bottom with filter paper, and a thin layer (1 cm height) of activated silica gel was added to provide a first purification step. A second filter was added on the top of the silica, and 5 g of snails mixed with “Fontainebleau” sand was added. Finally, a filter was added at the top of the cell, which was sealed well. Extraction was carried out with acetonitrile (100%), and the program was as follows: heating the cell for 7 min, 10 min of static cycle, temperature 100 °C, pressure 1500 psi, flushing 100%, and purging for 300 s. Around 30 min was required for each extracting cell.

ASE final extracts were collected in ASE bottles and prepared for SPE purification. Extracts were filtered and then diluted to 1000 mL with acidified (pH 3) ultrapure water.

2.3.1.2. SPE

The SPE procedure was as follows: conditioning of the cartridge with 5 mL of MeOH followed by 5 mL of ultrapure water, flushing 1000 mL of the sample into the cartridge at 10 mL min⁻¹, and drying by N₂ flushing for 30 min. Extracts were sequentially eluted with 2 mL of each of the following solvents: EA, TOL, and acetonitrile. The obtained extract was sampled in a 10 mL glass tube and then evaporated to 100 μ L. Around 1 h was needed for each sample to be extracted by SPE.

2.3.2. QuEChERS-based extraction

This extraction procedure was based on the work of Al Alam et al. in 2017 for the analysis of organic pollutant residues in honey [41].

First, 10 mL of acetonitrile was added to 5 g of fortified snails, and the tubes were shaken. When the mixture was homogeneous, 10 mL of QuEChERS citrate buffered extraction salts

was added. The tubes were immediately shaken by hand, vortexed for 1 min, and centrifuged for 10 min at 5000 rpm. Second, the supernatant was added to the 15 mL PSA tube. This tube was immediately shaken by hand, vortexed for around 30 s, and centrifuged for 10 min at 5000 rpm. Finally, the obtained extract was sampled in a 10 mL glass tube and then evaporated to 100 μ L. Around 20 min was needed for the whole procedure.

2.3.3. Extract reconstitution

Once evaporated, the collected extracts were reconstituted with acetonitrile to 1 mL to prepare them for chromatographic analysis.

About 100 μ L of the final extract was transferred to an LC vial, where 10 μ L of the appropriated IS solution was added. The extract was then analyzed by LC–MS/MS.

The remaining 900 μ L was diluted to 20 mL with salted water (1.5% NaCl) to promote their adsorption on the SPME fiber. Subsequently, 10 μ L of both appropriated GC IS solutions was added prior to this latest purification and extraction.

2.3.4. SPME and concentration

SPME fibers were soaked in 20 mL of the salted prepared solution and heated at 60 °C under agitation (500 rpm) for 40 min for the PA fiber and at 80 °C for 40 min for the PDMS fiber.

SPME was carried out by direct immersion in which the fiber was directly immersed into the liquid sample, and the analytes were divided between the fiber and the liquid sample. After extraction, the SPME fiber was transferred to the GC injection port where desorption of the analyte occurred and analysis was carried out.

2.4. Sample analysis

2.4.1. LC–MS/MS

The system used was an LC system (Thermo Scientific, Surveyor pump and autosampler) coupled with a tandem MS/MS system (TSQ Quantum Access Max equipped with a Hyper Quads Driven) operating in electrospray ionization (ESI) mode. Chromatographic

separation was performed on a Macherey-Nägel Nucleodur C₁₈ Pyramid column (150 mm × 3 mm; 3 μm) thermostated at 25 °C. The chromatographic system was also equipped with an autosampler (Accela Autosampler) and a Surveyor LC Pump Plus (Thermo Scientific). The flow rate was 300 μL min⁻¹. Samples were analyzed using a mobile phase of acetonitrile/water (0.05% formic acid). The gradient started with 30:70 (v/v) for 5 min, followed by 50:50 (v/v) for 6 min and then 80:20 (v/v) for 7 min to achieve 95:5 (v/v) for 10 min. Finally, a ratio of 30:70 (v/v) for 8 min was recommended to stabilize the column for a new injection. The injection volume was 20 μL.

Details on the analyzed pesticide figure are illustrated in supplementary materials S1.

2.4.2. GC-MS/MS

A Thermo Scientific Trace GC coupled with an MS/MS system (ITQ 700, Temperature source: 210 °C, transfer line temperature: 300 °C) operating in electron impact (EI) mode was used. The considered POPs (20 OCPs, 16 PAHs, and 22 PCBs) and the 70 remaining semi-volatile pesticides were analyzed on an XLB (50% phenyl/ 50% methylsiloxane) capillary column (30 m × 0.25 mm internal diameter and 0.25 μm as film thickness). Injections were made in splitless mode at 250 °C for 15 min. Helium was used as a carrier gas at a flow rate of 1 mL min⁻¹.

2.4.2.1. POP separation and analysis

Samples were injected by thermal desorption of the PDMS SPME fiber. The initial oven temperature was set at 50 °C for 3 min, followed by a linear ramp to 255 °C at a rate of 10 °C min⁻¹ and another increase to 330 °C at a rate of 20 °C min⁻¹, where it was maintained for 18 min. The total run time was 45.25 min.

Details on the analyzed POP figure are in supplementary materials S2.

2.4.2.2. Semi-volatile pesticide separation and analysis

Samples were injected by thermal desorption of the PA SPME fiber. The initial oven temperature was set at 50 °C for 3 min, followed by a linear ramp to 160 °C at a rate of 36.6 °C min⁻¹ and another ramp to 300 °C at a rate of 5.8 °C min⁻¹, where it was maintained for 10 min. The total run time was 41 min.

Details of the semi-volatile pesticides analyzed by GC-MS/MS are shown in supplementary materials S3.

2.5. Method validation

2.5.1. Validation parameters

The method was validated for all quantification parameters. First, fortified samples with a concentration range of 5–3000 ng g^{-1} were extracted in triplicate to determine linearity. Five samples of spiked matrix with three concentration levels (10, 100, and 1000 ng g^{-1}) were extracted for three successive days to determine intermediate precision and repeatability. The intra and inter-day precision and accuracy were estimated by analyzing five replicates at three different QC levels (10, 100, and 1000 ng g^{-1}). The intra-day precision of the assay was estimated by calculating the relative standard deviation (RSD) for the analysis of QC samples in five replicates, and inter-day precision was determined by the analysis of five replicates of QC samples on three consecutive days. This parameter was determined by varying processes conditions, which were the different days of extraction, different solvent bottles, and different extraction kits used. The intra-day and inter-day precision were evaluated by their corresponding RSD (%).

Concerning method validation limits, the limit of detection (LOD) and limit of quantification (LOQ) were calculated using the signal produced over the background noise obtained. These limits were determined using $LOD = 3 \times [\text{min}] S/N$ and $LOQ = 10 \times [\text{min}] S/N$, where $[\text{min}]$ = minimal concentration at which a signal was obtained, S = signal intensity obtained at this concentration, and N = noise intensity obtained at this concentration.

The recoveries were determined at the same levels as precision following the equation:

$$\text{Recovery (\%)} = (A \text{ extracted spiked sample} / A \text{ standard solution}) \times 100$$

where A = peak area obtained.

2.5.2. Matrix-matched calibration curves

To overcome the possible matrix effect and to obtain reliable data of analyzed samples, matrix-matched calibration curves were developed. Ten calibration points were prepared

using the validated extraction procedure. Homogenate matrices were spiked with standard solutions to cover a pollutant range between 5 and 3000 ng g⁻¹, extracted, and analyzed. The calibration points used included the following concentrations (in ng g⁻¹): 5, 10, 25, 50, 100, 500, 1000, 1500, 2000, and 3000.

Calibration curves were validated for their linearity by calculating the determination coefficient R^2 .

Finally, the validation of each compound included several criteria such as fragmentations, retention time, and ion ratios for nonvolatile compounds. Furthermore, the extraction performance was determined following the signal areas of the IS solutions in each analyzed sample.

Calibrations and quantification analysis were conducted using XCalibur software.

3. Results and discussion

3.1. Method development

To obtain a concentrated extract, a concentration step by evaporation and reconstitution was added prior to LC analysis. Moreover, SPME was added prior to GC analysis for arranging and discriminating volatile compounds. Among different SPME fibers, the PDMS fiber was used as it is well known for its usefulness for the extraction of analytes with high partition coefficients such as PAHs, PCBs, and OCPs, whereas PA was used for the extraction of pesticides due to its efficiency in extracting polar compounds. PA is a moderated polar coating characterized by a stronger hydrogen bond than PDMS fiber, which makes it ideal for polar compounds with moderate hydrophobicity compared with other fibers [42]. These concentration steps were used following the two extraction procedures previously detailed. Both extractions were compared to choose the method enabling the highest recovery with the lowest RSD%.

Chromatograms of the assessed nonvolatile pesticides, volatile pesticides, PAHs, PCBs, and OCPs are shown in Fig. 1, Fig. 2, Fig. 3 in supplementary materials S4. These obtained chromatograms showed good separation of all sought compounds, which allowed the identification of the assessed pollutants on the basis of each parameter previously shown in Tables 1s–3 s.

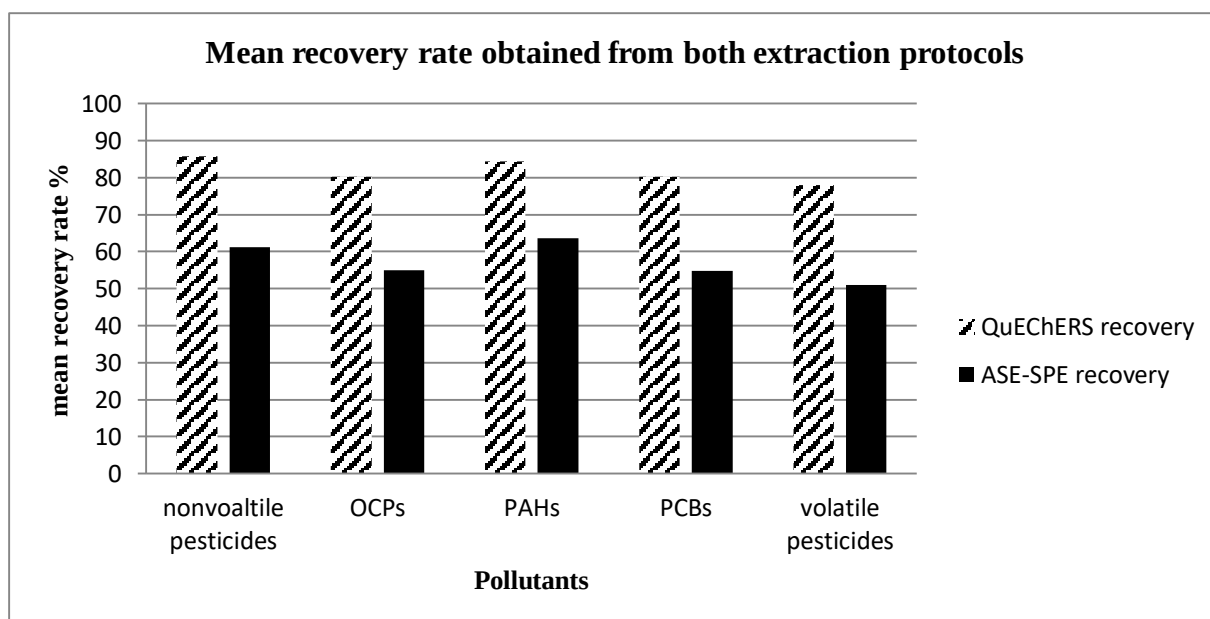


Fig. 1. Mean recovery rate obtained from both extraction protocols.

Table 1. Compounds detected in real samples: percentage of samples contaminated and the average quantified concentration.

	Average concentration (ng g ⁻¹)	Percentage of samples contaminated
Carbendazim	38.5	100
Chloridazon	0.56	66.6
Pymetrozine	30.6	100
Acetamiprid	0.43	66.6
Terbutryn	2.17	66.6
Metalaxyl-M	3.4	66.6
Sulcotrione	85.6	100
Trifluralin	26.6	100
Chlorpropham	33.5	66.6
Tebutam	8.63	33.3
Clomazone	53.71	100
Propyzamide	59.1	100
Naphthalene	8.54	100
Acenaphthene	6.82	100
Fluorene	9.16	66.6
Anthracene	42.9	100
Phenanthrene	18.73	100
Fluoranthene	314.33	100

3.1.1. Influence of the extraction procedure

Fortified snails at a mid-level concentration of 1000 ng g^{-1} of each pollutant mixture were extracted by ASE-SPE-based extraction and QuEChERS-based extraction. The two extractions were followed by a pre-concentration step using SPME prior to GC-MS/MS analysis.

All extractions were carried out in triplicate, and RSD% was calculated for each compound.

Fig. 1 shows the recoveries obtained by the two methods at a concentration of 1000 ng g^{-1} . This figure shows the average recovery rate for each type of considered pollutant.

The obtained results showed that the recoveries obtained with ASE-SPE-based extraction were lower than those obtained with QuEChERS-based extraction. For semi-volatile pesticides, the recoveries obtained with ASE-SPE-based extraction were between 20% and 94% with a mean recovery of 61.24%, whereas those obtained with QuEChERS-based extraction were between 65% and 107% with a mean recovery of 85.72%. For OCP recoveries, results obtained with ASE-SPE-based extraction were between 17% and 82% with a mean recovery of 54.93%, whereas those obtained with QuEChERS-based extraction were between 62% and 104% with a mean recovery of 80.24%. For PAH recoveries, results obtained with ASE-SPE-based extraction were between 56% and 71% with a mean recovery of 63.39%, whereas those obtained with QuEChERS-based extraction were between 64% and 96% with a mean recovery of 84.31%. For PCB recoveries, results obtained with ASE-SPE-based extraction were between 17% and 110% with a mean recovery of 54.75%, whereas those obtained with QuEChERS-based extraction were between 50% and 117% with a mean recovery of 80.2%. For the remaining volatile pesticides, high recovery rates were obtained with QuEChERS-based extraction. Results obtained with ASE-SPE-based extraction were between 14% and 127% with a mean recovery of 51.06%, whereas those obtained with QuEChERS-based extraction were between 55% and 114% with a mean recovery of 78.06%.

These results clearly demonstrated the influence of the extraction procedure on pollutant analysis from the same matrix. These results were also proven by Blasco et al. in 2011,

who proved that QuEChERS-based extraction allows higher recovery rates in comparison with PLE and SPE [43].

The RSD% of the two developed methods was also assessed. The RSD% of each pollutant type obtained using the two compared extraction methods is shown in Fig. 2.

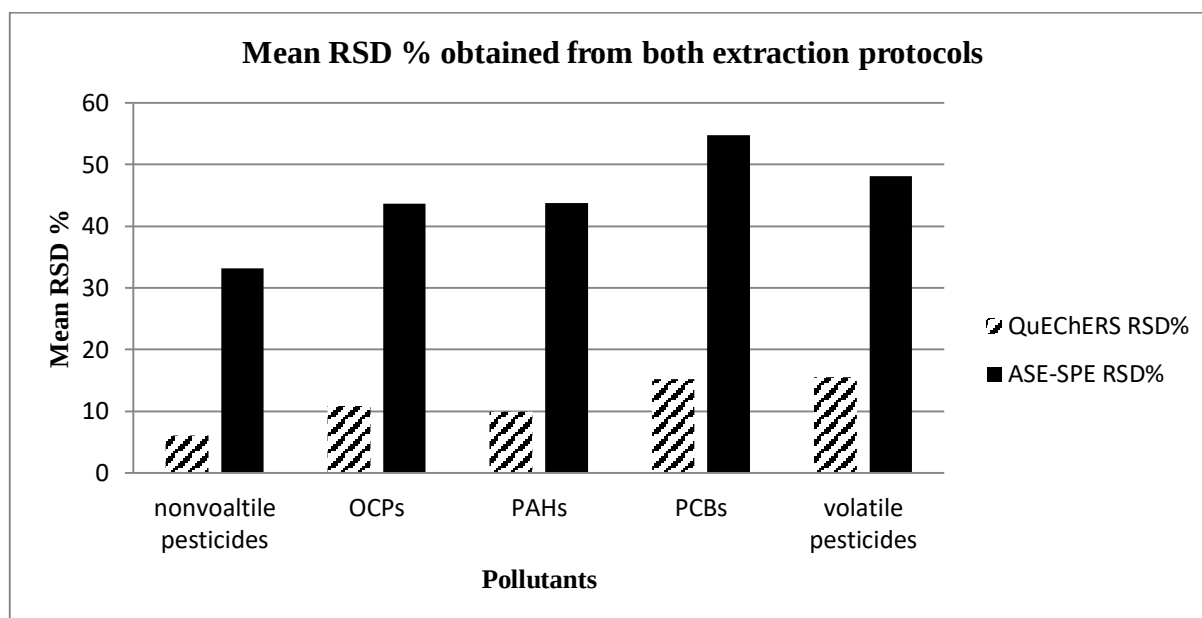


Fig. 2. Mean RSD% obtained from both extraction protocols.

The results obtained from the analysis of the RSD% of both extraction protocols favored extraction based on QuEChERS to that based on ASE–SPE. The RSD% obtained with ASE–SPE was higher than 20% for all the analyzed pollutants, whereas this value was lower than 15% for all compounds extracted using QuEChERS. The mean RSDs% for ASE–SPE-based extraction were 33.12%, 43.66%, 43.71%, 54.71%, and 48.06% for the determination of nonvolatile pesticides, OCPs, PAHs, PCBs, and remaining volatile pesticides, respectively. These values were far lower for QuEChERS-based extraction; the RSDs% were 6.11%, 10.82%, 9.91%, 15.16%, and 15.55% for nonvolatile pesticides, OCPs, PAHs, PCBs, and remaining volatile pesticides, respectively.

The results provided by the calculation of the recoveries and the RSD% proved that QuEChERS-based extraction is a method of choice for the analysis of multiresidual pollutants from such matrices, providing high recovery rates with a low RSD. Compared with ASE-based methods, QuEChERS-based extraction is rapid and easy to use, providing better recoveries with fewer losses of volatile compounds [44].

For all these reasons, QuEChERS-based extraction was proved to be the method of choice in this work. Furthermore, several crucial points, such as the high cost of equipment, large volume used for cell rinsing and preparation before extraction, high temperature leading to low recoveries and decomposition of thermally instable analytes, and several extraction steps increasing RSD% [45, 46], reduce ASE–SPE-based extraction's efficiency and give high credibility to QuEChERS-based extraction for the analysis of multi-residues of environmental pollutants.

3.1.2. Influence of the nature of the extraction solvent

As QuEChERS seems to be the most efficient extraction procedure, a selection of an appropriate extraction solvent for liquid–liquid extraction is crucial to improve recovery. The extraction solvent plays a main role in any extraction step as incomplete extraction and matrix effects can lead to an underestimation of the actual concentration in the sample [47]. The organic solvent chosen must be highly polar, miscible in water, and able to induce phase separation following the addition of the appropriate extraction salts. Moreover, the suitable salt must not be soluble in the extraction solvent [29]. Acetonitrile and EA were tested on fortified snails with the same precision level, and the extraction efficiency of both solvents was compared. To choose the best extraction solvent, recoveries were calculated. Fig. 3 shows the recovery results obtained with the use of acetonitrile and EA as QuEChERS extraction solvent.

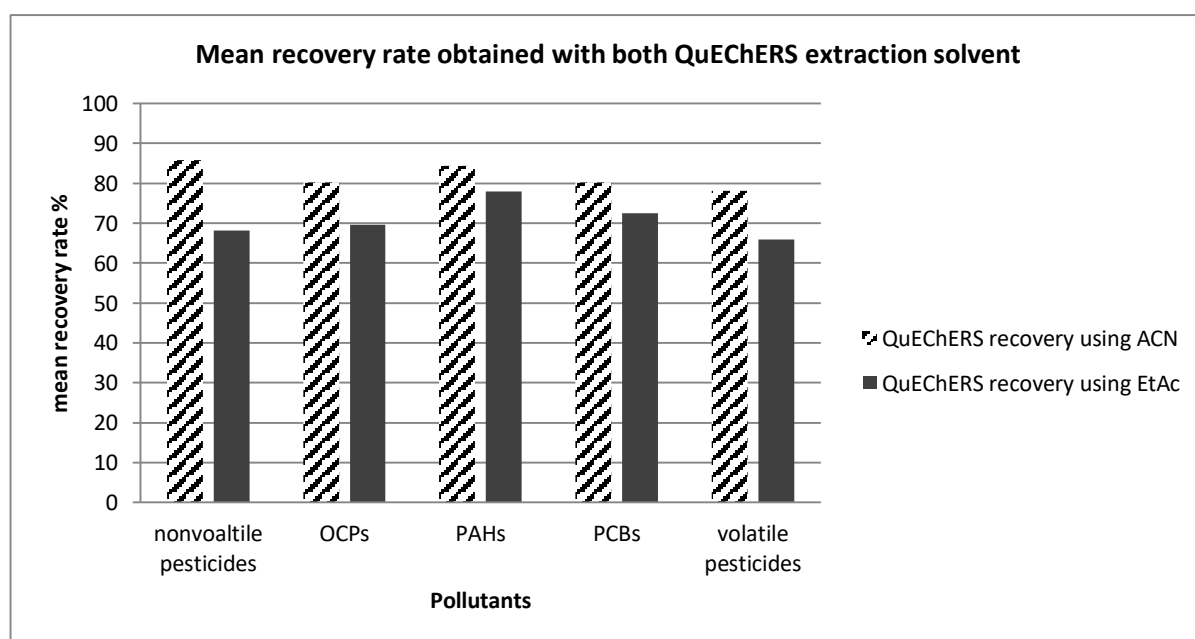


Fig. 3. Mean recovery rate obtained with both QuEChERS extraction solvent.

As observed in Fig. 3, the use of EA showed recoveries lower than those obtained with acetonitrile. For nonvolatile pesticides, the recovery obtained was 68.09% for the use of EA and 85.62% with the use of acetonitrile; for OCPs, the use of EA gave a mean recovery of 69.59%, whereas this value was 80.24% with the use of acetonitrile. For PAHs and PCBs, the mean recoveries were 77.91 and 72.49% with EA, respectively, and 84.31 and 80.2% with acetonitrile, respectively. Likewise, the recovery rate of semi-volatile pesticides increased from 65.95% with the use of EA as QuEChERS extraction solvent to 78.06% with the use of acetonitrile.

The use of acetonitrile has proven its efficiency in several multiresidue analyses in biological samples in comparison with EA, exhibiting better recoveries and lower standard deviations between replicates [29, 48, 49].

For matrix effects, the analysis of biological matrices, such as snails, can lead to the co-extraction of a certain quantity of other compounds, such as lipids, sugars, and organic acids; their elimination prior to the final determination step is crucial [48].

Studies have shown that PSA allows the removal of all polar organic acids, polar pigments, sugars, and fatty acids from the extracts, whereas C₁₈ allows the elimination of nonpolar interfering substances such as lipids [34, 50].

The analyzed extracts showed good separation among all searched compounds, which allowed the identification of 120 pesticides, 16 PAHs, and 22 PCBs on the basis of the properties of each parameter previously shown in Tables 1s, 2 s, and 3 s.

3.2. Method validation

Once chosen and developed, the method should be validated as efficient and useful. The validation proves that the used analytical methodology is accurate, specific, reproducible, and robust over the specified range that a compound will be analyzed [51]. Several parameters were tested: repeatability and reproducibility for method accuracy, LOD and LOQ for method limits, linearity for the ability of the method to elicit test results that are directly proportional to analyte concentration within a range between 5 and 3000 ng g⁻¹, and recovery for extraction efficiency. To include the error due to the matrix effect in

measurements, matrix-matched calibrations were conducted. Figure 4 s in supplementary material S5 shows the calibration curves of some analyzed compounds.

Table 4 s represents the results of the validation parameters for nonvolatile pesticides analyzed by LC-MS/MS.

For the nonvolatile pesticides analyzed by LC-MS/MS, all target compounds were validated with good linearity expressed by a regression coefficient higher than 0.99 for the 30 targeted pesticides. LOD and LOQ were lower than 15 ng g^{-1} for all compounds, except lufenuron having an LOQ of about 20 ng g^{-1} . The 30 pesticides were detected with high precision with RSD% lower than 20% for inter- and intra-day analyses. Furthermore, the method showed good recoveries higher than 65% for all targeted compounds.

For semi-volatile compounds, Tables 5 s and 6 s represent the validation parameters for OCPs, PAHs, PCBs, and volatile pesticide analyzed by GC-MS/MS.

For the semi-volatile compounds analyzed with GC-MS/MS, compounds were validated for their good linearity expressed by regression coefficients higher than 0.99 for the majority of the compounds sought. Low LOD and LOQ were determined for the analyzed compounds with an RSD lower than 20% for most of them. By contrast, the calculated RSD% was higher than the one obtained with the nonvolatile pesticides. The main explanation for these results is the introduction of SPME as a pre-concentration step; excluding this step increased the error and decreased the recovery rates [52]. Moreover, some fiber's saturation problems could be the main reasons for the high RSD% and low recovery observed with some compounds (PCB-169, PCB-157, methoxychlor, *o,p'*-DDT, and bupirimate).

3.3. Application to real samples

The samples collected were analyzed in accordance with the previously developed method. Residue levels were calculated using Xcalibur software based on the previously plotted calibration curves.

Most of the assessed samples were contaminated with pesticides and PAHs, whereas no important PCB residues were found for all the samples analyzed.

Table 1 shows the most relevant pesticides and the PAH residues found in the samples analyzed.

4. Conclusion

The analytical method developed in this paper enabled the simultaneous analysis of 158 emerging environmental pollutants from the terrestrial gastropod *H. aspersa*. The combination of a simple extraction method such as QuEChERS with an SPME process coupled with chromatographic analytical techniques allowed the extraction of this wide number of different types of pollutants. The comparison of the QuEChERS–SPME-based method with ASE–SPE–SPME proved the efficiency of the first method, especially in terms of being efficient and ecofriendly. The validation of the developed method yielded positive results, indicating good performance in terms of linearity, accuracy, and precision.

The presented extraction method followed by chromatographic analysis via LC–MS/MS and GC–MS/MS presents a reliable tool for the routine analysis of a large range of compounds at trace level in snails. The proposed method may be applied in further studies focusing on environmental biomonitoring.

References

- [1] S.I. Korfali, H. Karaki, Speciation of metals in soils and water: risk assessment, *Environ. Process* 5 (2018) 101–125, doi:[10.1007/s40710-018-0328-1](https://doi.org/10.1007/s40710-018-0328-1).
- [2] M. Wazne, S. Korfali, Spatial and temporal assessment of metal pollution in the sediments of the Qaraoun reservoir, Lebanon, *Environ. Sci. Pollut. Res.* 23 (2016) 7603–7614, doi:[10.1007/s11356-015-6022-1](https://doi.org/10.1007/s11356-015-6022-1).
- [3] S. Saha, R. Mistri, B.C. Ray, Determination of pyridine, 2-picoline, 4-picoline and quinoline from mainstream cigarette smoke by solid-phase extraction liquid chromatography/electrospray ionization tandem mass spectrometry, *J. Chromatogr. A* 1217 (2010) 307–311, doi:[10.1016/j.chroma.2009.11.050](https://doi.org/10.1016/j.chroma.2009.11.050).
- [4] S. Saha, R. Mistri, B.C. Ray, Rapid and sensitive method for simultaneous determination of six carcinogenic aromatic amines in mainstream cigarette smoke by liquid chromatography/electrospray ionization tandem mass spectrometry, *J. Chromatogr. A* 1216 (2009) 3059–3063, doi:[10.1016/j.chroma.2009.01.069](https://doi.org/10.1016/j.chroma.2009.01.069).
- [5] Z. Yang, B. Chen, L. Li, B. Zheng, L. Liu, International conference on ecological informatics and ecosystem conservation (ISEIS 2010) biomonitoring and bioindicators used for river ecosystems: definitions, approaches and trends, *Procedia Environ. Sci* 2 (2010) 1510–1524, doi:[10.1016/j.proenv.2010.10.164](https://doi.org/10.1016/j.proenv.2010.10.164).
- [6] M. Marc, M. Tobiszewski, B. Zabiegała, M. de la Guardia, J. Namiesnik, Current air quality analytics and monitoring: a review, *Anal. Chim. Acta* 853 (2015) 116–126, doi:[10.1016/j.aca.2014.10.018](https://doi.org/10.1016/j.aca.2014.10.018).
- [7] B. Wolterbeek, Biomonitoring of trace element air pollution: principles, possibilities and perspectives, *Environ. Pollut.* 120 (2002) 11–21, doi:[10.1016/S0269-7491\(02\)00124-0](https://doi.org/10.1016/S0269-7491(02)00124-0).
- [8] H.T. Wolterbeek, P. Bode, State of the art of trace element determinations in plant matrices strategies in sampling and sample handling in the context of large-scale plant biomonitoring surveys of trace element air pollution, *Sci. Total Environ.* 176 (1995) 33–43, doi:[10.1016/0048-9697\(95\)04825-1](https://doi.org/10.1016/0048-9697(95)04825-1).
- [9] S. Pérez, P. Sánchez-Marín, J. Bellas, L. Viñas, V. Besada, N. Fernández, Limpets (*Patella* spp. Mollusca, Gastropoda) as model organisms for biomonitoring environmental quality, *Ecol. Indic.* 101 (2019) 150–162, doi:[10.1016/j.ecolind.2019.01.016](https://doi.org/10.1016/j.ecolind.2019.01.016).
- [10] A. Berlioz-Barbier, R. Baudot, L. Wiest, M. Gust, J. Garric, C. Cren-Olivé, A. Buleté, MicroQuEChERS–nanoliquid chromatography–nanospray–tandem mass spectrometry for the detection and quantification of trace pharmaceuticals in benthic invertebrates, *Talanta* 132 (2015) 796–802, doi:[10.1016/j.talanta.2014.10.030](https://doi.org/10.1016/j.talanta.2014.10.030).
- [11] M. Gust, T. Buronfosse, O. Geffard, R. Mons, H. Queau, J. Mouthon, J. Garric, In situ biomonitoring of freshwater quality using the New Zealand mudsnail *Potamopyrgus antipodarum* (Gray) exposed to waste water treatment plant (WWTP) effluent discharges, *Water Res.* 44 (2010) 4517–4528, doi:[10.1016/j.watres.2010.06.019](https://doi.org/10.1016/j.watres.2010.06.019).
- [12] A. Beeby, L. Richmond, Evaluating *Helix aspersa* as a sentinel for mapping metal pollution, *Ecol. Indic.* 1 (2002) 261–270, doi:[10.1016/S1470-160X\(02\)00022-5](https://doi.org/10.1016/S1470-160X(02)00022-5).
- [13] R. Laskowski, S.P. Hopkin, Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*): implications for predators, *Environ. Pollut.* 91 (1996) 289–297, doi:[10.1016/0269-7491\(95\)00070-4](https://doi.org/10.1016/0269-7491(95)00070-4).

- [14] A. De Vaufléury, M. Coeurdassier, P. Pandard, R. Scheifler, C. Lovy, N. Crini, P.M. Badot, How terrestrial snails can be used in risk assessment of soils, *Env.-iron. Toxicol. Chem.* 25 (2006) 797–806, doi:[10.1897/04-560R.1](https://doi.org/10.1897/04-560R.1).
- [15] B. Pauget, F. Gimbert, M. Coeurdassier, N. Crini, G. Pérès, O. Faure, F. Douay, Richard, C. Grand, A. de Vaufléury, Assessing the in situ bioavailability of trace elements to snails using accumulation kinetics, *Ecol. Indic.* 34 (2013) 126–135, doi:[10.1016/j.ecolind.2013.04.018](https://doi.org/10.1016/j.ecolind.2013.04.018).
- [16] A. Gomot-de Vaufléury, A. Bispo, Methods for toxicity assessment of contaminated soil by oral or dermal uptake in land snails. 1. Sublethal effects on growth, *Environ. Sci. Technol.* 34 (2000) 1865–1870, doi:[10.1021/es9907212](https://doi.org/10.1021/es9907212).
- [17] K.S. El-Gendy, M.A. Radwan, A.F. Gad, A.E. Khamis, E.H. Eshra, Use of multiple endpoints to investigate the ecotoxicological effects of abamectin and thi-amethoxam on *Theba pisana* snails, *Ecotoxicol. Environ. Saf.* 167 (2019) 242–249, doi:[10.1016/j.ecoenv.2018.10.027](https://doi.org/10.1016/j.ecoenv.2018.10.027).
- [18] D. Kowalczyk-Pecka, S. Pecka, E. Kowalczyk-Vasilev, Changes in fatty acid metabolism induced by varied micro-supplementation with zinc in snails *Helix pomatia* (Gastropoda Pulmonata), *Ecotoxicol. Environ. Saf.* 138 (2017) 223–230, doi:[10.1016/j.ecoenv.2016.12.033](https://doi.org/10.1016/j.ecoenv.2016.12.033).
- [19] J.L. Capinera, K. Dickens, Some effects of copper-based fungicides on plant-feeding terrestrial molluscs: a role for repellents in mollusc management, *Crop Prot.* 83 (2016) 76–82, doi:[10.1016/j.cropro.2016.01.018](https://doi.org/10.1016/j.cropro.2016.01.018).
- [20] F.R.d. Silva, B. Erdtmann, T. Dalpiaz, E. Nunes, A. Ferraz, T.L. Martins, J.F. Dias, D.P.d. Rosa, M. Porawskie, S. Bona, Genotoxicity of *Nicotiana tabacum* leaves on *Helix aspersa*, *Genet. Mol. Biol.* 36 (2013) 269–275, doi:[10.1590/S1415-47572013005000020](https://doi.org/10.1590/S1415-47572013005000020).
- [21] M.R. de Souza, F.R. da Silva, C.T. de Souza, L. Niekraszewicz, J.F. Dias, S. Pre-moli, D.S. Corrêa, M.d.C. Soares, N.P. Marroni, M.I. Morgam-Martins, J. da Silva, Evaluation of the genotoxic potential of soil contaminated with mineral coal tailings on snail *Helix aspersa*, *Chemosphere* 139 (2015) 512–517, doi:[10.1016/j.chemosphere.2015.07.071](https://doi.org/10.1016/j.chemosphere.2015.07.071).
- [22] C. Druart, M. Millet, R. Scheifler, O. Delhomme, C. Raeppl, A. de Vaufléury, Snails as indicators of pesticide drift, deposit, transfer and effects in the vineyard, *Sci. Total Environ.* 409 (2011) 4280–4288, doi:[10.1016/j.scitotenv.2011.07.006](https://doi.org/10.1016/j.scitotenv.2011.07.006).
- [23] A.G.d. Vaufléury, F. Pihan, Methods for toxicity assessment of contaminated soil by oral or dermal uptake in land snails: metal bioavailability and bioaccumulation, *Environ. Toxicol. Chem.* 21 (2002) 820–827, doi:[10.1002/etc.5620210419](https://doi.org/10.1002/etc.5620210419).
- [24] A. De Vaufléury, M. Coeurdassier, P. Pandard, R. Scheifler, C. Lovy, N. Crini, P.M. Badot, How terrestrial snails can be used in risk assessment of soils, *Env.-iron. Toxicol. Chem.* 25 (2006) 797–806, doi:[10.1897/04-560R.1](https://doi.org/10.1897/04-560R.1).
- [25] A.D. Campoy-Diaz, M.A. Arribére, S.R. Guevara, I.A. Vega, Bioindication of mercury, arsenic and uranium in the apple snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae): bioconcentration and depuration in tissues and symbiotic corpuscles, *Chemosphere* 196 (2018) 196–205, doi:[10.1016/j.chemosphere.2017.12.145](https://doi.org/10.1016/j.chemosphere.2017.12.145).
- [26] M. Ianistcki, J. Dallarosa, C. Sauer, C. Teixeira, J. Da Silva, Genotoxic effect of polycyclic aromatic hydrocarbons in the metropolitan area of Porto Alegre, Brazil, evaluated by *Helix aspersa* (Müller, *Environ. Pollut.* 157 (2009) 1774) 2037–2042, doi:[10.1016/j.envpol.2009.02.025](https://doi.org/10.1016/j.envpol.2009.02.025).
- [27] F. Pihan, A. De Vaufléury, The snail as a target organism for the evaluation of industrial waste dump contamination and the efficiency of its remediation, *Ecotoxicol. Environ. Saf.* 46 (2000) 137–147, doi:[10.1006/eesa.1999.1891](https://doi.org/10.1006/eesa.1999.1891).
- [28] F. Regoli, S. Gorbi, D. Fattorini, S. Tedesco, A. Notti, N. Machella, R. Bocchetti, M. Benedetti, F. Piva, Use of the land snail *Helix aspersa* as sentinel organism for monitoring ecotoxicological effects of urban pollution: an integrated approach, *Environ. Health Perspect.* (2006) 63–69, doi:[10.1289/ehp.8397](https://doi.org/10.1289/ehp.8397).
- [29] A. Berlioz-Barbier, A. Buleté, J. Faburé, J. Garric, C. Cren-Olivé, E. Vulliet, Multi-residue analysis of emerging pollutants in benthic invertebrates by modified micro-quick-easy-cheap-efficient-rugged-safe extraction and nanoliquid chromatography-nanospray-tandem mass spectrometry analysis, *J. Chromatogr. A* 1367 (2014) 16–32, doi:[10.1016/j.chroma.2014.09.044](https://doi.org/10.1016/j.chroma.2014.09.044).
- [30] J.L. Gómez-Ariza, M. Bujalance, I. Giráldez, A. Velasco, E. Morales, Determination of polychlorinated biphenyls in biota samples using simultaneous pressurized liquid extraction and purification, *J. Chromatogr. A* 946 (2002) 209–219, doi:[10.1016/S0021-9673\(01\)01534-5](https://doi.org/10.1016/S0021-9673(01)01534-5).
- [31] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: a critical review (Part I), *Trends Anal. Chem.* 80 (2016) 641–654, doi:[10.1016/j.trac.2015.08.015](https://doi.org/10.1016/j.trac.2015.08.015).
- [32] J. Li, Y.-B. Wang, K.-Y. Li, Y.-Q. Cao, S. Wu, L. Wu, Advances in different configurations of solid-phase microextraction and their applications in food and environmental analysis, *Trends Anal. Chem.* 72 (2015) 141–152, doi:[10.1016/j.trac.2015.04.023](https://doi.org/10.1016/j.trac.2015.04.023).
- [33] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce, *J. AOAC Int.* 86 (2003) 412–431.
- [34] A. Albinet, S. Tomaz, F. Lestremau, A really quick easy cheap effective rugged and safe (QuEChERS) extraction procedure for the analysis of particle-bound PAHs in ambient air and emission samples, *Sci. Total Environ.* 450 (2013) 31–38, doi:[10.1016/j.scitotenv.2013.01.068](https://doi.org/10.1016/j.scitotenv.2013.01.068).
- [35] F.P. Costa, S.S. Caldas, E.G. Primel, Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in canned and fresh peach, *Food Chem.* 165 (2014) 587–593, doi:[10.1016/j.foodchem.2014.05.099](https://doi.org/10.1016/j.foodchem.2014.05.099).
- [36] P.-L. Cloutier, F. Fortin, P.E. Groleau, P. Brousseau, M. Fournier, M. Desrosiers, QuEChERS extraction for multi-residue analysis of PCBs, PAHs, PBDEs and PCDD/Fs in biological samples, *Talanta* 165 (2017) 332–338, doi:[10.1016/j.talanta.2016.12.080](https://doi.org/10.1016/j.talanta.2016.12.080).
- [37] M. Mattarozzi, M. Milioli, F. Bianchi, A. Cavazza, S. Pigozzi, A. Milandri, M. Careri, Optimization of a rapid QuEChERS sample treatment method for HILIC-MS2 analysis of paralytic shellfish poisoning (PSP) toxins in mussels, *Food Control* 60 (2016) 138–145, doi:[10.1016/j.foodcont.2015.07.027](https://doi.org/10.1016/j.foodcont.2015.07.027).
- [38] D. Megson, E.J. Reiner, K.J. Jobst, F.L. Dorman, M. Robson, J.-F. Focant, A re-view of the determination of persistent organic pollutants for environmental forensics investigations, *Anal. Chim. Acta* (2016), doi:[10.1016/j.aca.2016.08.027](https://doi.org/10.1016/j.aca.2016.08.027).
- [39] M.W. Kujawski, Z. Barganska, K. Marciniak, E. Miedzianowska, J.K. Kujawski, M. Słeboda, J. Namiesnik, Determining pesticide contamination in honey by LC-ESI-MS/MS—Comparison of pesticide recoveries of two liquid-liquid extraction based approaches, *LWT-Food Sci. Technol.* 56 (2014) 517–523, doi:[10.1016/j.lwt.2013.11.024](https://doi.org/10.1016/j.lwt.2013.11.024).
- [40] P.A. Souza Tette, L. Rocha Guidi, M.B. de Abreu Glória, C. Fernandes, Pesticides in honey: a review on chromatographic analytical methods, *Talanta* 149 (2016) 124–141, doi:[10.1016/j.talanta.2015.11.045](https://doi.org/10.1016/j.talanta.2015.11.045).
- [41] J. Al-Alam, Z. Fajloun, A. Chbani, M. Millet, A multiresidue method for the analysis of 90 pesticides, 16 PAHs, and 22 PCBs in honey using QuEChERS-SPME, *Anal. Bioanal. Chem.* 409 (2017) 5157–5169, doi:[10.1007/s00216-017-0463-y](https://doi.org/10.1007/s00216-017-0463-y).
- [42] Solid Phase Microextraction: Recent developments and applications, 2016.
- [43] C. Blasco, P. Vazquez-Roig, M. Onghena, A. Masia, Y. Picó, Analysis of insecticides in honey by liquid chromatography-ion trap-mass spectrometry: comparison of different extraction procedures, *J. Chromatogr. A* 1218 (2011) 4892–4901, doi:[10.1016/j.chroma.2011.02.045](https://doi.org/10.1016/j.chroma.2011.02.045).

- [44] F. Rouvière, A. Buleté, C. Cren-Olivé, C. Arnaudguilhem, Multiresidue analysis of aromatic organochlorines in soil by gas chromatography-mass spectrometry and QuEChERS extraction based on water/dichloromethane partitioning, Comparison with accelerated solvent extraction, *Talanta* 93 (2012) 336–344, doi:[10.1016/j.talanta.2012.02.048](https://doi.org/10.1016/j.talanta.2012.02.048).
- [45] H. Sun, X. Ge, Y. Lv, A. Wang, Application of accelerated solvent extraction in the analysis of organic contaminants, bioactive and nutritional compounds in *food and feed*, *J. Chromatogr. A* 1237 (2012) 1–23, doi:[10.1016/j.chroma.2012.03.003](https://doi.org/10.1016/j.chroma.2012.03.003).
- [46] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: a critical review. Part II, *Trends Anal. Chem.* 80 (2016) 655–667, doi:[10.1016/j.trac.2015.08.014](https://doi.org/10.1016/j.trac.2015.08.014).
- [47] K. Sharmili, S. Jinap, R. Sukor, Development, optimization and validation of QuEChERS based liquid chromatography tandem mass spectrometry method for determination of multimycotoxin in vegetable oil, *Food Control* 70 (2016) 152–160, doi:[10.1016/j.foodcont.2016.04.035](https://doi.org/10.1016/j.foodcont.2016.04.035).
- [48] M. Saraiva, J. Cavalheiro, L. Lanceleur, M. Monperrus, Synthetic musk in seafood products from South Europe using a quick, easy, cheap, effective, rugged [and safe extraction method](#), *Food Chem* 200 (2016) 330–335, doi:[10.1016/j.foodchem.2016.01.017](https://doi.org/10.1016/j.foodchem.2016.01.017).
- [49] S. Ramos, V. Homem, L. Santos, Development and optimization of a QuEChERS–GC–MS/MS methodology to analyse ultraviolet-filters and synthetic [musks in sewage sludge](#), *Sci. Total Environ.* 651 (2019) 2606–2614, doi:[10.1016/j.scitotenv.2018.10.143](https://doi.org/10.1016/j.scitotenv.2018.10.143).
- [50] A. Wilkowska, M. Biziuk, Determination of pesticide residues in food matrices using the QuEChERS methodology, *Food Chem* 125 (2011) 803–812, doi:[10.1016/j.foodchem.2010.09.094](https://doi.org/10.1016/j.foodchem.2010.09.094).
- [51] G.A. Shabir, Validation of high-performance liquid chromatography methods for pharmaceutical analysis: understanding the differences and similarities between validation requirements of the US food and drug administration, the US pharmacopeia and the international conference on harmonization, *J. Chromatogr. A* 987 (2003) 57–66, doi:[10.1016/S0021-9673\(02\)01536-4](https://doi.org/10.1016/S0021-9673(02)01536-4).
- [52] K.M. Dimpe, P.N. Nomngongo, Current sample preparation methodologies for analysis of emerging pollutants in different environmental matrices, *Trends Anal. Chem.* 82 (2016) 199–207, doi:[10.1016/j.trac.2016.05.023](https://doi.org/10.1016/j.trac.2016.05.023).

Supplementary Materials S1

Supplementary materials S1- table 1s: LC-MS/MS method parameters

Pesticides	RT (mn)	Parent ion	Daughter ion	MRM ratio
pymetrozine	1.80	218 100	105 226	1.5-3.6
		218 101	79 188	
carbendazim	1.85	192 100	160 119	0.4-2.0
		192 101	132 162	
chloridazon	5.14	222 000	104 290	0.5-1.3
		222 001	77 360	
acetamiprid	6.10	223 000	99 207	0.3
		223 002	126 022	
thiacloprid	8.25	253 000	126 262	6.4-6.9
		253 001	99 234	
nicosulfuron	7.70	411 100	181 891	1.9-2.1
		411 101	212 896	
chlortoluron	11.10	213 100	72 296	4.8-7.5
		213 102	140 033	
carbetamide	8.20	237 101	192 087	4.2-4.8
		237 102	120 227	
terbutryn	10.05	242 000	186 210	25.0-56.0
		242 001	68 390	
spinosad A	10.50	732 500	142 071	6.0-18.8
		732 501	98 152	
isoproturon	11.70	207 100	72 361	2.7-4.4
		207 101	46 503	
diuron	12.05	233 001	72 306	4.0-4.5
		233 002	46 598	
metalaxyl-M	11.40	280 001	220 036	1.0-1.6
		280 002	192 088	
spinosad D	11.15	746 774	141 977	1.2-833.3
		746 775	98 457	
dimethenamid-p	14.35	276 100	244 011	2.9-3.3
		276 101	168 034	
penconazole	15.47	284 000	159 070	1.3-1.8
		284 001	70 390	
isoxadifen	16.62	296 000	232 120	1.3-1.5
		296 001	204 100	
tebuconazole	15.77	308 000	70 380	3.9-5.0
		308 001	125 190	

diflubenzuron	15.50	311 000	157 946	0.9-1.6
		311 001	140 999	
epoxiconazole	14.44	330 000	221	24.0-27.5
		330 001	119 200	
propiconazole	15.89	342 000	159 090	5.6
		342 001	123 120	
prothioconazole	15.75	344 000	326 005	0.4-2.7
		344 001	153 994	
chlorfenvinphos	16.17	359 000	170 020	1.5
		359 001	99 180	
triflurosulfuron methyl	14.75	493 100	263 907	4.4-6.4
		493 101	96 088	
pendimethalin	20.50	282 100	211 986	11.0-11.4
		282 101	193 934	
cyazofamid	16.60	325 000	225 974	0.5-3.0
		325 001	224 974	
pyraclostrobin	17.26	388 000	194 100	1.1-1.8
		388 001	163 150	
diflufenican	18.01	395 000	266 060	5.0
		395 001	246 030	
flufenoxuron	19.70	489 001	157 980	1.2
		489 002	141 049	
lufenuron	19.00	511 000	157 901	1.6-2.0
		511 001	141 040	

Supplementary materials S2- table 2s: GC-MS/MS method parameters for OCPs-PAHs and PCBs

Compound	Parent ion	Daughter ions	Collision energy (ev)	RT (mn)
OCPs				
aldrin	263	191/227	1.8	21.56
cisclordane	373	301/337	1.4	23.28
dieldrin	279	241/206	1.6	23.8
heptachlor	272	237/235	1.5	20.9
heptachlor epoxide A	183	155/119	1.5	22.62
heptachlor epoxide B	353	253/317	1.5	22.51
hexachlorobenzene	284	249/214	1.4	18.98
methoxychlor	227	169	1.12	25.67
o.p'-DDD	235	199/165	1.4	23.83
o.p'-DDE	246	176/150	1.9	22.93
o.p'-DDT	235	199/165	1.5	24.36
p.p'-DDD	235	199	1.5	24.57
p.p'-DDE	246	176/150	1.9	23.66
p.p'-DDT	235	165/199	1.5	25.01
transclordane	373	301/264	1.4	23.17
α -endosulfan	241	170/204	1.7	23.33
α -HCH	183	147/109	1.6	18.82
β -endosulfan	195	159/123	1.8	24.69
β -HCH	183	147/109	1.6	20.3
γ -HCH	183	147/109	1.6	19.57
δ -HCH	183	147/109	1.6	20.8

PAHs				
acenaphthene	153	150/151	1.3	16.14
anthracene	178	152/176	1.2	20.06
benz[a]anthracene	228	226/202	1.2	25.99
benzo[a]pyrene	252	250/226	1.3	28.19
benzo[b]fluoranthene	252	250/226	1.3	27.66
benzo[e]pyrene	252	250/226	1.3	28.09
benzo[g,h,i]perylene	276	274	1.4	30.87
benzo[k]fluoranthene	252	250/226	1.3	27.7
chrysene	228	226/202	1.2	26.06
dibenzo[a,h]anthracene	278	276	1.5	30.21
fluoranthene	202	200	1.3	22.96
fluorene	165	163/139	1.2	17.48
indeno[1,2,3-c,d]pyrene	276	274	1.4	30.25
naphthalene	128	102/126	1.2	11.73
Phenanthrene	178	152/176	1.2	19.9
pyrene	202	200	1.2	23.52
PCBs				
PCB 101	326	291/254	1.2	23.11
PCB 105	326	256/254	1.4	24.56
PCB 114	326	256/254	1.3	24.41
PCB 118	326	256/254	1.3	24.38
PCB 123	326	256/254	1.3	24.29
PCB 126	326	256/254	1.4	24.81
PCB 138	360	325/288	2.1	25.05
PCB 149	360	288/290	2	24.14
PCB 153	360	290/288	2	24.6
PCB 156	360	290/288	1.5	25.84
PCB 157	360	290/288	1.5	25.89
PCB 167	360	290/288	2	25.52
PCB 169	360	290/288	1.5	26.4
PCB 18	256	186/221	1.4	19.57
PCB 180	396	361/324	1.8	25.94
PCB 189	396	326/324	2.1	26.74
PCB 28	256	186/150	1.7	20.8
PCB 31	256	186/150	1.7	20.73
PCB 44	292	257/220	1.9	21.74
PCB 52	292	257/220	1.9	21.35
PCB 70	292	220/185	1.3	22.68
PCB 81	292	220/185	2.1	23.89

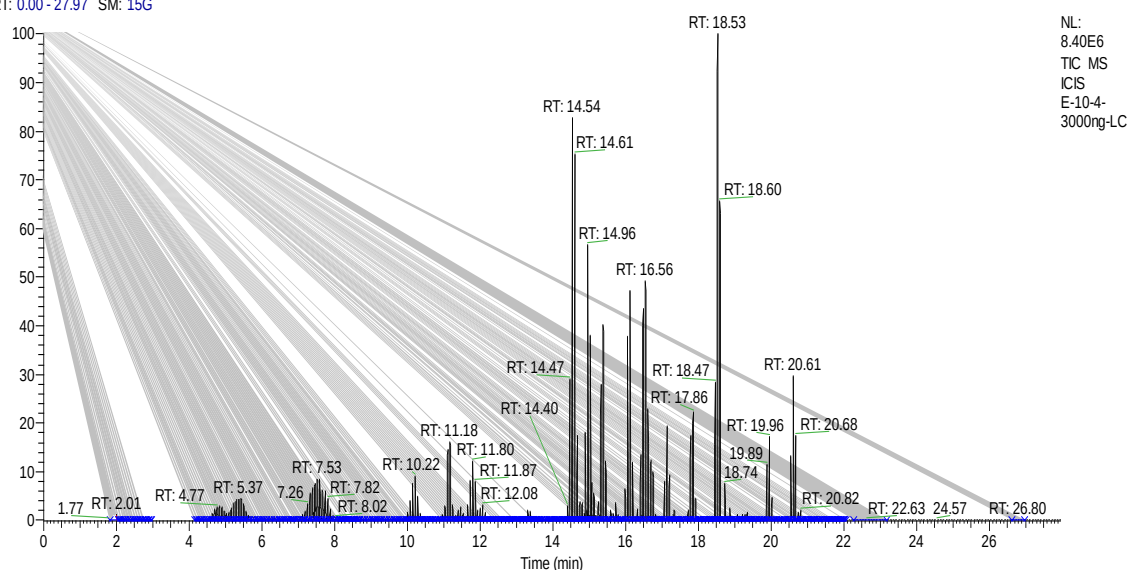
Supplementary materials S3- table 3s: GC-MS/MS method parameters for remained volatile pesticides

Pesticides	Parent ion	Daughter ions	Collision energy (ev)	RT(mn)
acetochlor	146	132/131	1.26	14.87
aclonifen	194	167/139	1.45	21.45
alachlor	160	132/117	1.4	15.16
azoxystrobin	344	329	1.6	31.77
bifenox	341	311/310	1.2	24.59
bifenthrin	181	165/166	1.1	23.38
boscalid	140	112/76	1.4	28.92
bromoxynil octanoate	127	109/67	0.76	22.13
bupirimate	193	165/109	1.4	19.71
buprofezin	175	132/117	1.2	19.68
captan	79	51/77	1.2	18.43
carbaryl	144	116/144	1.28	15.59
chloridazon	220	193/166	1.6	22.75
chlorpropham	127	100/92	1.46	11.49
chlorpyrifos	314	258/286	1.1	16.4
clofentezine	137	102/75/110	1.4	6.97
clomazone	204	107/174	1.6	12.97
cypermethrin	181	152	1.5	28.51
cyprodinil	224	208/197	1.9	17.53
deltamethrin	181	152	1.8	31.21
dichlobenil	171	136/100	1.5	8.31
diclofop-methyl	340	253/281	1.4	22.65
difenoconazole	265	249/202	1.45	31.06
diflufenican	266	246/238	1.6	22.8
dimethachlor	134	105/79	1.3	14.84
dimethenamid-P	154	137/111	1.22	14.81
dimethomorph	301	165/258	1.6	32.07
dimoxystrobin	116	89/63	1.2	23.42
diphenylamine	169	139/166	1.5	11.27
epoxiconazole	192	138/157	1.16	23.48
ethofumesate	161	133/105	1.4	15.91
etridiazole	211	183/140	1	9.03
fenarimol	139	111/75	1.3	26.05
fenoxycarb	186	157/158	1.4	23.89
fenpropidin	98	70/55	1.3	15.35
fenpropimorph	128	110/70	1.6	16.16
fluazinam	387	359/324	1.5	22.84
flurochloridone	174	127/145	1.4	17.52
flusilazole	233	165/152	1.85	20.19
folpet	260	260/232	1.2	18.66
indoxacarb	203	134/175	1.22	30.92
isoxaflutole	279	252/223	1.5	17.75

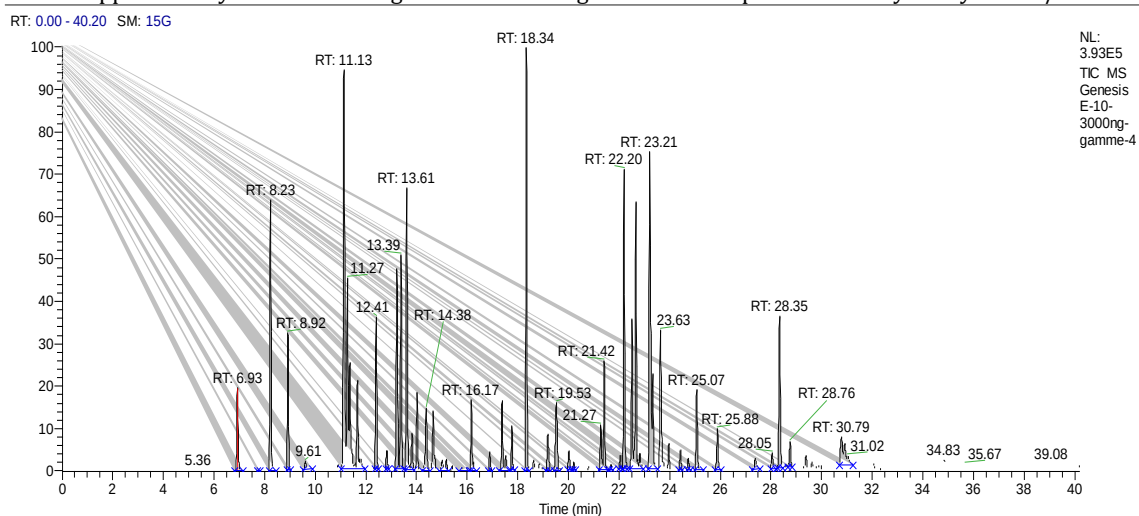
krersoxim-methyl	116	116/89	2.13	19.68
lambda cyhalothrin	181	152/161	1.5	25.25
lenacil	153	136/135	1.3	22.43
lindane	183	147/148	1.4	13.54
malathion	127	99	0.78	15.98
metamitron	202	174/186	0.9	20.94
metazachlor	209	132/174	1.2	17.75
metolachlor-S	162	133/134	1.3	16.36
myclobutanil	179	125/152	1.3	20.37
oxadiazon	258	275/146	1.6	19.34
penconazole	248	192/157	1.42	17.95
pendimethalin	252	208/191	1.2	17.7
prochloraz	180	138	1.2	27.53
procymidone	283	255/254	1.4	18.19
propiconazole	259	191/173	1.4	22.19
propyzamide	173	145/109	1.4	13.39
pyraclostrobin	132	104/77	1.3	26.89
Pyrimethanil	198	183/182	1.7	13.77
quinoxifen	237	208/181	1.9	22.35
spiroxamine	100	72/58	1.1	14.65/15.45
tebuconazole	250	163/153	1.4	23
tebufenpyrad	171	156/127	1.5	24.14
tebutam	91	65	1.4	11.82
tolyfluanid	137	122/109	1.4	17.73
trifloxystrobin	116	89/63	1.16	21.57
trifluralin	264	206/160	1.5	11.4

Supplementary materials S4-Figure 1s: chromatogram of nonvolatile pesticides analyzed by LC-MS/MS

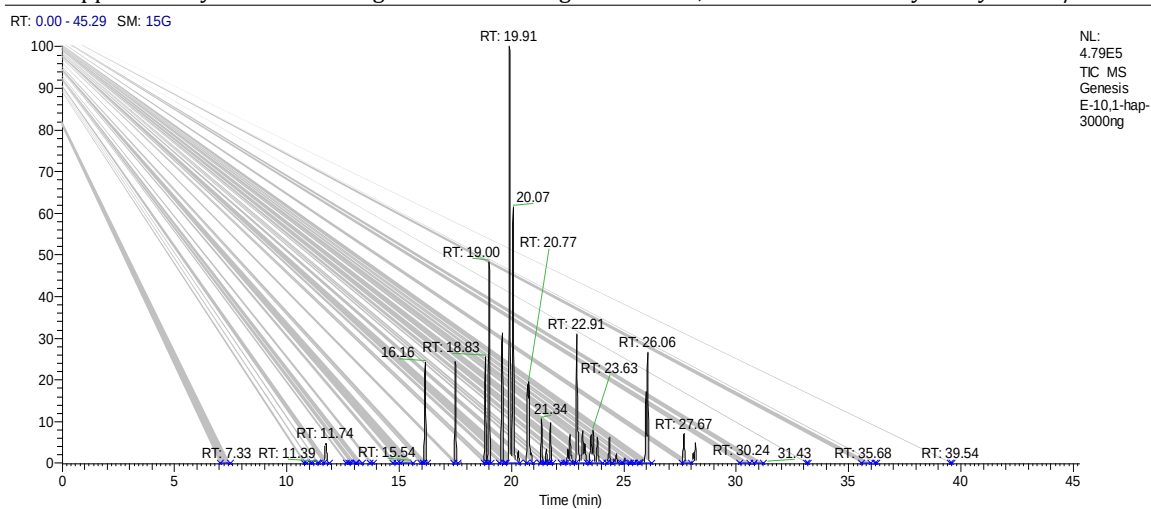
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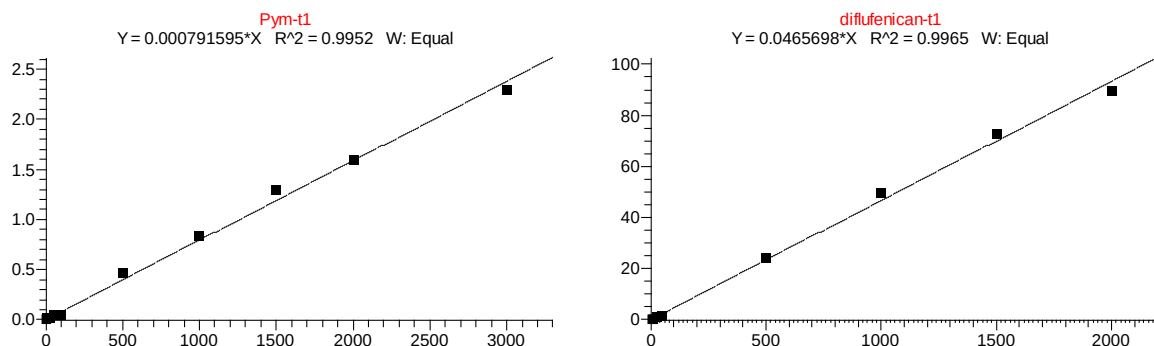
Supplementary materials S4-Figure 2s: chromatogram of volatile pesticides analyzed by GC-MS/MS

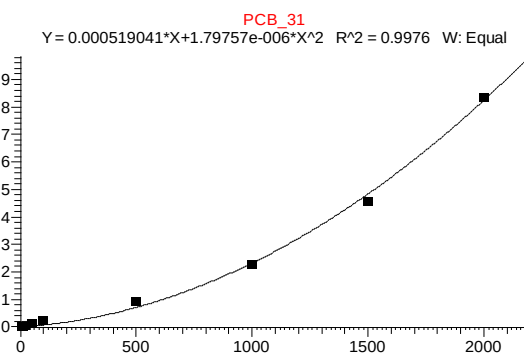
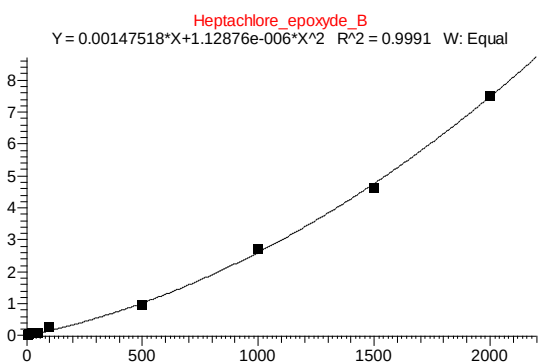
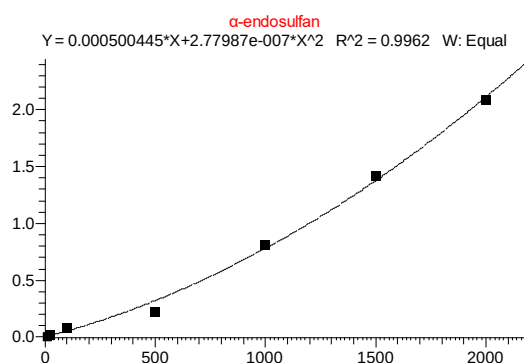
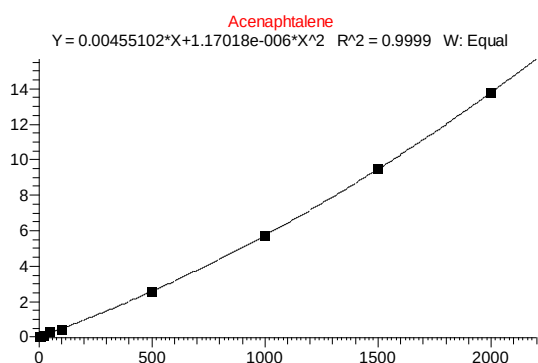
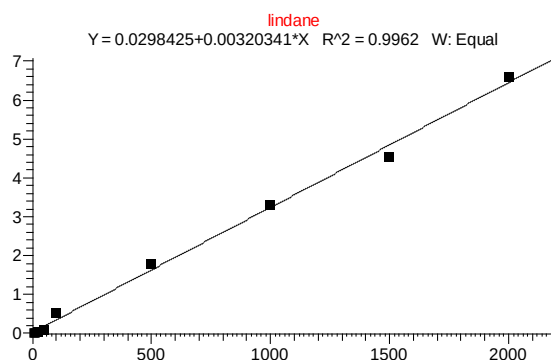
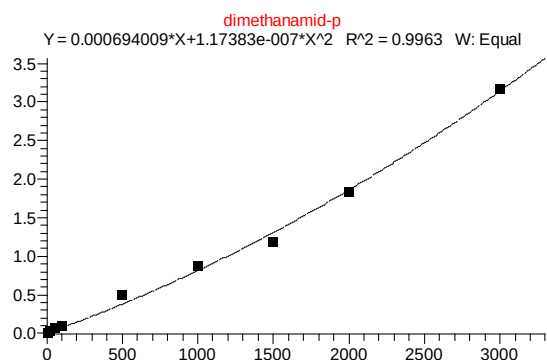


Supplementary materials S4-Figure 3s: chromatogram of PAHs, PCBs and OCPs analyzed by GC-MS/MS



Supplementary materials S5-Figure 4s: Calibration curves of some analyzed compounds





Supplementary Material S6: Table 4s: LC-MS/MS method performance and validation for pesticides analysis

Table 4s: LC-MS/MS method performance and validation for pesticides analysis

Compounds	Regression line equation	Regression coefficient	LOD (ng/g)	LOQ (ng/g)	Repeatability (intra-day RSD%)	Reproducibility (Inter-day RSD %)	Recovery (%)
acetamiprid	$Y = 0.0011 \cdot X$	0.998	5.13	17.1	0.932	5.728	74.728
carbendazim	$Y = 0.0040 \cdot X$	0.996	1.13	3.78	4.219	5.1854	72.086
carbetamide	$Y = 0.0073 \cdot X$	0.997	2.56	8.58	3.212	5.458	67.373
chloridazon	$Y = 0.0021 \cdot X$	0.997	1.01	3.39	0.550	4.147	85.532
chlortoluron	$Y = 0.010 \cdot X$	0.998	1.98	6.61	1.805	5.577	74.034
clofenvinphos	$Y = 0.0045 \cdot X$	0.998	3.32	11.08	2.501	6.267	73.500

cyazofamid	$Y = 8.8e-005*X$	0.995	2.48	8.26	0.746	7.813	98.249
diflufenican	$Y = 0.0465*X$	0.997	1.03	3.44	0.827	6.026	98.500
dimethenamid-P	$Y = 0.1194*X$	0.997	0.46	1.55	1.013	4.381	92.870
diuron	$Y = 0.0050*X$	0.999	0.52	1.73	1.426	4.791	77.791
DPMU	$Y = 0.0060*X$	0.997	1.73	5.77	Nd	Nd	Nd
epoxiconazole	$Y = 0.0715*X$	0.998	1.06	3.53	0.059	6.738	87.001
flufenoxuron	$Y = 0.0087*X$	0.995	1.11	3.71	2.598	5.717	95.493
foramsulfuron	$Y = 0.0014*X$	0.998	2.71	9.03	4.060	4.422	97.402
isoproturon	$Y = 0.0244*X$	0.998	1.19	3.95	0.087	5.901	79.0373
isoxadifen	$Y = 0.0076*X$	0.998	1.76	5.85	1.604	4.932	77.045
lufenuron	$Y = 0.0007*X$	0.997	5.87	19.57	0.628	6.867	97.529
metalaxyl-M	$Y = 0.0043*X$	0.996	4.42	14.7	1.927	6.344	69.119
nicosulfuron	$Y = 0.0009*X$	0.997	5.22	17.41	Nd	Nd	Nd
penconazole	$Y = 0.0145*X$	0.998	1.42	4.73	1.835	6.853	97.682
pendimethalin	$Y = 0.0160*X$	0.998	1.68	5.6	0.788	5.251	95.630
propiconazole	$Y = 0.0302*X$	0.997	3.73	12.43	1.332	5.598	94.1038
pymetrozine	$Y = 0.0008*X$	0.995	2.63	8.8	4.588	8.744	67.800
pyraclostrobin	$Y = 0.0154*X$	0.992	0.79	2.63	0.0027	5.771	77.279
spinosad A	$Y = 0.0294*X$	0.996	4.19	14	0.479	5.813	72.433
spinosad D	$Y = 0.0067*X$	0.996	0.16	0.55	0.607	4.971	82.214
tebuconazole	$Y = 0.0188*X$	0.994	0.94	3.13	0.491	5.711	92.611
terbutryn	$Y = 0.0242*X$	0.993	1.99	6.64	0.096	3.960	84.026
thiacloprid	$Y = 0.010*X$	0.995	1.96	6.53	1.45	4.827	79.263
triflu-Methyl	$Y = 0.0185*X$	0.999	1.51	5.04	0.130	4.868	101.649

Supplementary Material S7: Table 5s: GC-MS/MS method performance and validation for OCPs, PAHs and PCBs analysis

Table 5s: GC-MS/MS method performance and validation for OCPs, PAHs and PCBs analysis

Compounds	Regression line equation	Regression coefficient	LOD (ng/g)	LOQ (ng/g)	intra-day RSD%	inter-day RSD %	Recovery %
aldrin	$Y = 9.73604e-005*X - 6.54446e-009*X^2$	0.9901	5	16.5	1.144	7.443	85.934
cisclordane	$Y = 0.0001*X - 1.92e-008*X^2$	0.994	2.14	7.14	1.980	8.98	79.222
dieldrin	$Y = 6.5e-006*X + 3.48e-008*X^2$	0.990	10	33	4.311	5.036	74.108
heptachlor	$Y = 5.7e-005*X + 2.02e-010*X^2$	0.994	5	16.5	2.630	8.016	65.285
heptachlor epoxide A	$Y = 0.0002*X + 2.3e-008*X^2$	0.994	5.77	19.2	2.600	8.23	78.809
heptachlor epoxide B	$Y = 0.0015*X + 1.12e-006*X^2$	0.991	1.67	5.56	1.729	6.916	95.174
hexachlorobenzene	$Y = 0.00181147*X - 1.94583e-007*X^2$	0.996	0.41	1.39	0.970	8.516	75.210
methoxychlor	$Y = 0.0005*X - 3.4e-009*X^2$	0.998	1.33	4.42	9.945	5.416	55.071
o,p'-DDD	$Y = 3.36e-006*X + 1.07e-006*X^2$	0.997	7.8	26.2	1.595	4.496	67.107
o,p'-DDE	$Y = 0.003*X - 6.23e-007*X^2$	0.992	7.5	24.7	3.772	6.733	71.643

o,p'-DDT	$Y = 0.0003 \cdot X - 4.79e-008 \cdot X^2$	0.975	2.14	7.14	Nd	Nd	Nd
p,p'-DDD	$Y = 0.0003 \cdot X - 1.02e-007 \cdot X^2$	0.990	1.58	5.26	2.003	2.683	81.609
p,p'-DDE	$Y = 0.0001 \cdot X + 1.8e-007 \cdot X^2$	0.998	10	33	3.033	7.313	66.746
p,p'-DDT	$Y = 0.0002 \cdot X + 3.9e-008 \cdot X^2$	0.994	6.43	21.4	4.436	7.006	73.259
transchlordanane	$Y = 7.3e-005 \cdot X - 6.94e-009 \cdot X^2$	0.994	1.36	4.55	2.877	9.343	78.379
α -endosulfan	$Y = 0.0005 \cdot X + 2.7e-007 \cdot X^2$	0.996	5	16.5	2.65	5.696	83.161
α -HCH	$Y = 0.0004 \cdot X + 1.9e-008 \cdot X^2$	0.996	5	16.5	1.130	8.493	94.241
β -endosulfane	$Y = 2.2e-005 \cdot X + 5.1e-008 \cdot X^2$	0.995	5	16.5	2.320	6.633	71.868
β -HCH	$Y = 8.4e-005 + 9.8e-005 \cdot X$	0.995	10	33	0.686	7.06	75.011
γ -HCH	$Y = 0.0004 \cdot X + 2.5e-008 \cdot X^2$	0.996	1.5	5	2.300	10.726	94.471
δ -HCH	$Y = 0.0019 + 0.0001 \cdot X$	0.997	10	33	0.301	7.246	86.838
acenaphthylene	$Y = 0.0045 \cdot X + 1.17e-006 \cdot X^2$	0.999	6.89	23	1.093	6.691	95.424
anthracene	$Y = 0.030 + 0.001 \cdot X$	0.995	2.25	7.5	1.299	7.388	94.27
benz[a]anthracene	$Y = 4.48e-005 \cdot X + 2.25e-008 \cdot X^2$	0.993	2.55	8.33	0.350	7.545	85.948
benzo[a]pyrene	$Y = 0.0023 + 3.81e-005 \cdot X$	0.996	3.75	12.5	1.208	9.759	82.76
benzo[b]fluoranthene	$Y = 0.0001 \cdot X - 1.2e-008 \cdot X^2$	0.996	1.76	5.88	2.913	8.952	64.959
benzo[e]pyrene	$Y = 0.0013 + 4.33e-005 \cdot X$	0.997	3.75	12.5	1.389	11.934	75.425
benzo[g,h,i]perylene	$Y = 1.3e-005 \cdot X + 2.13e-009 \cdot X^2$	0.998	10	33	1.048	7.322	65.809
benzo[k]fluoranthene	$Y = 3.04e-006 \cdot X - 5.7e-011 \cdot X^2$	0.981	8.57	28.6	0.830	9.240	82.479
chrysene	$Y = 8.12e-005 \cdot X + 5.27e-009 \cdot X^2$	0.993	10	33	0.474	7.555	93.436
dibenz[a,h]anthracene	$Y = 2.7e-005 + 1.32e-005 \cdot X$	0.994	10	33	1.152	7.048	85.956
fluoranthene	$Y = 0.011 + 0.0006 \cdot X$	0.993	10	33	2.370	4.821	94.225
fluorene	$Y = 0.0005 \cdot X - 7.9e-008 \cdot X^2$	0.999	0.41	1.39	3.604	6.706	83.667
indeno[1,2,3-c,d]pyrene	$Y = 0.0001 + 2.6e-005 \cdot X$	0.995	10	33	1.169	4.532	94.314
naphthalene	$Y = 2.6e-005 \cdot X + 2.51e-008 \cdot X^2$	0.997	0.68	2.27	1.930	7.020	76.183
phenanthrene	$Y = 0.002 \cdot X - 1.89e-007 \cdot X^2$	0.995	1.43	4.75	0.397	7.016	86.821
pyrene	$Y = 0.0030 \cdot X + 2.62e-006 \cdot X^2$	0.996	10	33	2.497	7.128	91.629
PCB 101	$Y = 4.16e-005 \cdot X + 8.92e-008 \cdot X^2$	0.97	5	16.7	1.005	9.352	67.768
PCB 105	$Y = 2.85e-005 \cdot X + 6.8e-009 \cdot X^2$	0.998	3.75	12.5	1.982	9.739	97.349
PCB 114	$Y = 2.88e-005 \cdot X + 2.52e-009 \cdot X^2$	0.997	3.33	11.1	1.635	9.813	64.887
PCB 118	$Y = 0.0002 \cdot X - 8.03e-008 \cdot X^2$	0.996	1.15	3.85	0.591	10.257	88.054
PCB 123	$Y = 2.34e-006 \cdot X + 1.22e-008 \cdot X^2$	0.996	2.31	7.69	3.094	7.657	83.191
PCB 126	$Y = 3.4e-006 \cdot X + 1.3e-008 \cdot X^2$	0.994	1.88	6.25	4.785	8.897	68.091

PCB 138	$Y = 1.22e-005*X + 1.2e-008*X^2$	0.837	5	16.7	5.047	10.237	68.711
PCB 149	$Y = 4.85e-006*X - 9.17e-010*X^2$	0.985	10	33	1.822	5.157	84.150
PCB 153	$Y = 2.01e-006*X + 1.50e-010*X^2$	0.995	10	33	1.710	9.759	95.652
PCB 156	$Y = 1.26e-005*X + 2.90e-009*X^2$	0.998	5	16.7	2.100	7.991	66.098
PCB 157	$Y = 1.20e-005*X + 3.1e-009*X^2$	0.998	5	16.7	0.566	6.673	64.389
PCB 167	$Y = 7.39e-006*X + 1.31e-008*X^2$	0.9874	10	33	1.570	9.553	96.663
PCB 169	$Y = 0.0008 + 4.29e-006*X$	0.967	10	33	3.007	6.911	63.673
PCB 18	$Y = 0.00014*X + 2.1e-006*X^2$	0.995	2.14	7.14	2.194	7.252	101.876
PCB 180	$Y = 2.09e-005*X + 6.39e-009*X^2$	0.999	7.5	25	1.572	8.215	77.136
PCB 189	Nd	Nd	Nd	Nd	Nd	Nd	Nd
PCB 28	$Y = 0.0002*X - 1.63e-008*X^2$	0.991	5	16.5	0.798	8.089	76.086
PCB 31	$Y = 0.0005*X + 1.80e-006*X^2$	0.997	1.07	3.57	0.939	9.807	84.419
PCB 44	$Y = 0.0001*X - 1.58e-008*X^2$	0.991	3.75	12.5	1.787	9.160	100.273
PCB 52	$Y = 0.0001*X - 1.6e-008*X^2$	0.995	1.67	5.56	0.986	5.085	95.532
PCB 70	$Y = 4.73e-006*X + 8.12e-008*X^2$	0.981	10	33	1.522	10.716	75.614
PCB 81	$Y = 1.63e-005*X + 3.09e-008*X^2$	0.991	7.5	25	Nd	Nd	Nd

Supplementary Material S8: Table 6s: GC-MS/MS method performance and validation for pesticides analysis

Table 6s: GC-MS/MS method performance and validation for pesticides analysis

Compounds	Regression line equation	Regression coefficient	LOD (ng/g)	LOQ (ng/g)	Intra-day RSD %	Inter-day RSD %	Recovery %
acetochlor	$Y = 0.010*X + 3.00e-005*X^2$	0.996	13.53	45.11	5.940	10.492	78.6075
aclonifen	$Y = 1.53 + 0.023*X$	0.961	9.43	31.43	3.730	10.076	71.033
alachlor	$Y = 0.0009*X + 1.39e-006*X^2$	0.991	5	16.7	1.788	11.941	86.351
azoxystrobin	$Y = 0.084 + 0.0017*X$	0.996	2.73	9.09	5.243	10.684	84.474
bifenox	$Y = 0.0005*X + 1.5e-006*X^2$	0.999	12.39	41.3	9.561	8.841	75.197
bifenthrin	$Y = 0.127*X + 0.0001*X^2$	0.988	9.95	33.2	4.631	8.834	79.233
boscalid	$Y = 0.006*X + 1.18e-005*X^2$	0.998	2.34	7.81	6.742	10.124	81.896
bromoxynil octanoate	$Y = 0.007*X + 2.3e-006*X^2$	0.997	13.93	46.43	Nd	Nd	Nd
bupirimate	$Y = 0.099 + 0.005*X$	0.996	11.63	38.75	8.140	11.334	82.638
buprofezin	$Y = 0.002*X + 1.39e-005*X^2$	0.994	7.5	25	3.916	12.575	76.552
captan	$Y = 0.0049*X - 3.35e-007*X^2$	0.998	13.91	46.38	3.740	8.676	92.039
carbaryl	$Y = 0.28 + 0.0049*X - 2.7e-007*X^2$	0.991	5.63	18.8	4.318	11.197	82.26
chloridazon	$Y = 0.0001*X + 2.49e-007*X^2$	0.997	5	16.7	0.0468	11.475	81.324
chlorpropham	$Y = 0.056*X + 1.18e-005*X^2$	0.998	5.12	17.07	9.478	8.902	78.738

chlorpyrifos	$Y = 0.003 \cdot X + 7.64e-008 \cdot X^2$	0.999	4.09	13.6	6.220	9.865	89.600
clofentezine	$Y = 0.030 \cdot X - 1.82e-006 \cdot X^2$	0.994	3.75	12.5	4.5082	12.307	91.468
clomazone	$Y = 0.008 \cdot X + 5.21e-006 \cdot X^2$	0.993	5	16.7	6.483	7.739	81.769
cypermethrin	$Y = 0.185 + 0.005 \cdot X$	0.997	12	40	6.618	7.444	79.834
cyprodinil	$Y = 0.0212 \cdot X + 1.13e-005 \cdot X^2$	0.994	5	16.7	1.127	11.405	83.997
deltamethrin	$Y = 0.0001 \cdot X + 6.25e-007 \cdot X^2$	0.998	7.5	25	5.050	7.214	82.597
dichlobenil	$Y = 0.080 \cdot X + 4.68e-005 \cdot X^2$	0.996	0.27	0.89	3.3514	9.880	86.521
diclofop-methyl	$Y = 0.068 \cdot X + 6.27e-005 \cdot X^2$	0.988	1.22	4.07	3.165	11.345	84.519
difenoconazole	$Y = 0.0053 \cdot X + 7.872e-006 \cdot X^2$	0.998	15.1	50.3	6.594	9.885	76.077
diflufenicanil	$Y = 0.062 \cdot X + 0.0003 \cdot X^2$	0.980	0.88	2.94	8.270	11.91271	72.194
dimethachlor	$Y = 0.0039 \cdot X + 9.18e-006 \cdot X^2$	0.999	4.17	13.89	5.909	10.607	81.9438
dimethenamid-P	$Y = 0.0007 \cdot X + 1.17e-007 \cdot X^2$	0.996	10.53	35.11	1.136	10.119	79.296
dimethomorph	$Y = 0.0006 \cdot X - 4.97e-009 \cdot X^2$	0.993	6.25	20.8	5.291	7.557	83.363
dimoxystrobin	$Y = 0.150 \cdot X + 4.36e-005 \cdot X^2$	0.997	1.86	6.19	3.948	9.717	92.639
diphenylamine	$Y = 0.321 \cdot X + 0.0001 \cdot X^2$	0.998	2.31	7.69	7.586	10.029	80.576
epoxiconazole	$Y = 0.0027 \cdot X + 2.14e-007 \cdot X^2$	0.990	10.73	35.77	5.862	9.914	90.869
ethofumesate	$Y = 0.13 + 0.001 \cdot X + 2.25e-006 \cdot X^2$	0.997	9.38	31.3	0.259	12.287	82.124
etridiazole	$Y = 0.076 \cdot X + 3.66e-006 \cdot X^2$	0.998	5.64	18.8	7.608	9.858	79.617
fenarimol	$Y = 0.152 + 0.001 \cdot X$	0.998	8.33	27.77	14.707	8.485	70.454
fenoxycarb	$Y = 0.0003 \cdot X + 6.07e-008 \cdot X^2$	0.991	6	20	4.533	12.623	90.464
fenpropidin	$Y = 0.0019 \cdot X + 3.27e-006 \cdot X^2$	0.992	20	66.67	8.236	10.786	77.830
fenpropimorph	$Y = 0.002 \cdot X + 3.78e-007 \cdot X^2$	0.996	7.5	25	7.287	8.0123	80.885
fluazinam	$Y = 9.20e-005 \cdot X + 1.84e-007 \cdot X^2$	0.995	12	40	2.435	9.879	79.209
flurochloridone	$Y = 0.022 \cdot X - 4.32e-006 \cdot X^2$	0.993	12.27	40.9	6.803	9.228	82.256
flusilazole	$Y = 0.014 \cdot X - 8.51e-007 \cdot X^2$	0.991	2.47	8.22	12.171	10.196	76.448
folpet	$Y = 4.76e-005 \cdot X + 2.89e-008 \cdot X^2$	0.981	11.7	38.9	6.119	9.269	80.267
indoxacarb	$Y = 0.0009 \cdot X + 3.29e-006 \cdot X^2$	0.998	8.72	29.61	Nd	Nd	Nd
isoxaflutole	$Y = 0.0002 \cdot X + 9.52e-008 \cdot X^2$	0.993	5	16.7	2.570	8.014	91.416
kresoxim-methyl	$Y = 0.0001 \cdot X - 1.41e-008 \cdot X^2$	0.998	11.25	37.5	7.943	9.487	80.226
lambda cyhalothrin	$Y = 0.0013 \cdot X + 1.06e-005 \cdot X^2$	0.989	8.18	27.3	2.906	10.647	92.189
lenacil	$Y = 0.0016 \cdot X + 5.41e-007 \cdot X^2$	0.998	14.4	48.1	5.638	9.308	74.172
lindane	$Y = 0.03 + 0.0032 \cdot X$	0.996	0.65	2.17	7.394	9.726	81.818
malathion	$Y = 0.002 \cdot X - 6.17e-008 \cdot X^2$	0.996	9.46	31.5	3.321	9.710	89.062

metamitron	$Y = 0.0006 \cdot X + 5.45e-008 \cdot X^2$	0.995	7.5	25	Nd	Nd	Nd
metazachlor	$Y = 0.0028 \cdot X - 3.23e-007 \cdot X^2$	0.997	19.3	64.8	8.908	11.361	85.043
metolachlor-S	$Y = 0.053 \cdot X + 2.27e-005 \cdot X^2$	0.991	6.43	21.43	2.930	12.256	84.939
myclobutanil	$Y = 0.0027 \cdot X + 3.41e-006 \cdot X^2$	0.998	11.78	39.29	3.148	10.562	89.468
oxadiazon	$Y = 0.0018 \cdot X - 3.6e-007 \cdot X^2$	0.991	5	16.7	6.828	10.340	84.740
penconazole	$Y = 0.048 \cdot X + 4.63e-005 \cdot X^2$	0.972	0.22	0.72	4.730	8.113	84.940
pendimethalin	$Y = 0.0012 \cdot X + 3.38e-006 \cdot X^2$	0.996	7.06	23.53	1.898	9.549	85.314
prochloraz	$Y = 0.0023 \cdot X + 2.43e-006 \cdot X^2$	0.997	14.4	48	6.628	9.646	79.559
procymidone	$Y = 0.0011 \cdot X + 1.80e-007 \cdot X^2$	0.997	18.6	62.1	4.164	10.795	90.772
propiconazole	$Y = 0.008 \cdot X + 2.7e-005 \cdot X^2$	0.995	1.82	6.06	3.208	11.852	79.902
Propyzamide	$Y = 0.076 \cdot X + 7.17e-005 \cdot X^2$	0.996	7.33	24.43	1.851	8.176	77.863
pyraclostrobin	$Y = 0.359 + 0.010 \cdot X$	0.996	9.16	30.55	6.578	11.067	81.051
pyrimethanil	$Y = 0.085 \cdot X + 7.43e-005 \cdot X^2$	0.997	2.5	8.33	2.789	8.048	84.718
quinoxifen	$Y = 0.017 \cdot X + 1.52e-005 \cdot X^2$	0.991	0.44	1.47	5.911	7.662	89.660
spiroxamine	$Y = 0.0007 \cdot X + 3.04e-007 \cdot X^2$	0.983	6.36	21.2	8.575	7.765	79.443
tebuconazole	$Y = 0.013 + 0.0004 \cdot X$	0.998	13.12	43.75	7.505	11.455	82.666
tebufenpyrad	$Y = 0.0013 \cdot X - 2.02e-007 \cdot X^2$	0.999	0.61	2.04	1.441	11.570	82.150
tebutam	$Y = 0.0817 \cdot X - 4.09e-006 \cdot X^2$	0.996	5.27	17.57	2.307	10.5094	82.069
tolyfluanid	$Y = 0.0003 \cdot X + 2.76e-007 \cdot X^2$	0.995	5	16.7	2.786	11.622	84.136
trifloxystrobin	$Y = 0.016 \cdot X + 3.34e-005 \cdot X^2$	0.991	9.5	31.7	6.239	11.409	84.214
trifluralin	$Y = 0.008 \cdot X + 2.74e-005 \cdot X^2$	0.999	0.83	2.78	1.129	11.909	76.932

III. Conifers as environmental biomonitors: A multi-residue method for the concomitant quantification of pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls by LC-MS/MS and GC-MS/MS

Résumé

Ces recherches, présentées dans cet article publié dans « *Microchemical Journal* », consiste au développement d'une méthode multi-résidus pour l'analyse des pesticides, HAPs et des PCBs à partir des aiguilles de conifères de type *Pinus nigra*.

L'utilisation de la végétation pour la surveillance de l'environnement peut être considérée comme une technique de surveillance simple, efficace et rentable pour détecter et évaluer sa pollution. Parmi les différentes espèces végétales, les aiguilles de conifères peuvent jouer un rôle important en tant qu'échantillonneurs passifs. Ils ont été largement utilisés pour la biosurveillance consécutive de plusieurs polluants et se caractérisent par une forte capacité à accumuler un ou plusieurs polluants dans leurs tissus.

Dans cette étude, une stratégie d'extraction multi-résidus optimisée basée sur l'approche QuEChERS-SPME pour le criblage simultané de 134 pesticides, 22 PCBs et 16 HAPs suivie de leur analyse par LC-MS/MS et GC-MS/MS est rapportée. L'extraction a été réalisée sur QuEChERS en utilisant l'ACN, suivie d'un lavage par extraction en phase solide dispersive en utilisant du PSA et de sorbants octadécyl (C_{18}). La technique d'extraction utilisée est suivie d'une étape de préconcentration pour les composés volatils en utilisant une SPME, préalablement à leur analyse en GC. Les composés non volatils, quant à eux, ont été directement analysés en LC après leur extraction. La procédure d'analyse validée a révélé une bonne récupération comprise entre 60 et 121% pour tous les composés cibles. De plus, tous ces composés ont été validés avec une bonne linéarité exprimée par un R^2 supérieur à 0.98 et des LOD et des LOQ inférieures à 15 ng g^{-1} .

Ainsi, le développement d'outils d'extraction pour l'analyse des traces de polluants organiques dans une telle matrice semble efficace afin d'établir un contrôle environnemental spécifique. Les résultats ont prouvé que le protocole QuEChERS-SPME en combinaison avec les techniques chromatographiques peut être utilisé comme un outil de surveillance environnementale. Les méthodes développées ont été appliquées avec succès dans l'analyse d'échantillons réels collectés dans 15 régions du Liban.

Highlights

- Application of QuEChERS for the analysis of pesticides, PAHs and PCBs in conifer needles.
- QuEChERS-SPME was shown to be a reliable extraction procedure for the multi-residue's analysis of organic pollutants in conifer needles.
- Conifers were used as biomonitor candidates to evaluate atmospheric contamination in Lebanon.

Abstract

The conifer is a natural vascular land plant composed of very particular leaves called needles that are able to accumulate, throughout years, a wide range of non-volatile, semi-volatile and volatile compounds of different structure and polarity such as pesticides and persistent organic pollutants. However, the extraction of these compounds from such matrix remains mainly uncommitted. In this paper, a QuEChERS based extraction procedure was developed for the concomitant extraction and analysis of 134 pesticides, 22 polychlorinated biphenyls and 16 polycyclic aromatic hydrocarbons residues from conifer needles. The method included a liquid-liquid extraction using acetonitrile (ACN) followed by a clean-up step using, Primary Secondary Amine (PSA), graphitized carbon black (GCB) and C₁₈ particles, in the presence of salts. The obtained extracts were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for their contamination by non-volatile pesticides. However, volatile pesticides, polychlorinated biphenyls, organochlorine pesticides and polycyclic aromatic hydrocarbons were subjected to a concentration step using solid-phase microextraction (SPME) prior to their analysis by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). The method was developed and validated, and the obtained results revealed a recovery rate ranged between 60% and 121% for all the targeted compounds. In addition, the method showed high sensitivity and precision with detection and quantification limits less than 20 ng g⁻¹ for most target compounds and low RSD for both inter and intra-day analysis. Once developed the method was applied on conifer samples collected from 15 different sites in northern Lebanon. The analysis of the

collected samples showed a persistence of the different assessed pollutants depending of the characteristics of each site.

Keywords : Conifers; Environmental pollution; QuEChERS; Pesticides; Polycyclic aromatic hydrocarbons; Polychlorinated biphenyls.

1. Introduction

Environmental pollution is a global common problem to both developed and developing countries, which attracts human attention to its severe long-term consequences [1]. Therefore, the monitoring of this pollution becomes a worldwide necessity. Among the different monitoring techniques used, figures the biomonitoring. In fact, biomonitoring requires the use of responses from natural species at multiple levels to identify or predict environmental changes and to observe their evolution as a function of time [2]. Consequently, biological monitoring can be defined as the measurement of the response of living organisms to changes in their environment [3]. In terrestrial environments, many species or groups of species can be used for monitoring purposes [4,5]. For instance, lichens, mosses, vascular plants and fungi have been widely used as passive biomonitors for air pollution [6,7].

As a follow, the use of vegetation for environmental monitoring can be considered as a simple, efficient and cost-effective monitoring technique for detecting and assessing environmental pollution [8,9]

Among the different vegetation species, conifer needles can play an important role as passive samplers. They have been widely used for the consecutive biomonitoring of several pollutants and are characterized by a high capacity to accumulate one or more pollutants in their tissues such as pesticides [9,10], PAHs [11,12] and PCBs [13,14]. Moreover, conifers are widespread and can be found in a large area that can be difficult to access [15]. Furthermore, conifers are characterized by a waxy layer allowing the adsorption and the accumulation of organic air contaminants through the years and therefore, making them efficient for environmental passive sampling [16].

For all of these reasons, the development of extraction tools for the analysis of traces of organic pollutants in such matrix seems efficient in order to establish a specific environmental control.

In fact, a large variety of approaches have been used for determination of environmental pollutants in vegetables [17]. For instance, some modern techniques such as ultrasound-assisted extraction (UAE) [18,19], accelerated solvent extraction (ASE) [20-23], Soxhlet [11], ultrasonic solvent extraction [24], or pressurized liquid extraction [11] were used.

However, all these traditional methods present some major drawbacks and practical limitations which are mainly overcome by the multi residues extraction method QuEChERS. In fact, this method known as quick, easy, cheap, effective, rugged, and safe extraction procedure, has proved its efficiency for the extraction of organic pollutants, including non-polar and polar pesticides, in several matrix. [25,26]. This technique is divided in two steps, the first considered as a soft extraction method using ACN as extraction agent while the second as an optional clean-up procedure by dispersive solid-phase extraction (d-SPE) [27]. However, even that QuEChERS extraction procedure has proven its efficiency in many environmental studies, this method, was to the best of our knowledge never applied on conifer needles.

Following their extraction, the analytical control of pesticides and POPs residues in environmental matrices requires their analysis by efficient analytical instruments such as tandem mass spectrometry (MS/MS) associated with gas chromatography (GC) or liquid chromatography (LC) [28,29]. The use of such analytical tools plays a key role and provides the most effective and efficient means to assess hundreds of analytes in a variety of matrices in one run. In fact, it is important to note that while GC-MS/MS is specifically targeted at non-polar and semi-polar, volatile and semi-volatile compounds, LC-MS/MS is more suitable for polar and semi-polar, non-volatile and thermolabile compounds [30,31].

For all these reasons, this manuscript proposes the development of QuEChERS, a multi-residue analytical method for the extraction of 134 pesticides, 22 PCBs and 16 PAHs followed by their analysis using LC-MS/MS and GC-MS/MS. The application of this

method to the analysis of coniferous matrices is considered to be challenging as well. In fact, the introduction of the QuEChERS method provides a high-throughput multi-residual approach to the routine pollutant monitoring of conifers samples. The extraction technique used was followed by a pre-concentration step for volatile compounds using SPME prior for their analysis by GC-MS/MS while non-volatile compounds were directly analyzed, after their extraction, by LC-MS/MS. In fact, the extraction techniques used and the wide number and pollutants assessed, were, to the best of our knowledge, never reported before. The developed method presents, therefore, a new contribution to this field. Moreover, the developed method was applied on real samples collected from several regions in northern Lebanon in order to biomonitor the environmental state in these regions.

2. Experimental

2.1. Materials and reagents

For LC-MS/MS pesticides analysis, the 31 non-volatile pesticides (Sigma Aldrich, St, Quentin Fallavier, France) were:

Acetamiprid, Carbendazim, Carbetamide, Chlorfenvinphos, Chloridazone, Chlortoluron, Cyazofamid, Diflubenzuron, Diflufenican, Dimethenamid-P, Diuron, Epoxyconazole, Flufenoxuron, Formasulfuron, Isoproturon, Isoxadifen, Metalaxyl-M, Nicosulfuron, Penconazole, Pendimethalin, Propiconazole, Prothioconazole, Pymetrozine, Pyraclostrobine, Spinosad-A, Spinosad-D, Sulcotrione, Tebuconazole, Terbutryn, Thiacloprid and Triflurosulfuron-methyl.

A stock solution of each of these standards at 1 g L⁻¹ was prepared in ACN.

For GC-MS/MS pesticides analysis, the 82 volatile pesticides except OCPs (Sigma Aldrich, St, Quentin Fallavier, France) were:

2,4-MPCA, Acetochlor, Aclonifen, Alachlor, Azinphos-ethyl, Azoxystrobin, Benoxacor, Bifenox, Bifenthrin, Boscalid, Bromoxynil-octanoate, Bupirimate, Buprofezine, Captan, Carbaryl, Chlorothalonil, Chlorpropham, Chlorpyrifos, Chlorpyrifos-Methyl, Clofentezin, Clomazone, Cypermethrin, Cyproconazole, Cyprodinil, Deltamethrin, Dicamba, Diclobenil, Diclofop-methyl, Diflufenicanil, Dimethachlor, Dimethanamid-P, Dimetomorph,

Dimoxystrobin, Diphenylamine, Epoxyconazole, Ethofumesate, Etridiazole, Fenarimol, Fenoxycarb, Fenpropidin, Fluazinam, Fludioxynil, Flumioxazin, Flurochloridon, Flusilazole, Folpet, Indoxacarb, Iprovalicarb, Isoxaflutole, Kerosym-methyl, Lambda-cyhalothrin, Lenacil, Lindane, Malathion, Mecroprop-P, Metamitron, Metazachlor, Metolachlor-S, Myclobutanil, Oxadiazon, Penconazol, Pendimethalin, Picloram Piperonil-butoxide, Prochloraz, Procymidon, Propiconazole, Propyzamid, Prosulfocarb, Pyraclostrobin, Pyrimethanil, Quinoxifen, Spiroxamine, Tebuconazole, Tebufenpyrad, Tebutam, Tetraconazole, Tolyfluanid, Triadimenol, Trifloxystrobin, Trifluralin and Zoxamide.

A stock solution of each of these standards at 1 g L⁻¹ was prepared in ACN.

For OCPs analysis, a solution at 0.1 g L⁻¹ of 21 OCPs including: Aldrine, *cis*-chlordane, *trans*-chlordane, Dieldrine, α -Endosulfan, β -Endosulfan, Heptachlore, Heptachlore-epoxyde A, Heptachlore-epoxyde B, Hexachlorobenzene, Metoxychlore, o,p'-DDD, o,p'-DDE, o,p'-DDT, p,p'-DDD, p,p'-DDE, p,p'-DDT, α -HCH, β -HCH, γ -HCH and δ -HCH was purchased from Cluzeau Info Labo, St, Croix la Grande, France.

For PAHs analysis, a solution at 0.1 g L⁻¹ of 16 PAHs including: Acenaphthene, Anthracene, Benzo(a)anthracene, Benzo(a)pyrene, Benzo(b)fluoranthrene, Benzo(e)pyrene, Benzo(g,h,i)perylene, Benzo(k)fluoranthrene, Chrysene, Dibenzo(a,h)anthracene, Fluoranthrene, Fluorene, Indenol(1,2,3)pyrene, Naphtalene, Phenanthrene and Pyrene was purchased from Cluzeau Info Labo, St, Croix la Grande, France.

For PCBs analysis, a solution at 0.1 g L⁻¹ of 22 PCBs including: PCB 18, PCB 28, PCB 31, PCB 44, PCB 52, PCB 70, PCB 81, PCB 101, PCB 105, PCB 114, PCB 118, PCB 123, PCB 126, PCB 138, PCB 149, PCB 153, PCB 156, PCB 157, PCB 167, PCB 169, PCB 180 and PCB 189 was purchased from Cluzeau Info Labo, St, Croix la Grande, France.

Internal standards for LC-MS/MS were obtained from Sigma Aldrich, St, Quentin Fallavier, France. These standards included: Carbendazim-d⁴ (99.3%), Diuron-d⁶ (99.8%), Pendimethalin-d⁵ (99%), and Nicosulfuron-d⁶ (99%). A standard solution of each compound at 1 g L⁻¹ in ACN was prepared. A mixture of these standards at 0.01 g L⁻¹ in ACN was also prepared for their alternative use as IS.

Internal standards for GC-MS/MS were obtained from Sigma Aldrich, St, Quentin Fallavier, France except Naphtalene-d⁸ obtained from Cambridge Isotope Laboratories. A mixture of Trifluralin-d¹⁴, 4-Nitrophenol-d⁴, 2,4-D-d³, Atrazine-d⁵, Pendimethalin-d⁵ and *trans*-Cypermethrin-d⁵ at 1 g L⁻¹ in ACN was used as IS for pesticides analysis except OCPs, while a mixture of Naphtalene-d⁸, Phenanthrene-d¹⁰, Chrysene-d¹² and Perylene-d¹² at 1 g L⁻¹ in ACN was used for OCPs, PAHs and PCBs analysis. A mixture of these two standard solutions at 0.01 g/L in ACN was prepared.

All prepared solutions were stored at -18 °C.

Kits for QuEChERS (RESTEK France-EN 1566 method) were purchased as ready to use containing 4 g MgSO₄, 1 g NaCl, 1 g trisodium citrate dehydrate and 0.5 g disodium hydrogen citrate sesquihydrate. For clean-up, the kits (AOAC 2007 method) containing 1.2 g MgSO₄, 400 mg PSA, 400 mg C₁₈ and 400 mg GCB were used.

LC-MS grade ACN, LC-MS grade water and formic acid ≥ 99% were purchased from Avantor®, United States. The ACN and water solutions are prepared by mixing 500 mL of each solution with 0.1% (0.5 mL) of formic acid. The solvents used were all HPLC grade and the ultra-pure water was obtained through a Milli-Q system (18 MΩ cm) from Merck, Germany.

2.2. Sample collection

Blank conifer matrix samples (*Pinus nigra*) were collected from the botanical garden of the university of Strasbourg, campus of Cronenbourg, France and only the terminal parts of the branches, newly obtained and not exposed to environmental pollutants were collected. The samples were transported in polyethylene bags to the laboratory where they were finely cut and then washed with ACN for 15 min under the hood. This washing is repeated twice in order to remove all traces of contaminants on their surfaces. After washing, the conifers were well dried, and were kept at -18 °C until their analysis. These needles were then analyzed by GC-MS/MS and LC-MS/MS in order to assess any previous contamination that may interfere with targeted pollutants. The results showed a complete absence of such interference and therefore these needles were used as for matrix matched calibration curves and for method development as well. Other conifers samples were collected from 15 different sites in northern of Lebanon (Bsharri, Abdine, Akkar, Koura

and Tripoli) in August 2018. The samples were transported in polyethylene bags and stored frozen (- 18 °C) until analysis [23].

A map showing the location of the different sampling sites is shown in figure 1 in supplementary materials (S3).

2.3. Extraction procedures

Five grams of homogenized samples of washed conifer needles were weighed in 50 mL centrifuge tube then fortified with different concentrations of each mixture's solution (5, 10, 25, 50, 100, 500, 1000, 1500, 2000 and 3000 ng g⁻¹). The samples were kept at 4 °C overnight, and then extracted using the QuEChERS extraction procedure cited below. All extractions were done in triplicate.

The protocol chosen was as follows: to 5 gs of homogenized matrix, 15 mL of ACN were added and the tubes were shaken. After stirring, QuEChERS citrate buffered extraction salts were added, then the tubes were vortexed for 1 min and then centrifuged for 10 min at 5000 rpm. Afterwards, the supernatant was added to the 15 mL of PSA tube then vortexed and centrifuged for 10 min at approximately 5000 rpm. Finally, the obtained extract (around 3 mL), was sampled in a 10 mL glass tube, and then evaporated at approximately 100 µL.

2.3.1. Extract reconstitution

Once evaporated, the collected extracts were reconstituted with ACN to 1 mL, in order to prepare them to chromatographic analysis.

100 µL of this solution were transferred to LC vials and were directly injected into an LC-MS/MS system after the addition of 10 µL of the appropriate internal standards. The remained 900 µL were derivatized then pre concentrated by SPME prior to their analysis with GC-MS/MS.

2.3.1.1. Derivatization by silylation

A silylation reaction from N-methyl-N-tert-butyldimethylsilyltrifluoroacetamide (noted MTBSTFA) was used. The remained 900 µL were brought in contact with 50 µL of MTBSTFA at 80 °C for 1 h After the derivation reaction, the obtained solution was diluted

to 20 mL using of acidified water (pH 3) and 10 μ L of both appropriated GC internal standards. The acidified water solution is prepared by mixing 30 gs of sodium chloride >99,8% with 250 μ L of nitric acid 68% and 2 L ultrapure water.

2.3.2. Concentration and injection by solid-phase microextraction (SPME)

Due to the large number of compounds with different chemical families, characteristics and polarities, two SPME fibers were used. The first was coated with polydimethylsiloxane (PDMS) of 100 μ m and was used for the extraction of PAHs, PCBs, OCPs. The second was coated with polyacrylate (PA) at 85 μ m and was used for the extraction of the remained semi-volatile pesticides. The immersion time for both fibers in the solution was 40 min.

2.4. Extract analysis

Extracted samples were analyzed, according to their properties, using LC-MS/MS for the 31 non-volatile pesticides and GC-MS/MS for the 103 volatile pesticides, the 22 PCBs and the 16 PAHs.

2.4.1. Liquid chromatography coupled to a tandem mass spectrometer

A thermo Scientific TSQ Vantage Triple Quadrupole Mass Spectrometer coupled with a Surveyor pump and autosampler (Accela Autosampler) operating in electrospray ionization mode (ESI) was used. The sampler is equipped with a 20 μ L injection loop and the samples were kept at a temperature of 1 $^{\circ}$ C. The analysis was performed on a Nucleodur C₁₈ Pyramid column (150 mm $\times$ 3 mm, 3 μ m) thermostated at 25 $^{\circ}$ C. Samples were analyzed using a mobile phase ACN/water (0.1% formic acid) with a flow rate of 0.3 mL min⁻¹. The gradient started with 30:70 (v/v) for 5 min, followed by 50:50 (v/v) for 6 min, then 80:20 (v/v) for 7 min, to achieve 95:5 (v/v) for 10 min, finally a ratio of 30:70 (v/v) for 8 min was set in order to stabilize the column for any new injection.

The LC-MS/MS parameters for non-volatile pesticides analysis figure in supplementary materials S1-1.

2.4.2. Gas chromatography coupled to a tandem mass spectrometer

A GC–MS/MS Trace GC Ultra/ITQ 700 coupling equipped with a Combi PAL equipped with SPME fiber was used for the analysis of semi volatile pesticides, PAHs, PCBs and OCPs. The analysis was carried out on an XLB (50% phenyl/ 50% methylsiloxane) capillary column of 30 m × 0.25 mm, 0.25 µm film thickness. Injection was done in splitless mode at 250 °C for 15 min. The transfer line was maintained at 300 °C and the source of the MS at 210 °C. Helium was used as carrier gas at a flow rate of 1 mL min⁻¹.

2.4.2.1. Semi volatile pesticides separation and analysis

Injection of the sample was done by thermal desorption of the polyacrylate fiber. Initial oven temperature was set at 50 °C for 3 min, followed by a linear ramp to 160 °C at a rate of 36.6 °C min⁻¹, followed by a ramp to 300 °C at a rate 5.8 °C min⁻¹, where it was maintained for 10 min, leading to a total run time of 41 min.

The GC–MS/MS parameters for semi volatile pesticides analysis figure in supplementary materials S1-2.

2.4.2.2. POPs separation and analysis

Injection of the sample was done by thermal desorption of the polydimethylsiloxane fiber. Initial oven temperature was set at 50 °C for 3 min, followed by a linear ramp to 255 °C at a rate of 10 °C min⁻¹, followed by a ramp to 330 °C at a rate 20 °C min⁻¹, where it was maintained for 18 min, leading to a total run time of 45.25 min.

The GC–MS/MS parameters for POPs analysis figure in supplementary materials S1-3.

2.5. Validation of the method

Once developed, the method was validated for all analytical parameters. First, triplicate extraction of fortified samples with concentrations ranged from 5 to 3000 ng g⁻¹ (5–10–25–50–100–500–1000–1500–2000–3000) was performed to determine linearity. Then, five samples of spiked matrix with three level concentration (10, 100 and 1000 ng g⁻¹) were extracted for three successive days in order to determine intermediate precision and repeatability. These two precision parameters were evaluated by their correspondent relative standard deviation (RSD%).

In fact, matrix-matched calibration curves were performed using the washed conifer needles. Matrix effect was assessed by the use of internal standards which gave the same intensity for both washed and real matrix and therefore we assumed that the wash had no effect on the matrix composition. For this, quantification was done using the matrix matched calibration curves developed on the washed conifer needles in order to ensure that the matrix was exactly the same as the samples. Concerning method validation limits, the limit of detection and quantification were calculated as the lowest concentration for which precision and accuracy has been demonstrated and which responds to the relationship respectively: $\text{signal/noise} \geq 3$ and $\text{signal/noise} \geq 10$. In fact, the method limit of detection (LOD) was determined as the analyte concentration that produced a peak signal of three times the background noise from the chromatogram, and the method limit of quantification (LOQ) was determined as the analyte concentration that produced a peak signal of ten times the background noise from the chromatogram. These limits were then determined graphically with: $\text{LOD} = 3 \times [\text{min}] \text{ S/N}$ and $\text{LOQ} = 10 \times [\text{min}] \text{ S/N}$ [32,33].

Regarding the recoveries, they were also determined at the same three levels of concentrations as precision (10, 100 and 1000 ng g⁻¹). The recoveries were considered as the ratio of the area of the spiked samples to the area of the standard following the equation:

$$\text{Recovery\%} = (\text{Sample concentration} / \text{Standard solution concentration}) * 100.$$

Results

3.1. Method development

A concentration step by evaporation and reconstitution was added before the liquid and gas chromatographic analysis to obtain a concentrated extract. For the volatile compounds, this process was followed by solid-phase microextraction extraction/concentration step prior to gas chromatographic analysis using the polyacrylate and polydimethylsiloxane fiber for the extraction of semi volatile pesticides and PAHs, PCBs, OCPs respectively [34,35]. A validation procedure was performed, which showed good results for suitability, recovery and repeatability. The developed method was applied to the determination of real samples that some pollutants were detected.

3.2. Method validation

Once identified and developed, the method was validated and verified in order to ensure its reliability and efficiency. Validation shows that the analytical method used to evaluate a component is reliable, accurate, reproducible and robust over the specific range and it is suitable for its intended purpose [36]. Typical characteristics of validation to be considered were: accuracy, linearity, precision: repeatability and reproducibility, detection limit, quantitation limit and recovery.

All target compounds were validated with good linearity expressed by a regression coefficient higher than 0.98. LOD were lower than 15 ng g⁻¹ for all compounds except Indoxacarb and LOQ were lower than 15 ng g⁻¹ for all non-volatile pesticides and for the majority of volatile compounds analyzed by GC-MS/ MS. Moreover, results showed that all these pollutants were detected with high precision with RSD% lower than 20% for inter and intra-day analysis except nicosulfuron and diflubenzuron. Furthermore, the method showed good recoveries between 60.48 to 98.31%, 62.75 to 107.14%, 71.29 to 121.92%, 73.38 to 99.97% and 61.04 to 98.89% for non-volatile pesticides, volatile pesticides, OCPs, PCBs and PAHs respectively.

The validation parameters for non-volatile pesticides analyzed by LC-MS/MS, volatile pesticides and POPs analyzed by GC-MS/MS figure in supplementary materials S2-1, S2-2 and S2-3 respectively.

4. Application to real samples

Once developed and validated, the method was applied on real samples. These samples were collected from several regions in Lebanon and were analyzed according to the previously developed method. Residue levels were calculated with Xcalibur using the previously plotted calibration curves.

Table 1 shows the majority of the pesticides, PAHs, OCPs and PCBs residues found in the samples analyzed.

Table 1. Concentration of compounds (ng g⁻¹) detected in real samples.

Compounds	Average concentration	Lowest concentration	Highest concentration
Chloridazon	2.66	0.3	6.72
Metalaxyl-M	38.66	3.64	51.24
Pendimethalin	3.93	1.59	11.91
Fluoranthrene	372.18	48.16	984.44
Boscalid	87.93	65.71	208.36
Naphtalene	11.68	4.51	39.6
Diflufenican	158.6	23.9	612.81
Acenaphtalene	7.21	13.39	82.19
Hexachlorobenzene	523.7	256.92	826.04
Terbutryn	5.33	0.81	56.22
Fenpropidin	238	75.27	918.68
Sulcotrione	78.23	4.72	287.18

5. Discussion

To the best of our knowledge, this is the first article on the combination of QuEChERS-SPME extraction with LC-MS/MS and GC-MS/MS analysis for simultaneous quantification and confirmation analysis for 134 pesticides, 16 PAHs and 22 PCBs from coniferous matrix.

The major advantage of the proposed method is the low volume of the organic solvent used allowing the extraction of a wide range of pollutants (172 pollutants) with a short time of sample preparation. Concerning the analytical procedure, it is more complicated to implement splitless injection. The temperature of the oven, the solvent and the splitless time must be carefully selected. However, this method is suitable for trace analysis since the complete sample is introduced into the column. Once optimized, the methodology is easy to use, fairly robust and easy to automate [37].

In fact, the results provided by the developed method overcome the drawbacks presented by the traditional used extraction methods. For instance, this method proved to be fast, efficient, environmental friendliness and reliable mainly due to the reduction of the sample weight (5 g) and the amount of solvents used [25]. Moreover, recoveries obtained using QuEChERS appear to be even higher than those obtained by accelerated solvent

extraction or solid-phase extraction especially with taking into consideration the high amount of solvent used in these latest techniques [38]. For instance, the recoveries obtained with our developed method were higher than those provided for the analysis of pesticides in conifer needles using S-PLE. In fact, the recoveries obtained for these two methods were for chlorpyrifos 73 and 50%, for trifluralin 86 and 70%, for Aldrin 96 and 65%, for heptachlor 87 and 70%, 86 and 70% for PCB 105 and for β -Endosulfan 96 and 65% respectively [39].

Furthermore, the developed method was evaluated and compared in terms of extraction time, accuracy, precision, sensitivity and versatility, with other procedures. The main differences between the developed procedures and other published methods for determination of pollutants residues in conifer samples pertain to the time of the stage of extraction and clean-up [40], [41].

Based on the data presented in the literature it can be concluded that the developed methodology introduces a new trend in the process of determination of a wide range of pollutants (pesticides, PCBs, PAHs and OCPs) in conifer samples in comparison with other already published methods such as ASE/S-PLE for pesticides and PCBs analysis [39], ASE mainly used for the analysis of PAHs [42], Soxhlet for the OCPs [43], polychlorinated dibenzo-p-dioxins/- furans [44] and ultrasonic extraction for PAHs [45].

Many researchers reported influence of different extraction solvents on the content of pollutants in matrix [46]. Efficiency of solvents and methods are strongly dependent on plant matrix used [47]. Solvents, such as methanol, ethanol, dichloromethane, ACN and ethyl acetate have been commonly used for the extraction of pollutants in conifer. Another main advantage between the QuEChERS-SPME procedure and other published methods for determination of pollutants residues in conifer samples is the number and the volume of solvents used for extraction. In the developed methodology, needles were extracted using ACN as solvent extraction while in other methods several solvents such dichloromethane, toluene, ethyl acetate and methanol were required for the extraction of pesticides and POPs from conifer needles [23,48,49].

In addition, the range of QuEChERS applications is very wide and allowed its comparison with reference methods in different applications such as liquid-liquid extraction, solid-

liquid extraction, solid-phase extraction, accelerated solvent extraction, microwave assisted extraction and ultrasound assisted extraction that depend essentially by the nature of the analytes to extract and the complexity of the matrix. In fact, QuEChERS is a high-performance reasonable choice able to provide similar or better analytical performance without the drawbacks of the other methods, as well as the need for specific devices such as microwave or ultrasound [50]. For instance, Di et al. in 2015, analyzed organochlorine pesticides and confirmed that QuEChERS and microwave-assisted extraction methods generated higher results compared to the accelerated solvent extraction and ultrasound assisted extraction [51]. Moreover, the QuEChERS method showed better performance for the determination of pesticides in honey and honey bees [52] and in roots and rhizomes of herbal medicines [53] than liquid-liquid extraction and solid-phase extraction.

A comparison of the %RSD for pesticides, PCBs and PAHs in the pine needle using conventional pressurized liquid extraction (PLE), selective pressurized liquid extraction (S-PLE) and QuEChERS method developed in our work showed that this latest provided higher precision than PLE and S-PLE with lowest % RSD for both α -Endosulfan and α -HCH [39].

In addition, the comparison of the developed method with the work of AL ALAM et al., 2017 in which ASE-SPE-SPME was used for the multi-residue's organic pollutants in conifer needles, showed that even if no big differences were found in term of analytical parameters, it is clear that the amount of solvent and time used in QuEChERS-SPME favor this latest and make it the method of choice for such studies [23,54].

On the other side, the analysis of organic pollutants residues in conifer by GC-MS/MS and LC-MS/MS proved the efficiency of these analytical tools in multi- residues analysis. In fact, results showed that the combination of the separating power of liquid and gas chromatography with the highly sensitive and selective mass analysis capability of MS/MS solve most of the problems associated with assessing pollutant residues such as the small amount of sample that can be detected, the relative analysis time, the precision, the broad range of samples, the continuous operation on a large scale and the simplicity of equipment [55,56]. In fact, chromatography is used in a wide range of applications due to its possibility to separate different components of a complex mixture based on polarity,

molecular weight and ionic mobility. However, both techniques of chromatography are necessary in trace analysis for the determination of a wide spectrum of environmental pollutants such as polynuclear aromatic hydrocarbons, dioxins, PCBs, chlorinated pesticides, and some other pesticides [57-59].

The results proved that the QuEChERS-SPME protocol in combination with LC and GC techniques can be used as a tool in environmental monitoring. The developed methods were successfully applied in monitoring real samples collected from 15 districts in Lebanon. Some pollutant concentrations in the conifer samples are summarized in Table 1. The concentrations were expressed in ng g^{-1} . Several studies have shown the currency of the use of conifer as an environmental biomonitor for pesticide and POPs pollution. Detectable pollutants have been found in most samples with varying residual levels by area and by different factors such as temperature, humidity, altitude and precipitation that can have a significant impact on the concentration of these pollutants [60]. In their study, Al-Alam et al., 2017 showed a direct effect of meteorological effects on pollutant concentrations found in conifer [23]. The levels of pesticides found in our study were comparable to those reported for honey samples from same sites in Northern Lebanon (Bsharri and Akkar) studied by Al Alam et al., 2017. However, the concentrations of diflufenican, fenpropidin, hexachlorobenzene and other pesticides present in this study were significantly identical as those above [61]. The results of the PAHs showed that the 15 sites tested were in accordance with the results provided by Al Alam et al., 2019 for determination of 16 PAHs and 22 PCBs in honey samples from different regions of Lebanon [62].

6. Conclusion

This study aimed to develop an analytical method for the extraction and quantification of pesticides, PCBs and PAHs' residues from conifer needles. The protocol chosen consisted of the use of QuEChERS based extraction followed by SPME for extraction and concentration of multi- residues organic pollutants. Chromatographic analysis was done using salts with LC-MS/MS and GC-MS/MS. The developed method proves its efficiency especially being fast, simple and covering a large majority of the pollutants assessed. The results showed satisfactory quantification and detection limits, good recoveries rates with a reduction of the analysis time and the solvent consumption as well.

References

- [1] G. E. Bergmann, "Impacts of Pesticide Pollution on Soil Microbial Communities, Ecosystem Function and Human Health," in Reference Module in Earth Systems and Environmental Sciences, Elsevier, 2019.
- [2] De Temmerman, L., Bell, N.B., Garrec, J.-P., Klumpp, A., Krause, G.H.M., Tonneijck, A.E.G., 2001. Biomonitoring of air pollutants with plants - Considerations for the future. Urban Air Pollution, bioindication and Environmental Awareness.
- [3] S. Mandic-Rajcevic, F. M. Rubino, E. Ariano, D. Cottica, S. Negri, and C. Colosio, "Exposure duration and absorbed dose assessment in pesticide-exposed agricultural workers: Implications for risk assessment and modeling," *International Journal of Hygiene and Environmental Health*, vol. 222, no. 3, pp. 494–502, Apr. 2019.
- [4] M. Shahid, C. Dumat, S. Khalid, E. Schreck, T. Xiong, and N. K. Niazi, "Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake," *Journal of Hazardous Materials*, vol. 325, pp. 36–58, Mar. 2017.
- [5] F. Şevik, İ. Tosun, and K. Ekinci, "The effect of FAS and C/N ratios on co-composting of sewage sludge, dairy manure and tomato stalks," *Waste Management*, vol. 80, pp. 450–456, Oct. 2018.
- [6] Y. Jiang, M. Fan, R. Hu, J. Zhao, and Y. Wu, "Mosses Are Better than Leaves of Vascular Plants in Monitoring Atmospheric Heavy Metal Pollution in Urban Areas," *Int J Environ Res Public Health*, vol. 15, no. 6, Jun. 2018.
- [7] S. Cen, "Biological Monitoring of Air Pollutants and Its Influence on Human Beings," *Open Biomed Eng J*, vol. 9, pp. 219–223, Aug. 2015.
- [8] N. Ratola et al., "Biomonitoring of pesticides by pine needles — Chemical scoring, risk of exposure, levels and trends," *Science of The Total Environment*, vol. 476–477, pp. 114–124, Apr. 2014.
- [9] D. Paustenbach and D. Galbraith, "Biomonitoring and Biomarkers: Exposure Assessment Will Never Be the Same," *Environ Health Perspect*, vol. 114, no. 8, pp. 1143–1149, Aug. 2006.
- [10] A. Hellström, H. Kylin, W. M. Strachan, and S. Jensen, "Distribution of some organochlorine compounds in pine needles from Central and Northern Europe," *Environmental Pollution*, vol. 128, no. 1–2, pp. 29–48, Mar. 2004.
- [11] N. Ratola, S. Lacorte, A. Alves, and D. Barceló, "Analysis of polycyclic aromatic hydrocarbons in pine needles by gas chromatography-mass spectrometry," *Journal of Chromatography A*, vol. 1114, no. 2, pp. 198–204, May 2006.
- [12] J. M. Amigo, N. Ratola, and A. Alves, "Study of geographical trends of polycyclic aromatic hydrocarbons using pine needles," *Atmospheric Environment*, vol. 45, no. 33, pp. 5988–5996, Oct. 2011.
- [13] J. O. Grimalt and B. L. van Drooge, "Polychlorinated biphenyls in mountain pine (*Pinus uncinata*) needles from Central Pyrenean high mountains (Catalonia, Spain)," *Ecotoxicology and Environmental Safety*, vol. 63, no. 1, pp. 61–67, Jan. 2006.
- [14] E. J. Al Dine et al., "Concomitant evaluation of atmospheric levels of polychlorinated biphenyls, organochlorine pesticides, and polycyclic aromatic hydrocarbons in Strasbourg (France) using pine needle passive samplers," *Environ Sci Pollut Res*, vol. 22, no. 22, pp. 17850–17859, Nov. 2015.
- [15] J. Wittsiepe, P. Schrey, F. Lemm, G. Eberwein, and M. Wilhelm, "Polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and organochlorine pesticides in human blood of pregnant women from Germany," *J. Toxicol. Environ. Health Part A*, vol. 71, no. 11–12, pp. 703–709, 2008.
- [16] J. Burkhardt and S. Pariyar, "Particulate pollutants are capable to 'degrade' epicuticular waxes and to decrease the drought tolerance of Scots pine (*Pinus sylvestris* L.)," *Environmental Pollution*, vol. 184, pp. 659–667, Jan. 2014.
- [17] E. K. Match, N. G. Chavez Soria, D. S. Aga, and G. E. Atilla-Gokcumen, "Applications of metabolomics in assessing ecological effects of emerging contaminants and pollutants on plants," *Journal of Hazardous Materials*, vol. 373, pp. 527–535, Jul. 2019.
- [18] E. Beristain-Montiel, R. Villalobos-Pietrini, G. E. Arias-Loaiza, S. L. Gómez-Arroyo, and O. Amador-Muñoz, "An innovative ultrasound assisted extraction micro-scale cell combined with gas chromatography/mass spectrometry in negative chemical ionization to determine persistent organic pollutants in air particulate matter," *Journal of Chromatography A*, vol. 1477, pp. 100–107, Dec. 2016.
- [19] Z. Lianfu and L. Zelong, "Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes," *Ultrasonics Sonochemistry*, vol. 15, no. 5, pp. 731–737, Jul. 2008.
- [20] X. Huang et al., "Simultaneous determination of 50 residual pesticides in *Flos Chrysanthemi* using accelerated solvent extraction and gas chromatography," *Journal of Chromatography B*, vol. 967, pp. 1–7, Sep. 2014.
- [21] G. Wang et al., "Determination of thiamphenicol, florfenicol and florfenicol amine residues in poultry meat and pork via ASE-UPLC-FLD," *Journal of Food Composition and Analysis*, vol. 81, pp. 19–27, Aug. 2019.
- [22] A. F. Diefendorf, D. T. Sberna, and D. W. Taylor, "Effect of thermal maturation on plant-derived terpenoids and leaf wax n-alkyl components," *Organic Geochemistry*, vol. 89–90, pp. 61–70, Dec. 2015.
- [23] J. Al-Alam, Z. Fajloun, A. Chbani, and M. Millet, "The use of conifer needles as biomonitor candidates for the study of temporal air pollution variation in the Strasbourg region," *Chemosphere*, vol. 168, pp. 1411–1421, Feb. 2017.
- [24] T. A. Tomashuk, T. M. Truong, M. Mantha, and A. E. McGowin, "Atmospheric polycyclic aromatic hydrocarbon profiles and sources in pine needles and particulate matter in Dayton, Ohio, USA," *Atmospheric Environment*, vol. 51, pp. 196–202, May 2012.
- [25] M. Á. González-Curbelo, B. Socas-Rodríguez, A. V. Herrera-Herrera, J. González-Sálamo, J. Hernández-Borges, and M. Á. Rodríguez-Delgado, "Evolution and applications of the QuEChERS method," *TrAC Trends in Analytical Chemistry*, vol. 71, pp. 169–185, Sep. 2015.
- [26] L. Kim, D. Lee, H.-K. Cho, and S.-D. Choi, "Review of the QuEChERS method for the analysis of organic pollutants: Persistent organic pollutants, polycyclic aromatic hydrocarbons, and pharmaceuticals," *Trends in Environmental Analytical Chemistry*, vol. 22, p. e00063, Apr. 2019.
- [27] M. Anastassiades, S. J. Lehotay, D. Stajnbaher, and F. J. Schenck, "Fast and easy multiresidue method employing acetonitrile extraction/partitioning and 'dispersive solid-phase extraction' for the determination of pesticide residues in produce," *J AOAC Int*, vol. 86, no. 2, pp. 412–431, Apr. 2003.
- [28] F. Vaiano, G. Serpelloni, M. Focardi, A. Fioravanti, F. Mari, and E. Bertol, "LC-MS/MS and GC-MS methods in propofol detection: Evaluation of the two analytical procedures," *Forensic Science International*, vol. 256, pp. 1–6, Nov. 2015.
- [29] H. Kwon, S. J. Lehotay, and L. Geis-Asteggiant, "Variability of matrix effects in liquid and gas chromatography-mass spectrometry analysis of pesticide residues after QuEChERS sample preparation of different food crops," *J Chromatogr A*, vol. 1270, pp. 235–245, Dec. 2012.
- [30] G. Purcaro, S. Moret, and L. Conte, "Sample pre-fractionation of environmental and food samples using LC-GC multidimensional techniques," *TrAC Trends in Analytical Chemistry*, vol. 43, pp. 146–160, Feb. 2013.
- [31] W. J. Griffiths and Y. Wang, "Analysis of neurosteroids by GC-MS and LC-MS/MS," *Journal of Chromatography B*, vol. 877, no. 26, pp. 2778–2805, Sep. 2009.

- [32] J. M. Montiel-León et al., "Occurrence of pesticides in fruits and vegetables from organic and conventional agriculture by QuEChERS extraction liquid chromatography tandem mass spectrometry," *Food Control*, vol. 104, pp. 74–82, Oct. 2019.
- [33] S. Meyer et al., "Development, validation and application of an ICP-MS/MS method to quantify minerals and (ultra-)trace elements in human serum," *Journal of Trace Elements in Medicine and Biology*, vol. 49, pp. 157–163, Sep. 2018.
- [34] Ruiz del Castillo, M.L., Rodríguez-Valenciano, M., Flores, G., Blanch, G.P., 2019. New method based on Solid Phase Microextraction and Multidimensional gas chromatography-mass spectrometry to determine pesticides in strawberry jam. *LWT* 99, 283–290. <https://doi.org/10.1016/j.lwt.2018.09.063>.
- [35] Müller, L., Górecki, T., Pawliszyn, J., 2006. Solid-Phase Microextraction in Analysis of Pollutants in the Field, in: *Encyclopedia of Analytical Chemistry*. American Cancer Society. <https://doi.org/10.1002/9780470027318.a0922>.
- [36] Tiwari, G., Tiwari, R., 2010. Bioanalytical method validation: An updated review. *Pharm Methods* 1, 25–38. <https://doi.org/10.4103/2229-4708.72226>.
- [37] Korytár, P., Janssen, H.-G., Matisová, E., Brinkman, U.A.T., 2002. Practical fast gas chromatography: methods, instrumentation and applications. *TrAC Trends in Analytical Chemistry* 21, 558–572. [https://doi.org/10.1016/S0165-9936\(02\)00811-7](https://doi.org/10.1016/S0165-9936(02)00811-7).
- [38] Garcia, C.V., Gotah, A., 2017. Application of QuEChERS for Determining Xenobiotics in Foods of Animal Origin [WWW Document]. *Journal of Analytical Methods in Chemistry*. <https://doi.org/10.1155/2017/2603067>.
- [39] K. S. Lavin and K. J. Hageman, "Selective pressurised liquid extraction of halogenated pesticides and polychlorinated biphenyls from pine needles," *Journal of Chromatography A*, vol. 1258, pp. 30–36, Oct. 2012.
- [40] Albinet, A., Tomaz, S., Lestremau, F., 2013. A really quick easy cheap effective rugged and safe (QuEChERS) extraction procedure for the analysis of particle-bound PAHs in ambient air and emission samples. *Science of The Total Environment* 450–451, 31–38. <https://doi.org/10.1016/j.scitotenv.2013.01.068>.
- [41] Kim, L., Lee, D., Cho, H.-K., Choi, S.-D., 2019. Review of the QuEChERS method for the analysis of organic pollutants: Persistent organic pollutants, polycyclic aromatic hydrocarbons, and pharmaceuticals. *Trends in Environmental Analytical Chemistry* 22, e00063. <https://doi.org/10.1016/j.teac.2019.e00063>.
- [42] Y. Kuang, J. Li, and E. Hou, "Lipid-content-normalized polycyclic aromatic hydrocarbons (PAHs) in the xylem of conifers can indicate historical changes in regional airborne PAHs," *Environmental Pollution*, vol. 196, pp. 53–59, Jan. 2015.
- [43] D. Xu, W. Zhong, L. Deng, Z. Chai, and X. Mao, "Regional distribution of organochlorinated pesticides in pine needles and its indication for socioeconomic development," *Chemosphere*, vol. 54, no. 6, pp. 743–752, Feb. 2004.
- [44] E. Holt, A. Kočan, J. Klánová, A. Assefa, and K. Wiberg, "Polychlorinated dibenzo- p -dioxins/furans (PCDD/Fs) and metals in scots pine (*Pinus sylvestris*) needles from Eastern and Northern Europe: Spatiotemporal patterns, and potential sources," *Chemosphere*, vol. 156, pp. 30–36, Aug. 2016.
- [45] B. L. van Drooge, G. Garriga, and J. O. Grimalt, "Polycyclic aromatic hydrocarbons in pine needles (*Pinus halepensis*) along a spatial gradient between a traffic intensive urban area (Barcelona) and a nearby natural park," *Atmospheric Pollution Research*, vol. 5, no. 3, pp. 398–403, Jul. 2014.
- [46] J. A. Michiels, C. Kevers, J. Pincemail, J. O. Defraigne, and J. Dommes, "Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices," *Food Chemistry*, vol. 130, no. 4, pp. 986–993, Feb. 2012.
- [47] G. Spigno, L. Tramelli, and D. M. De Faveri, "Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics," *Journal of Food Engineering*, vol. 81, no. 1, pp. 200–208, Jul. 2007.
- [48] M. Chropeňová et al., "Pine needles and pollen grains of *Pinus mugo* Turra – A biomonitoring tool in high mountain habitats identifying environmental contamination," *Ecological Indicators*, vol. 66, pp. 132–142, Jul. 2016.
- [49] Araújo, R., Ratola, N., Moreira, J., Santos, L., Alves, A., 2012. Different extraction approaches for the biomonitoring of pesticides in pine needles. *Environmental technology* 33, 2359–68. <https://doi.org/10.1080/09593330.2012.668946>.
- [50] Łozowicka, B., Rutkowska, E., Jankowska, M., 2017. Influence of QuEChERS modifications on recovery and matrix effect during the multi-residue pesticide analysis in soil by GC/MS/MS and GC/ECD/NPD. *Environ Sci Pollut Res Int* 24, 7124–7138. <https://doi.org/10.1007/s11356-016-8334-1>.
- [51] Di, S., Shi, S., Xu, P., Diao, J., Zhou, Z., 2015. Comparison of Different Extraction Methods for Analysis of 10 Organochlorine Pesticides: Application of MAE-SPE Method in Soil from Beijing. *Bull Environ Contam Toxicol* 95, 67–72. <https://doi.org/10.1007/s00128-015-1538-0>.
- [52] Calatayud-Vernich, P., Calatayud, F., Simó, E., Picó, Y., 2016. Efficiency of QuEChERS approach for determining 52 pesticide residues in honey and honey bees. *MethodsX* 3, 452–458. <https://doi.org/10.1016/j.mex.2016.05.005>.
- [53] Liu, J., Tong, L., Li, D., Meng, W., Sun, W., Zhao, Y., Yu, Z., 2016. Comparison of two extraction methods for the determination of 135 pesticides in *Corydalis Rhizoma*, *Chuanxiong Rhizoma* and *Angelica Sinensis Radix* by liquid chromatography-triple quadrupole-mass spectrometry. Application to the roots and rhizomes of Chinese herbal medicines. *Journal of Chromatography B* 1017–1018, 233–240. <https://doi.org/10.1016/j.jchromb.2016.03.003>.
- [54] G. Ouyang, "8 - SPME and Environmental Analysis," in *Handbook of Solid Phase Microextraction*, J. Pawliszyn, Ed. Oxford: Elsevier, 2012, pp. 251–290.
- [55] R. Yang, T. Yao, B. Xu, G. Jiang, and X. Zheng, "Distribution of organochlorine pesticides (OCPs) in conifer needles in the southeast Tibetan Plateau," *Environmental Pollution*, vol. 153, no. 1, pp. 92–100, May 2008.
- [56] N. Ratola, A. Alves, L. Santos, and S. Lacorte, "Pine needles as passive bio-samplers to determine polybrominated diphenyl ethers," *Chemosphere*, vol. 85, no. 2, pp. 247–252, Sep. 2011.
- [57] N. Ratola, J. M. Amigo, M. S. N. Oliveira, R. Araújo, J. A. Silva, and A. Alves, "Differences between *Pinus pinea* and *Pinus pinaster* as bioindicators of polycyclic aromatic hydrocarbons," *Environmental and Experimental Botany*, vol. 72, no. 2, pp. 339–347, Sep. 2011.
- [58] E. M. Noth, S. Katharine Hammond, G. S. Biging, and I. B. Tager, "Mapping and modeling airborne urban phenanthrene distribution using vegetation biomonitoring," *Atmospheric Environment*, vol. 77, pp. 518–524, Oct. 2013.
- [59] R. Fernández-Varela, N. Ratola, A. Alves, and J. M. Amigo, "Relationship between levels of polycyclic aromatic hydrocarbons in pine needles and socio-geographic parameters," *Journal of Environmental Management*, vol. 156, pp. 52–61, Jun. 2015.
- [60] Yang, R., Yao, T., Xu, B., Jiang, G., Zheng, X., 2008. Distribution of organochlorine pesticides (OCPs) in conifer needles in the southeast Tibetan Plateau. *Environmental Pollution, Chelating Agents in the Environment* 153, 92–100. <https://doi.org/10.1016/j.envpol.2007.07.033>.
- [61] Al Alam, J., Fajloun, Z., Chabni, A., Millet, M., 2017. The use of honey as environmental biomonitor of pesticides contamination in northern Lebanon. *Euro-Mediterr J Environ Integr* 2, 23. <https://doi.org/10.1007/s41207-017-0034-9>.
- [62] Al-Alam, J., Fajloun, Z., Chbani, A., Millet, M., 2019. Determination of 16 PAHs and 22 PCBs in honey samples originated from different region of Lebanon and used as environmental biomonitor sentinel. *Journal of Environmental Science and Health, Part A* 54, 9–15. <https://doi.org/10.1080/10934529.2018.1500782>.

Supplementary Materials S1: Chapter II-II - Supplementary Materials - Table 1, 2 and 3.

Supplementary Materials S2

Supplementary materials S2-1: Validation parameters for LC-MS/MS method for semi-volatile pesticides analysis

Pesticide - LC	Regression line equation	Regression coefficient	LOD (ng/g)	LOQ (ng/g)	% RSD Intra-Day	% RSD Inter-Day	Recovery %
Carbendazim	$Y = 3.94081e-005 * X$	0.99	0.57	1.92	13.40	14.84	90.46
Chloridazone	$Y = 9.73219e-008 * X$	0.97	2.50	8.32	13.95	13.70	87.26
Pymetrozine	$Y = 5.53601e-005 * X$	0.99	0.62	2.08	10.99	14.47	68.73
Acetamiprid	$Y = 2.49889e-005 * X$	0.99	2.14	7.13	12.58	14.11	97.28
Chlortoluron	$Y = 1.80165e-005 * X$	0.99	0.42	1.42	14.62	16.06	80.36
Nicosulfuron	$Y = 2.73063e-005 * X$	0.99	1.15	3.84	16.33	20.08	91.39
Formasulfuron	$Y = 5.6901e-005 * X$	0.99	1.88	6.24	13.09	14.65	89.17
Thiacloprid	$Y = 0.000302437 * X$	0.99	1.07	3.56	10.06	11.55	98.31
Carbetamide	$Y = 0.000217453 * X$	0.99	1.76	5.87	12.48	13.51	92.31
Terbutryn	$Y = 7.81516e-005 * X$	0.99	0.31	1.06	11.74	13.95	84.53
Metalaxyl-M	$Y = 0.00334545 * X$	0.99	0.17	0.59	10.36	11.01	66.31
Sulcotrione	$Y = 0.000704102 * X$	0.99	0.15	0.51	13.20	13.42	81.77
Spinosade-A	$Y = 0.00243457 * X$	0.99	0.57	1.90	7.70	10.28	78.00
Isoproturon	$Y = 0.00946096 * X$	0.99	0.06	0.19	6.82	9.41	63.69
Diuron	$Y = 3.75718e-006 * X$	0.99	5.00	16.65	16.22	15.65	85.16
Spinosade-D	$Y = 1.51239e-005 * X$	0.99	0.57	1.92	8.32	11.13	90.77
Dimethenamid-P	$Y = 0.00284929 * X$	0.99	0.02	0.06	9.45	7.84	62.74
Epoxyconazole	$Y = 0.0138056 * X$	0.99	3.33	11.10	6.99	10.59	73.14
Trisulfuron-Methyl	$Y = 0.000191594 * X$	0.99	0.06	0.21	7.73	11.11	71.66
Tebuconazole	$Y = 0.00979492 * X$	0.99	0.20	0.67	14.96	13.26	68.43
Diflubenzuron	$Y = 0.000213112 * X$	0.99	1.07	3.56	15.67	21.26	95.60
Prothioconazole	$Y = 0.000482435 * X$	0.99	1.25	4.16	14.94	14.82	60.49
Penconazole	$Y = 0.0134616 * X$	0.99	0.07	0.24	9.11	10.92	95.66
Propioconazole	$Y = 0.0273699 * X$	0.99	1.76	5.87	8.80	9.91	76.18
Chlorfenvinphos	$Y = 0.00371447 * X$	0.99	0.41	1.36	12.74	12.84	66.98
Cyazofamid	$Y = 1.0503e-006 * X$	0.98	0.55	1.85	5.26	10.78	71.33
Isoxadifen	$Y = 0.00234394 * X$	0.99	0.45	1.51	11.68	12.45	71.48
Pyraclostrobin	$Y = 0.00110839 * X$	0.99	0.08	0.29	14.68	17.24	92.45
Diflufenican	$Y = 0.0012687 * X$	0.99	0.31	1.04	14.06	17.19	88.91
Flufenoxuron	$Y = 0.00045246 * X$	0.99	1.78	5.94	11.79	13.11	72.62
Pendimethalin	$Y = 0.00518177 * X$	0.99	0.22	0.73	12.02	13.07	84.10

Supplementary materials S2-2: GC-MS/MS method performance and validation for pesticides analysis

Pesticide - GC	Regression line equation	Regression coefficient	LOD (ng/g)	LOQ (ng/g)	% RSD Intra- Day	% RSD Inter- Day	Recovery %
2,4-MCPA	$Y = 3.33042e-006X + 1.2134e-009X^2$	0.99	3.55	11.82	11.11	11.14	68.47
Acetochlore	$Y = 0.000135324X + 3.30875e-007X^2$	0.99	0.46	1.55	16.58	14.46	93.58
Aclonifen	$Y = 0.00363907X - 5.29763e-007X^2$	0.99	0.25	0.83	8.36	10.70	91.94
Alachlore	$Y = 0.000125231X + 2.00607e-007X^2$	0.99	0.24	0.82	12.04	14.34	82.55
Azinphos-Ethyl	$Y = 0.00054823X + 1.29837e-007X^2$	0.99	6.25	20.81	11.76	12.70	89.00
Azoxystrobine	$Y = 0.000685443X + 6.87898e-007X^2$	0.99	11.75	39.14	9.15	9.25	97.88
Benoxacore	$Y = 0.00106206X + 6.19542e-007X^2$	0.99	10.22	34.03	11.10	11.45	102.30
Bifenox	$Y = 2.44507e-005X + 1.146e-007X^2$	0.99	5.00	16.65	10.44	12.49	94.09
Bifenthrin	$Y = 0.00079064X - 9.04425e-008X^2$	0.99	0.07	0.23	10.01	12.25	86.93
Boscalid	$Y = 1.43451e-005X + 2.09261e-008X^2$	0.99	7.50	24.97	8.74	10.00	94.81
Bromoxynil- Octanoate	$Y = 0.0129879X + 3.86195e-006X^2$	0.99	3.33	11.10	8.10	8.90	82.12
Bupirimate	$Y = 9.36501e-005X + 1.77803e-008X^2$	0.99	0.98	3.29	12.04	13.28	80.12
Buprofezine	$Y = 0.000329971X + 3.42977e-007X^2$	0.99	0.88	2.93	11.96	10.42	86.13
Captane	$Y = 0.000627018X - 7.95707e-008X^2$	0.98	7.50	24.97	8.97	9.40	89.36
Carbaryl	$Y = 2.5009e-006X + 1.70182e-008X^2$	0.97	1.32	4.39	16.15	15.49	65.81

Chlorothalonil	$Y = 0.000167542 \cdot X + 2.35736e-007 \cdot X^2$	0.99	2.32	7.75	8.94	9.85	71.39
Chlorpropham	$Y = 0.000219185 \cdot X - 6.94502e-009 \cdot X^2$	0.99	3.75	12.48	14.88	12.74	94.36
Chlorpyrifos	$Y = 0.000245862 \cdot X + 1.01725e-008 \cdot X^2$	0.96	0.17	0.56	15.38	14.41	73.82
Chlorpyrifos-Methyl	$Y = 0.00463647 \cdot X + 2.90356e-007 \cdot X^2$	0.99	1.43	4.79	13.36	15.97	95.27
Clofentezine	$Y = 0.00190331 \cdot X - 1.10233e-007 \cdot X^2$	0.99	1.60	5.48	6.38	10.64	89.37
Clomazone	$Y = 2.95923e-005 \cdot X + 9.46942e-009 \cdot X^2$	0.99	7.50	24.97	10.45	11.00	86.75
Cypermethrine	$Y = 0.00019814 \cdot X + 3.07773e-008 \cdot X^2$	0.99	9.63	32.07	12.33	12.54	98.28
Cyproconazole	$Y = 8.50295e-005 \cdot X + 3.5732e-008 \cdot X^2$	0.99	10.00	33.30	13.71	13.31	97.59
Cyprodinil	$Y = 4.11151e-006 \cdot X + 2.85062e-008 \cdot X^2$	0.98	12.90	43.20	17.08	14.70	84.94
Deltamethrin	$Y = 1.29209e-005 \cdot X - 2.45532e-009 \cdot X^2$	0.99	7.50	24.97	11.47	11.68	74.02
Dicamba	$Y = 3.72468e-006 \cdot X + 3.80604e-009 \cdot X^2$	0.99	7.50	24.97	14.07	14.74	82.31
Diclobenil	$Y = 0.000742132 \cdot X + 9.29598e-008 \cdot X^2$	0.99	1.87	6.24	11.73	11.52	82.14
Diclofop-Methyl	$Y = 4.39875e-005 \cdot X + 7.6866e-007 \cdot X^2$	0.99	0.06	0.21	12.27	12.14	98.26
Diflufenical	$Y = 0.000426293 \cdot X + 9.46314e-007 \cdot X^2$	0.99	3.39	11.29	10.91	11.48	80.80
Dimethachlore	$Y = 0.000158889 \cdot X + 1.11615e-007 \cdot X^2$	0.99	0.21	0.72	11.79	13.43	96.56
Dimethanamid-P	$Y = 2.29185e-005 \cdot X + 2.3419e-007 \cdot X^2$	0.99	0.17	0.58	10.16	11.89	91.03
Dimetomorph	$Y = 1.8938e-006 \cdot X + 1.61434e-009 \cdot X^2$	0.99	8.82	29.38	11.75	8.61	71.63
Dimoxystrobin	$Y = 0.0527932 \cdot X - 2.38672e-006 \cdot X^2$	0.97	1.25	4.16	11.37	9.58	107.14

Diphenylamine	$Y = 0.00122086 \cdot X + 1.22776e-006 \cdot X^2$	0.99	0.39	1.31	13.18	11.26	98.41
Epoxyconazole	$Y = 0.000942167 \cdot X + 9.97056e-008 \cdot X^2$	0.99	7.50	24.97	12.02	12.10	95.91
Ethofumesate	$Y = 0.00642255 \cdot X - 9.23822e-007 \cdot X^2$	0.98	4.83	16.11	11.91	13.86	88.81
Etridiazole	$Y = 0.000883778 \cdot X + 1.0261e-006 \cdot X^2$	0.99	0.60	1.99	8.92	10.39	90.36
Fenarimol	$Y = 0.0011558 \cdot X + 8.50419e-006 \cdot X^2$	0.99	3.75	12.48	8.32	8.93	92.45
Fenoxycarb	$Y = 0.00290547 \cdot X + 3.72671e-006 \cdot X^2$	0.99	1.17	3.89	14.31	12.97	98.70
Fenpropidine	$Y = 0.00379713 \cdot X + 2.18485e-006 \cdot X^2$	0.99	2.14	8.32	11.46	13.92	95.84
Fluazinam	$Y = 0.000170003 \cdot X + 3.03901e-008 \cdot X^2$	0.99	3.86	12.87	13.76	14.18	85.77
Fludioxynil	$Y = 0.000601692 \cdot X + 9.77059e-007 \cdot X^2$	0.99	0.75	2.49	11.42	11.55	92.95
Flumioxazin	$Y = 2.30478e-005 \cdot X + 3.96962e-007 \cdot X^2$	0.99	3.75	12.48	8.62	10.90	95.03
Flurochloridon	$Y = 0.000130154 \cdot X + 2.44659e-007 \cdot X^2$	0.99	1.62	5.42	11.48	12.42	85.93
Flusilazole	$Y = 9.80292e-005 \cdot X + 1.33274e-007 \cdot X^2$	0.99	9.38	31.21	9.15	8.50	95.55
Folpet	$Y = 6.23031e-005 \cdot X + 1.16141e-007 \cdot X^2$	0.99	9.68	32.26	11.35	11.79	96.37
Indoxacarb	$Y = 0.0002264 \cdot X + 2.77684e-008 \cdot X^2$	0.99	16.66	55.50	13.88	14.31	84.32
Iprovolicarbe	$Y = 6.99259e-005 \cdot X - 1.00861e-008 \cdot X^2$	0.98	15.00	49.95	13.21	13.45	84.04
Isoxaflutole	$Y = 0.000161381 \cdot X + 2.15252e-007 \cdot X^2$	0.99	7.50	24.97	14.73	14.05	90.08

Kerosym-Methyl	$Y = 0.0123359X + 1.28294e-005X^2$	0.99	4.54	15.13	11.78	11.23	98.92
Lambda-Cyhalothrine	$Y = 0.00295526X - 5.35227e-007X^2$	0.99	0.46	1.53	15.35	15.16	99.14
Lenacile	$Y = 7.05185e-005X + 2.21503e-007X^2$	0.99	0.11	0.36	11.62	13.05	89.55
Lindane	$Y = 0.00848122X + 5.99064e-006X^2$	0.99	3.99	13.30	9.42	10.73	98.11
Malathion	$Y = 5.15061e-005X + 2.16532e-007X^2$	0.99	2.14	8.32	19.61	15.66	95.53
Mercop-P	$Y = 7.35567e-006X + 1.41146e-009X^2$	0.98	5.00	16.65	15.35	13.55	98.68
Metamitrone	$Y = 4.60381e-005X + 6.93347e-008X^2$	0.99	5.00	16.65	7.64	10.75	92.97
Metazachlore	$Y = 0.000108082X - 7.86706e-009X^2$	0.99	8.46	28.19	9.51	10.39	97.00
Metolachlore-S	$Y = 0.0127101X + 9.91357e-006X^2$	0.99	2.50	8.32	8.86	11.71	90.50
Myclobutinil	$Y = 4.59712e-005X + 2.10983e-007X^2$	0.99	4.28	14.27	11.05	12.81	89.56
Oxadiazone	$Y = 0.000735906X + 5.54908e-007X^2$	0.99	3.75	12.48	9.34	10.83	90.24
Penconazole	$Y = 0.000183874X + 2.86178e-007X^2$	0.99	0.24	0.81	10.52	11.29	86.50
Pendimethalin	$Y = 0.0297307X + 1.10702e-005X^2$	0.98	0.77	2.58	13.46	12.64	77.82
Picloram	$Y = 3.7084e-005X - 6.46461e-010X^2$	0.99	15.00	49.95	9.41	12.96	69.25
Piperonil-Butoxide	$Y = 0.00653124X + 6.6018e-006X^2$	0.98	7.50	24.97	13.01	11.08	91.88
Prochloraze	$Y = 1.75474e-005X + 3.63795e-009X^2$	0.99	4.87	16.24	10.28	11.69	94.35
Procymidone	$Y = 1.28136e-005X + 7.66863e-009X^2$	0.99	6.35	21.16	14.72	12.97	99.45

Propioconazole	$Y = 0.000803894 \cdot X + 7.42073 \cdot 10^{-8} \cdot X^2$	0.99	0.27	0.89	12.68	12.72	82.12
Propoxur	$Y = 1.09538 \cdot 10^{-5} \cdot X + 2.34395 \cdot 10^{-8} \cdot X^2$	0.99	5.00	16.65	9.82	11.99	62.95
Propyzamid	$Y = 0.000258477 \cdot X + 4.48032 \cdot 10^{-7} \cdot X^2$	0.99	5.00	16.65	11.19	9.64	79.38
Prosulfocarb	$Y = 0.029353 \cdot X + 1.11883 \cdot 10^{-5} \cdot X^2$	0.98	1.59	5.30	11.14	13.20	80.39
Pyraclostrobin	$Y = 0.000610163 \cdot X + 1.09317 \cdot 10^{-7} \cdot X^2$	0.99	10.00	33.30	11.01	13.17	88.59
Pyrimethanil	$Y = 9.71542 \cdot 10^{-7} \cdot X + 2.83044 \cdot 10^{-9} \cdot X^2$	0.98	0.88	2.93	13.18	12.81	66.12
Quinoxifen	$Y = 0.00833408 \cdot X + 3.86209 \cdot 10^{-6} \cdot X^2$	0.99	7.50	24.97	10.61	10.72	95.52
Spiroxamine	$Y = 0.000128826 \cdot X + 5.14422 \cdot 10^{-8} \cdot X^2$	0.99	5.00	16.65	12.93	15.23	85.63
Tebuconazole	$Y = 7.12736 \cdot 10^{-5} \cdot X + 1.09577 \cdot 10^{-8} \cdot X^2$	0.99	15.00	49.95	13.06	13.60	87.13
Tebufenpyrad	$Y = 0.000706886 \cdot X + 1.09483 \cdot 10^{-6} \cdot X^2$	0.99	3.75	12.48	11.72	11.45	90.00
Tebutam	$Y = 0.0109834 \cdot X - 1.7285 \cdot 10^{-6} \cdot X^2$	0.99	2.14	7.13	11.74	12.48	93.16
Tetraconazole	$Y = 1.58667 \cdot 10^{-5} \cdot X + 5.09125 \cdot 10^{-8} \cdot X^2$	0.99	1.87	6.24	7.86	8.97	99.18
Tolyfluamid	$Y = 0.00184088 \cdot X + 3.07644 \cdot 10^{-6} \cdot X^2$	0.99	0.27	0.92	9.87	9.77	96.29
Triadimenol	$Y = 4.9824 \cdot 10^{-5} \cdot X + 4.05058 \cdot 10^{-8} \cdot X^2$	0.98	5.00	16.65	14.75	12.47	94.14
Trifloxystrobin	$Y = 0.00154587 \cdot X + 3.81657 \cdot 10^{-7} \cdot X^2$	0.99	0.32	1.07	11.57	11.34	94.57
Trifluarine	$Y = 6.49394 \cdot 10^{-5} \cdot X + 1.4133 \cdot 10^{-8} \cdot X^2$	0.99	2.50	8.33	14.25	14.36	86.91
Zoxamide	$Y = 0.00124399 \cdot X + 5.18609 \cdot 10^{-6} \cdot X^2$	0.99	1.25	4.16	9.02	10.34	98.90

Supplementary materials S2-3: Validation parameters for the POPs analyzed by GC-MS/MS

Compounds	Regression line equation	Regression coefficient	LOD (ng/g)	LOQ (ng/g)	% RSD Intra-Day	% RSD Inter-Day	Recovery %
Acenaphthalene	$Y = 0.0537161 \cdot X + 3.71474 \times 10^{-6} \cdot X^2$	0.99	0.46	1.56	13.87	12.04	91.74
Anthracene	$Y = 0.000323432 \cdot X + 4.0943 \times 10^{-8} \cdot X^2$	0.99	0.75	2.49	14.25	13.49	76.70
Benzo-a-Anthracene	$Y = 0.00142064 \cdot X + 8.07338 \times 10^{-8} \cdot X^2$	0.99	1.36	4.54	13.07	11.82	98.76
Benzo-a-Pyrene	$Y = 0.495489 + 0.0030427 \cdot X$	0.99	0.93	3.12	13.01	13.55	82.82
Benzo-b-Fluoranthrene	$Y = 0.00192698 \cdot X + 1.69011 \times 10^{-7} \cdot X^2$	0.99	0.57	1.92	14.46	13.70	97.62
Benzo-e-Pyrene	$Y = 0.00196019 \cdot X + 1.90739 \times 10^{-7} \cdot X^2$	0.99	0.83	2.77	14.25	15.87	75.69
Benzo-g,h,i-Perylene	$Y = 0.000253964 \cdot X - 5.00956 \times 10^{-9} \cdot X^2$	0.99	3.75	12.48	15.89	16.01	78.49
Benzo-k-Fluoranthrene	$Y = 0.00197205 \cdot X + 1.83325 \times 10^{-7} \cdot X^2$	0.99	2.30	7.68	13.79	13.19	94.51
Chrysene	$Y = 0.00122762 \cdot X + 6.97952 \times 10^{-8} \cdot X^2$	0.99	3.75	12.48	9.46	10.32	98.89
Dibenzo-a,h-Anthracene	$Y = 0.000633564 \cdot X + 4.70987 \times 10^{-8} \cdot X^2$	0.99	3.75	12.48	14.00	14.06	70.14
Fluoranthrene	$Y = 0.000299562 \cdot X + 2.1121 \times 10^{-8} \cdot X^2$	0.99	5.00	16.65	13.47	14.78	93.11
Fluorene	$Y = 0.0229726 \cdot X + 2.17022 \times 10^{-5} \cdot X^2$	0.99	0.44	1.46	10.40	10.65	75.16
Indenol-1,2,3-Pyrene	$Y = 0.000409942 \cdot X + 4.8782 \times 10^{-8} \cdot X^2$	0.99	5.00	16.65	7.96	10.12	95.75
Naphtalene	$Y = 0.0029162 \cdot X - 5.06184 \times 10^{-8} \cdot X^2$	0.99	0.78	2.62	11.24	11.42	97.38
Phenanthrene	$Y = 4.21984 \times 10^{-5} \cdot X + 4.5812 \times 10^{-9} \cdot X^2$	0.99	2.00	6.66	14.46	13.35	83.35
Pyrene	$Y = 0.000260029 \cdot X + 4.78073 \times 10^{-8} \cdot X^2$	0.99	5.00	16.65	14.61	12.56	91.06
PCB-101	$Y = 0.000701049 \cdot X + 3.45565 \times 10^{-7} \cdot X^2$	0.99	3.75	12.48	13.14	17.54	88.19
PCB-105	$Y = 0.000119678 \cdot X + 3.17369 \times 10^{-6} \cdot X^2$	0.99	0.93	3.12	13.93	12.73	88.14
PCB-114	$Y = 4.30403 \times 10^{-6} \cdot X + 8.81449 \times 10^{-10} \cdot X^2$	0.96	3.23	10.77	13.47	13.22	73.39
PCB-118	$Y = 1.05549 \times 10^{-5} \cdot X + 2.60827 \times 10^{-8} \cdot X^2$	0.99	0.88	2.93	13.24	15.38	81.74
PCB-123	$Y = 3.79015 \times 10^{-6} \cdot X + 1.31646 \times 10^{-9} \cdot X^2$	0.97	3.65	12.16	14.94	15.51	76.59
PCB-126	$Y = 0.000119678 \cdot X + 3.17369 \times 10^{-6} \cdot X^2$	0.99	3.75	12.48	13.78	13.04	88.02

PCB-138	$Y = 0.000478556X + 1.96386e-006X^2$	0.96	0.29	0.99	11.80	8.81	78.15
PCB-149	$Y = 0.000156911X + 9.33819e-007X^2$	0.99	0.15	0.53	16.28	14.72	80.45
PCB-153	$Y = 0.000478556X + 1.96386e-006X^2$	0.96	1.76	5.87	11.19	9.49	78.15
PCB-156	$Y = 2.21915e-005X + 1.25456e-007X^2$	0.94	0.28	0.96	11.83	12.28	95.13
PCB-157	$Y = 3.47673e-005X + 1.0468e-007X^2$	0.95	0.69	2.29	11.91	12.34	94.87
PCB-167	$Y = 0.000218079X + 2.71961e-007X^2$	0.99	1.25	4.16	15.12	15.51	86.30
PCB-169	$Y = 0.000143801X + 1.65032e-007X^2$	0.97	0.95	3.18	10.14	11.31	99.98
PCB-18	$Y = 8.74162e-007X + 2.61352e-010X^2$	0.99	3.75	12.48	12.03	11.54	74.57
PCB-180	$Y = 2.98888e-005X + 1.35514e-007X^2$	0.99	8.64	28.80	13.01	15.84	75.19
PCB-189	$Y = 2.8135e-005X + 7.65629e-009X^2$	0.98	9.05	30.15	10.18	10.59	82.35
PCB-28	$Y = 0.000194352X + 1.43348e-007X^2$	0.99	0.17	0.58	10.49	9.91	83.82
PCB-31	$Y = 0.000191618X + 1.40464e-007X^2$	0.99	0.27	0.91	10.74	10.15	84.82
PCB-44	$Y = 7.09343e-005X + 5.17817e-008X^2$	0.99	0.30	1.00	13.22	14.60	91.74
PCB-52	$Y = 7.62608e-005X + 4.97312e-008X^2$	0.99	0.13	0.44	13.46	14.97	87.94
PCB-70	$Y = 9.93025e-006X + 4.79887e-008X^2$	0.99	0.09	0.31	13.27	10.83	86.15
PCB-81	$Y = 9.93025e-006X + 4.79887e-008X^2$	0.99	0.35	1.17	14.64	14.79	86.72
Aldrine	$Y = 6.16015e-006X + 1.14803e-008X^2$	0.99	1.06	3.54	9.70	10.76	96.70
Cisclordane	$Y = 9.5917e-005X + 2.58647e-011X^2$	0.98	1.50	4.99	13.10	14.07	86.83
Dieldrine	$Y = 1.71891e-006X + 4.60978e-009X^2$	0.96	3.50	11.89	14.21	15.19	79.95
Heptachlore	$Y = 1.09775e-006X + 2.60916e-009X^2$	0.99	2.50	8.33	6.38	9.18	87.10
Heptachlore-Epoxyde-A	$Y = 2.32972e-005X + 1.54059e-008X^2$	0.99	1.88	6.24	8.25	9.27	103.79
Heptachlore-Epoxyde-B	$Y = 3.57522e-005X + 1.30002e-008X^2$	0.99	1.66	5.55	12.09	14.01	99.17
Hexachlorobenzene	$Y = 0.000604155X + 1.10654e-007X^2$	0.99	0.57	1.92	9.88	10.00	91.17
Metoxychlore	$Y = 0.000557597X + 1.53462e-006X^2$	0.99	7.50	24.97	9.37	9.05	121.82
O-P'-DDD	$Y = 0.00312545X + 2.00744e-006X^2$	0.99	5.00	16.65	15.05	15.62	98.23
O-P'-DDE	$Y = 2.05729e-005X + 2.04773e-008X^2$	0.97	10.00	33.30	15.06	13.29	71.29

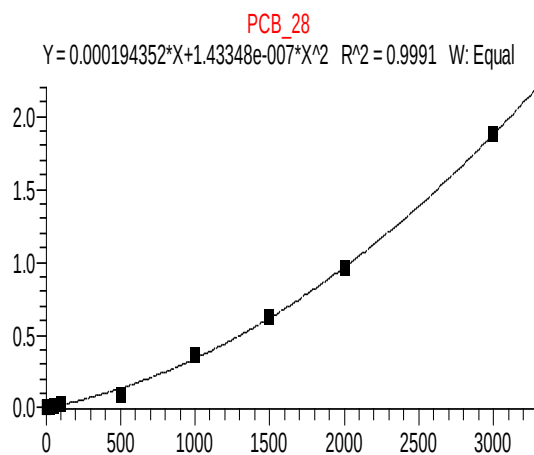
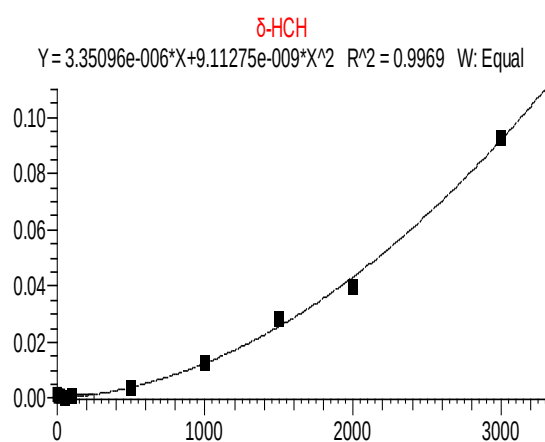
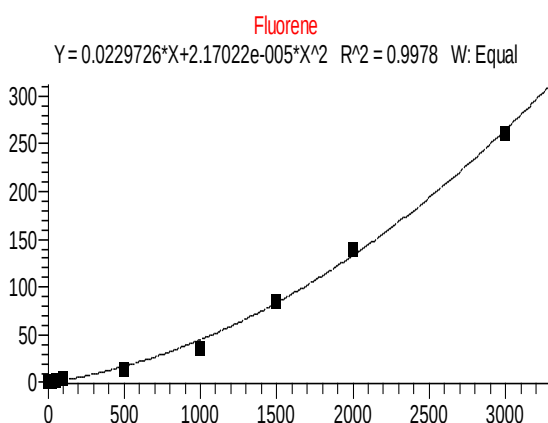
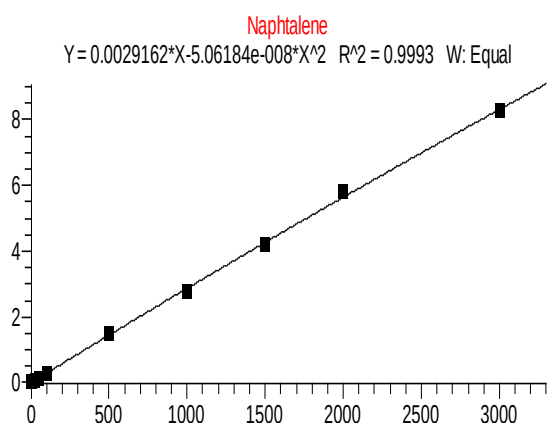
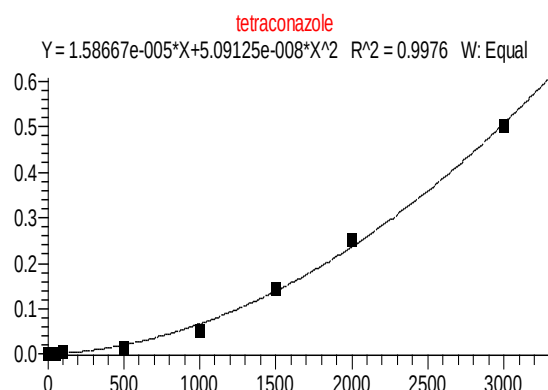
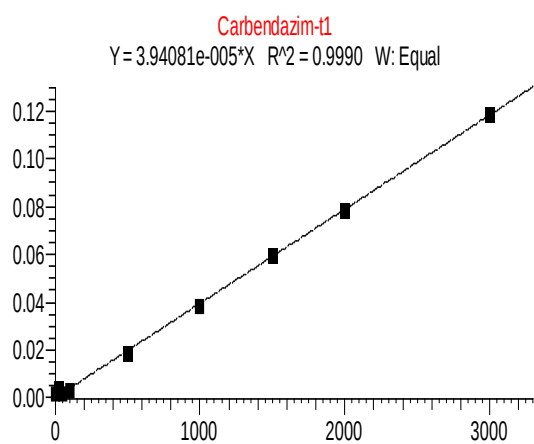
O-P'-DDT	$Y = 0.00322982X + 2.01513e-006X^2$	0.99	2.50	8.33	13.98	13.14	89.86
P-P'-DDD	$Y = 0.00107107X + 3.7361e-006X^2$	0.99	0.55	1.85	12.77	13.84	93.94
P-P'-DDE	$Y = 0.0115345X + 1.25002e-005X^2$	0.99	0.26	0.89	12.38	12.67	92.11
P-P'-DDT	$Y = 0.00316545X + 2.03424e-006X^2$	0.99	6.97	23.23	13.74	15.50	99.02
Transchlordan	$Y = 0.000650758X + 8.43452e-008X^2$	0.99	3.75	12.48	16.39	16.55	84.96
α -Endosulfan	$Y = 7.06931e-006X + 2.44469e-008X^2$	0.99	6.81	22.70	10.83	10.28	78.00
α -HCH	$Y = 8.04401e-006X + 6.4409e-009X^2$	0.99	5.00	16.65	8.27	7.26	95.64
β -Endosulfan	$Y = 5.44398e-006X + 2.10728e-009X^2$	0.99	7.50	24.97	14.05	15.83	96.02
β -HCH	$Y = 3.1446e-006X + 9.15863e-009X^2$	0.99	3.75	12.48	7.91	7.44	98.32
γ -HCH	$Y = 4.8628e-006X + 8.58973e-009X^2$	0.99	1.30	4.32	7.81	8.05	99.94
σ -HCH	$Y = 3.35096e-006X + 9.11275e-009X^2$	0.99	5.00	16.65	9.29	9.02	96.58

Supplementary materials S3

Supplementary materials S3-Figure 1: Sampling sites.

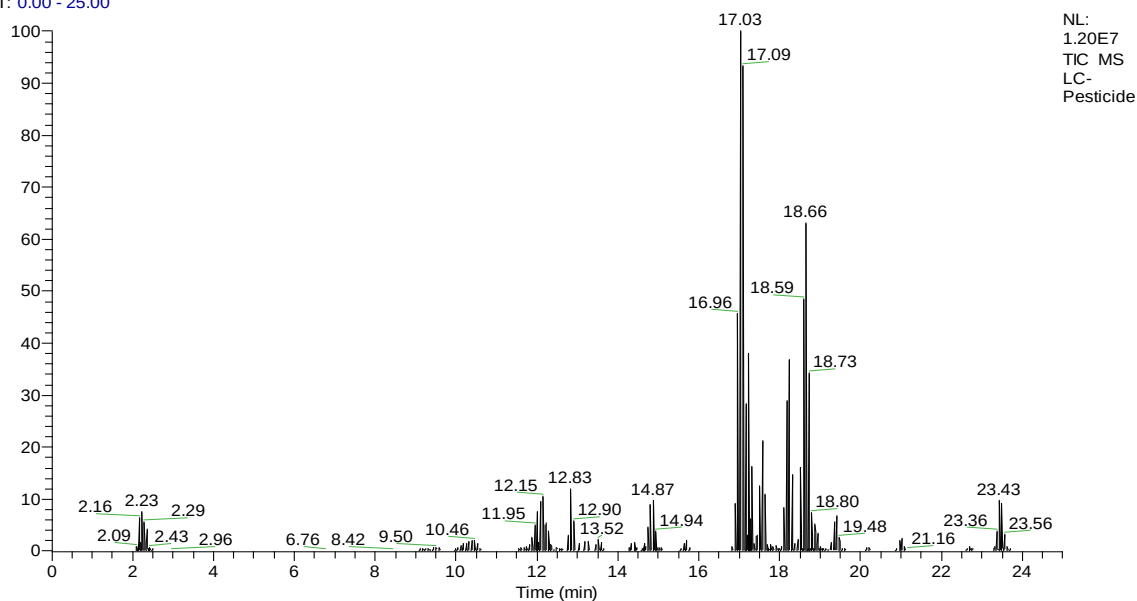


Supplementary materials S3-Figure 2: Calibration curves of some analyzed compounds.



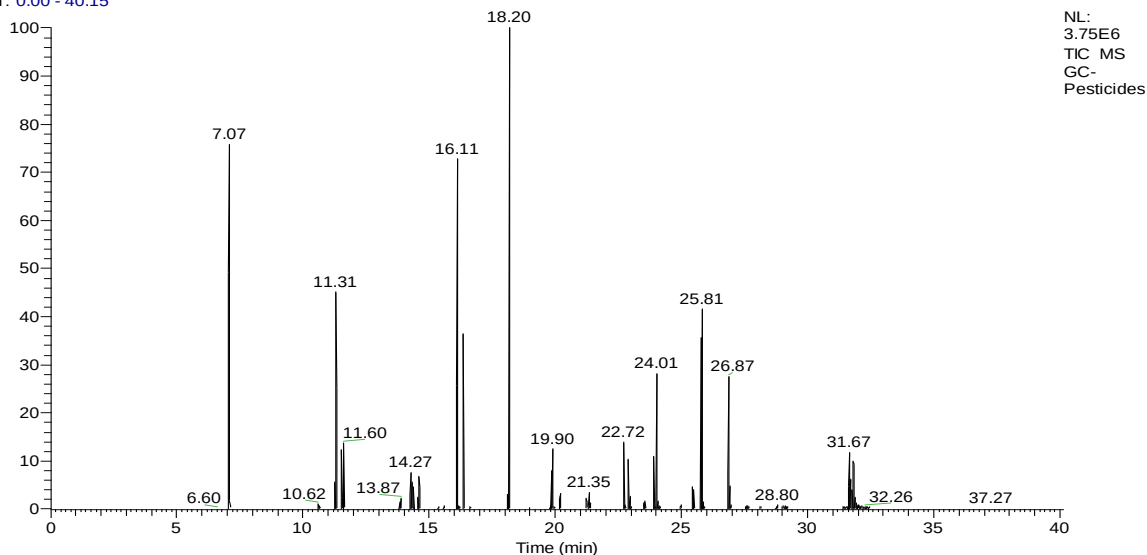
Supplementary materials S3-Figure 3: Chromatogram of nonvolatile pesticides analyzed by LC-MS/MS.

RT: 0.00 - 25.00



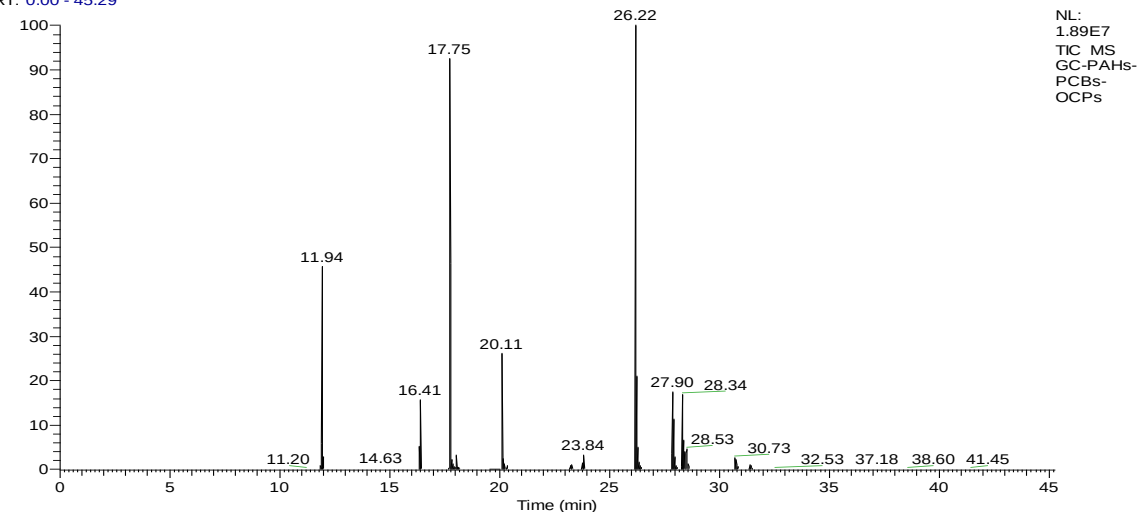
Supplementary materials S3-Figure 4: Chromatogram of volatile pesticides analyzed by GC-MS/MS.

RT: 0.00 - 40.15



Supplementary materials S3-Figure 5: Chromatogram of PAHs, PCBs and OCPs analyzed by GC-MS/MS.

RT: 0.00 - 45.29



IV. The use of *Pinus nigra* as a biomonitor of pesticides and polycyclic aromatic hydrocarbons in Lebanon

Résumé

Ce résultat, présenté sous forme d'un article publié dans « *Environmental Science and Pollution Research* », présente une première application de la méthode d'analyse multi-résidus développée sur les échantillons de conifères. Dans cette étude, 15 échantillons de conifères collectés dans différentes régions du Liban nord ont été analysés pour déterminer leur contamination environnementale.

En effet, parmi les différentes espèces de végétation, les conifères connus par leurs propriétés accumulatrices des polluants, leur teneur élevée en lipides et leur couche cireuse spécifique jouent un rôle important en tant que biomonitor de la pollution de l'air. Au total, 127 pesticides et 16 HAPs ont été analysés afin d'étudier la pollution de l'air dans ces différentes régions du nord du Liban : Tripoli, Koura, Bcharre et Akkar. L'extraction multi-résidus a été basée sur la méthode QuEChERS - SPME, suivie par des analyses chromatographiques par LC-MS/MS et GC-MS/MS.

Les résultats ont montré la présence des résidus de plusieurs pesticides et d'HAPs avec des taux variables selon les régions dans la plupart des échantillons analysés. Les échantillons collectés dans les régions d'Akkar et de Tripoli, largement connues pour leur agriculture, étaient les plus contaminés. Les concentrations totales dans les conifères récupérés à Akkar et à Tripoli étaient respectivement de 231 ng g⁻¹ et 192 ng g⁻¹ pour les pesticides et de 422 ng g⁻¹ et 370 ng g⁻¹ pour les HAPs. Cependant les échantillons provenant de la région de Bcharre dédiée aux productions organiques étaient les moins concentrés en pesticides et en HAPs avec une concentration totale de 50 et 66 ng g⁻¹. Ces résultats signifient que la pollution de l'air dans cette zone, qui est généralement consacrée aux cultures biologiques, est étroitement affectée par le trafic et/ou l'activité économique.

En conclusion, les résultats obtenus au cours de cette étude ont bien validé le fait que les conifères de type *Pinus nigra* utilisés comme échantillonneur passif jouent le rôle de

biomonitoring efficaces des niveaux de contamination dans l'air pour les pesticides et les HAPs.

Highlights

- Conifer needles were extracted using modified QuEChERS protocol coupled to LC-MS/MS and GC-MS/MS analysis.
- Presence of pesticides and PAHs residues in all of the assessed regions.
- *Pinus nigra* are suggested to be effective biomonitors of pollution levels.

Abstract

Among the various species of vegetation, conifers play an important role as a biomonitor of air pollution. The current study presents the determination of pesticides and polycyclic aromatic hydrocarbons in 15 conifer samples collected in August 2018 (summer season) from different regions in north Lebanon (Tripoli, Koura, Bcharre, and Akkar). Pollutants were extracted based on QuEChERS-SPME followed by liquid and gas chromatography-tandem mass spectrometry. Results showed that the samples collected from Bcharre region had the lowest concentration in both pesticides and polycyclic aromatic hydrocarbons with a total concentration of 50 and 66 ng g⁻¹, while the samples collected from the regions widely known by their agriculture (Akkar, Tripoli, and Koura areas) were the most polluted with concentrations of 231 and 422 ng g⁻¹, 192 and 370 ng g⁻¹, and 127 and 98 ng g⁻¹ for pesticides and polycyclic aromatic hydrocarbons respectively. This study revealed that conifers are suggested to be efficient biomonitors of contamination levels in the air.

Keywords: Air pollution . Biomonitoring . *Pinus nigra* . Organic pollutants . QuEChERS . Chromatography . Mass spectrometry.

Introduction

Air pollution has become a major public health issue. It is described as the introduction into the air of pollutants that are hazardous to the environment and humans. These pollutants are toxic liquids, solids, or gases emitted at higher than normal concentrations that decrease the quality of our climate. Due to their persistence and bioaccumulation, organic pollutants are considered as silent killers. They are present all across our atmosphere, including human, animals, air, water, and plants (Alharbi et al. 2018). Current epidemiological, biological, and toxicological studies confirm that exposure to air pollutants has short- and long-term effects on health and major impacts on ecosystems and crops (Baroudi et al. 2020a, b; Kim et al. 2018; Ghorani-Azam et al. 2016). The concentrations of pollutants are present in any individual age group with increased levels in the aging persons. Exposure to these pollutants causes various severe health issues, such as cancer, hormonal disorders, obesity, respiratory diseases, diabetes, cardiovascular diseases, and reproductive disorders (Hamanaka and Mutlu 2018; Petrakis et al. 2017). In addition to harming human health, air pollution can cause a variety of environmental impacts such as eutrophication, acid rain, haze, ozone depletion, crop and forest damage, global climate change, and effects on wildlife (Manisalidis et al. 2020).

The emission of harmful organic pollutants into the environment makes it necessary to determine their concentrations to qualitatively indicate air contamination levels. For instance, the use of pesticides including organochlorine pesticides (OCPs) known for their intensive agricultural activities has increased significantly and can therefore contaminate different environmental components including the soil, the water, and the food chain due to their dispersion throughout the air (Özkara et al. 2016). The absorption of pesticides from soils by plants is determined by transpiration, which is influenced by different climatic and ecological variables, including humidity, temperature, air movement, and sunlight intensity (Li 2020). In addition, polycyclic aromatic hydrocarbons (PAHs) emitted to the air through combustion processes are considered as potential contaminants for the outdoor air due to their environmental persistence, bioaccumulation, and toxicity potential (Elaridi et al. 2020). The absorption processes of PAHs in the plant are complicated and several aspects have an impact on accumulation activities, including ecological condition, biological properties of the plant, and

physicochemical characteristics of these pollutants (Luo et al. 2020; AbdelShafy and Mansour 2016).

The use of vegetation as a biomonitor has proven to be an interesting approach for the assessment of air contamination by pesticides and PAHs and to highlight the impregnation of the environment (AL-Alam et al. 2019; Parmar et al. 2016; Cuny 2012; Maatoug et al. 2012). Among different vegetation biomonitors, evergreen tree species such as conifers gained much importance as biomonitors in pollution studies due to their longterm usage, their needles characterized by a variety of criteria as high lipid content, and specific waxy layer allowing them to accumulate pollutants for several years, as well as their wide geographical distribution providing long-term information on emissions of pollutants (Baroudi et al. 2020a, b; Ratola et al. 2014; Ratola et al. 2011a, b). A wide variety of methods have been used to identify the air pollutants in conifers such as Soxhlet (Bukhanko et al. 2020), accelerated solvent extraction-solid phase extraction (Al-Alam et al. 2017; Lévy et al. 2016), microwave digestion (Alexandrino et al. 2020), and QuEChERS method (Baroudi et al. 2020a, b) that proved its efficiency for the extraction of pollutants in such matrix. In fact, the QuEChERS extraction method has been widely applied for the analysis of various contaminants including pesticides, persistent organic pollutants (POPs), hormones, and antibiotics in food and environmental matrices. Compared to the cited commonly known extraction techniques, QuEChERS allows the highest recovery rates with the lowest solvent consumption and sample preparation steps (Musarurwa et al. 2019; Perestrelo et al. 2019).

Accordingly, acetonitrile used in the QuEChERS method is appropriate for the extraction of pesticides and PAHs and has a significant level of specificity and selectivity in gas chromatography and liquid chromatography combined with mass spectrometry. Therefore, pollutants were selected in order to cover a wide range of pollutants that may be present in the environment. Pesticides were chosen in a way that may cover the maximum of the pesticides that could be found in the environment based on an assessment of the crop production in the assessed regions as well as based on an investigation of the local pesticide market. For PAHs, the choice was made due to the fact that the chosen 16 PAHs have been designated high priority pollutants by the Environmental Protection Agency (EPA).

For all these reasons, this study aims to investigate the accumulation of pesticides and PAHs in northern Lebanon based on 15 conifer samples used as biomonitor candidates. The chosen biomonitor was tested for the possible aggregation of pesticides and PAHs. These contaminants were subjected to a multi-residue extraction procedure based on QuEChERS followed by a chromatographic analysis coupled with tandem mass spectrometry.

Materials and methods

Chemicals and reagents

The pesticide analysis included 99 semi-volatile compounds, including 21 organochlorine pesticides (OCPs) analyzed by GC-MS/MS and 28 non-volatile compounds analyzed by LC-MS/MS. All pesticides, except OCPs, were purchased from Sigma-Aldrich, St. Quentin Fallavier, France. For OCP and PAH analysis, a mixture at 0.1 g L⁻¹ of 21 OCPs and 16 PAHs of each pollutants was purchased from Cluzeau Info Labo (St. Foy la Grande, France). The LC-MS/MS and GCMS/MS parameters for pesticide and PAH analysis are figured in Appendix A.

LC-MS/MS grade water and acetonitrile (ACN) were purchased from VWR Prolabo, France. Ultrapure water was obtained through a Milli-Q system (18 MΩ cm) from Elga Veolia, France.

QuEChERS extraction kits (EN 1566 method) containing 4 g of magnesium sulfate, 1 g of sodium chloride, 0.5 g of disodium hydrogen citrate sesquihydrate, and 1 g of trisodium citrate dehydrate and dSPE cleanup kits (AOAC 2007 method) containing 1.2 g of magnesium sulfate, 400 mg of primary and secondary amine (PSA), 400 mg of octadecyl (C₁₈), and 400 mg of graphitized carbon black were purchased (GCB), as ready to use, from RESTEK, France.

Internal standards for LC-MS/MS (carbendazim-d⁴, diuron-d⁶, pendimethalin-d⁵, and nicosulfuron-d⁶) and GC-MS/MS (trifluralin-d¹⁴, 4-nitrophenol-d⁴, atrazine-d⁵, pendimethalin-d⁵, trans-cypermethrin-d⁵, naphthalene-d⁸, phenanthrene-d¹⁰, chrysene-d¹², and perylene-d¹²) were obtained from Sigma-Aldrich, St. Quentin Fallavier, France.

Sample collection

For the sample analysis, conifer samples were collected from four different regions of northern Lebanon. These regions included Akkar, Tripoli, Koura, and Bcharre. Northern Lebanon is divided into districts (Batroun, Bcharre, Koura, Miniyeh-Danniyeh, Tripoli, Zgharta, and Akkar) and covers an area of 2024.8 km² with a population of 1,197,203. Akkar is bounded by the Syrian governorates and constitutes the second largest agricultural region after the Bekaa Valley. Koura district consists of a series of foothills surrounding a low-lying plain where olive trees are cultivated and considered among the most extensive in Lebanon. Tripoli, the capital of North Governorate and the second capital of Lebanon, is characterized by a relatively high population density (600/km²) and with a wide variety of crop production mainly based on citrus fruits and olives. Lebanon is characterized by an unsustainable road transport sector, an uncontrolled private sector of diesel generators, an insufficient database of pesticide used, and poor air quality control. Diesel emissions (Bcharre), petrol emissions (Tripoli), fossil fuel combustion (Tripoli and Koura), and the combustion of coal, wood, and grass (Bcharre and Akkar) are the main sources of PAHs in these studied regions, while the absence of all industrial activities in these areas specified for agricultural production is the main reason for the disappearance of polychlorinated biphenyls and other residues of pollutants issues from industrial waste. The Bcharre district is a mountainous district, with an altitude up to 3088 m and generally dedicated to organic crops. The altitude of Bcharre ranged from 1450 to 3088 m which is relatively higher than that of Akkar (700 m), Koura (290 m), and Tripoli (80 m). The geographical repartition of these sites over Lebanon is illustrated in Fig. 1.

The conifer "*Pinus nigra*" is widely distributed and easily identified in northern Lebanon, and the samples used were young needles collected from the same conifer species. These needles were used according to the work previously developed by Baroudi et al. in 2020 (Baroudi et al. 2020a, b). Approximately 50 g of conifers of the same age ranges from 6 months to 1 year, with only the terminal sections of the branches collected in August 2018. These samples were wrapped in aluminum foil, stored individually in zip lock bags, and placed in an iced cooler during transport, then placed at – 18 °C in the laboratory (Al-Alam et al. 2017).



Fig. 1 Map showing the sampling sites

Analytical procedure The QuEChERS-SPME extraction procedure and the analysis of the obtained extract by the LC-MS/MS or by GC-MS/MS used were based on the work of Baroudi et al. (2020a, b) for the study of pesticide and PAH residues in conifers (Baroudi et al. 2020a, b). A schematic description of the extraction procedure used is represented in Fig. 2. The validation parameters obtained for the non-volatile pesticides analyzed by LC-MS/MS and volatile pesticides, OCPs, and PAHs analyzed by GC-MS/MS are reported in Appendix B.

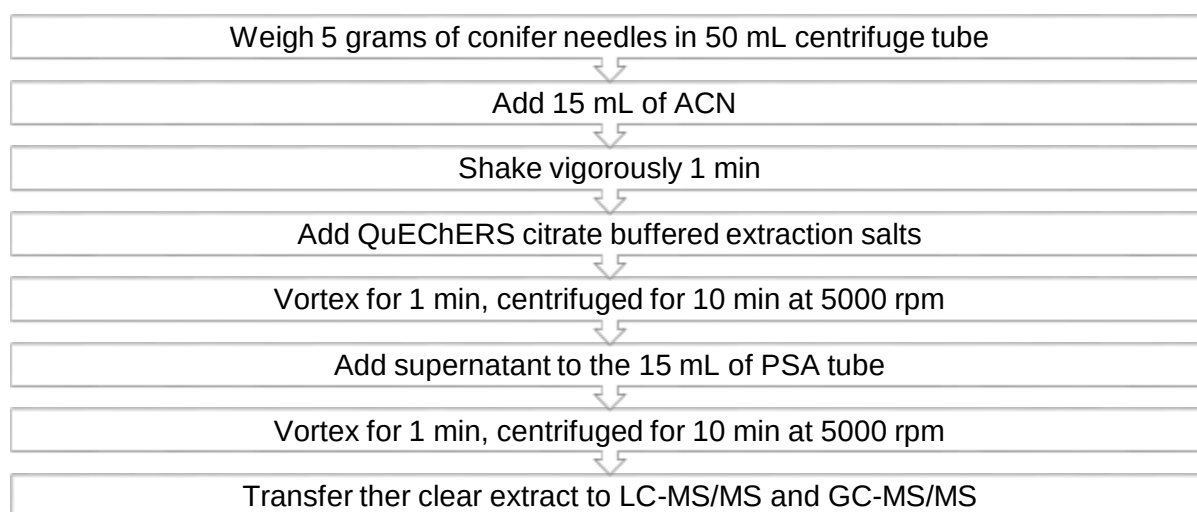


Fig. 2 Flow chart of the modified QuEChERS extraction procedure

Results

Among the 127 pesticides and 16 PAHs, only 19 of all pollutants sought (penconazole, pendimethalin, diflufenican, hexachlorobenzene, heptachlor, fenpropidin, deltamethrin, lambda-cyhalothrin, ethofumesate, clofentezine, naphthalene, acenaphthylene, phenanthrene, fluorene, pyrene, anthracene, fluoranthene, benzo[a]anthracene, and chrysene) were found in most of the samples with varying rates among regions. Moreover, results showed that the samples collected from the Bcharre district usually devoted to organic crops were the less contaminated with pesticide and PAH residues, while the highest concentration was found in Akkar valley followed by the region of Tripoli and Koura. All these results are shown in Table 1 and in Fig. 3.

Table 1 Sum of pesticide and PAH concentrations in conifer samples (ng g⁻¹)

Pollutants, <i>n</i>	Bcharre (<i>n</i> = 2)	Akkar (<i>n</i> = 4)	Tripoli (<i>n</i> = 4)	Koura (<i>n</i> = 5)
Pesticides				
Penconazole	3.5	8.8	6.2	9.7
Pendimethalin	0.8	16.9	8.1	4.2
Diflufenican	18.7	22.7	11.4	5.8
Hexachlobenzene	2.6	53.7	48.8	25.7
Heptachlor	-	20.6	10.5	3.5
Fenpropidin	9.6	34.9	12.8	6.7
Deltamethrin	-	24.3	43.5	27.3
Lambda-Cyhalothrin	2.9	17.9	30.9	35.7
Ethofumesate	-	12.5	8.7	3.9
Clofentezine	12.7	19.4	11.8	5.2
Sum (Pesticides), <i>n</i>	50.7	231.5	192.5	127.6
PAHs				
Naphthalene	14.9	6.9	10.5	7.3
Acenaphthylene	7.7	9.6	7.5	1.4
Fluorene	9.7	43.6	52.8	7.7
Phenanthrene	5.3	71.1	56.0	52.2
Anthracene	4.9	68.5	37.4	11.2
Fluoranthene	13.1	85.9	68.5	6.3
Pyrene	6.1	65.2	79.3	9.1
Chrysene	1.5	7.2	14.5	1.0
Benzo[a]anthracene	3.0	64.1	43.8	2.7
Sum (PAHs), <i>n</i>	66.1	422.1	370.2	98.9

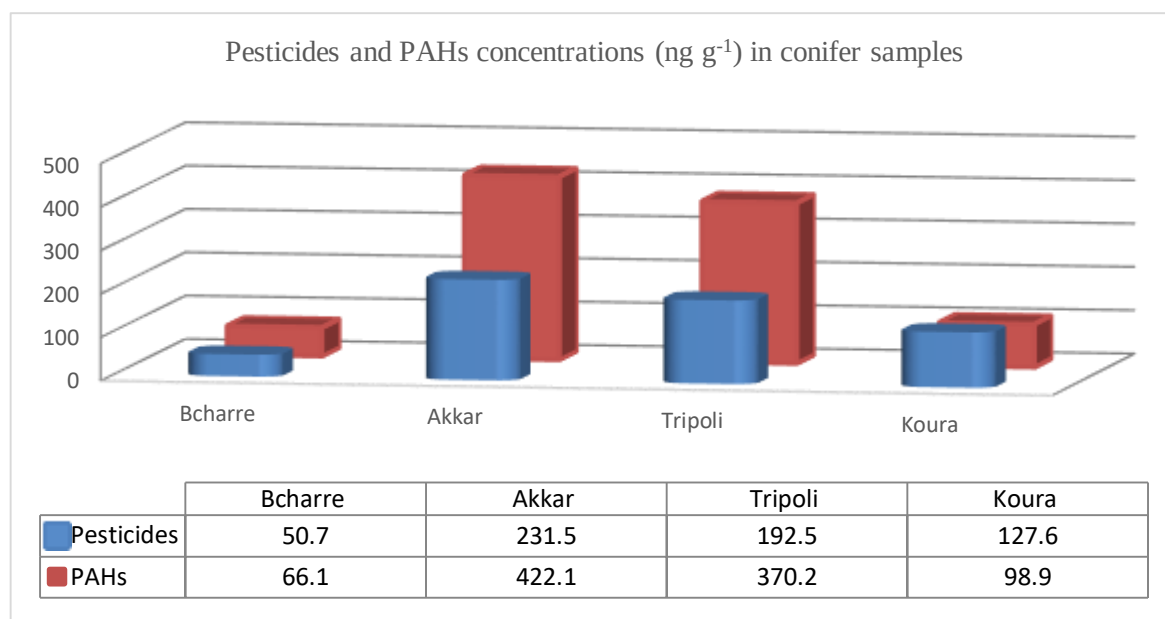


Fig. 3 Pesticide and PAH concentrations (ng g⁻¹) in conifer samples

Discussion

The QuEChERS-SPME extraction and cleanup procedure was applied for the analysis of 15 conifer samples collected from four different areas in Lebanon in order to detect the presence of pesticide and PAH residues. In fact, *Pinus nigra* species are characterized by a wide geographical distribution allowing them to accumulate pollutants for several years. Their needles are well known for their high lipid content and specific waxy layer (Ratola et al. 2011). The abundance of pollutants in conifer was the result of long-term accumulation associated with exposure period of needle in the air. The presence of a limited number of pollutants is related to the young age of the conifers analyzed between 6 months and 1 year of age, as higher concentrations of pollutants were identified in older needles than in younger ones, and these concentrations indicated a more significant increase in needle age (increase with concentration by a factor of 3 in the first year, then only by one-third between the first and the second year) (Di Guardo et al. 2003; Klánová et al. 2009; Kylin et al. 2017). The analysis of the accumulation of pesticides and PAHs in conifers was carried out during the month of August, however in the absence of meteorological variations between seasons, such as precipitation and temperature, which affect the phenomena of volatilization and degradation of pollutants. In addition, these results show that the accumulation of pesticides in the collected conifers has occurred after 4 months as the main pesticide pulverization season in March.

Pesticide levels in collected samples

The majority of the investigated sites detect the presence of some pesticide residues in most of the samples with rates varying among regions. Each of the studied regions located in a rural area was known for its specific agricultural products.

As seen from the results obtained from this sampling campaign, Akkar valley appears to be the most polluted area with pesticide residues. The highest pesticide residues found belong to diflufenican, hexachlorobenzene, and fenpropidin. Furthermore, diflufenican is usually used for preemergence and postemergence of foliar absorbed herbicide to control annual weeds in cereal crops, while fenpropidin is a foliar fungicide which is used for protective, curative, and eradivative activity as a fungicide for cereals, whereas the persistence and bioaccumulation of hexachlorobenzene are due to its resistance to degradation and high solubility. The agricultural plain of Akkar that makes the region suitable for the cultivation of various crops such as cereals, vegetables, tobacco, and olive explains the abundance of these pesticides (Helou et al. 2019; EL-Osmani et al. 2014).

For Tripoli and Koura, the highest pesticide residues belong to deltamethrin (43 ng g^{-1} and 27 ng g^{-1}) and lambda-cyhalothrin (30 ng g^{-1} and 35 ng g^{-1}) respectively. The detection of these pesticides in those regions is clarified by the agricultural production in this area that is well known for the presence of olive trees and the production of olive oil (Amvrazi and Albanis 2008). Deltamethrin is an agricultural insecticide classified as very toxic and harmful, which used to control a large variety of diseases in olives trees such as the fighting pests (Jaabiri 2013). The results suggest that the less polluted with pesticide residues were the samples collected from the regions of Bcharre. These facts mean that the air pollution in this area, which is usually devoted to organic crops, is closely affected by traffic volume or economic activity.

Lebanon, being not producer of pesticides, is dependent on legal imports of these products in order to fulfill its needs for pests' protections (Youssef et al. 2015). Unfortunately, there are many obstacles to agricultural protection in Lebanon despite the availability of legislation. First, there is a lack of compliance requirements for the use and identification of pollutants and there is a deficiency of research facilities for detecting their residues and effects, while legislative and policy control measures still need to be

established. Certain deficiencies in the control of pollutants are that the farmers do not have sufficient databases of pollutant use and a large number of them prefer to have high efficiency and low cost instead of environmental effects (El-Osmani et al. 2014). As the risk of acute and chronic contamination increases due to the lack of precision of pesticide targets, the farmers have limited knowledge across pollutants regarding their persistence in the air and their harmful effects for human and animal (Chaza et al. 2018).

PAH levels in collected samples

One of the aspects for the analysis of pollutants at different sites is the assessment of the variations in concentration along altitude gradients. The study of Davidson et al. (2003) found that the concentrations of volatile organic compounds (VOCs) increased at higher altitudes (Davidson et al. 2003), while other studies failed to detect the relation between altitude and differences in some VOC concentrations (Yang et al. 2013). These inconsistencies are caused by the proximity of the sampling sites to the sources, the different properties of compounds, and the environmental factors (Pompa-García et al. 2017). These latest observations are mainly behind the results obtained in this sampling campaign for the assessment of conifer needles sampled from several regions at different altitudes (Tripoli, Koura, Bcharre, and Akkar). As previously said, the altitudes of Bcharre ranged from 1450 to 3088 m, which were relatively higher than those of Akkar, Koura, and Tripoli (700 m, 290 m, and 80 m respectively), while the overall concentration of PAHs for Bcharre was lower compared to that for other regions with concentrations of 66 ng g⁻¹, 422 ng g⁻¹, 99 ng g⁻¹, and 370 ng g⁻¹ respectively. The spatial distribution of PAHs influenced by their physicochemical properties, their photochemical degradation, and their emission density reflects regional differences in accordance to population density. For example, PAH concentrations in Akkar are higher than in Bcharre due to the unbalanced development and the population growth (389.899 and 76.831 respectively) (Peng et al. 2016).

To identify the source of pollution by PAHs for the four selected regions, the low molecular weight PAHs (LMW, 2–3 rings) and high molecular weight PAHs (HMW, 4–6 rings) produced in low- and high-temperature processes respectively (Al-Alam et al. 2017; Choi et al. 2010) were compared with those provided by Tobiszewski and Namieśnik in 2012 (Tobiszewski and Namieśnik 2012). The distributions of the sum of the PAHs (Σ PAHs (ng

$\text{g}^{-1}) = \Sigma \text{LMW} + \Sigma \text{HMW}$) indicate that the concentration of the LMW PAHs in Bcharre and Koura exceeds the concentration of the HMW PAHs, whereas the inverse is observed in Tripoli and Akkar. In comparison, the study reveals that the anthracene/(anthracene + phenanthrene) ratio for all regions studied was higher than 0.1, which indicates the presence of pyrogenic sources in these areas studied. The analysis of fluorene/(fluorene + pyrene) enabled to conclude that car and road emissions are the sources of the diesel emissions detected in the Bcharre area, while petrol emissions have been detected in Tripoli due to the present port, recognizing that petrol and diesel emissions cannot be separated and should be considered together. The fluoranthene/(fluoranthene + pyrene) ratio has shown that pyrogenic emissions in Bcharre and Akkar are due in particular to the combustion of coal, wood, and grass, whereas in Tripoli and Koura, they are due to fossil fuel combustion. The diagnostic ratios for PAHs for the selected areas are presented in Table 2.

Table 2 Diagnostic ratios of PAHs for selected areas based on the work of Tobiszewski and Namieśnik in 2012

	Bcharre	Akkar	Tripoli	Koura
Sum PAHs, n (ng g^{-1})	66.1	422.1	370.2	98.9
Sum LMW	42.5	199.6	164.2	79.9
Sum HMW	23.6	222.4	206	19
FL/(FL + PYR)				
<0.5 Petrol emissions	0.61	0.4	0.39	0.45
>0.5 Diesel emission				
ANT/(ANT + PHE)				
<0.1 Petrogenic	0.48	0.49	0.4	0.17
>0.1 Pyrogenic				
FLA/(FLA + PYR)				
<0.4 Petrogenic	0.68	0.56	0.46	0.4
0.4–0.5 Fossil fuel combustion				
>0.5 Grass, wood, coal combustion				

$\Sigma \text{PAHs (ng g}^{-1}) = \Sigma \text{LMW} + \Sigma \text{HMW}$

An anthracene, Fla fluoranthene, F fluorene, Phe phenanthrene, Pyr pyrene

Several studies have described significant variations in the spatial distribution of outdoor PAHs. The results showed that the highest concentrations of PAHs were measured in high traffic areas (Tripoli and Akkar), followed by urban and rural areas (Bcharre) (Lovinsky-Desir et al. 2016; Jaward et al. 2004). Inside the city, concentrations of outdoor atmospheric PAHs can also differ significantly, and higher atmospheric PAH concentrations measured along main roads (Tripoli and Akkar) can be reached compared to concentrations measured in a private street (Koura and Bcharre), many kilometers

away (Choi et al. 2007). These results can be clarified by variations in proximity to roads and highways and emissions from traffic sources on a relatively large scale. Our results also presented a diversity in atmospheric PAH concentrations where the concentrations of phenanthrene and anthracene respectively in Bcharre (5.3 ng g^{-1} and 4.9 ng g^{-1}) with a daily temperature in August of 16°C are lower compared to the concentrations of Akkar (71 ng g^{-1} and 68.5 ng g^{-1}), Tripoli (56 ng g^{-1} and 37 ng g^{-1}), and Koura (52 ng g^{-1} and 11 ng g^{-1}) with a mean temperature of 26°C . On the other side, Akkar seems to be highly contaminated by phenanthrene due to the fact that the increase in temperature (28°C) also will cause the transfer and accumulation of phenanthrene in plant tissues, which rises rapidly and reaches the maximum accumulation within a few hours (Salehi-Lisar et al. 2015; Shen et al. 2019). Indeed, it was shown that plants such as conifers can absorb phenanthrene and some of its compounds through their needles if it is in the gaseous and/or particulate phase, but also via their roots when it is in the soil (Srogi 2007).

Conclusion

The QuEChERS-SPME method has allowed us to analyze 15 samples of conifers from northern Lebanon in order to assess the air quality in this region based on a biomonitoring process. *Pinus nigra* was used as a passive sampler and the results obtained suggest that these species play the role of effective biomonitors of contamination levels in the air for pesticides and PAHs.

The total concentration of 10 pesticides detected in conifers from 15 sampling sites in Lebanon ranged from 50 to 231 ng g^{-1} . Insecticides were the main family of chemicals followed by fungicides and herbicides. In general, their distribution in the regions studied revealed that the pesticides used in each of them could be different. Hexachlorobenzene, a commonly used pesticide in Lebanon, presents the highest risk of toxicity, while penconazole and pendimethalin present a very low risk due to their low concentrations in some of the analyzed samples and absence in others. The total concentrations of pesticides and PAHs in conifers recovered from Akkar and Tripoli were higher than the samples from Bcharre due to its location in the mountain of Lebanon and its organic production. Overall, it has been shown that conifer can serve as accurate pesticide biomonitors in sample, but further research strategies are required to analyze their actions further thoroughly.

References

- Abdel-Shafy, H. I., & Mansour, M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, 25(1), 107–123. <https://doi.org/10.1016/j.ejpe.2015.03.011>
- Al Alam, J., Fajloun, Z., Chbani, A., & Millet, M. (2018). Determination of 16 PAHs and 22 PCBs in honey samples originated from different region of Lebanon and used as environmental biomonitoring sentinel. *Journal of Environmental Science and Health Part A*, 54. <https://doi.org/10.1080/10934529.2018.1500782>
- AL-Alam, J., Chbani, A., Fajloun, Z., & Millet, M. (2019). The use of vegetation, bees, and snails as important tools for the biomonitoring of atmospheric pollution—a review. *Environmental Science and Pollution Research*, 26(10), 9391–9408. <https://doi.org/10.1007/s11356-019-04388-8>
- Al-Alam, J., Fajloun, Z., Chbani, A., & Millet, M. (2017). The use of conifer needles as biomonitor candidates for the study of temporal air pollution variation in the Strasbourg region. *Chemosphere*, 168, 1411–1421. <https://doi.org/10.1016/j.chemosphere.2016.11.103>
- Alexandrino, K., Viteri, F., Rybarczyk, Y., Guevara Andino, J. E., & Zalakeviciute, R. (2020). Biomonitoring of metal levels in urban areas with different vehicular traffic intensity by using Araucaria heterophylla needles. *Ecological Indicators*, 117, 106701. <https://doi.org/10.1016/j.ecolind.2020.106701>
- Alharbi, O.M.L., Basheer, A.A., Khattab, R.A., Ali, I., 2018. Health and environmental effects of persistent organic pollutants. *Journal of Molecular Liquids* 263, 442–453. <https://doi.org/10.1016/j.molliq.2018.05.029>
- Amvrazi, E., & Albanis, T. (2008). Multiclass Pesticide Determination in Olives and Their Processing Factors in Olive Oil: Comparison of Different Olive Oil Extraction Systems. *Journal of Agricultural and Food Chemistry*, 56, 5700–5709. <https://doi.org/10.1021/jf703783u>
- Baroudi, F., Al Alam, J., Fajloun, Z., & Millet, M. (2020). Snail as sentinel organism for monitoring the environmental pollution; a review. *Ecological Indicators*, 113, 106240. <https://doi.org/10.1016/j.ecolind.2020.106240>
- Baroudi, F., Al-Alam, J., Chimjarn, S., Delhomme, O., Fajloun, Z., & Millet, M. (2020). Conifers as environmental biomonitoring: A multi-residue method for the concomitant quantification of pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls by LC-MS/MS and GC-MS/MS. *Microchemical Journal*, 154, 104593. <https://doi.org/10.1016/j.microc.2019.104593>
- Bukhanko, N., Attard, T., Arshadi, M., Eriksson, D., Budarin, V., Hunt, A. J., Geladi, P., Bergsten, U., & Clark, J. (2020). Extraction of cones, branches, needles and bark from Norway spruce (*Picea abies*) by supercritical carbon dioxide and Soxhlet extractions techniques. *Industrial Crops and Products*, 145, 112096. <https://doi.org/10.1016/j.indcrop.2020.112096>
- Chaza, C., Sopheak, N., Mariam, H., David, D., Baghdad, O., & Moomen, B. (2018). Assessment of pesticide contamination in Akkar groundwater, northern Lebanon. *Environmental Science and Pollution Research International*, 25(15), 14302–14312. <https://doi.org/10.1007/s11356-017-8568-6>
- Choi, H., Harrison, R., Komulainen, H., & Saborit, J. M. D. (2010). *Polycyclic aromatic hydrocarbons*. World Health Organization. <https://www.ncbi.nlm.nih.gov/books/NBK138709/>
- Choi, S.-D., Baek, S.-Y., Chang, Y.-S., 2007. Influence of a large steel complex on the spatial distribution of volatile polycyclic aromatic hydrocarbons (PAHs) determined by passive air sampling using membrane-enclosed copolymer (MECOP). *Atmospheric Environment* 41, 6255–6264. <https://doi.org/10.1016/j.atmosenv.2007.03.058>
- Cuny, D. (2012). [Air pollution biomonitoring with plants and fungi: concepts and uses]. *Annales Pharmaceutiques Françaises*, 70(4), 182–187. <https://doi.org/10.1016/j.pharma.2012.05.003>
- Davidson, D., Wilkinson, and, Blais, J., Kimpe, L., McDonald, K., & Schindler, D. (2003). Orographic Cold-Trapping of Persistent Organic Pollutants by Vegetation in Mountains of Western Canada. *Environmental Science & Technology*, 37, 209–215. <https://doi.org/10.1021/es020605q>
- Di Guardo, A., Zaccara, S., Cerabolini, B., Acciarri, M., Terzaghi, G., Calamari, D., 2003. Conifer needles as passive biomonitoring of the spatial and temporal distribution of DDT from a point source. *Chemosphere* 52, 789–797. [https://doi.org/10.1016/S0045-6535\(03\)00256-X](https://doi.org/10.1016/S0045-6535(03)00256-X)
- Elaridi, J., Fakhro, M., Yamani, O., Dimassi, H., Othman, H., Attieh, Z. 2020. GC-MS analysis of polycyclic aromatic hydrocarbons in bottled olive oil marketed in Lebanon. *Toxicological Research*, 1-10. <https://doi.org/10.1007/s43188-019-00015-3>
- EL-OSMANI, R., NET, S., Dumoulin, D., Baroudi, M., H., B., & Ouddane, B. (2014). Solid Phase Extraction of Organochlorine Pesticides Residues in Groundwater (Akkar Plain, North Lebanon). *International Journal of Environmental Research*, 8.
- Ghorani-Azam, A., Riahi-Zanjani, B., & Balali-Mood, M. (2016). Effects of air pollution on human health and practical measures for prevention in Iran. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*, 21. <https://doi.org/10.4103/1735-1995.189646>
- Hamanaka, R.B., Mutlu, G.M., 2018. Particulate Matter Air Pollution: Effects on the Cardiovascular System. *Front Endocrinol (Lausanne)* 9. <https://doi.org/10.3389/fendo.2018.00680>
- Helou, K., Harmouche-Karak, M., Karake, S., & Narbonne, J.-F. (2019). A review of organochlorine pesticides and polychlorinated biphenyls in Lebanon: Environmental and human contaminants. *Chemosphere*, 231, 357–368. <https://doi.org/10.1016/j.chemosphere.2019.05.109>
- Jaabiri, I. (2013). Development and method validation for determination of Deltamethrin residue in olive oil using a reversed-phase high performance liquid chromatography. *IOSR Journal of Applied Chemistry*, 6(3), 01–08. <https://doi.org/10.9790/5736-0630108>
- Jaward, F.M., Barber, J.L., Booi, K., Jones, K.C., 2004. Spatial distribution of atmospheric PAHs and PCNs along a north-south Atlantic transect. *Environmental Pollution* 132, 173–181. <https://doi.org/10.1016/j.envpol.2004.03.029>
- Kim, D., Chen, Z., Zhou, L.-F., & Huang, S.-X. (2018). Air pollutants and early origins of respiratory diseases. *Chronic Diseases and Translational Medicine*, 4(2), 75–94. <https://doi.org/10.1016/j.cdtm.2018.03.003>
- Klánová, J., Čupr, P., Baráková, D., Šeda, Z., Anděl, P., Holoubek, I., 2009. Can pine needles indicate trends in the air pollution levels at remote sites? *Environmental Pollution, Persistent Organic Pollutants in Mountainous Areas* 157, 3248–3254. <https://doi.org/10.1016/j.envpol.2009.05.030>
- Kylin, H., Svensson, T., Jensen, S., Strachan, W.M.J., Franich, R., Bouwman, H., 2017. The trans-continental distributions of pentachlorophenol and pentachloroanisole in pine needles indicate separate origins. *Environmental Pollution* 229, 688–695. <https://doi.org/10.1016/j.envpol.2017.07.010>
- Lévy, M., Fournier, E., Heyrich, Y., & Millet, M. (2016). Coupling ASE, SPE and SPME for the Extraction and Quantification of PAH in Passive Samplers and Biological Materials (Pine Needles). *Polycyclic Aromatic Compounds*. <https://doi.org/10.1080/10406638.2016.1253595>

- Li, Z., 2020. Spatiotemporal pattern models for bioaccumulation of pesticides in common herbaceous and woody plants. *Journal of Environmental Management* 276, 111334. <https://doi.org/10.1016/j.jenvman.2020.111334>
- Lovinsky-Desir, S., Miller, R.L., Bautista, J., Gil, E.N., Chillrud, S.N., Yan, B., Camann, D., Perera, F.P., Jung, K.H., 2016. Differences in Ambient Polycyclic Aromatic Hydrocarbon Concentrations between Streets and Alleys in New York City: Open Space vs. Semi-Closed Space. *Int J Environ Res Public Health* 13. <https://doi.org/10.3390/ijerph13010127>
- Luo, Y., Sun, J., Wang, P., Li, Y., Li, H., Xiao, K., Yang, R., Zhang, Q., Jiang, G., 2020. Age dependence accumulation of organochlorine pesticides and PAHs in needles with different forest types, southeast Tibetan Plateau. *Science of The Total Environment* 716, 137176. <https://doi.org/10.1016/j.scitotenv.2020.137176>
- Manisalidis, I., Stavropoulou, E., Stavropoulos, A., Bezirtzoglou, E., 2020. Environmental and Health Impacts of Air Pollution: A Review. *Front Public Health* 8. <https://doi.org/10.3389/fpubh.2020.00014>
- Maatoug, M., Taïbi, K., Akermi, A., Achir, M., & Mestrari, M. (2012). *Bio-Monitoring of Air Quality Using Leaves of Tree and Lichens in Urban Environments*. <https://doi.org/10.5772/31963>
- Musarurwa, H., Chimuka, L., Pakade, V. E., & Tavengwa, N. T. (2019). Recent developments and applications of QuEChERS based techniques on food samples during pesticide analysis. *Journal of Food Composition and Analysis*, 84, 103314. <https://doi.org/10.1016/j.jfca.2019.103314>
- Özkara, A., Akyıl, D., & Konuk, M. (2016). Pesticides, Environmental Pollution, and Health. *Environmental Health Risk - Hazardous Factors to Living Species*. <https://doi.org/10.5772/63094>
- Parmar, T. K., Rawtani, D., & Agrawal, Y. K. (2016). Bioindicators: the natural indicator of environmental pollution. *Frontiers in Life Science*, 9(2), 110–118. <https://doi.org/10.1080/21553769.2016.1162753>
- Peng, C., Wang, M., & Chen, W. (2016). Spatial Analysis of PAHs in Soils along an Urban–Suburban–Rural Gradient: scale effect, distribution patterns, diffusion and influencing factors. *Scientific Reports*, 6. <https://doi.org/10.1038/srep37185>
- Perestrelo, R., Silva, P., Porto-Figueira, P., Pereira, J. A. M., Silva, C., Medina, S., & Câmara, J. S. (2019). QuEChERS - Fundamentals, relevant improvements, applications and future trends. *Analytica Chimica Acta*, 1070, 1–28. <https://doi.org/10.1016/j.aca.2019.02.036>
- Petrakis, D., Vassilopoulou, L., Mamoulakis, C., Psycharakis, C., Anifantaki, A., Sifakis, S., Docea, A.O., Tsiaoussis, J., Makrigiannakis, A., Tsatsakis, A.M., 2017. Endocrine Disruptors Leading to Obesity and Related Diseases. *Int J Environ Res Public Health* 14. <https://doi.org/10.3390/ijerph14101282>
- Pompa-García, M., Sánchez-Salguero, R., & Camarero, J. J. (2017). Observed and projected impacts of climate on radial growth of three endangered conifers in northern Mexico indicate high vulnerability of drought-sensitive species from mesic habitats. *Dendrochronologia*, 45, 145–155. <https://doi.org/10.1016/j.dendro.2017.08.006>
- Ratola, N., Alves, A., Santos, L., & Lacorte, S. (2011). Pine needles as passive bio-samplers to determine polybrominated diphenyl ethers. *Chemosphere*, 85(2), 247–252. <https://doi.org/10.1016/j.chemosphere.2011.06.005>
- Ratola, N., Amigo, J. M., Oliveira, M. S. N., Araújo, R., Silva, J. A., & Alves, A. (2011). Differences between Pinus pinea and Pinus pinaster as bioindicators of polycyclic aromatic hydrocarbons. *Environmental and Experimental Botany*, 72(2), 339–347. <https://doi.org/10.1016/j.envexpbot.2011.04.012>
- Ratola, N., Homem, V., Silva, J. A., Araújo, R., Amigo, J. M., Santos, L., & Alves, A. (2014). Biomonitoring of pesticides by pine needles — Chemical scoring, risk of exposure, levels and trends. *Science of The Total Environment*, 476–477, 114–124. <https://doi.org/10.1016/j.scitotenv.2014.01.003>
- Salehi-Lisar, S. Y., Deljoo, S., & Harzandi, A. M. (2015). Fluorene and Phenanthrene Uptake and Accumulation by Wheat, Alfalfa and Sunflower from the Contaminated Soil. *International Journal of Phytoremediation*, 17(12), 1145–1152. <https://doi.org/10.1080/15226514.2015.1045123>
- Shen, Y., Sheng, Y., Li, J., Zhu, J., Shi, S., & Zhan, X. (2019). The role of temperature in phenanthrene transfer and accumulation in crop leaves. *Environmental Pollution (Barking, Essex: 1987)*, 258, 113827. <https://doi.org/10.1016/j.envpol.2019.113827>
- Srogi, K. (2007). Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. *Environmental Chemistry Letters*, 5(4), 169–195. <https://doi.org/10.1007/s10311-007-0095-0>
- Tobiszewski, M., & Namieśnik, J. (2012). PAH diagnostic ratios for the identification of pollution emission sources. *Environmental Pollution*, 162, 110–119. <https://doi.org/10.1016/j.envpol.2011.10.025>
- Yang, J.-J., Liu, C.-C., Chen, W.-H., Yuan, C.-S., & Lin, C. (2013). Assessing the altitude effect on distributions of volatile organic compounds from different sources by principal component analysis. *Environmental Science. Processes & Impacts*, 15(5), 972–985. <https://doi.org/10.1039/c3em00034f>
- Yang, R., Yao, T., Xu, B., Jiang, G., & Zheng, X. (2008). Distribution of organochlorine pesticides (OCPs) in conifer needles in the southeast Tibetan Plateau. *Environmental Pollution*, 153(1), 92–100. <https://doi.org/10.1016/j.envpol.2007.07.033>
- Youssef, L., Younes, G., Kouzayha, A., & Jaber, F. (2015). Occurrence and levels of pesticides in South Lebanon water. *Chemical Speciation & Bioavailability*, 27(2), 62–70. <https://doi.org/10.1080/09542299.2015.1023092>
- Wang, C., Wang, X., Gong, P., Yao, T., 2018. Long-term trends of atmospheric organochlorine pollutants and polycyclic aromatic hydrocarbons over the southeastern Tibetan Plateau. *Science of The Total Environment* 624, 241–249. <https://doi.org/10.1016/j.scitotenv.2017.12.140>

Appendix A : Chapter II-II - Supplementary Materials S1 - Table 1, 2 and 3.

Appendix B : Chapter II-III - Supplementary Materials S2 - Table 1, 2 and 3.

V. The use of *Helix aspersa* and *Pinus nigra* as environmental biomonitors for the study of temporal air pollution variation in Northern Lebanon

Résumé

Ce résultat présenté sous forme d'un article soumis dans « *Environmental Chemistry Letters* », présente une étude basée sur l'utilisation des escargots et des aiguilles de conifères pour réaliser l'évaluation de la qualité de l'air dans cinq régions au nord du Liban. Les escargots reflètent la qualité de l'air en raison de leur large distribution en accumulant certains polluants toxiques et peuvent être utilisés pour évaluer la biodisponibilité des polluants atmosphériques en mesurant leur concentration sur une période de temps spécifiée. Les aiguilles de conifères sont également utilisées comme biomoniteurs passifs pour étudier divers polluants organiques dans l'air en raison de leur forte teneur en lipides, leur répartition sur une grande surface géographique et leur fort potentiel d'accumulation de polluants organiques.

Dans cette étude, une méthode combinant QuEChERS avec SPME suivie d'une analyse par LC-MS/MS et GC-MS/MS a été utilisée pour analyser les pesticides, y compris les OCPs et les HAPs. Cette méthode a été appliquée à deux matrices différentes (*Helix aspersa* et *Pinus nigra*) pour étudier leur rôle en tant que biomoniteur de la variation spatio-temporelle de la pollution de l'air dans la région du nord du Liban (Tripoli, Batroun, Akkar, Koura et Bcharre). Elle visait également à évaluer si les conditions météorologiques affectent la contamination de ces matrices, en ayant considéré plusieurs périodes différentes de prélèvement allant de juillet 2019 à décembre 2019, période de fortes variations, afin de détecter les variations spatiales temporelles de différents polluants.

Les résultats obtenus pour les échantillons des escargots et des aiguilles de conifères collectés pendant six mois consécutifs avec les mêmes procédures d'échantillonnage et d'analyse, montrent que pour ces deux matrices différentes, l'absorption des pesticides et des HAPs ne seraient pas similaire. L'étude des deux matrices montre une différence significative entre les résultats des échantillons et que les concentrations de pesticides et des HAPs dans les sites d'Akkar sont plus élevées que celles des sites de Batroun et de Bcharre.

En conclusion, les escargots et les aiguilles de conifères ont servi comme un agent de biomonitoring pour l'analyse de la qualité de l'air par l'accumulation de polluants organiques. Les variations météorologiques jouent un rôle important dans l'accumulation des polluants, et les données obtenues à partir des deux matrices confirment que les niveaux de pollution semblent varier différemment.

Highlights

- Pesticides and polycyclic aromatic hydrocarbons uptake ability was tested in snails (*Helix aspersa*) and pine needles (*Pinus nigra*) for six months in seven sites of Northern Lebanon.
- *Helix aspersa* and *Pinus nigra* evidenced difference accumulation trends over the study area.
- *Helix aspersa* and *Pinus nigra* highlighted similar pollution sources based on specific diagnostic ratios.
- The exposure time, the structure and morphology of *Helix aspersa* and *Pinus nigra* biomonitors seems a key factor to estimate accumulation rates of pollutants.

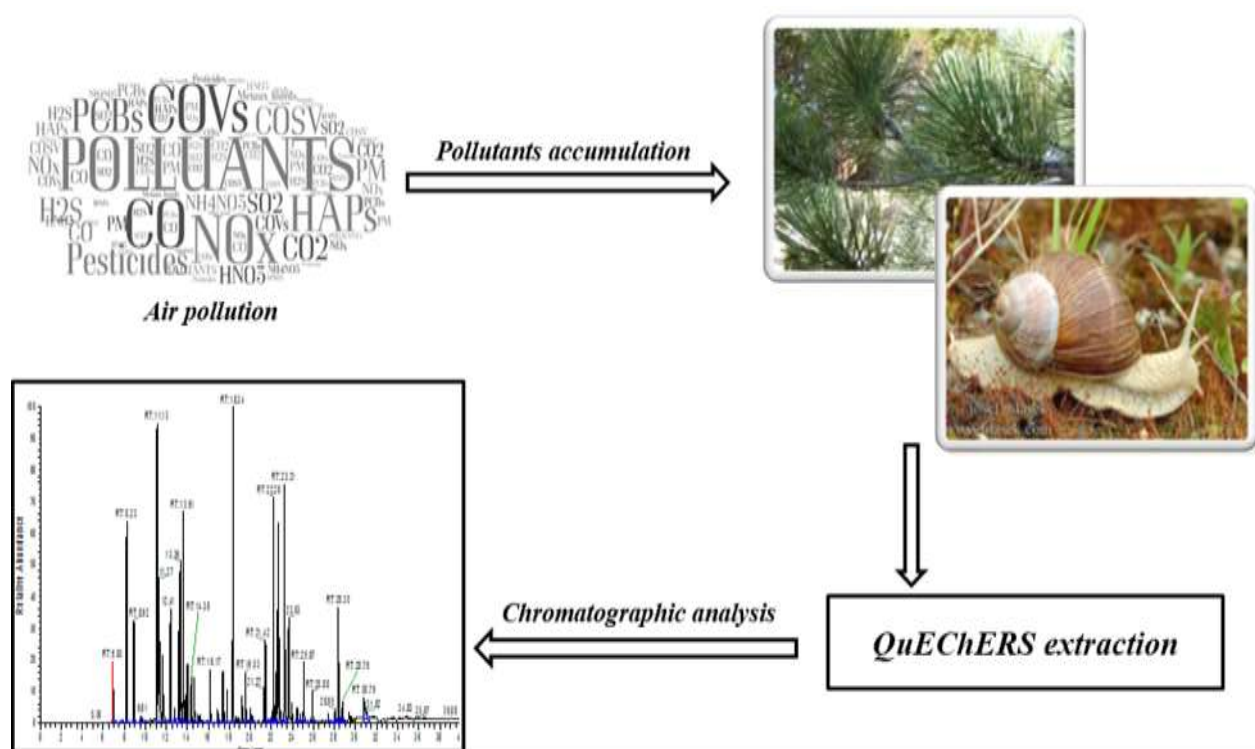
Abstract

Land snails *Helix aspersa* and pine needles *Pinus nigra*, known as biomonitors of air pollution, are often used to assess contamination by organic pollutants. In this regard, a sampling campaign was conducted for the first time in Lebanon from June to December 2019, in five different regions in Northern Lebanon. For this, snails and pine needles samples were collected monthly. The extraction of pollutants was based on QuEChERS followed by a pre-concentration step of volatile compounds using solid phase microextraction. Results showed that for *Pinus nigra*, a high concentration of pollution was observed during the first four months of the analysis followed by a noticeable decrease at the end of the study, while for *Helix aspersa*, an increasing of pollution concentration was observed during the sampling period. Moreover, results showed that Akkar and Tripoli could be regarded as a highly contaminated areas by pollutants whereas Batroun and Bcharre had the lowest concentration. Furthermore, meteorological data analysis proved the effect of climatic conditions on the bioaccumulation of pollutants

by the chosen biomonitors. This is the first time that a comparison between two different biomonitors is conducted while such comparison is highly required for a better environmental biomonitoring assessment.

Keywords: Snail; Pine needle; Air pollution; Meteorological condition; Pesticide; Polycyclic aromatic hydrocarbon.

Graphical abstract



1. Introduction

In the last twenty years, studies have associated air pollution to respiratory diseases like pneumonia, cardiovascular diseases such as arrhythmias and metabolic disorders such as hepatic encephalopathy (Jiang et al. 2016; Lee et al. 2014). Air pollution consists of a variety of contaminants issued from natural and synthetic sources and formed by the photochemical transformation process including volatile and non-volatile organic compounds, particulate matter and gaseous products, each causing harmful effects on human health (Tran et al. 2020).

Pesticides, for instance, pollute the air through various processes and approximately 30 to 50 % of the quantities applied are present in the air depending on the physicochemical characteristics of the pesticides, the climatic conditions in particular wind and temperature and the processing equipment and procedures (Damalas and Eleftherohorinos 2011). In general, the concentrations of pesticides in the air are consistently higher for pesticides with high volatile characteristics, in agricultural areas and during exposure periods (Schummer et al. 2010). Polycyclic aromatic hydrocarbons (PAHs) are a diverse class of persistent lipophilic organic pollutants that are generally divided based of their physico-chemical properties into low and high molecular weight (Dudhagara and Dave 2018; Souza et al. 2015). PAHs and their polar compounds that arise in the air are generated mainly from natural or anthropogenic sources and disperse far from their source through contaminated air masses in different forms (ash or smoke) and then are dispersed in natural and animal matrices (Elaridi et al. 2020; Hasheminassab et al. 2013).

The wide distribution of PAHs and pesticides including organochlorine pesticides (OCPs) in the air is of great concern to the researchers now, which has contributed to the careful monitoring of their critical studies by active samplers that have been used widely in air monitoring (Gaga et al. 2012). However, these samplers have been recently replaced by passive sampler among which figure the natural species such as conifer needles and snails (Al-Alam et al. 2019). In fact, pine needles are biomonitors used to investigate various pollutants in the air including PAHs (Wang et al. 2019; Oishi, 2018), pesticides (Luo et al. 2020; Ratola et al. 2014) and polychlorinated biphenyls (Cindoruk et al. 2020). Owing to their high lipid content and wide specific surface area, they are spread widely in several

regions and have a high potential to accumulate organic pollutants on a wide geographical range (Likus-Cieřlik et al. 2020; Wierzbicka et al. 2019). As well, snails, also known as environmental biomonitors, reflect the air quality in their surroundings due to their wide distribution by accumulating certain toxic pollutants and can be used to assess bioavailability of air pollutants by measuring their concentration over a specified period of time (Baroudi et al. 2020; Sturba et al. 2020). They can be easily collected and are in contact with various organic pollutants that they absorb respiratory, digestively and transcutaneous from plants, water and soil such as pesticides (Girones et al. 2020), PAHs (Al-Alam et al. 2020) and polychlorinated biphenyls (Wu et al. 2019).

In this study, *Helix aspersa* and *Pinus nigra* were used as potential biomonitors of air pollution in Northern Lebanon (Tripoli, Batroun, Akkar, Koura and Bcharre). The two selected biomonitors were assessed for their potential accumulation of pesticides and PAHs. These pollutants were subjected to a multi-residue extraction procedure based on QuEChERS followed by chromatographic analysis coupled to tandem mass spectrometry. Moreover, the impact of meteorological conditions on the contamination of these matrices was also assessed, since the study was performed in different time periods from June to December 2019 during which the weather widely varied. It is worth noting that, to the best to the authors' knowledge, a comparison between two different biomonitors was never reported before and accordingly such comparison is highly required for a better environmental biomonitoring assessment.

2. Experimental

2.1 Materials

Acetonitrile grade for liquid chromatography, water for chromatography, formic acid and acetonitrile were purchased from Sigma-Aldrich (L'Isle D'Abeau, France). Ultrapure water used was obtained through a Milli-Q system (18 M Ω cm) from Merck, Germany. Polyacrylate (85 μ m) and polydimethylsiloxane (100 μ m) coated fibers used for solid-phase microextraction were purchased from Supelco-Sigma Aldrich (Saint Martin d'Hère France).

2.2 Study region

Northern Lebanon (34°26'N 35°51'E), covering an area of 2024.8 km² with a population of 1197203, is divided into multiple districts Batroun, Bcharre, Koura, Minieh-Dennie, Tripoli, Akkar and Zgharta district. The described study was conducted on five monitoring regions (seven sites) spread over the north of Lebanon included: Akkar (Araa, Beit Hajj), Koura (Beshmezzine, Haykaliyeh), Bcharre (Abdine), Batroun (Tannourine) and Tripoli (Abi Samra).

Tripoli, the capital of the North Governorate and the second largest city in Lebanon, is characterized by high population density and a wide range of crop production mainly focused on olives and citrus fruits. Koura district is particularly known for its olive tree cultivation and olive oil production. Akkar is bordered by the Governorates of Syria and is the second largest agricultural area after Bekaa valley. Tannourine and Bcharre dedicated to organic agriculture in general are mountainous districts and considered one of Lebanon's largest and densest cedar forests. The geographic location of sampling sites over the north of Lebanon is illustrated in Supplementary Information (SI-1.1) (Fig. 1).

2.3 Sampling

The certified *Helix aspersa* blank snails used for this experiment were purchased from a farm at Bkeftine, Lebanon. All snails were kept in fully protected cages using sieve allowing the air free flow within the cage. The cages are kept in the lands of different studied regions where there is vegetation and besides the pine needles trees that are part of this study. Snails were fed on pure water and cabbage purchased from farms specific in organic products. Sampling was done monthly from July till December 2019, during which 3 snails of each cages and 10 grams of one-year old needles were collected. All samples were freshly frozen in propylene bags at -18 °C until analysis.

2.4 Sample preparation and analysis

The QuEChERS extraction method was based on the work of Al-Alam et al. in 2020 for the study of pesticides and PAHs residues in snails (Al-Alam et al. 2020) and the work of Baroudi et al. in 2020 for the study of these organic pollutant's residues in pine needles (Baroudi et al. 2020). A schematic representation of the methods used to extract pesticides and PAHs from matrices is shown in Supplementary Information (SI-1.1) (Fig.

2). Chromatographic parameters for all assessed pollutants are also shown in Supplementary Information (SI-1.2).

3. Results and Discussion

3.1 *Helix aspersa* and *Pinus nigra* contamination

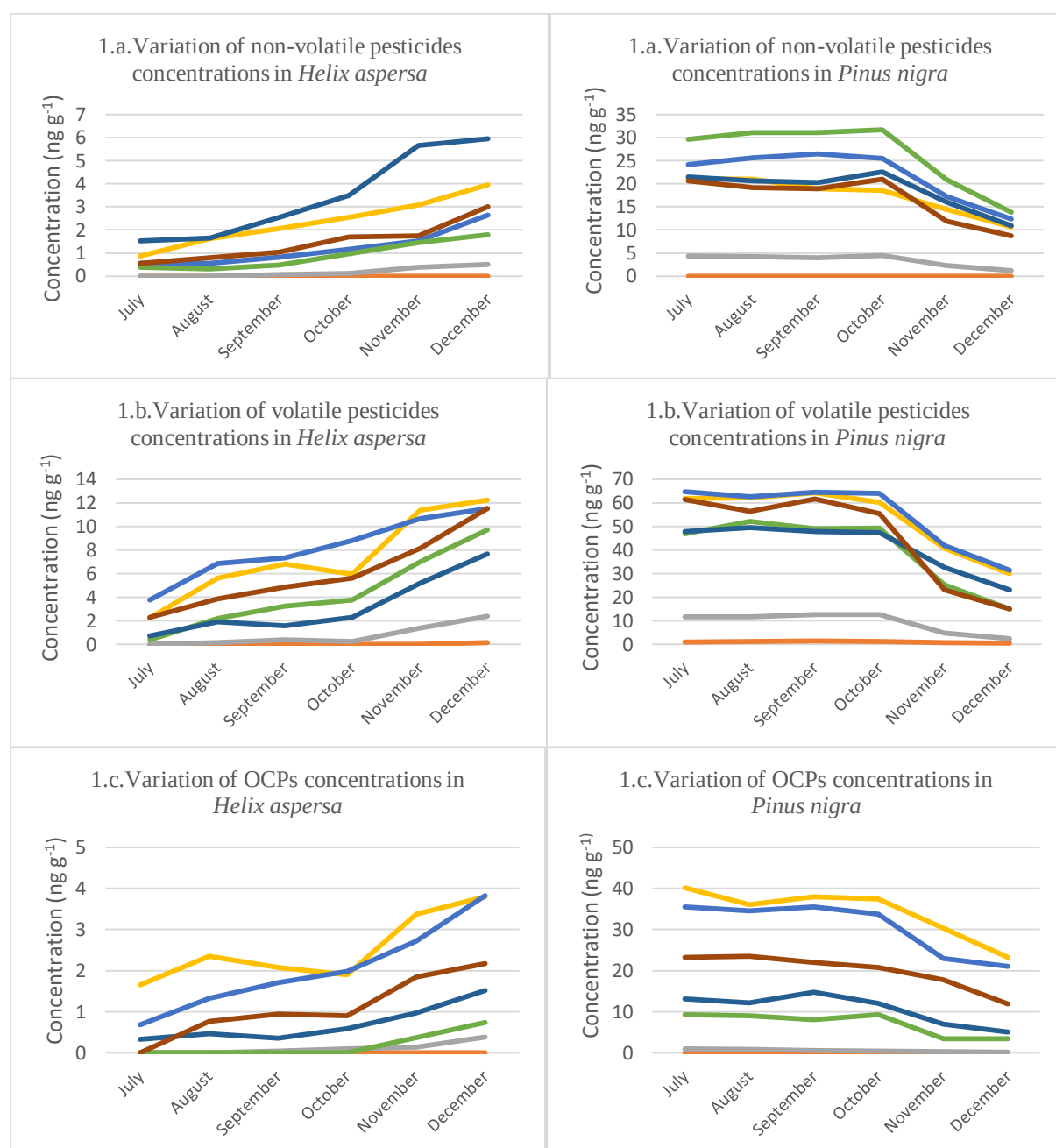
For LC-MS/MS, the detected non-volatile pesticides were carbendazim, diflufenican, diuron, penconazole and pendimethalin. The analysis of the variation of the concentrations of these pesticides for six months shows that the maximum concentrations obtained were in the months of November and December for snails' analysis and in the months of July, August, September and October for pine needles analysis. Figure 1.a shows the distribution of the concentrations of non-volatile pesticides (ng g^{-1}) found in *Helix aspersa* and *Pinus nigra* matrices.

For volatile pesticides, fenpropidin, acetochlor, chlorpropham, clofentezine, deltamethrin, diclobenil, diphenylamine, ethofumesate, kresoxim-methyl, lambda-cyhalothrin and pyraclostrobin were detected during the six months of analysis in all the regions studied. As for non-volatile pesticides, the maximum concentration of pesticides was found at the last month of the campaign for snails. However, for pine needles, comparable concentration was shown for the first four months followed by a significant drop on December. Figure 1.b shows the distribution of the concentrations of volatile pesticides (ng g^{-1}) found in *Helix aspersa* and *Pinus nigra* matrices.

Regarding the OCPs, results showed that heptachlor, hexachlorobenzene and α -endosulfan were the only compounds detected over the assessed regions. The variation in the concentration of these OCPs, known by their high persistence, shows an increasing concentration for the snails to obtain a maximum at the last months. For pine needles, a similar accumulation was seen in the first four months, followed by a considerable decrease in December. Among the OCPs assessed, none of them were found in Tannourine for both the analyzed matrices. Figure 1.c shows the distribution of the concentrations of OCPs (ng g^{-1}) found in *Helix aspersa* and *Pinus nigra* matrices.

On the other side, the detected PAHs were naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, benz[a]anthracene, benzo[e]pyrene,

benzo(k)fluoranthene, chrysene and fluoranthene. For pine needles, results showed a high PAHs concentration during the first 4 months of the study for Akkar and Tripoli (Abi Samra), followed by a decrease in the fifth and sixth months of the study. For snails, the highest concentrations were observed in Akkar and Tripoli with an increasing concentration over time. Figure 1.d shows the distribution of the concentrations of PAHs (ng g^{-1}) found in *Helix aspersa* and *Pinus nigra* matrices.



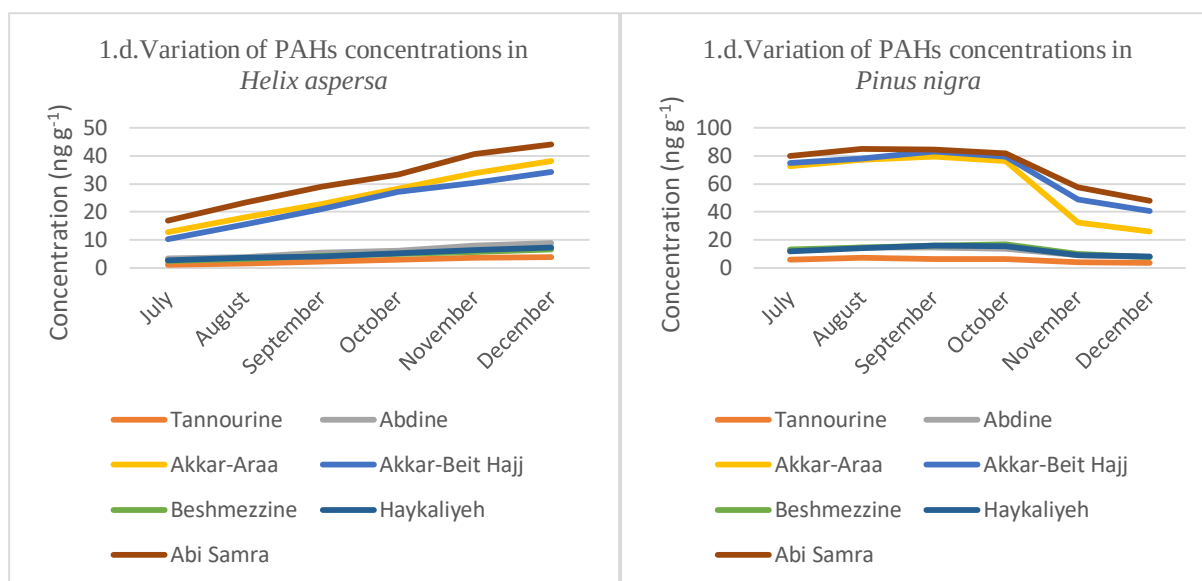


Fig. 1 Monthly concentrations of pesticides and polycyclic aromatic hydrocarbons in studied areas reporting increase of the pollutants assessed in *Helix aspersa* during the total duration of the study and the decline of these pollutants during the last two months of the study in the assessed *Pinus nigra*

The results show that the majority of regions contain residues of pesticides with concentrations varying between these regions. Akkar appears to be the most polluted region with residues of pesticides also known as an agricultural area, while Batroun was the less polluted with residues of pesticides, considered one of the principal densest and largest cedar forests in Lebanon. For PAHs, the high emissions levels were observed in Akkar and Tripoli while the regions of Batroun, Koura and Bcharre were the least contaminated. The seasonal difference, heating activities in winter, variation in temperature, petrogenic and pyrogenic sources may be attributed to the variation in deposition and accumulation of PAHs in each matrices. The most relevant pesticides and PAHs residues found in the samples analyzed are shows in Supplementary Information (SI-1.3).

3.2 Meteorological effects on pollutant concentrations

The pollutants' uptake by the two assessed biomonitors showed, despite being different, a similar trend in accumulation for each matrix.

Pinus nigra

For *Pinus nigra*, as indicated in our analysis, the concentration of both PAHs and pesticides are approximately constant in the first four months of study that showed a high

concentration of pollutants (July, August, September and October), then the concentration decreased during the last two months of analysis (November and December) (Figure 1). In order to find a reasonable explanation for this difference, meteorological conditions were assessed and recorder during the total duration of the campaign. According to the Lebanon Weather sites (source for weather conditions in Lebanon), the first four months of the sampling (July - October) were categorized by favorable climatic conditions in which no precipitation was noticeable, and the temperature was the highest compared to the last two months (November - December) which had significant rainfall, accompanied by severe storms, with a decrease in temperatures. In fact, weather and climate changes during this period of year were very normal occurrences, which may be the cause for the decrease of pollutants at the end of the study. Variations in climatic conditions (July 15 - December 15, 2019) given by LebWeather.net are shown in figure 2.

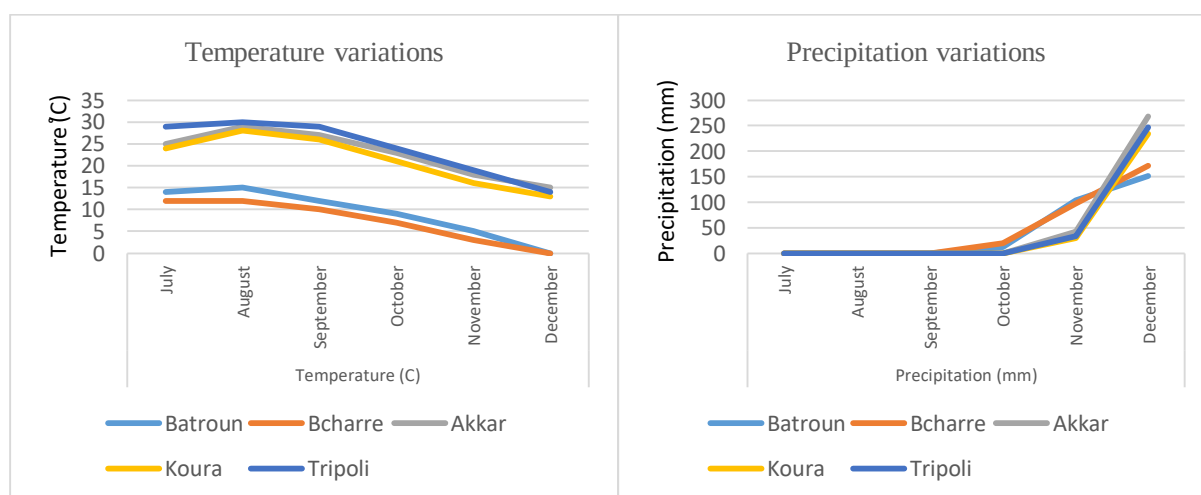


Fig. 2 Monthly levels of air temperature and precipitation in selected locations across the Northern Lebanon reporting decrease temperature and significant increase of precipitation during July and December 2019

The first reason of these results in pine needles is supposed to be the young age of the needles chosen (< 1 year) which in the presence of successive heavy rains, these new needles having a low accumulation of pollutants over the time are washed in abundance before storing these pollutants and can be directly influenced by meteorological variations (Luo et al. 2020; Al-Alam et al. 2017).

The Σ pesticides (non-volatile and volatile pesticides, OCPs) of seven sites between the first and last month in Lebanon city were 1.04 - 0.35 ng g⁻¹ in Tannourine area, 16.96 - 3.77 ng g⁻¹ in Abdine area, 123.17 - 64.03 ng g⁻¹ in Araa area, 124.2 - 64.79 ng g⁻¹ in Beit

Hajj area, 85.85 - 32.12 ng g⁻¹ in Beshmezzine area, 82.41 - 39.08 ng g⁻¹ in Haykaliyeh area and 105.31 - 35.76 ng g⁻¹ in Abi Samra area, respectively. Moreover, the high concentration of pesticides observed in the first four months are probably due to the accumulation of these pollutants by the needles, the high concentration of which is generally observed from the end of spring to the summer after pulverization (Luo et al. 2020; Rybicki and Jungmann 2018). In addition, many studies suggested a relation between climate changes and concentrations of pesticides. Estellano et al. (2015) showed that the highest concentration of pesticides in Italy were identified in samples collected during spring and summer (Estellano et al. 2015). As well, it was shown that OCPs concentrations depend principally on seasonal variations in which the greatest concentration was observed during dry season while the lowest appear during the wet season (Li et al. 2014). In addition, obtained results correlate with those reported by Scheyer et al. (2005), indicating that the highest concentration was observed in Strasbourg during spring and summer (Scheyer et al. 2005) while lowest concentrations are observed during rainy seasons (Sauret et al. 2009).

It was also shown that the climatic conditions of the studied areas are the main causes of PAHs variations (Cao et al. 2019; Zheng et al. 2019). The concentrations of Σ PAHs from July to December were 5.96 to 3.52 ng g⁻¹, 12.09 to 7.84 ng g⁻¹, 72.52 to 25.8 ng g⁻¹, 75.05 to 40.62 ng g⁻¹, 13.2 to 7.42 ng g⁻¹, 11.59 to 8.23 ng g⁻¹ and 79.68 to 47.69 ng g⁻¹ in Tannourine, Abdine, Araa, Beit Hajj, Beshmezzine, Haykaliyeh and Abi Samra, respectively. Many studies show that climatic parameters as well as the source emissions can contribute to varying concentration of PAHs between winter and summer (Tan et al. 2006). Furthermore, the precipitation and repeated washing-out effect for pollutants result in minimum concentrations of particulates and thus allow the reduction of PAHs (Chang et al. 2006; Zheng et al. 2019).

Helix aspersa

Snails can be contaminated by three sources: soil or water (ingestion and cutaneous contact), air (respiration and skin contact), and plants (digestion) (Girones et al. 2020). The accumulation of pollutants in snails depends on the duration and time of exposure, which means that wild snails are highly polluted due to their chronic exposure to environmental contaminants. During the sampling period, an accumulation of pesticides

and PAHs was observed in the snails, with an increase in concentration during the six months to achieve a maximum peak reached in the sixth month (Figure 1).

The concentration of Σ pesticides of the first sampling in July were 0 ng g⁻¹ for Tannourine and Abdine, 4.78 ng g⁻¹ for Araa, 4.98 ng g⁻¹ for Beit Hajj, 0.77 ng g⁻¹ for Beshmezzine, 2.58 ng g⁻¹ for Haykaliyeh and 2.81 ng g⁻¹ for Abi Samra, respectively. Overall pesticides levels increased during this period and ranged about several orders of magnitude from 0.16 ng g⁻¹, 3.25 ng g⁻¹, 19.96 ng g⁻¹, 17.98 ng g⁻¹, 12.22 ng g⁻¹, 15.12 ng g⁻¹ and 16.66 ng g⁻¹, respectively. The transfer of pesticides in snails may be due to many different exposure routes starting with their direct application and then through respiration and skin contact especially to the air (Baroudi et al. 2020). The highest concentrations obtained over the last two months dependent on the interaction of the snails with the pesticide-polluted soil (Druart et al. 2011) present in high concentrations due to precipitation effects and continuous washing of pesticides from the plants and trees especially the pine needles and accumulation of these pesticides in the soil (Gill and Garg 2014). These results correspond with those reported by Zhao et al. in 2020, showing that the pesticide residues in soils currently planted are higher than soils previously planted or no planted. The variations in concentrations between regions are usually due to the different pesticide application of local farmers to improve effective harvest, the different half-life and the degree of degradation of these pesticides in the agricultural soils, the soil pH and the soil properties (Zhao et al. 2020).

Many researchers suggest that meteorological conditions and sources emissions, can lead to variations in PAH concentrations between summer and winter (Zheng et al. 2019). The total concentrations of PAHs (Σ PAHs) in Tannourine is ranging from 1.09 to 3.91 ng g⁻¹, from 3.28 to 8.94 ng g⁻¹ in Abdine, from 12.76 to 38.16 ng g⁻¹ in Araa, from 10.2 to 34.19 ng g⁻¹ in Beit Hajj, from 2.37 to 6.61 ng g⁻¹ in Beshmezzine, from 2.66 to 7.33 ng g⁻¹ in Haykaliyeh and from 16.96 to 44.15 ng g⁻¹ in Abi Samra. These lowest concentrations of PAHs in summer were likely caused by photo-degradation and the increased air dispersion, while the highest concentrations of PAHs in winter were mostly caused by the highly contaminated air movement and sources of local emissions (Chang et al. 2006; Guo et al. 2003). Bae et al. (2002), also found that concentrations of PAHs are higher in winter than those in spring due to the significant weather conditions and the highest emission of stationary and mobile sources (Bae et al. 2002). It is supposed that the PAHs

concentration is the highest during November and December due to the decreased air temperature, slower radical degradation of PAHs and photolysis, the increased use of coal and the inverse temperature in winter which can produce higher quantities of PAHs that contributes to the difficulty of pollutants spread which can aggravate the pollution of PAHs in the air (Wang et al. 2018; Zheng et al. 2019). Several studies have suggested that a highest concentration of PAHs in the raining season can be due to an increased condensation of PAHs at low temperatures in the gaseous phase, to the increased consumption of fossil fuel and the limited photochemical degradation of some PAHs in winter through solar radiation (Saeed et al. 2011; Hytönen et al. 2009).

Potential factors on accumulation of pesticides and PAHs in Helix aspersa and Pinus nigra

A multiple linear regression was performed for pesticides and PAHs concentrations with temperature and precipitation in order to classify the control factors for pesticides and PAHs aggregation in various matrices. The results showed that the parameters above were significant factors in describing the variation in the concentrations of pesticides and PAHs in pine needles and snails samples ($P < 0.05$). The P-value for concentrations (ng g^{-1}) in samples with temperature and precipitation of the sampling sites, were shown in Supplementary Information (SI-1.3).

3.3 Residual levels of pesticides in *Helix aspersa* and *Pinus nigra*

In both matrices obtained from northern Lebanon, nineteen pesticides comprising five non-volatile, twenty-one volatile pesticides and three OCPs were detected. The residual levels of these pesticides differed significantly among the seven studied areas. These findings indicate that the least polluted with pesticide residues were the samples obtained from Tannourine, one of the largest cedar forests in Lebanon followed by the area of Abdine which is usually devoted to organic crops. The results of the analysis show that the concentrations of Tannourine and Abdine for pesticides and OCPs were at extremely low concentrations, apparently due to reduced human activity (Xing et al. 2020).

In addition, Akkar, well-known for its largest agricultural production, appears to be the most polluted region with residues from pesticides. Moreover, results showed the presence of OCPs such as heptachlor, hexachlorobenzene and α -endosulfan which are considered persistent in the environment, resistance to degradation and accumulated in

the air of Araa and Beit Hajj due to its high lipid solubility (Qu et al. 2015). These pesticides are used in this area with very lower control limit for the reason of its existence close to the borders of Syria where farmers try to obtain high productivity with low cost against the backdrop of environmental effects. Helou et al. (2019) show the presence of OCPs in the Akkar district groundwater indicating the use of these pesticides in this agricultural area (Helou et al. 2019). The presence of OCPs in the air appear to accumulate in species, food chain and soil and can be transmitted into the surface water and groundwater by surface runoff and agricultural drainage. Many factors influence the fate of OCPs in soils, including the soil types, physiochemical properties and climatic parameters (Qu et al. 2016; Yu et al. 2013).

For Koura and Tripoli, known by their agricultural production, the highest detection of agricultural insecticide such as deltamethrin and lambda-cyhalothrin residues is confirmed by the olive trees characterizing these regions (López-Blanco et al. 2018; Maalej et al. 2017). In fact, deltamethrin is used frequently to control a wide range of diseases in olive trees like pest control (Jaabiri et al. 2013). The agricultural productions of Koura area, known for its vegetables and fruits productions, justify the highest residues of pesticides found like penconazole and diflufenican. The presence of these pesticides in abundance in the regions of Beshmezzine and Haykaliyeh, is explain by the control of penconazole for various molds and mildews in agricultural crops and its application to the prevention of a variety of vegetable and fruit diseases while diflufenican is an herbicide used for pre and post-crop emergence against herbicide for the weed control in winter (Szpyrka et al. 2015; Zhang et al. 2019).

3.4 Residual levels of PAHs in *Helix aspersa* and *Pinus nigra*

Diagnostic ratios of PAHs have been commonly used for source distribution (Ali et al., 2016). PAHs from various sources have different molecular compositions, and some reports have shown that pyrogenic PAHs are more concentrated in higher molecular weight (HMW) components, whereas petrogenic components include a higher percentage of lower molecular weight (LMW) PAHs (Stogiannidis and Laane 2015). To assess the sources of PAHs emission for the seven selected sites, ratios of low molecular weight PAHs (LMW, 2-3 rings: naphthalene: NP, acenaphthene: ACE, fluorene: FL, phenanthrene: PHE, anthracene: ANT) produced in low-temperature processes (domestic wood burning) over

high molecular weight PAHs (HMW, 4-6 rings: pyrene: PYR, benz[a]anthracene: BaA, benzo[e]pyrene: BeP, benzo(k)fluoranthene: BkF, chrysene: CHR, fluoranthene: FLA) produced in high-temperature processes (fuels combustions in engines) were compared to literature (Table 1) (Al-Alam et al. 2019; Tobiszewski and Namieśnik 2012).

For both matrices, the comparison of the ratio $\Sigma \text{LMW} / \Sigma \text{HMW}$ for the five selected regions confirm that the main reasons of pollutions by PAHs in Batroun, Bcharre and Koura are the road combustions considered as petrogenic source ($\Sigma \text{LMW} / \Sigma \text{HMW} > 1$), while the principal origins of PAHs in Akkar are the pyrogenic source ($\Sigma \text{LMW} / \Sigma \text{HMW} < 1$) (Zhang et al. 2008).

The study of the ratio $\text{FL} / (\text{FL} + \text{PYR})$ for snails and pine needles identify that diesel emissions (> 0.5) issued from road and vehicle emissions are the responsible of petrogenic pollution in the areas of Bcharre, Akkar and Koura areas, while for Tripoli a petrol emission (< 0.5) was detected due to the existence of the port considered to be responsible for the petrol emission (Ravindra et al. 2008).

In contrast, the results of the $\text{ANT} / (\text{ANT} + \text{PHE})$ ratio of all sampling sites in the two matrices were higher than 0.1, meaning that pyrogenic sources are present in the five areas tested (Pies et al., 2008), while the $\text{FLA} / (\text{FLA} + \text{PYR})$ ratio higher than 0.5 for all samples implied that these pyrogenic emissions are due in particular to the combustion of wood, coal and grass (De La Torre-Roche et al. 2009).

The $\text{BaA} / (\text{BaA} + \text{CHR})$ ratio, which for the five regions was higher than 0.35 in snails as in pine needles, confirms the importance of vehicle emissions in the environmental contamination process by PAHs, which means that the emissions of petrol and diesel cannot be separated and must be considered together (Akyüz and Çabuk 2010).

Table 1 Diagnostic ratios of polycyclic aromatic hydrocarbons in selected areas reporting the petrogenic and pyrogenic source during July and December 2019 based on the work of Tobiszewski and Namiesnik in 2012

	Tannourine		Abdine		Araa		Beit Hajj		Beshmezzine		Haykaliyeh		Abi Samra	
	Snail	Pine needle	Snail	Pine needle	Snail	Pine needle	Snail	Pine needle	Snail	Pine needle	Snail	Pine needle	Snail	Pine needle
$\Sigma \text{LMW} / \Sigma \text{HMW}$	1.9	1.8	2.4	2.2	0.6	0.5	0.7	0.4	1.4	2.4	1.6	2.5	0.5	0.6
$\text{Fl} / (\text{Fl} + \text{Pyr})$	-	-	0.7	0.6	0.5	0.6	0.6	0.6	0.5	0.8	0.5	0.8	0.3	0.4
$\text{An} / (\text{An} + \text{Phe})$	1.0	1.0	0.6	0.6	0.5	0.4	0.5	0.4	0.4	0.5	0.3	0.6	0.4	0.4
$\text{Fla} / (\text{Fla} + \text{Pyr})$	0.5	0.5	0.5	0.4	0.6	0.5	0.6	0.5	0.6	0.59	0.5	0.5	0.6	0.5
$\text{BaA} / (\text{BaA} + \text{CHR})$	-	-	0.5	0.5	0.9	0.9	0.8	0.9	0.3	0.6	0.3	0.6	0.4	0.5

4. Conclusion

Land snails '*Helix aspersa*' and pine needles '*Pinus nigra*', considered as biomonitors of air pollution, are used for the first time in Lebanon to monitor for pesticide and persistent organic pollutants contamination. This study shows that snails and pine needles are well suitable for pesticides and PAHs detection of pollution inputs in different areas. Pine needles accumulated significantly higher concentrations for all pesticides and PAHs compared to snails. The better accumulation of the needles reflects on its long-term persistence in the study areas relative to snails maintained at the time of study. The specific behavior of the two matrices in pesticides and PAHs accumulation depends on their different characteristics, specifically the high lipid content and wide specific surface area of the pine needles that favored the storage of the pollutants. Exposure time appears to be a crucial factor in estimating accumulation activities, as knowledge of the structure and morphology of plant and animal species is a critical prerequisite for choosing organisms for target contaminants in order to obtain accurate results in environmental studies.

References

- Akyüz M, Çabuk H (2010) Gas-particle partitioning and seasonal variation of polycyclic aromatic hydrocarbons in the atmosphere of Zonguldak, Turkey. *Science of The Total Environment* 408, 5550–5558. <https://doi.org/10.1016/j.scitotenv.2010.07.063>
- Al-Alam J, Baroudi F, Chbani A, Fajloun Z, Millet M (2020) A multiresidue method for the analysis of pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in snails used as environmental biomonitors. *Journal of Chromatography A* 1621, 461006. <https://doi.org/10.1016/j.chroma.2020.461006>
- Al-Alam J, Chbani A, Fajloun Z, Millet M (2019) The use of vegetation, bees, and snails as important tools for the biomonitoring of atmospheric pollution—A review. *Environmental Science and Pollution Research*, 26, 9391–9408. <https://doi.org/10.1007/s11356-019-04388-8>
- Al-Alam J, Fajloun Z, Chbani A, Millet M (2019b) Determination of 16 PAHs and 22 PCBs in honey samples originated from different region of Lebanon and used as environmental biomonitors sentinel. *Journal of Environmental Science and Health, Part A* 54, 9–15. <https://doi.org/10.1080/10934529.2018.1500782>
- Al-Alam J, Fajloun Z, Chbani A, Millet M (2017) The use of conifer needles as biomonitor candidates for the study of temporal air pollution variation in the Strasbourg region. *Chemosphere* 168, 1411–1421. <https://doi.org/10.1016/j.chemosphere.2016.11.103>
- Ali N, Ismail I.M.I, Khoder M, Shamy M, Alghamdi M, Costa M, Ali L.N, Wang W, Eqani S.A.M.A.S (2016) Polycyclic aromatic hydrocarbons (PAHs) in indoor dust samples from Cities of Jeddah and Kuwait: Levels, sources and non-dietary human exposure. *Science of The Total Environment* 573, 1607–1614. <https://doi.org/10.1016/j.scitotenv.2016.09.134>
- Bae S.Y, Yi S.M, Kim Y.P (2002) Temporal and spatial variations of the particle size distribution of PAHs and their dry deposition fluxes in Korea. *Atmospheric Environment*, 12th Clean Air & Environment Congress and Exhibition 36, 5491–5500. [https://doi.org/10.1016/S1352-2310\(02\)00666-0](https://doi.org/10.1016/S1352-2310(02)00666-0)
- Baroudi F, Al Alam J, Fajloun Z, Millet M (2020) Snail as sentinel organism for monitoring the environmental pollution; a review. *Ecological Indicators* 113, 106240. <https://doi.org/10.1016/j.ecolind.2020.106240>
- Baroudi F, Al-Alam J, Chimjarn S, Delhomme O, Fajloun Z, Millet M (2020) Conifers as environmental biomonitors: A multi-residue method for the concomitant quantification of pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls by LC-MS/MS and GC-MS/MS. *Microchemical Journal* 154, 104593. <https://doi.org/10.1016/j.microc.2019.104593>
- Cao Z, Wang M, Chen Q, Zhu C, Jie J, Li X, Dong X, Miao Z, Shen M, Bu Q (2019) Spatial, seasonal and particle size dependent variations of PAH contamination in indoor dust and the corresponding human health risk. *Science of The Total Environment* 653, 423–430. <https://doi.org/10.1016/j.scitotenv.2018.10.413>
- Chang K.-F, Fang G.-C, Chen J.-C, Wu Y.-S (2006) Atmospheric polycyclic aromatic hydrocarbons (PAHs) in Asia: A review from 1999 to 2004. *Environmental Pollution* 142, 388–396. <https://doi.org/10.1016/j.envpol.2005.09.025>
- Cindoruk S.S, Sakin A.E, Tasdemir Y (2020) Levels of persistent organic pollutants in pine tree components and ambient air. *Environmental Pollution* 256, 113418. <https://doi.org/10.1016/j.envpol.2019.113418>

- Damalas C.A, Eleftherohorinos I.G (2011) Pesticide Exposure, Safety Issues, and Risk Assessment Indicators. *Int J Environ Res Public Health* 8, 1402–1419. <https://doi.org/10.3390/ijerph8051402>
- De La Torre-Roche R.J, Lee W.-Y, Campos-Díaz S.I (2009) Soil-borne polycyclic aromatic hydrocarbons in El Paso, Texas: Analysis of a potential problem in the United States/Mexico border region. *Journal of Hazardous Materials* 163, 946–958. <https://doi.org/10.1016/j.jhazmat.2008.07.089>
- Druart C, Millet M, Scheifler R, Delhomme O, Raeppl C, de Vaufléury A (2011) Snails as indicators of pesticide drift, deposit, transfer and effects in the vineyard. *Science of The Total Environment* 409, 4280–4288. <https://doi.org/10.1016/j.scitotenv.2011.07.006>
- Dudhagara D.R, Dave B.P (2018) Mycobacterium as Polycyclic Aromatic Hydrocarbons (PAHs) Degradar. *Mycobacterium - Research and Development*. <https://doi.org/10.5772/intechopen.73546>
- Elaridi J, Fakhro M, Yamani O, Dimassi H, Othman H, Attieh Z (2020) GC–MS analysis of polycyclic aromatic hydrocarbons in bottled olive oil marketed in Lebanon. *Toxicol Res*. 36, 211–220. <https://doi.org/10.1007/s43188-019-00015-3>
- Estellano V.H, Pozo Karla, Efstathiou C, Pozo Katherine, Corsolini S, Focardi S (2015) Assessing levels and seasonal variations of current-use pesticides (CUPs) in the Tuscan atmosphere, Italy, using polyurethane foam disks (PUF) passive air samplers. *Environmental Pollution* 205, 52–59. <https://doi.org/10.1016/j.envpol.2015.05.002>
- Gaga E, Dogeroglu T, Ozden O, Ari A, Yay O, Altug H, Akyol N, Ornektekin S, Van Doorn W (2012) Evaluation of air quality by passive and active sampling in an urban city in Turkey: Current status and spatial analysis of air pollution exposure. *Environmental science and pollution research international* 19, 3579–96. <https://doi.org/10.1007/s11356-012-0924-y>
- Gill H.K, Garg H (2014) Pesticides: Environmental Impacts and Management Strategies. *Pesticides - Toxic Aspects*. <https://doi.org/10.5772/57399>
- Girones L, Arias A.H, Oliva A.L, Recabarren-Villalon T, Marcovecchio J.E (2020) Occurrence and spatial distribution of organochlorine pesticides in the southwest Buenos Aires using the freshwater snail *Chilina parhappii* as environmental biomonitor. *Regional Studies in Marine Science* 33, 100898. <https://doi.org/10.1016/j.rsma.2019.100898>
- Guo H, Lee S.C, Ho K.F, Wang X.M, Zou S.C (2003) Particle-associated polycyclic aromatic hydrocarbons in urban air of Hong Kong. *Atmospheric Environment* 37, 5307–5317. <https://doi.org/10.1016/j.atmosenv.2003.09.011>
- Hasheminassab S, Daher N, Schauer J.J, Sioutas C (2013) Source apportionment and organic compound characterization of ambient ultrafine particulate matter (PM) in the Los Angeles Basin. *Atmospheric Environment* 79, 529–539. <https://doi.org/10.1016/j.atmosenv.2013.07.040>
- Helou K, Harmouche-Karaki M, Karake S, Narbonne J.-F (2019) A review of organochlorine pesticides and polychlorinated biphenyls in Lebanon: Environmental and human contaminants. *Chemosphere* 231, 357–368. <https://doi.org/10.1016/j.chemosphere.2019.05.109>
- Hytönen K, Yli-Pirilä P, Tissari J, Gröhn A, Riipinen I, Lehtinen K.E.J, Jokiniemi J (2009) Gas–Particle Distribution of PAHs in Wood Combustion Emission Determined with Annular Denuders, Filter, and Polyurethane Foam Adsorbent. *Aerosol Science and Technology* 43, 442–454. <https://doi.org/10.1080/02786820802716743>
- Jaabiri I, Belhaj D, Turki N, Kallel M, Ayadi H, Ksantini M, Bouzid J, Gargouri R (2013) Development and method validation for determination of Deltamethrin residue in olive oil using a reversed-phase high performance liquid chromatography. <https://doi.org/10.9790/5736-0630108>
- Jiang X.-Q, Mei X.-D, Feng D (2016) Air pollution and chronic airway diseases: what should people know and do? *J Thorac Dis* 8, E31–E40. <https://doi.org/10.3978/j.issn.2072-1439.2015.11.50>
- Lee B.-J, Kim B, Lee K (2014) Air Pollution Exposure and Cardiovascular Disease. *Toxicol Res* 30, 71–75. <https://doi.org/10.5487/TR.2014.30.2.071>
- Li Y, Niu J, Shen Z, Zhang C, Wang Z, He T (2014) Spatial and seasonal distribution of organochlorine pesticides in the sediments of the Yangtze Estuary. *Chemosphere* 114, 233–240. <https://doi.org/10.1016/j.chemosphere.2014.04.112>
- Likus-Ciešlik J, Socha J, Gruba P, Pietrzykowski M (2020) The current state of environmental pollution with sulfur dioxide (SO₂) in Poland based on sulfur concentration in Scots pine needles. *Environmental Pollution* 258, 113559. <https://doi.org/10.1016/j.envpol.2019.113559>
- López-Blanco R, Moreno-González D, Nortes-Méndez R, García-Reyes J.F, Molina-Díaz A, Gilbert-López B (2018) Experimental and theoretical determination of pesticide processing factors to model their behavior during virgin olive oil production. *Food Chemistry* 239, 9–16. <https://doi.org/10.1016/j.foodchem.2017.06.086>
- Luo Y, Sun J, Wang P, Li Y, Li H, Xiao K, Yang R, Zhang Q, Jiang G (2020) Age dependence accumulation of organochlorine pesticides and PAHs in needles with different forest types, southeast Tibetan Plateau. *Science of The Total Environment* 716, 137176. <https://doi.org/10.1016/j.scitotenv.2020.137176>
- Maalej A, Mahmoudi A, Bouallagui Z, Fki I, Marrekchi R, Sayadi S (2017) Olive phenolic compounds attenuate deltamethrin-induced liver and kidney toxicity through regulating oxidative stress, inflammation and apoptosis. *Food and Chemical Toxicology* 106, 455–465. <https://doi.org/10.1016/j.fct.2017.06.010>
- Oishi Y (2018) Comparison of moss and pine needles as bioindicators of transboundary polycyclic aromatic hydrocarbon pollution in central Japan. *Environmental Pollution* 234, 330–338. <https://doi.org/10.1016/j.envpol.2017.11.035>
- Qu C, Albanese S, Chen W, Lima A, Doherty A.L, Piccolo A, Arienzo M, Qi S, De Vivo B (2016) The status of organochlorine pesticide contamination in the soils of the Campanian Plain, southern Italy, and correlations with soil properties and cancer risk. *Environmental Pollution* 216, 500–511. <https://doi.org/10.1016/j.envpol.2016.05.089>
- Qu C, Qi S, Yang D, Huang H, Zhang J, Chen W, Yohannes H.K, Sandy E.H, Yang J, Xing X (2015) Risk assessment and influence factors of organochlorine pesticides (OCPs) in agricultural soils of the hill region: A case study from Ningde, southeast China. *Journal of Geochemical Exploration* 149, 43–51. <https://doi.org/10.1016/j.gexplo.2014.11.002>
- Ratola N, Homem V, Silva J.A, Araújo R, Amigo J.M, Santos L, Alves A (2014) Biomonitoring of pesticides by pine needles — Chemical scoring, risk of exposure, levels and trends. *Science of The Total Environment* 476–477, 114–124. <https://doi.org/10.1016/j.scitotenv.2014.01.003>
- Ravindra K, Wauters E, Van Grieken R (2008) Variation in particulate PAHs levels and their relation with the transboundary movement of the air masses. *Science of The Total Environment* 396, 100–110. <https://doi.org/10.1016/j.scitotenv.2008.02.018>
- Rybicki M, Jungmann D (2018) Direct and indirect effects of pesticides on a benthic grazer during its life cycle. *Environ Sci Eur* 30. <https://doi.org/10.1186/s12302-018-0165-x>
- Saeed T, Ali L.N, Al-Bloushi A, Al-Hashash H, Al-Bahloul M, Al-Khabbaz A, Al-Khayat A (2011) Effect of environmental factors on photodegradation of polycyclic aromatic hydrocarbons (PAHs) in the water-soluble fraction of Kuwait crude oil in seawater. *Marine Environmental Research* 72, 143–150. <https://doi.org/10.1016/j.marenvres.2011.07.004>

- Sauret N, Wortham H, Strekowski R, Herckès P, Nieto L.I (2009) Comparison of annual dry and wet deposition fluxes of selected pesticides in Strasbourg, France. *Environmental Pollution* 157, 303–312. <https://doi.org/10.1016/j.envpol.2008.06.034>
- Scheyer A, Graeff C, Morville S, Mirabel P, Millet M (2005) Analysis of some organochlorine pesticides in an urban atmosphere (Strasbourg, east of France). *Chemosphere* 58, 1517–1524. <https://doi.org/10.1016/j.chemosphere.2004.10.013>
- Schummer C, Mothiron E, Appenzeller B.M.R, Rizet A.-L, Wennig R, Millet M (2010) Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg, France. *Environmental Pollution* 158, 576–584. <https://doi.org/10.1016/j.envpol.2009.08.019>
- Souza H.M.L, Taniguchi S, Bícago M.C, Oliveira L.A, Oliveira T.C.S, Barroso H.S, Zanotto S.P, Souza H.M.L, Taniguchi S, Bícago M.C, Oliveira L.A, Oliveira T.C.S, Barroso H.S, Zanotto S.P (2015) Polycyclic Aromatic Hydrocarbons in Superficial Sediments of the Negro River in the Amazon Region of Brazil. *Journal of the Brazilian Chemical Society* 26, 1438–1449. <https://doi.org/10.5935/0103-5053.20150112>
- Stogiannidis E, Laane R (2015) Source Characterization of Polycyclic Aromatic Hydrocarbons by Using Their Molecular Indices: An Overview of Possibilities, in: Whitacre, D.M. (Ed.), *Reviews of Environmental Contamination and Toxicology, Reviews of Environmental Contamination and Toxicology*. Springer International Publishing, Cham, pp. 49–133. https://doi.org/10.1007/978-3-319-10638-0_2
- Sturba L, Fattorini N, Liberatori G, Vannuccini M.L, Nannoni F, Protano G, Tursi A, Corsi I (2020) Multi-model inference analysis of toxicological responses and levels of heavy metals in soft tissue of land snail *Cornu aspersum* caged in proximity to an industrial setting. *Ecological Indicators* 117, 106688. <https://doi.org/10.1016/j.ecolind.2020.106688>
- Szpyrka E, Kurdziel A, Matyaszek A, Podbielska M, Rupar J, Słowik-Borowiec M (2015) Evaluation of pesticide residues in fruits and vegetables from the region of south-eastern Poland. *Food Control, Recent Advances of Food Analysis* 48, 137–142. <https://doi.org/10.1016/j.foodcont.2014.05.039>
- Tan J.-H, Bi X.-H, Duan J.-C, Rahn K.A, Sheng G.-Y, Fu J.-M (2006) Seasonal variation of particulate polycyclic aromatic hydrocarbons associated with PM₁₀ in Guangzhou, China. *Atmospheric Research* 80, 250–262. <https://doi.org/10.1016/j.atmosres.2005.09.004>
- Tobiszewski M, Namieśnik J (2012) PAH diagnostic ratios for the identification of pollution emission sources. *Environ. Pollut.* 162, 110–119. <https://doi.org/10.1016/j.envpol.2011.10.025>
- Tran V.V, Park D, Lee Y.-C (2020) Indoor Air Pollution, Related Human Diseases, and Recent Trends in the Control and Improvement of Indoor Air Quality. *Int J Environ Res Public Health* 17. <https://doi.org/10.3390/ijerph17082927>
- Wang R, Liu G, Sun R, Yousaf B, Wang J, Liu R, Zhang H (2018) Emission characteristics for gaseous- and size-segregated particulate PAHs in coal combustion flue gas from circulating fluidized bed (CFB) boiler. *Environmental Pollution* 238, 581–589. <https://doi.org/10.1016/j.envpol.2018.03.051>
- Wang X.-T, Zhou Y, Hu B.-P, Fu R, Cheng H.-X (2019) Biomonitoring of polycyclic aromatic hydrocarbons and synthetic musk compounds with Masson pine (*Pinus massoniana* L.) needles in Shanghai, China. *Environmental Pollution* 252, 1819–1827. <https://doi.org/10.1016/j.envpol.2019.07.002>
- Wierzbicka A, Dyderski M.K, Kamczyc J, Rączka G, Jagodziński A.M (2019) Responses of soil mite communities (Acari: Oribatida, Mesostigmata) to elemental composition of mosses and pine needles and long-term air pollution in Scots pine (*Pinus sylvestris* L.) stands. *Science of The Total Environment* 691, 284–295. <https://doi.org/10.1016/j.scitotenv.2019.07.138>
- Wu J.-P, Chen X.-Y, Wu S.-K, Tao L, She Y.-Z, Luo X.-J, Mai B.-X (2019) Polychlorinated biphenyls in apple snails from an abandoned e-waste recycling site, 2010–2016: A temporal snapshot after the regulatory efforts and the bioaccumulation characteristics. *Science of The Total Environment* 650, 779–785. <https://doi.org/10.1016/j.scitotenv.2018.09.074>
- Xing X, Mao Y, Hu T, Tian Q, Chen Z, Liao T, Zhang Z, Zhang J, Gu Y, Qi S (2020) Spatial distribution, possible sources and health risks of PAHs and OCPs in surface soils from Dajiuhe Sub-alpine Wetland, central China. *Journal of Geochemical Exploration* 208, 106393. <https://doi.org/10.1016/j.gexplo.2019.106393>
- Yu H.-Y, Li F.-B, Yu W.-M, Li Y.-T, Yang G.-Y, Zhou S.-G, Zhang T.-B, Gao Y.-X, Wan H.-F (2013) Assessment of organochlorine pesticide contamination in relation to soil properties in the Pearl River Delta, China. *Science of The Total Environment* 447, 160–168. <https://doi.org/10.1016/j.scitotenv.2012.12.070>
- Zhang W, Zhang S, Wan C, Yue D, Ye Y, Wang X (2008) Source diagnostics of polycyclic aromatic hydrocarbons in urban road runoff, dust, rain and canopy throughfall. *Environmental Pollution* 153, 594–601. <https://doi.org/10.1016/j.envpol.2007.09.004>
- Zhang X, Wang X, Luo F, Sheng H, Zhou L, Zhong Q, Lou Z, Sun H, Yang M, Cui X, Chen Z (2019) Application and enantioselective residue determination of chiral pesticide penconazole in grape, tea, aquatic vegetables and soil by ultra performance liquid chromatography-tandem mass spectrometry. *Ecotoxicology and Environmental Safety* 172, 530–537. <https://doi.org/10.1016/j.ecoenv.2019.01.103>
- Zhao L, Li Y, Ren W, Huang Y, Wang X, Fu Z, Ma W, Teng Y, Luo Y (2020) Pesticide residues in soils planted with *Panax notoginseng* in south China, and their relationships in *Panax notoginseng* and soil. *Ecotoxicology and Environmental Safety* 201, 110783. <https://doi.org/10.1016/j.ecoenv.2020.110783>
- Zheng L, Ou J, Liu M, Chen Y, Tang Q, Hu Y (2019) Seasonal and spatial variations of PM₁₀-bounded PAHs in a coal mining city, China: Distributions, sources, and health risks. *Ecotoxicology and Environmental Safety* 169, 470–478. <https://doi.org/10.1016/j.ecoenv.2018.11.063>

Supplementary Information (SI)

Supplementary Information (SI-1.1)



Fig. S1 Geographic location of the seven sampling sites

Kits for QuEChERS (EN 1566 method): 0.5g disodium hydrogen citrate sesquihydrate, 1g trisodium citrate dihydrate, 1g sodium chloride, 4g magnesium sulfate.

Dispersive solid phase extraction clean-up kits (AOAC 2007.01 method): 1.2g MgSO_4 , 400 mg primary secondary amine, 400 mg graphitized carbon black, 400 mg octadecyl.

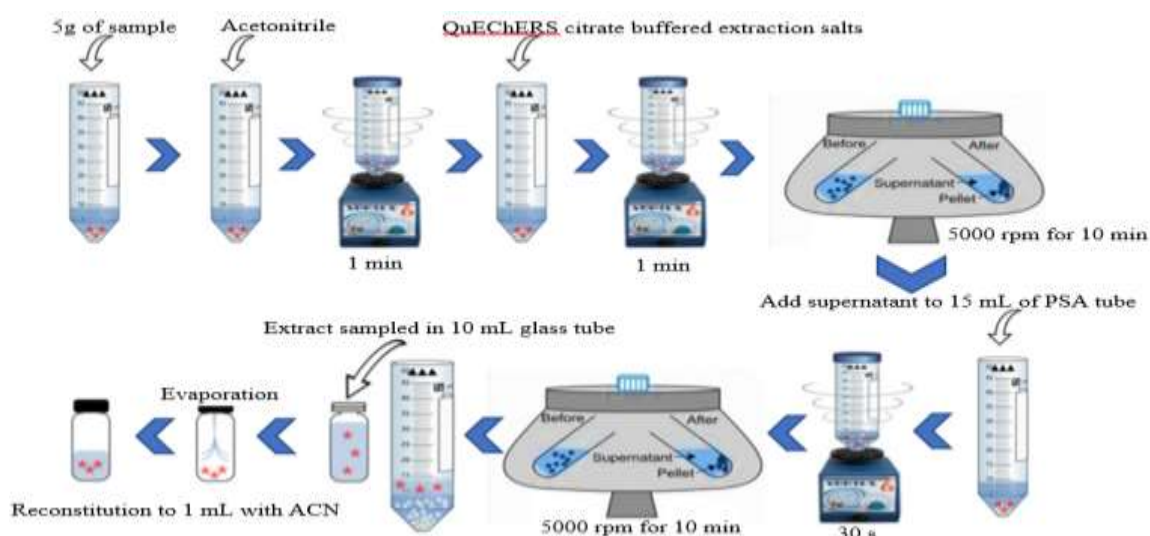


Fig. S2 Schematic representation of the QuEChERS procedure used for the analysis of pesticides and polycyclic aromatic hydrocarbons

Supplementary Information (SI-1.2)

Part I: Sample analysis: Chapter II-II - Supplementary Materials - Table 1, 2 and 3.

Part II: Snails '*Helix aspersa*' : Chapter II-II - Supplementary Materials - Table 4, 5 and 6.

Part III: Pine needles '*Pinus nigra*' : Chapter II-III - Supplementary Materials S2 - Table 1, 2 and 3.

Supplementary Information (SI-1.3)

Table S10. Variation of pesticides and polycyclic aromatic hydrocarbons concentrations in Tannourine

	July	August	September	October	November	December	July	August	September	October	November	December
	Snails						Pine needles					
Carbendazim	0	0	0	0	0	0	0	0	0	0	0	0
Diflufenican	0	0	0	0	0	0	0	0	0	0	0	0
Diuron	0	0	0	0	0	0	0	0	0	0	0	0
Penconazole	0	0	0	0	0	0	0	0	0	0	0	0
Pendimethalin	0	0	0	0	0	0	0	0	0	0	0	0
Fenpropidin	0	0	0	0	0	0	0	0	0	0	0	0
Acetochlor	0	0	0	0	0	0	0	0	0	0	0	0
Chlorpropham	0	0	0	0	0	0	0	0	0	0	0	0
Clofentezine	0	0	0	0	0	0.164	0.866	1.032	1.316	0.97	0.566	0.272
Deltamethrin	0	0	0	0	0	0	0	0	0	0	0	0
Diclobenil	0	0	0	0	0	0	0	0	0	0	0	0
Diphenylamine	0	0	0	0	0	0	0	0	0	0	0	0
Ethofumesate	0	0	0	0	0	0	0	0	0	0	0	0
Kresoxim-methyl	0	0	0	0	0	0	0	0	0	0	0	0
Lambda-cyhalothrin	0	0	0	0	0	0	0	0	0	0	0	0
Pyraclostrobin	0	0	0	0	0	0	0.182	0.192	0.226	0.166	0.102	0.086
Heptachlor	0	0	0	0	0	0	0	0	0	0	0	0
Hexachlorobenzene	0	0	0	0	0	0	0	0	0	0	0	0
α-Endosulfan	0	0	0	0	0	0	0	0	0	0	0	0
Total Pesticides:	0	0	0	0	0	0.164	1.048	1.224	1.542	1.136	0.668	0.358
Naphthalene	0.564	0.658	0.982	0.904	1.086	1.22	1.46	1.926	1.498	1.47	1.098	1.028
Acenaphthene	0.172	0.354	0.396	0.742	0.816	0.996	1.542	1.964	1.832	1.688	1.204	1.254
Fluorene	0	0	0	0	0	0	0	0	0	0	0	0
Phenanthrene	0	0	0	0	0	0	0	0	0	0	0	0
Anthracene	0	0	0.072	0.322	0.392	0.52	0.69	0.798	0.822	0.864	0.384	0.188
Pyrene	0.132	0.25	0.382	0.55	0.57	0.476	0.99	1.03	0.964	1.032	0.582	0.254
Benz[a]anthracene	0	0	0	0	0	0	0	0	0	0	0	0
Benzo[e]pyrene	0	0	0	0	0	0	0	0	0	0	0	0
Benzo(k)fluoranthene	0	0	0	0	0	0	0	0	0	0	0	0
Chrysene	0	0	0	0	0	0	0	0	0	0	0	0
Fluoranthene	0.228	0.35	0.476	0.42	0.636	0.704	1.254	1.408	1.226	1.316	0.762	0.796
Total PAHs:	1.096	1.612	2.308	2.938	3.5	3.916	5.936	7.126	6.342	6.37	4.03	3.52

Table S11. Variation of pesticides and polycyclic aromatic hydrocarbons concentrations in Abdine

	July	August	September	October	November	December	July	August	September	October	November	December
	Snails						Pine needles					
Carbendazim	0	0	0	0	0	0	0	0	0	0	0	0
Diflufenican	0	0	0.062	0.124	0.388	0.504	2.954	2.92	2.68	3.054	1.06	0.502
Diuron	0	0	0	0	0	0	1.006	0.89	0.762	0.824	0.724	0.21
Penconazole	0	0	0	0	0	0	0.388	0.438	0.57	0.61	0.446	0.426
Pendimethalin	0	0	0	0	0	0	0	0	0	0	0	0
Fenpropidin	0	0	0.182	0.248	0.402	0.718	3.328	3.136	3.052	3.068	1.018	0.586
Acetochlor	0	0	0	0	0	0	1.378	1.318	1.424	1.344	0.494	0.366
Chlorpropham	0	0	0	0	0	0	0	0	0	0	0	0
Clofentezine	0	0	0	0	0.276	0.546	2.55	3.112	3.328	3.534	1.592	0.656
Deltamethrin	0	0.126	0.214	0	0.566	0.718	2.692	2.67	2.528	2.836	0.882	0.408
Diclobenil	0	0	0	0	0	0	0	0	0	0	0	0
Diphenylamine	0	0	0	0	0	0	0	0	0	0	0	0
Ethofumesate	0	0	0	0	0	0	0	0	0	0	0	0
Kresoxim-methyl	0	0	0	0	0	0	0	0	0	0	0	0
Lambda-cyhalothrin	0	0	0	0	0.12	0.24	1.538	1.23	1.984	1.564	0.594	0.352
Pyraclostrobin	0	0	0	0	0	0.146	0.21	0.232	0.188	0.208	0.112	0.076
Heptachlor	0	0	0	0	0	0	0	0	0	0	0	0
Hexachlorobenzene	0	0	0.038	0.102	0.144	0.384	0.918	0.794	0.544	0.482	0.23	0.192
α -Endosulfan	0	0	0	0	0	0	0	0	0	0	0	0
Total Pesticides:	0	0.126	0.496	0.474	1.896	3.256	16.962	16.74	17.06	17.524	7.152	3.774
Naphthalene	0.502	0.656	0.838	0.986	1.14	1.384	2.08	2.46	2.324	2.204	1.328	1.242
Acenaphthene	0.994	0.848	1.196	1.112	1.426	1.508	1.93	2.57	2.346	2.536	1.64	1.704
Fluorene	0.348	0.724	0.716	0.846	1.756	1.826	2.232	2.914	2.712	2.778	1.852	1.786
Phenanthrene	0.156	0.234	0.366	0.428	0.544	0.546	0.76	0.836	0.99	0.818	0.654	0.368
Anthracene	0.37	0.428	0.712	0.782	0.91	1.144	1.388	1.046	1.362	1.268	0.98	0.522
Pyrene	0.29	0.224	0.352	0.572	0.43	0.656	1.206	1.586	1.672	1.51	0.836	0.594
Benz[a]anthracene	0.13	0.23	0.36	0.378	0.596	0.632	0.832	0.862	0.758	0.792	0.504	0.536
Benzo[e]pyrene	0	0	0	0	0	0	0	0	0	0	0	0
Benzo(k)fluoranthene	0	0	0	0	0	0	0	0	0	0	0	0
Chrysene	0.156	0.178	0.32	0.386	0.384	0.428	0.636	0.702	0.736	0.802	0.568	0.246
Fluoranthene	0.334	0.298	0.616	0.648	0.79	0.824	1.034	1.456	1.494	1.092	0.816	0.842
Total PAHs:	3.28	3.82	5.476	6.138	7.976	8.948	12.098	14.432	14.394	13.8	9.178	7.84

Table S12. Variation of pesticides and polycyclic aromatic hydrocarbons concentrations in Araa

	July	August	September	October	November	December	July	August	September	October	November	December
	Snails						Pine needles					
Carbendazim	0	0	0	0	0	0	9.232	9.502	8.308	7.602	6.086	4.65
Diflufenican	0	0.364	0.542	0.614	0.744	1.03	4.28	4.108	3.666	3.888	3.06	2.52
Diuron	0	0.126	0.256	0.428	0.496	0.702	1.45	1.486	1.342	1.386	0.93	0.412
Penconazole	0.368	0.536	0.624	0.656	0.952	0.856	2.644	2.294	2.516	2.386	1.87	1.288

Pendimethalin	0.508	0.596	0.634	0.856	0.894	1.368	3.48	3.568	3.124	3.308	2.52	1.846
Fenpropidin	0.12	0.526	0.55	0.29	0.426	0.652	13.876	13.082	14.606	14.054	8.082	6.354
Acetochlor	0	0.184	0	0.128	0.376	0.634	4.258	4.414	4.114	4.62	3.422	2.71
Chlorpropham	0.42	0	0.412	0	0.668	0	4.232	4.04	4.68	4.37	2.212	1.814
Clofentezine	0.256	1.186	1.446	2.15	2.856	3.704	4.974	5.508	5.898	4.616	3.704	3.05
Deltamethrin	0	0	0.414	0.658	1.436	1.366	8.288	9.48	10.066	7.138	5.934	3.848
Diclobenil	0.14	0.228	0.24	0.34	0.564	0.64	2.15	1.844	2.366	2.212	1.59	0.952
Diphenylamine	0.18	1.04	1.78	0.82	2.24	1.808	3.904	4.252	4.554	4.174	2.942	2.146
Ethofumesate	0	0.388	0.64	0	0.7	0.96	5.848	5.96	4.854	5.306	3.206	2.726
Kresoxim-methyl	0.74	1.44	1.18	0.94	1.22	1.26	8.046	7.692	7.328	7.746	5.29	3.828
Lambda-cyhalothrin	0.4	0.64	0.156	0.596	0.7	0.96	5.972	5.624	5.296	5.582	4.172	2.594
Pyraclostrobin	0	0	0	0	0.164	0.226	0.384	0.236	0.418	0.348	0.044	0.038
Heptachlor	0.368	0.572	0.68	0.84	1.064	0.938	5.076	5.628	4.686	4.934	3.65	2.384
Hexachlorobenzene	0.72	0.984	0.834	1.06	1.364	1.722	15.732	14.718	16.314	17.178	11.482	9.122
α -Endosulfan	0.568	0.796	0.562	0	0.946	1.138	19.346	15.698	16.93	15.312	15.172	11.748
Total Pesticides:	4.788	9.606	10.95	10.376	17.81	19.964	123.172	119.134	121.066	116.16	85.368	64.03
Naphthalene	0.594	0.432	0.706	0.876	0.814	0.84	0.824	1.282	1.094	1.4	0.766	0.708
Acenaphthene	0.106	0.192	0.32	0.202	0.248	0.326	0.342	0.624	0.694	0.804	0.34	0.226
Fluorene	0.872	1.398	1.476	1.862	2.47	3.296	5.008	4.902	5.674	5.944	3.184	3.046
Phenanthrene	1.274	2.176	3.24	4.36	5.09	5.614	10.014	9.24	10.16	9.264	4.562	3.274
Anthracene	2.006	2.57	3.806	4.476	5.156	5.734	8.37	10.02	9.142	9.746	3.94	2.744
Pyrene	0.744	0.924	1.232	1.758	2.136	2.344	3.16	3.494	3.928	3.822	1.64	1.928
Benz[a]anthracene	0.962	1.876	2.286	3.254	4.304	5.016	7.78	7.25	7.486	8.25	4.108	3.608
Benzo[e]pyrene	3.804	5.274	5.342	6.93	7.984	8.826	29.044	31.674	32.408	27.978	10.054	7.962
Benzo(k)fluoranthene	0.546	0.836	1.134	1.498	1.794	2.118	2.56	2.832	2.664	2.852	1.964	0.638
Chrysene	0.068	0.158	0.322	0.378	0.462	0.59	0.742	0.794	0.984	1.024	0.258	0.15
Fluoranthene	1.792	2.1	2.93	2.592	3.422	3.464	4.68	5.008	5.146	5.324	1.626	1.52
Total PAHs:	12.768	17.936	22.794	28.186	33.88	38.168	72.524	77.12	79.38	76.408	32.442	25.804

Table S13. Variation of pesticides and polycyclic aromatic hydrocarbons concentrations in Beit Hajj

	July	August	September	October	November	December	July	August	September	October	November	December
	Snails						Pine needles					
Carbendazim	0	0	0	0	0	0	12.81	14.304	16.688	13.85	9.44	6.366
Diflufenican	0	0	0	0	0	0	4.474	4.79	3.134	4.686	2.942	1.774
Diuron	0	0	0.078	0.112	0.164	0.434	1.462	1.094	1.056	1.384	0.876	0.548
Penconazole	0	0	0	0	0	0.722	2.01	2.098	1.898	2.328	1.88	1.23
Pendimethalin	0.552	0.562	0.736	1.046	1.362	1.498	3.364	3.316	3.646	3.25	2.094	2.462
Fenpropidin	0	0.182	0	0.484	0.508	0.806	15.454	16.502	15.04	14.098	10.37	7.802
Acetochlor	0.194	0.332	0.484	0.536	0.59	0.734	5.566	5.048	6.09	5.432	3.302	2.554
Chlorpropham	0.262	0.294	0.366	0	0.648	0.748	6.48	6.36	6.94	5.948	4.26	3.248
Clofentezine	1.504	2.128	3.284	4.162	3.504	4.346	9.502	7.832	8.534	8.126	5.866	4.156
Deltamethrin	0	0.92	0.638	0.702	1.308	1.164	6.296	5.638	7.15	9.83	5.726	4.058
Diclobenil	0	0.236	0.374	0.48	0.674	0.9	1.278	1.378	1.51	1.008	0.742	0.406

Diphenylamine	0.14	0.5	0.296	0.438	0.744	0.862	2.868	3.07	2.65	2.776	2.05	1.596
Ethofumesate	0	0	0	0	0	0	4.488	3.948	3.696	3.74	2.502	1.812
Kresoxim-methyl	1.64	2.26	1.62	1.536	1.9	1.698	5.516	5.102	5.954	5.56	2.406	2.884
Lambda-cyhalothrin	0	0	0.26	0.48	0.64	0	6.836	7.356	6.57	6.874	4.428	2.738
Pyraclostrobin	0	0	0	0	0.122	0.258	0.346	0.388	0.298	0.506	0.164	0.126
Heptachlor	0	0	0.128	0.254	0.572	0.84	5.034	4.104	5.226	4.57	2.408	2.048
Hexachlorobenzene	0.436	0.57	0.806	0.744	1.122	1.476	12.83	12.286	11.212	14.09	8.59	7.628
α -Endosulfan	0.254	0.75	0.774	0.984	1.03	1.502	17.59	18.128	19.1	15.088	11.942	11.356
Total Pesticides:	4.982	8.734	9.844	11.958	14.888	17.988	124.204	122.742	126.392	123.144	81.988	64.792
Naphthalene	0.34	0.508	0.564	0.674	0.946	0.824	1.24	1.824	1.876	1.782	1.384	1.006
Acenaphthene	0.204	0.19	0.264	0.366	0.422	0.548	0.57	0.852	1.01	0.912	0.628	0.61
Fluorene	1.076	1.572	1.706	2.016	2.392	2.92	4.462	5.586	5.438	5.592	2.742	2.848
Phenanthrene	0.858	1.936	2.472	3.67	4.166	4.428	10.368	10.472	11.276	9.886	5.396	3.828
Anthracene	2.284	2.644	3.522	4.238	4.774	5.188	7.33	8.342	8.898	8.252	5.14	3.42
Pyrene	0.422	0.952	1.062	1.68	1.782	1.744	2.848	3.004	3.366	3.556	2.46	1.964
Benz[a]anthracene	0.616	0.958	2.364	3.606	2.984	3.612	6.08	7.24	7.15	7.4	5.832	4.992
Benzo[e]pyrene	2.284	3.384	4.712	5.796	6.57	7.754	34.428	31.87	34.29	33.306	19.928	17.054
Benzo(k)fluoranthene	0.934	1.054	1.584	1.694	2.076	2.462	3.426	3.526	3.866	3.358	1.526	1.784
Chrysene	0.15	0.21	0.336	0.306	0.346	0.438	0.59	0.658	0.634	0.87	0.568	0.35
Fluoranthene	1.04	2.118	2.29	3.208	3.93	4.278	3.712	4.482	5.124	4.494	3.112	2.772
Total PAHs:	10.208	15.526	20.876	27.254	30.388	34.196	75.054	77.856	82.928	79.408	48.716	40.628

Table S14. Variation of pesticides and polycyclic aromatic hydrocarbons concentrations in Beshmezzine

	July	August	September	October	November	December	July	August	September	October	November	December
	Snails						Pine needles					
Carbendazim	0	0	0	0	0	0	7.952	9.178	10.036	9.49	4.756	3.42
Diflufenican	0.374	0.318	0.474	0.964	1.442	1.246	14.246	13.914	13.068	14.36	11.234	6.94
Diuron	0	0	0	0	0	0	2.058	2.186	1.896	2.176	1.376	0.804
Penconazole	0	0	0	0	0	0	4.43	4.928	5.256	4.782	3.208	2.352
Pendimethalin	0	0	0	0	0	0.55	0.984	0.892	0.858	0.906	0.312	0.256
Fenpropidin	0	0.35	0.422	0	0.504	0.578	8.224	7.858	7.324	7.582	3.246	1.972
Acetochlor	0	0	0.122	0.216	0.258	0.588	5.27	5.036	4.694	4.858	3.622	2.41
Chlorpropham	0	0.106	0.18	0.126	0.22	0.3	1.296	1.942	1.06	1.62	0.52	0.22
Clofentezine	0	0.42	0.588	1.368	1.584	1.73	1.39	1.846	1.96	1.614	0.766	0.406
Deltamethrin	0	0.74	0.96	1.248	1.4	1.72	10.772	14.306	15.192	12.508	5.738	1.972
Diclobenil	0	0	0	0	0.32	0.46	0.654	0.982	0.536	0.818	0.226	0.11
Diphenylamine	0.118	0.074	0.116	0.294	0.434	0.782	2.074	1.692	1.378	1.66	0.91	0.774
Ethofumesate	0	0.206	0.292	0	0.652	0.962	2.692	2.516	2.324	2.434	1.686	1.468
Kresoxim-methyl	0.1	0.076	0.286	0.528	0.98	1.54	4.424	4.972	4.676	4.714	3.044	2.134
Lambda-cyhalothrin	0.18	0.212	0.28	0	0.634	1.022	9.85	10.634	9.702	10.898	5.282	3.278
Pyraclostrobin	0	0	0	0	0	0	0.292	0.374	0.234	0.436	0.206	0.216
Heptachlor	0	0	0	0	0.368	0.742	2.012	1.596	1.708	1.596	0.698	0.862

Hexachlorobenzene	0	0	0	0	0	0	7.23	7.458	6.344	7.704	2.752	2.526
α -Endosulfan	0	0	0	0	0	0	0	0	0	0	0	0
Total Pesticides:	0.772	2.502	3.72	4.744	8.796	12.22	85.85	92.31	88.246	90.156	49.582	32.12
Naphthalene	0.61	0.776	0.986	1.052	1.348	1.306	1.896	2.278	2.408	2.274	1.562	1.214
Acenaphthene	0.152	0.224	0.27	0.374	0.354	0.378	0.334	0.438	0.628	0.676	0.278	0.19
Fluorene	0.168	0.328	0.384	0.528	0.42	0.546	4.128	4.834	4.772	4.648	4.012	3.184
Phenanthrene	0.328	0.384	0.46	0.686	0.7	0.98	1.38	1.26	1.486	1.714	0.728	0.604
Anthracene	0.194	0.234	0.406	0.55	0.484	0.566	1.756	1.454	1.89	1.664	0.928	0.54
Pyrene	0.136	0.296	0.33	0.366	0.522	0.618	0.894	1.084	0.834	1.196	0.59	0.238
Benz[a]anthracene	0.104	0.048	0.178	0.276	0.394	0.334	0.504	0.76	1.04	1.028	0.366	0.194
Benzo[e]pyrene	0	0	0	0	0	0	0	0	0	0	0	0
Benzo(k)fluoranthene	0.106	0.092	0.29	0.272	0.386	0.346	0.82	0.832	1.056	1.004	0.728	0.388
Chrysene	0.232	0.368	0.33	0.392	0.508	0.596	0.294	0.294	0.59	0.622	0.184	0.162
Fluoranthene	0.348	0.408	0.554	0.562	0.614	0.946	1.196	1.466	1.256	1.76	0.62	0.708
Total PAHs:	2.378	3.158	4.188	5.058	5.73	6.616	13.202	14.7	15.96	16.586	9.996	7.422

Table S15. Variation of pesticides and polycyclic aromatic hydrocarbons concentrations in Haykaliyeh

	July	August	September	October	November	December	July	August	September	October	November	December
	Snails						Pine needles					
Carbendazim	0.414	0.286	0.546	0.856	1.484	1.446	4.026	3.24	3.7	4.474	3.354	1.974
Diflufenican	0.204	0.312	0.448	0.572	0.63	0.752	10.776	10.548	9.93	10.842	7.85	5.62
Diuron	0.582	0.612	0.82	0.988	1.426	1.484	2.03	1.604	2.03	2.054	1.104	0.582
Penconazole	0.12	0.076	0.268	0.492	1.146	1.41	4.008	4.256	3.942	4.3	3.362	2.51
Pendimethalin	0.212	0.364	0.472	0.588	0.974	0.848	0.684	0.95	0.66	0.864	0.336	0.216
Fenpropidin	0	0	0.16	0.23	0.374	0.498	7.966	8.784	9.612	8.572	6.374	4.438
Acetochlor	0	0	0	0	0	0	3.07	3.364	3.68	3.284	2.74	1.968
Chlorpropham	0.17	0.258	0.316	0.53	0.654	0.964	3.16	2.58	2.1	2.53	1.746	1.16
Clofentezine	0.232	0.366	0.338	0	0.584	0.75	1.098	1.226	1.47	1.914	0.638	0.266
Deltamethrin	0.3	0.614	0	0.5	1.12	1.96	11.818	14.024	13.078	12.262	9.004	5.862
Diclobenil	0	0	0	0	0.346	0.498	1.598	1.304	1.062	1.28	0.238	0.282
Diphenylamine	0	0	0	0	0.36	0.64	0.85	1.274	0.956	1.062	0.54	0.344
Ethofumesate	0	0	0	0	0	0	1.758	1.772	1.898	1.626	0.816	0.944
Kresoxim-methyl	0	0.32	0.46	0.52	1.008	1.3	4.728	5.228	4.95	4.782	3.258	2.272
Lambda-cyhalothrin	0.022	0.328	0.278	0.508	0.74	0.98	11.438	9.708	8.57	9.738	7.148	5.444
Pyraclostrobin	0	0	0	0	0	0.074	0.344	0.272	0.392	0.292	0.136	0.168
Heptachlor	0	0	0	0	0	0	1.276	1.864	1.564	1.956	1.024	0.772
Hexachlorobenzene	0	0	0	0	0	0	4.628	4.144	5.376	3.986	2.096	1.766
α -Endosulfan	0.328	0.472	0.358	0.592	0.97	1.52	7.158	6.224	7.842	6.05	3.852	2.494
Total Pesticides:	2.584	4.008	4.464	6.376	11.816	15.124	82.414	82.366	82.812	81.868	55.616	39.082
Naphthalene	0.542	0.776	1.07	1.208	1.416	1.504	2.36	2.83	2.782	2.824	1.568	1.404
Acenaphthene	0.206	0.276	0.38	0.45	0.462	0.512	0.388	0.472	0.75	0.54	0.258	0.248
Fluorene	0.212	0.392	0.472	0.404	0.628	0.784	3.206	3.744	3.872	3.61	2.456	2.75

Phenanthrene	0.566	0.574	0.464	0.638	0.91	1.004	0.9	1.184	1.438	1.278	0.566	0.658
Anthracene	0.182	0.212	0.382	0.398	0.434	0.49	1.728	1.872	2.486	2.248	1.424	1.196
Pyrene	0.246	0.296	0.248	0.556	0.59	0.706	0.654	1.044	1.136	0.962	0.566	0.126
Benz[a]anthracene	0.148	0.17	0.194	0.236	0.378	0.512	0.586	0.69	0.962	1.34	0.61	0.294
Benzo[e]pyrene	0	0	0	0	0	0	0	0	0	0	0	0
Benzo(k)fluoranthene	0.138	0.162	0.188	0.178	0.294	0.308	0.56	0.742	0.798	0.844	0.682	0.764
Chrysene	0.17	0.346	0.374	0.524	0.642	0.794	0.41	0.378	0.546	0.696	0.188	0.342
Fluoranthene	0.254	0.368	0.352	0.502	0.624	0.722	0.806	0.95	1.224	1.204	0.568	0.454
Total PAHs:	2.664	3.572	4.124	5.094	6.378	7.336	11.598	13.906	15.994	15.546	8.886	8.236

Table S16. Variation of pesticides and polycyclic aromatic hydrocarbons concentrations in Abi Samra

	July	August	September	October	November	December	July	August	September	October	November	December
	Snails						Pine needles					
Carbendazim	0	0	0	0	0	0	10.244	9.422	9.26	10.824	6.998	5.004
Diflufenican	0.548	0.456	0.614	0.92	0.856	1.564	6.656	6.276	5.912	6.594	2.976	2.386
Diuron	0	0	0	0	0.146	0.25	0.844	0.922	0.78	0.762	0.246	0.162
Penconazole	0	0	0	0	0	0	0	0	0	0	0	0
Pendimethalin	0	0.328	0.434	0.764	0.742	1.188	2.87	2.498	2.964	2.834	1.632	1.162
Fenpropidin	0	0.142	0	0.296	0.48	0.552	5.142	4.532	5.378	5.206	3.872	2.582
Acetochlor	0	0	0.206	0.216	0.248	0.294	0.948	0.846	1.052	0.768	0.276	0.592
Chlorpropham	0.268	0.396	0.348	0	0.474	1.166	3.96	3.02	3.56	2.86	2.006	1.676
Clofentezine	0	0	0	0	0	0	5.732	5.724	7.424	6.31	0.958	0.712
Deltamethrin	0	0	0.192	0.214	0.56	1.9	16.942	17.36	18.154	15.744	5.086	1.604
Diclobenil	0	0	0	0	0.3	0.56	2.002	1.526	1.802	1.246	0.71	0.414
Diphenylamine	0	0	0.26	0.456	0.76	0.822	2.598	1.982	2.32	1.876	1.322	0.912
Ethofumesate	0.554	1.188	1.502	1.678	2.724	3.304	3.964	3.724	3.528	3.728	1.522	1.286
Kresoxim-methyl	1.44	2.12	2.36	2.74	2.6	2.9	2.868	3.23	3.122	2.91	1.854	1.498
Lambda-cyhalothrin	0	0	0	0	0	0	16.684	13.766	14.904	14.186	5.348	3.69
Pyraclostrobin	0	0	0	0	0	0	0.568	0.63	0.458	0.592	0.158	0.134
Heptachlor	0	0	0	0	0	0	5.274	4.788	4.55	3.898	3.796	2.328
Hexachlorobenzene	0	0.77	0.944	-	1.84	2.166	18.014	18.724	17.508	16.816	13.928	9.624
α -Endosulfan	0	0	0	0	0	0	0	0	0	0	0	0
Total Pesticides:	2.81	5.4	6.86	7.284	11.73	16.666	105.31	98.97	102.676	97.154	52.688	35.766
Naphthalene	0.374	0.56	0.854	1.006	1.08	1.18	1.26	1.856	1.672	1.894	1.208	1.19
Acenaphthene	0.142	0.302	0.396	0.402	0.598	0.48	0.342	0.602	0.996	0.716	0.398	0.28
Fluorene	1.912	1.976	2.314	2.572	3.276	3.812	8.384	8.764	9.004	8.726	6.04	5.162
Phenanthrene	2.574	3.254	2.722	4.36	4.872	5.294	10.46	11.162	11.834	10.802	6.41	5.476
Anthracene	0.944	1.694	2.592	3.79	4.318	4.698	8.96	9.81	10.236	9.136	7.068	4.858
Pyrene	2.136	4.292	4.624	3.712	5.95	6.528	13.086	14.068	13.288	12.632	9.026	6.17
Benz[a]anthracene	0.718	0.846	0.904	1.254	1.458	1.716	2.434	2.346	2.306	3.078	2.186	1.906
Benzo[e]pyrene	1.452	2.122	2.924	3.714	4.294	5.224	13.004	14.036	13.542	13.712	10.47	9.656
Benzo(k)fluoranthene	0.544	0.614	0.764	0.902	1.022	1.29	1.92	1.896	1.944	2.424	1.446	1.142

Chrysene	0.944	1.148	1.178	1.23	1.626	1.892	2.56	2.476	2.39	2.308	1.256	1.368
Fluoranthene	5.222	6.49	9.81	10.448	12.206	12.038	17.278	18.082	17.154	16.084	11.946	10.486
Total PAHs:	16.962	23.298	29.082	33.39	40.7	44.152	79.688	85.098	84.366	81.512	57.454	47.694

Table S17. P-value for concentrations in samples with temperature and precipitation of the sampling sites

		Tannourine	Abdine	Araa	Beit Hajj	Beshmezzine	Haykaliyeh	Abi Samra
Pine needle-	Temperature	0.03	0.01	0.007	0.007	0.008	0.009	0.002
Pesticides	Precipitation	0.008	0.0008	0.01	0.02	0.03	0.01	0.03
Pine needle-	Temperature	0.01	0.02	0.006	0.008	0.04	0.06	0.003
PAHs	Precipitation	0.002	0.006	0.05	0.04	0.04	0.13	0.03
Snail-	Temperature	0.06	0.002	0.04	0.03	0.08	0.05	0.01
Pesticides	Precipitation	0.06	0.0001	0.005	0.03	0.003	0.03	0.02
Snail-PAHs	Temperature	0.004	0.0005	0.02	0.04	0.02	0.01	0.01
	Precipitation	0.03	0.01	0.003	0.004	0.002	0.08	0.001

VI. Liquid-liquid based extraction for multiresidue determination of non-volatile pesticides in honey as environmental biomonitors

Résumé

Ce travail, présenté sous format d'un article soumis dans « *Journal of Environmental Science and Health, Part B* » contribue au développement d'une méthode multi-résidus dans le but d'analyser les pesticides non volatiles à partir du miel. L'efficacité de cette matrice dans la biosurveillance environnementale a été démontrée dans de nombreuses études en raison de ses propriétés médicinales.

En effet, en tant qu'indicateurs biologiques, les abeilles et leurs produits de la ruche peuvent grandement contribuer à la procédure de biosurveillance environnementale. Le miel largement utilisé à des fins thérapeutiques et nutritionnelles, est sujet à divers types de contamination. La contamination indirecte du miel par l'air, l'eau, le sol et les fleurs peut se produire lors de l'application de pesticides en agriculture pendant l'activité quotidienne de butinage des abeilles domestiques. Ainsi, une méthode multi-résidus basée sur l'extraction liquide-liquide à l'aide d'acétate d'éthyle suivie d'une analyse par LC-MS/MS pour l'analyse simultanée de 32 pesticides a été développée. Les résultats ont montré des écarts-types relatifs intra-journalier et inter-journalier inférieurs à 5%, avec des taux de récupérations compris entre 68 et 104%. En outre, la méthode a montré une précision et une sensibilité élevées pour tous les pesticides analysés, avec des LOD et des LOQ respectivement inférieures à 3 et 9 ng g⁻¹.

En conclusion, la méthode analytique développée nous permet de séparer et d'analyser les résidus de pesticides par une méthode rapide, simple, efficace et impliquant la préparation directe de la matrice de miel avec un solvant non miscible à l'eau. Les résultats des échantillons réels de miel collectés au Liban montrent le potentiel du miel en tant que biomoniteur pour évaluer la pollution de l'air.

Highlights

- Application of liquid-liquid extraction for the analysis of pesticides in honey coupled to LC-MS/MS.
- Liquid-liquid extraction was shown to be a reliable extraction procedure for the multi-residue's analysis of organic pollutants in honey.
- Honey is suggested to be effective biomonitors to evaluate atmospheric contamination in Lebanon.

Abstract

In recent years, bees' products (nectar, honey, beeswax, pollen) are considered as potential biomonitoring of air pollution. The efficacy of these matrices in environmental biomonitoring especially honey has been demonstrated in many studies due to its medicinal properties. A multi residue method based on liquid-liquid extraction using ethyl acetate followed by an analysis using liquid chromatography coupled to tandem mass spectrometry for the analysis of pesticides, was developed in this paper. Afterwards, the method was validated, and results showed that the intra-day and inter-day relative standard deviation analysis was below 5 %, and the recoveries obtained were generally ranged from 68 to 104 %. Furthermore, the method showed high precision and sensitivity for all target compounds, with detection and quantification limits lower 3 and 9 ng g⁻¹ respectively. Finally, the results of real honey samples collected from Lebanon show the potentiality of honey as a biomonitor for assessing air pollution.

Keywords: Honey; Biomonitoring; Organic pollutants; Sample extraction; Liquid chromatography-tandem mass spectrometry.

Introduction

Industrialization, transportation, agricultural practices and increasing population have contributed to pollution of the global environment with changes in its compositional and structural that have negatively impacted biodiversity, leading in behavioral damage and physiological to living organisms, such as bees. [1, 2]

Biomonitoring, a valuable tool for assessing air pollution which has gained increased attention, is defined as the detection of pollutants in the air by its effects on ecosystems and organisms. The air quality bioindication is the use of bioindicators (lichens, mosses, insects, etc....) that provide quantitative information on the contamination of the air and can evaluate directly the environmental impacts of pollutants. These study organisms also make it possible to control their spatio-temporal distribution. [3, 4]

In fact, as biological indicators, honeybees and their products can highly contribute to environmental biomonitoring procedure. [5] Although bee populations are increasing worldwide, multiple factors such as climate change, poisoning by chemical compounds, reduced flower diversity and infection with pathogens have caused colony losses. [6, 7] Bees are essential pollinators for worldwide agriculture and have been widely considered as biomonitors of pollutants from the air. [8] During their foraging activities, these organisms are exposed to pollutants, including pesticides, metals associated with particles of various sizes in the air, soil, vegetation and water. [9, 10]

Contaminants are transferred to the hives and can also be presented in apiary products including wax and honey. [11] In fact, honey widely used for therapeutic and nutritional purposes, is subject to various types of contamination. The indirect contamination of honey by air, water, soil and flowers may occur during pesticide application in agriculture during bees foraging activities. [12] Therefore, pesticides can be transferred into the hive where they can result to a high mortality level among bees and contaminate the honey unsuitable for human consumption. [13, 14] Recent surveys show that bees are highly exposed to pesticides used in crops. [15]

Organophosphate insecticides, pyrethroids and fungicides are the most common agrochemical residues collected by bees from treated crops. [16] Like any pollutant, the exposure of bees to sublethal doses of pesticides over long periods of time has the

potential to harm their immune system, making them much more sensitive to parasitic fungi and other pathogens and may also affect their products. [17, 18]

Several extraction methods have been used to investigate the contamination of honey, such as supercritical fluid extraction, [19] solid-phase extraction, [20] liquid-liquid extraction, [21] matrix solid phase dispersion, [22] pressurized solvent extraction [23] and QuEChERS. [24] Among all these currently used extraction procedures, LLE extraction is one of the oldest methods and most commonly used for the qualitative and quantitative survey of honey pesticides. [25]

For those reasons, the aim of this manuscript was to develop and validate a simple procedure for the assessment of 32 non-volatile pesticides in Acacia honey based on a liquid-liquid extraction followed by a liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. This validated method was applied to five real samples of honey collected from several regions in northern Lebanon.

Materials and methods

Chemicals and reagents

A solution of certified standard pesticides (1 g L⁻¹) including pymetrozine, foramsulfuron, fluroxypyr, spinosad-A, terbutryn, spinosad-D, sulcotrione, chloridazone, chlortoluron, isoproturon, metalaxyl-M, diuron, epoxiconazole, thiacloprid, triflurosulfuron-methyl, boscalid, anilazine, carbendazim, tebuconazole, diflubenzuron, nicosulfuron, penconazole, propiconazole, chlorfenvinphos, cyazofamid, carbetamide, isoxadifen, pyraclostrobin, lufenuron, acetamiprid, flufenoxuron and pendimethalin was prepared in acetonitrile (ACN).

Standard pesticides, internal standards (Carbendazim-d⁴, Pendimethalin-d⁵, Diuron-d⁶ and Nicosulfuron-d⁶), acetonitrile and PTFE membranes were obtained from Sigma Aldrich, St, Quentin Fallavier, France.

LC-MS/MS grade water and ACN, ethyl acetate and hydrochloric acid were obtained from VWR Prolabo, France. Ultrapure water was purchased through a Milli-Q system (18 MΩ cm) from Elga Veolia, France.

Sample collection

Organic Acacia honey was purchased from Lebanese local market for calibration and method development. For the real sample study, 5 honey samples were obtained from beekeepers in northern Lebanon (Bcharre, Akkar valley, Koura and Batroun). Samples were collected and frozen at -18°C in propylene tubes until analysis.

Figure 1 shows the geographical partition of the 5 sampling sites.



Figure 1. Geographical partition of sampling sites

Method development

Preparation of spiked samples

1 gram of the organic honey, weighed in plastic centrifuge tube (50 mL), was heated at 25°C for 15 min in a water bath to reduce its viscosity and then fortify the solution with specific concentrations of each mixture of pesticides (5, 10, 25, 50, 100, 200, 300 and 500 ng g^{-1}). Spiked honeys were kept in centrifuge tubes at 4°C until analysis for 24 h to best fit the pesticide to the matrix.

Extraction procedure

Organic and fortified samples underwent the modified liquid-liquid extraction method developed by Bernal et al. [26] for the extraction of carbendazim and benomyl in honey.

The procedure used was the following: 5 mL of ethyl-acetate and 1 mL of HCl (0.05 M) were added to 1 gram of honey. The organic layer was collected after a mechanical shake (15 min) and centrifugation (10 min at 4000 rpm). 5 mL of ethyl-acetate was added to the remaining solid layer in the tube, then centrifuged to collect the organic layer combined with the previous one. 5 mL of ethyl-acetate and 1 mL of NaOH (0.1 M) and were added to the remaining solid layer, the tube was mechanically shaken and then centrifuged for another 10 min in order to collect the organic layer. The three organic layers were combined, evaporated under the hood to obtain 1 mL as a final solution. Then, the mixture was filtered through a PTFE membrane of 0.50 μm pore size (Whatman syringe filter, 25 mm diameter) prior to chromatographic analysis.

Sample analysis

A Thermo Scientific Accela Liquid Chromatography Autosampler with Surveyor pump coupled to a TSQ Quantum Access Max triple quadrupole mass spectrometer was used. The chromatographic separation was performed on a Macherey-Nagel™ Nucleodur™ C₁₈ Pyramid HPLC Column (150 mm $\times$ 3 mm; 3 μm). The mobile phases consisted of 0.1% formic acid in acetonitrile and 0.1% formic acid in water. The flow rate of the mobile phase was maintained at 0.3 mL min⁻¹, the injection volume was 20 μL and the column was thermostated at 15 °C. Samples were separated using a 36 minutes gradient (30/70 for 5 min, 50/50 for 6 min, 80/20 for 7 min, 95/5 for 10 min and 30/70 for 8 min).

The LC-MS/MS analysis parameters for the assessed pesticide figure in supporting materials (Table 1S).

Method validation

For all parameters, including linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability and reproducibility standard deviation (%RSD), the method

developed has been validated. First, for linearity, matrix matched calibration curves were done in triplicate using samples spiked with 5, 10, 25, 50, 100, 200, 300 and 500 ng g⁻¹. LOD and LOQ were defined as the lowest concentrations where accuracy and precision were corresponded respectively to the ratio: signal/noise ≥ 3 and signal/noise ≥ 10 .

Then, five samples, spiked with three different levels of concentrations, 10, 100 and 300 ng g⁻¹, were extracted for three consecutive days to determine inter-day and intra-day precision (reproducibility and repeatability) that were assessed by their corresponding relative standard deviation.

The recoveries of spiked honey were calculated according to Equation 1.

$$R\% = (\text{Sample concentration} / \text{Standard solution concentration}) * 100 \quad (1)$$

Results and Discussion

The proposed LLE extraction procedure followed by liquid chromatographic analysis to determine pesticides in honey is of great importance in the assessment of air pollution. Results showed that all non-volatile pesticides revealed good linear regression higher than 0.99 with LOD and LOQ lower than 3 and 9 ng g⁻¹ respectively. Calibration curves of some analyzed pesticides figure in supporting materials (Figure.1S). Furthermore, results showed that for repeatability (intra-day) and intermediate precision (inter-day), all these pesticides were detected with high precision (%RSD < 5%) except fluroxypyr and good recoveries between 68 and 104 %. Table 1 presents the validation parameters for non-volatile pesticides analyzed by LC-MS/MS.

Table 1 Validation parameters for non-volatile pesticides analyzed by LC-MS/MS

Pesticide	Regression line equation	Regression coefficient	LOD (ng g ⁻¹)	LOQ (ng g ⁻¹)	% RSD Intra-Day	% RSD Inter-Day	Recovery %
Pymetrozine	Y = 0.000160239*X	0.9951	0.57	1.90	1.52	1.16	93.24
Carbendazim	Y = 0.0328428*X	0.9978	0.27	0.90	0.94	1.02	98.80
Chloridazone	Y = 0.00131559*X	0.9987	0.07	0.23	0.83	1.21	96.70
Acetamiprid	Y = 0.00172956*X	0.9957	2.30	7.67	1.51	2.25	86.99

Nicosulfuron	$Y = 0.000155547 * X$	0.9966	0.75	2.50	0.82	0.61	74.41
Thiacloprid	$Y = 0.033226 * X$	0.9990	0.65	2.17	2.75	4.73	83.05
Carbetamide	$Y = 0.0577388 * X$	0.9997	0.19	0.63	0.31	0.42	72.06
Foramsulfuron	$Y = 0.000570191 * X$	0.9976	0.39	1.30	0.94	2.23	92.69
Fluroxypyr	$Y = 0.00806344 * X$	0.9914	0.78	2.60	6.38	2.65	84.59
Spinosad-A	$Y = 0.000839845 * X$	0.9953	0.05	0.17	0.77	0.74	85.12
Terbutryn	$Y = 0.00455538 * X$	0.9980	1.87	6.23	0.84	1.23	104.51
Spinosad-D	$Y = 2.93436e-006 * X$	0.9915	2.14	7.13	1.12	1.85	90.96
Sulcotrione	$Y = 0.00202121 * X$	0.9985	1.76	5.87	0.93	4.71	69.44
Chlortoluron	$Y = 0.0146021 * X$	0.9945	0.93	3.10	1.68	4.89	74.10
Isoproturon	$Y = 0.0422272 * X$	0.9984	0.02	0.07	2.24	3.21	80.06
Metalaxyl-M	$Y = 0.000765717 * X$	0.9973	0.51	1.70	1.47	3.43	68.30
Diuron	$Y = 0.0165449 * X$	0.9969	0.64	2.13	3.39	4.47	84.47
Epoxiconazole	$Y = 0.127481 * X$	0.9972	0.20	0.67	0.76	3.81	81.4
Triflurosulfuron-Methyl	$Y = 0.0557256 * X$	0.9984	0.05	0.17	3.15	3.23	70.54
Boscalid	$Y = 0.0505686 * X$	0.9988	0.04	0.13	0.85	4.11	94.01
Anilazine	$Y = 0.00104083 * X$	0.9993	0.37	1.23	1.37	2.51	93.48
Tebuconazole	$Y = 0.0586595 * X$	0.9978	0.60	2.00	0.99	2.68	70.74

Diflubenzuron	$Y = 0.00471512 * X$	0.9995	0.24	0.80	1.49	3.92	72.03
Penconazole	$Y = 0.139982 * X$	0.9985	0.10	0.33	2.41	4.23	92.72
Propiconazole	$Y = 0.454928 * X$	0.9990	0.04	0.13	4.87	3.25	82.90
Chlorfenvinphos	$Y = 0.0755802 * X$	0.9989	2.50	8.33	0.75	2.33	81.31
Cyazofamid	$Y = 0.000428225 * X$	0.9905	2.14	7.13	2.85	3.27	nd
Isoxadifen	$Y = 0.00638442 * X$	0.9966	1.60	5.33	1.51	4.63	72.31
Pyraclostrobin	$Y = 0.000600441 * X$	0.9982	0.65	2.17	2.37	3.99	90.72
Lufenuron	$Y = 0.000330173 * X$	0.9961	0.53	1.77	3.21	4.33	83.18
Flufenoxuron	$Y = 0.00713239 * X$	0.9969	0.26	0.87	1.72	1.54	72.23
Pendimethalin	$Y = 0.00673314 * X$	0.9958	1.87	6.23	1.99	3.58	95.87

nd: not detected

The use of an LLE is governed by various physicochemical parameters depending on the solutions to be extracted which provides information on the pH, choice of solvent, type and concentration of reagents and how those choices affect the selectivity needed for sample clean-up. [27, 28] Several solvents such as acetonitrile, ethyl-acetate and methanol were used for the analysis of pesticide in honey that depend on the physicochemical characteristics of each pesticide. [29, 30] In this work, the non-volatile pesticides were extracted using ethyl-acetate, which yielded acceptable quantitative results. During extraction, hydrochloric acid (HCl) was used to increase the solubility of pesticides while sodium hydroxide (NaOH) was used to avoid the persistence of these compounds in this aqueous phase after the last extraction. [31]

In fact, LLE has been a technique of sample extraction for many years involving the direct preparation of the honey matrix with a water-immiscible solvent. [32] Among other multi

residue processes, the method developed has proved its effectiveness. Several studies using LLE extraction followed by liquid chromatography coupled to tandem mass spectrometry showed an improvement in the method's sensitivity. [25] For instance, compared to the reference method based on the study of Bernal et al. [26] for the analysis of benomyl and carbendazim only in honey by reversed phase high performance liquid chromatography (HPLC), the extraction protocol used followed by liquid chromatography coupled to tandem mass spectrometry analysis resulted in a greater number of extracted pesticides with better limits and recoveries. For 1 gram of honey fortified with 1000 ng g⁻¹, the % for recovery and precision for carbendazim respectively were 97.4% and 4.1 %, while by the presented developed method for the fortification of 1 gram by 100 ng g⁻¹ the results were 98.8% and 1.52 % respectively.

Furthermore, comparison of our results with those provided by the LLE extraction using acetonitrile containing 1% of formic acid followed by ultra-high-performance liquid chromatography (UHPLC), showed improvement in limits of detection of some compounds. For example, the LODs of boscalid and fluroxypyr analyzed using the developed method were respectively 0.04 and 0.78 ng g⁻¹, while these limits were respectively 50 and 25 ng g⁻¹ with the extraction using acetonitrile as solvent. [33] The use of different solvents may also affect extraction efficiency and the interferences including pigments and carbohydrates can be co-extracted and influence the recovery of the pesticides depending on the nature and properties of the solvent. [25] Ethyl acetate seems to be the appropriate and effective solvent for the extraction of pesticides in honey, and in all extractions the %RSD obtained were lower than 5%.

Moreover, all RSDs of repeatability and intermediate precision obtained within this developed method respect the validation norms for the honey matrix, [34] while the RSD% for some pesticides were higher than 20% in the study using the QuEChERS method followed LC-MS/MS. For example, the inter-day RSD % obtained from honey fortified at 10 ng g⁻¹ for these two methods were for carbendazim 3.33% and 10%, for penconazole 4.82% and 11%, for propiconazole 2.43% and 4% and for tebuconazole 4.34% and 22%. [35]

LC-MS/MS has also been used widely for the analysis of thermally labile pesticides in honey due to the possibility of separating several components based on molecular weight,

polarity and ionic mobility ^[36] and allows their detection in complex matrices at low concentrations by improving the sensitivity and the reduction of matrix interferences. ^[37]

Application to real samples

The five real samples purchased from four regions of northern Lebanon underwent the same method of extraction described above. Results showed that acetamiprid and sulcotrione residues were observed in all honey samples. Residues of pesticides detected in the samples analyzed are showed in table 2.

Table 2 Concentration of pesticides (ng g⁻¹) detected in real samples

	Akkar-Halba	Akkar-Fnaydek	Batroun	Bcharre	Koura
Acetamiprid	54	61	98	14	84
Sulcotrione	39	71	18	9	30
Cyazofamid	35	82	nd	nd	8
Fluroxypyr	nd	4	nd	nd	10
Metalaxyl-M	nd	nd	9	nd	12

nd= not detected

Bees and their products have been widely studied, the life cycle and geographical distribution of the bees as well as the activities and properties of honey are well documented. ^[38-40] Honey is increasingly used as a biomonitor of air pollution and has been employed for a wide range of environmental pollutants, ranging from pesticides, ^[41, 42] persistent organic pollutant, ^[43, 44] and heavy metals. ^[45]

Conclusion

It is well known that the detection of pesticide in honey matrix is essential due to its medicinal properties. Furthermore, honey is an important tool for determining contamination by pesticides in the environment. The method developed in this work followed by a liquid chromatography coupled to tandem mass spectrometry detector is generally the most appropriate method for simultaneous analysis of the selected 32 pesticides in honey. This method proved its efficiency, and the validation proved its good performance in terms of linearity, accuracy, precision, limit of detection, limit of quantitation. Tandem mass spectrometry detector fulfills such criteria in terms of high

sensitivity and selectivity, as well as reliable analyte identification at very low detection limits.

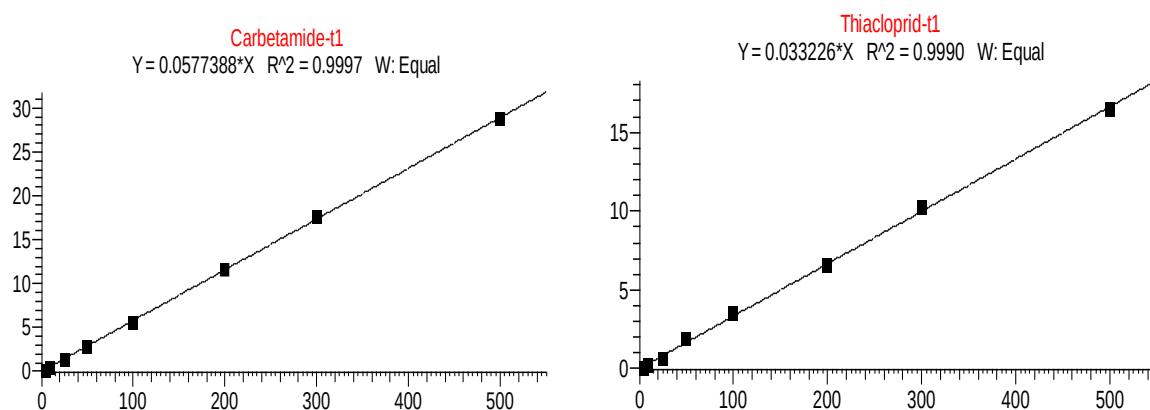
References

- [1] Hendry, A. P.; Gotanda, K. M.; Svensson, E. I. Human Influences on Evolution, and the Ecological and Societal Consequences. *Philos Trans R Soc Lond B Biol Sci* 2017, 372 (1712).
- [2] Lee, Y.-C.; Ahern, J.; Yeh, C.-T. Ecosystem Services in Peri-Urban Landscapes: The Effects of Agricultural Landscape Change on Ecosystem Services in Taiwan's Western Coastal Plain. *Landscape and Urban Planning* 2015, 139, 137–148.
- [3] Al Alam, J.; Chbani, A.; Fajloun, Z.; Millet, M. The Use of Vegetation, Bees, and Snails as Important Tools for the Biomonitoring of Atmospheric Pollution—a Review. *Environmental Science and Pollution Research* 2019, 26.
- [4] Dołęgowska, S.; Migaszwski, Z. Terrestrial Mosses as Trace Element Bioindicators: A Review; 2014; pp 25–69.
- [5] Bargańska, Ż.; Ślebioda, M.; Namieśnik, J. Honey Bees and Their Products: Bioindicators of Environmental Contamination. *Critical Reviews in Environmental Science and Technology* 2016, 46 (3), 235–248.
- [6] Belsky, J.; Joshi, N. K. Impact of Biotic and Abiotic Stressors on Managed and Feral Bees. *Insects* 2019, 10 (8).
- [7] Li, G.; Zhao, H.; Liu, Z.; Wang, H.; Xu, B.; Guo, X. The Wisdom of Honeybee Defenses Against Environmental Stresses. *Front Microbiol* 2018, 9.
- [8] Patel, V.; Pauli, N.; Biggs, E.; Barbour, L.; Boruff, B. Why Bees Are Critical for Achieving Sustainable Development. *Ambio* 2020.
- [9] Sáez, A.; Negri, P.; Viel, M.; Aizen, M. A. Pollination Efficiency of Artificial and Bee Pollination Practices in Kiwifruit. *Scientia Horticulturae* 2019, 246, 1017–1021.
- [10] Klein, S.; Pasquaretta, C.; He, X. J.; Perry, C.; Søvik, E.; Devaud, J.-M.; Barron, A. B.; Lihoreau, M. Honey Bees Increase Their Foraging Performance and Frequency of Pollen Trips through Experience. *Scientific Reports* 2019, 9 (1), 6778.
- [11] Al-Waili, N.; Salom, K.; Al-Ghamdi, A.; Ansari, M. J. Antibiotic, Pesticide, and Microbial Contaminants of Honey: Human Health Hazards. *ScientificWorldJournal* 2012, 2012.
- [12] Sánchez-Bayo, F.; Goka, K. Impacts of Pesticides on Honey Bees; 2016; pp 77–97.
- [13] Gregorc, A.; Alburaki, M.; Rinderer, N.; Sampson, B.; Knight, P. R.; Karim, S.; Adamczyk, J. Effects of Coumaphos and Imidacloprid on Honey Bee (Hymenoptera: Apidae) Lifespan and Antioxidant Gene Regulations in Laboratory Experiments. *Scientific Reports* 2018, 8 (1), 15003.
- [14] Ravoet, J.; Reybroeck, W.; de Graaf, D. C. Pesticides for Apicultural and/or Agricultural Application Found in Belgian Honey Bee Wax Combs. *Bull Environ Contam Toxicol* 2015, 94 (5), 543–548.
- [15] Zawislak, J.; Adamczyk, J.; Johnson, D. R.; Lorenz, G.; Black, J.; Hornsby, Q.; Stewart, S. D.; Joshi, N. Comprehensive Survey of Area-Wide Agricultural Pesticide Use in Southern United States Row Crops and Potential Impact on Honey Bee Colonies. *Insects* 2019, 10 (9).
- [16] Moniruzzaman, M.; Chowdhury, M. A. Z.; Rahman, M. A.; Sulaiman, S. A.; Gan, S. H. Determination of Mineral, Trace Element, and Pesticide Levels in Honey Samples Originating from Different Regions of Malaysia Compared to Manuka Honey. *Biomed Res Int* 2014, 2014.
- [17] Williamson, S. M.; Willis, S. J.; Wright, G. A. Exposure to Neonicotinoids Influences the Motor Function of Adult Worker Honeybees. *Ecotoxicology* 2014, 23 (8), 1409–1418.
- [18] Wood, T. J.; Goulson, D. The Environmental Risks of Neonicotinoid Pesticides: A Review of the Evidence Post 2013. *Environ Sci Pollut Res Int* 2017, 24 (21), 17285–17325.
- [19] Messina, C. M.; Panettieri, V.; Arena, R.; Renda, G.; Espinosa Ruiz, C.; Morghese, M.; Piccolo, G.; Santulli, A.; Bovera, F. The Inclusion of a Supercritical Fluid Extract, Obtained From Honey Bee Pollen, in the Diet of Gilthead Sea Bream (*Sparus Aurata*), Improves Fish Immune Response by Enhancing Anti-Oxidant, and Anti-Bacterial Activities. *Front. Vet. Sci.* 2020, 7.
- [20] Ruiz, P.; Ares, A. M.; Valverde, S.; Martín, M. T.; Bernal, J. Development and Validation of a New Method for the Simultaneous Determination of Spinetoram J and L in Honey from Different Botanical Origins Employing Solid-Phase Extraction with a Polymeric Sorbent and Liquid Chromatography Coupled to Quadrupole Time-of-Flight Mass Spectrometry. *Food Research International* 2020, 130, 108904.
- [21] Zhu, Z.; Zhang, Y.; Wang, J.; Li, X.; Wang, W.; Huang, Z. Sugaring-out Assisted Liquid-Liquid Extraction Coupled with High Performance Liquid Chromatography-Electrochemical Detection for the Determination of 17 Phenolic Compounds in Honey. *Journal of Chromatography A* 2019, 1601, 104–114.
- [22] Balsebre, A.; Báez, M. E.; Martínez, J.; Fuentes, E. Matrix Solid-Phase Dispersion Associated to Gas Chromatography for the Assessment in Honey Bee of a Group of Pesticides of Concern in the Apicultural Field. *Journal of Chromatography A* 2018, 1567, 47–54.
- [23] Chiesa, L. M.; Labella, G. F.; Giorgi, A.; Panzeri, S.; Pavlovic, R.; Bonacci, S.; Arioli, F. The Occurrence of Pesticides and Persistent Organic Pollutants in Italian Organic Honey from Different Productive Areas in Relation to Potential Environmental Pollution. *Chemosphere* 2016, 154, 482–490.
- [24] Zhang, C.; Deng, Y.; Zheng, J.; Zhang, Y.; Yang, L.; Liao, C.; Su, L.; Zhou, Y.; Gong, D.; Chen, L.; Luo, A. The Application of the QuEChERS Methodology in the Determination of Antibiotics in Food: A Review. *TrAC Trends in Analytical Chemistry* 2019, 118, 517–537.
- [25] Souza Tette, P. A.; Rocha Guidi, L.; de Abreu Glória, M. B.; Fernandes, C. Pesticides in Honey: A Review on Chromatographic Analytical Methods. *Talanta* 2016, 149, 124–141.
- [26] Bernal, J. L.; del Nozal, M. J.; Toribio, L.; Jiménez, J. J.; Atienza, J. High-Performance Liquid Chromatographic Determination of Benomyl and Carbendazim Residues in Apian Samples. *Journal of Chromatography A* 1997, 787 (1), 129–136.
- [27] Chemat, F.; Abert Vian, M.; Ravi, H. K.; Khadhraoui, B.; Hilali, S.; Perino, S.; Fabiano Tixier, A.-S. Review of Alternative Solvents for Green Extraction of Food and Natural Products: Panorama, Principles, Applications and Prospects. *Molecules* 2019, 24 (16).
- [28] Daso, A. P.; Okonkwo, O. Conventional Extraction Techniques: Soxhlet and Liquid-Liquid Extractions and Evaporation; 2015; pp 1437–1468.
- [29] Panzeri, S.; Catalano, A.; Giorgi, A.; Arioli, F.; Procopio, A.; Britti, D.; Chiesa, L. M. Occurrence of Pesticide Residues in Italian Honey from Different Areas in Relation to Its Potential Contamination Sources. *Food Control* 2014, 38, 150–156.

- [30] Salami, F. H.; Queiroz, M. E. C. Microextraction in Packed Sorbent for the Determination of Pesticides in Honey Samples by Gas Chromatography Coupled to Mass Spectrometry. *J Chromatogr Sci* 2013, 51 (10), 899–904.
- [31] Leng, P.; Zhang, Z.; Li, Q.; Zhao, M.; Pan, G. Microemulsion Formulation of Carbendazim and Its In Vitro Antifungal Activities Evaluation. *PLoS One* 2014, 9 (10).
- [32] Kuś, P.; Jerkovic, I. New Sample Preparation Method for Honey Volatiles Fingerprinting Based on Dehydration Homogeneous Liquid–Liquid Extraction (DHLLE). *Molecules* 2018, 23, 1769.
- [33] Gómez-Pérez, M. L.; Plaza-Bolaños, P.; Romero-González, R.; Martínez-Vidal, J. L.; Garrido-Frenich, A. Comprehensive Qualitative and Quantitative Determination of Pesticides and Veterinary Drugs in Honey Using Liquid Chromatography–Orbitrap High Resolution Mass Spectrometry. *Journal of Chromatography A* 2012, 1248, 130–138.
- [34] Tiwari, G.; Tiwari, R. Bioanalytical Method Validation: An Updated Review. *Pharmaceutical Methods* 2010, 1 (1).
- [35] Wiest, L.; Buleté, A.; Giroud, B.; Fratta, C.; Amic, S.; Lambert, O.; Pouliquen, H.; Arnaudguilhem, C. Multi-Residue Analysis of 80 Environmental Contaminants in Honeys, Honeybees and Pollens by One Extraction Procedure Followed by Liquid and Gas Chromatography Coupled with Mass Spectrometric Detection. *Journal of Chromatography A* 2011, 1218 (34), 5743–5756.
- [36] Stachniuk, A.; Fornal, E. Liquid Chromatography–Mass Spectrometry in the Analysis of Pesticide Residues in Food. *Food Anal. Methods* 2016, 9 (6), 1654–1665.
- [37] Sampaio, M. R. F.; Tomasini, D.; Cardoso, L. V.; Caldas, S. S.; Primel, E. G. Determination of Pesticide Residues in Sugarcane Honey by QuEChERS, and Liquid Chromatography. *Journal of the Brazilian Chemical Society* 2012, 23 (2), 197–205.
- [38] Thakur, M.; Nanda, V. Composition and Functionality of Bee Pollen: A Review. *Trends in Food Science & Technology* 2020, 98, 82–106.
- [39] Zięba, K.; Szostak, E.; Czekońska, K.; Miśkowiec, P.; Moos-Matysik, A.; Nyczyk-Malinowska, A.; Szentgyörgyi, H. Usefulness of Bee Bread and Capped Brood for the Assessment of Monocyclic Aromatic Hydrocarbon Levels in the Environment. *Environmental Pollution* 2020, 265, 114882.
- [40] Bodó, A.; Radványi, L.; Kőszegi, T.; Csepregi, R.; Nagy, D. U.; Farkas, Á.; Kocsis, M. Melissopalynology, Antioxidant Activity and Multielement Analysis of Two Types of Early Spring Honeys from Hungary. *Food Bioscience* 2020, 35, 100587.
- [41] Sgargi, D.; Adam, B.; Budnik, L. T.; Dinelli, G.; Moldovan, H. R.; Perry, M. J.; Scheepers, P. T.; Schlünsen, V.; Teixeira, J. P.; Mandrioli, D.; Belpoggi, F. Protocol for a Systematic Review and Meta-Analysis of Human Exposure to Pesticide Residues in Honey and Other Bees' Products. *Environmental Research* 2020, 186, 109470.
- [42] El-Nahhal, Y. Pesticide Residues in Honey and Their Potential Reproductive Toxicity. *Science of The Total Environment* 2020, 741, 139953.
- [43] Wang, W.; Zhang, S.; Li, Z.; Li, J.; Yang, X.; Wang, C.; Wang, Z. Construction of Covalent Triazine-Based Frameworks and Application to Solid Phase Microextraction of Polycyclic Aromatic Hydrocarbons from Honey Samples. *Food Chemistry* 2020, 322, 126770.
- [44] Villalba, A.; Maggi, M.; Ondarza, P. M.; Szawarski, N.; Miglioranza, K. S. B. Influence of Land Use on Chlorpyrifos and Persistent Organic Pollutant Levels in Honey Bees, Bee Bread and Honey: Beehive Exposure Assessment. *Science of The Total Environment* 2020, 713, 136554.
- [45] Ragab, M. A. A.; El-Yazbi, A. F.; El-Hawiet, A. Fast Economic Electrochemical Assay for Vitamins and Heavy Mineral Components in honey Samples of Different Botanical Origin. *Microchemical Journal* 2020, 155, 104770.

Supporting materials

Figure.1S Calibration curves of some analyzed pesticides figure in supporting materials



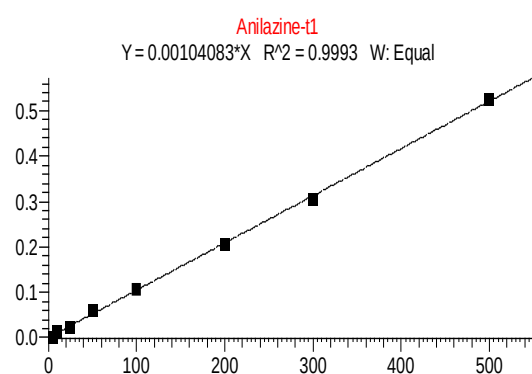
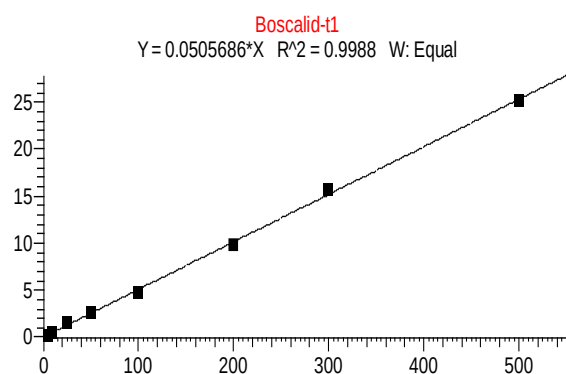


Table 1S. The LC-MS/MS analysis parameters for the assessed pesticide: Chapter II-II - Supplementary Materials - Table 1, 2 and 3.

Chapitre III : Discussion générale

Au Liban, les niveaux de polluants ont atteint les niveaux de « smog » visibles à l'œil nu qui se forme dans l'air lorsque la forte lumière du soleil réagit avec les oxydes d'azote et les composés organiques volatils, produits par la combustion de combustibles et par l'évaporation des combustibles liquides et des solvants. Le Liban n'a pas d'industries à impact significatif, mais le développement du secteur de l'immobilier et l'augmentation des automobiles conduisent à une aggravation de la pollution et ainsi à une dégradation de la qualité de l'air et à une augmentation des effets sur la santé (Badaro-Saliba et al., 2014). Au Liban, très peu d'études ont été menées pour tenter de générer des preuves d'une relation entre la pollution de l'air et les effets sur la santé. Cependant, aucun protocole général n'a encore été établi pour étudier les effets de la pollution de l'air sur la santé, compte tenu du manque de surveillance des données du système de santé et des conditions environnementales spécifiques, ce qui est courant dans les pays en développement. Les secteurs de l'agriculture, du transport et d'activités industrielles sont les causes principales de la mauvaise qualité de l'air surtout dans les quartiers urbains densément peuplés (Baayoun et al., 2019 ; Mokalled et al., 2018 ; Abi Ghanem, 2018).

Un inventaire des émissions a été établi pour le Liban en tenant compte des sources de monoxyde de carbone, de l'ozone troposphérique, de l'oxyde d'azote, du dioxyde de soufre, ainsi que des particules en suspension (PM₁₀ ; PM_{2.5}). Cette étude, qui reposait sur un comptage du trafic, en plus des informations spécifiques sur l'origine et la destination du trajet, et une estimation approximative des émissions des générateurs diesel à estimer la présence des concentrations de monoxyde de carbone, de dioxyde de soufre, d'ozone troposphérique (Abdallah et al., 2016 ; Waked et al., 2012), des OCPs, des PCBs (Merhaby et al., 2020 ; Helou et al., 2019) et des HAPs (Soukarieh et al., 2018). Une autre étude qui repose sur l'analyse de la qualité de l'eau au Liban estime la présence des pesticides dans les eaux de surface du à leur utilisation dans la culture d'agrumes, de fruits à pépins, de pommiers et de légumes (Jabali et al., 2019).

Dans cette thèse, le travail est divisé en deux parties étroitement liées. Dans un premier temps, une approche méthodologique de la biosurveillance et de l'analyse chimique des échantillons a été développée. Dans un second temps, l'utilisation d'aiguilles de conifères

et des escargots pour la détection de la pollution de l'air et de l'environnement, a été évaluée. Afin d'atteindre l'ensemble de nos objectifs tant au niveau de l'analyse de la concentration des polluants dans différentes régions, de l'identification des sources de pollution, qu'au niveau de la capacité de leurs accumulations dans les escargots et les aiguilles de conifères, six projets différents ont été entrepris et sont présentés ici.

1. Apports de l'approche méthodologique d'utilisation des aiguilles de conifères et des escargots comme des biomoniteurs pour l'étude de la pollution dans l'air

Les aiguilles de conifères se sont révélées être une matrice utile pour la surveillance de la pollution dans l'air car elles récupèrent les contaminants de l'atmosphère et les accumulent dans leurs tissus sur de longues périodes de temps. Pourtant, il y a un manque d'informations sur la dynamique de ces processus tout au long de la durée de vie des aiguilles de conifères. Le but de l'étude de cette matrice était de générer des données démontrant comment une absorption de produits chimiques sélectionnés dans les aiguilles (*Pinus nigra*), leur mobilité et dégradation se traduisent par les niveaux de ces composés déterminés dans les tissus des aiguilles à divers moments. Klánová et al. en 2009, ont montré qu'à long terme, la surveillance des aiguilles fournit des informations très similaires sur les tendances temporelles de la pollution atmosphérique, car la surveillance de l'air à grand volume fournit le même plan d'échantillonnage (âge des aiguilles, saison d'échantillonnage) et le même protocole d'analyse.

L'âge optimal des aiguilles et la meilleure saison d'échantillonnage sont cependant encore inconnus. Ces données devraient améliorer notre compréhension des fluctuations temporelles de leurs niveaux et de leurs modèles, et permettre une décision éclairée sur le meilleur âge des aiguilles et la saison optimale pour la collecte de ces dernières à des fins de surveillance. Des espèces végétales vertes telles que *Magnolia grandiflora*, *Cedrus deodara*, *Ginkgo biloba*, *Pinus nigra*, *Platanus acerifolia*, *Salix matsudana* et *Cinnamomum camphora* (Mukhopadhyay et al., 2020 ; Tian et al., 2019) qui comprennent à la fois des arbres à feuilles larges, ainsi que des arbres à feuilles persistantes et des conifères ont été étudiées par les auteurs pour évaluer la concentration de polluants organiques accumulés. Zhao et al. en 2018, ont rapporté que les profils de composition et de distribution des polluants dans les feuilles de *Salix matsudana* ont changé en réponse aux paramètres des feuilles et aux conditions atmosphériques et ont réussi à prouver leur

pouvoir cumulatif efficace. En revanche, Tian et al. en 2019, ont rapporté que la concentration de certains polluants dans les conifères (*Cedrus deodara* et *Sabina chinesis*) atteint une accumulation maximale par rapport à celle des espèces à feuilles larges. Par conséquent, les conclusions de beaucoup d'auteurs (Massimi et al., 2021, 2019 ; Mukhopadhyay et al., 2020 ; Odabasi et al., 2015 ; Tian et al., 2019) fournissent un cadre théorique pour l'initiation d'un système de surveillance de la qualité de l'air ambiant à base de plantes qui sont hautement capables d'adsorber les polluants en suspension dans l'air. Schrlau et al. en 2011, se sont également concentrés sur l'échantillonnage passif de l'air et ont évalué la présence de polluants semi-volatils dans les aiguilles de conifères et ont démontré que les aiguilles plates et larges pourraient accumuler jusqu'à 71 fois plus que les aiguilles étroites et rondes.

Plusieurs études ont rapporté la capacité des gastéropodes à accumuler les composés du milieu environnant (Mleiki et al., 2020 ; Emilia et al., 2016 ; Krupnova et al., 2018 ; Li et al., 2020). Dans une étude antérieure par Sturba et al. en 2018 sur l'escargot terrestre *Cornu aspersum*, et dans des conditions de laboratoire contrôlées, le chlorure de cadmium vaporisé a entraîné une accumulation dans divers tissus de l'escargot (pied < corps entier < hépatopacrées), ainsi qu'une augmentation significative de la cytotoxicité. Une altération de la capacité de reproduction et une inhibition de la croissance ont également été signalées chez des gastéropodes exposés à des métaux lourds à la fois sur le terrain et dans des conditions contrôlées en laboratoire (Schmielau et al., 2019). Diverses espèces d'escargots terrestres ont été utilisées comme espèces naturelles dans les études de biosurveillance menées dans les zones urbaines touchées par les métaux lourds (Abdel-Halim et al., 2013 ; Filippi et al., 2018). La translocation vers un site contaminé d'escargots sains en cage a permis de reconnaître les réponses biologiques liées à l'exposition et d'identifier les sources de contaminants. L'utilisation d'échantillons locaux fournit plus d'informations sur la distribution et l'accumulation des polluants sur une période déterminée et d'estimer les niveaux réels de contamination (Voua Otomo et al., 2011). L'escargot s'est avéré utile pour l'identification de la source de pollution de l'air, en accord avec des études antérieures menées avec des espèces de plantes, de mousses et de lichens comme biomoniteurs (Massini et al., 2019). On note également que les escargots sont impliqués dans de multiples chaînes alimentaires au Liban. Par conséquent, la contamination des escargots par les polluants peut être transférée aux consommateurs

entraînant un risque d'intoxication secondaire. Pour ces raisons, l'utilisation d'escargots sains en cage dans un programme de biosurveillance pour les dépôts de polluants organiques semble être un moyen idéal pour obtenir des informations appropriées et précises sur la qualité de l'air et l'évaluation des effets à l'échelle géographique.

2. Apports du développement d'une méthode multi-résidus pour l'analyse des pesticides, des hydrocarbures aromatiques polycycliques et des polychlorobiphényles chez les escargots utilisés comme biomoniteurs environnementaux

Les polluants organiques sont aujourd'hui considérés comme toxiques pour la santé humaine. Les teneurs maximales en résidus autorisées dans l'air, l'eau et les denrées alimentaires sont de plus en plus strictes. Par conséquent, des techniques analytiques sélectives et sensibles sont nécessaires pour leur quantification et leur identification. Le but de cette étude menée au cours de nos travaux de thèse était de développer pour la première fois une méthode analytique pour déterminer différentes classes de polluants dans les escargots. Il s'agit d'une procédure d'extraction QuEChERS modifiée. La méthode présentée en plus de sa nouveauté pour le dosage de pesticides et de polluants organiques persistants est simple, rapide, précise et robuste. Le dosage des 158 composés n'a pas été perturbé par les solvants utilisés et / ou par des effets de matrice. Pour les effets matriciels, l'analyse de matrices biologiques, comme les escargots, peut conduire à la co-extraction d'une certaine quantité d'autres composés (acides organiques, sucres, lipides), dont leur élimination avant l'étape de détermination finale est cruciale (Saraiva et al., 2016). L'utilisation de l'ACN a prouvé son efficacité dans plusieurs analyses multi-résidus dans des échantillons biologiques, présentant de meilleures récupérations (Al-Alam et al., 2020 ; DeArmond et al., 2015).

Des études antérieures ont montré que le C₁₈ permet l'élimination des substances interférentes non polaires telles que les lipides, tandis que l'amine primaire secondaire permet d'éliminer tous les pigments polaires, les acides organiques polaires, les acides gras et les sucres des extraits (Islam et al., 2019 ; Dimpe et al., 2016). La linéarité, la précision, l'exactitude, les LOD et LOQ, la sélectivité de la méthode et la stabilité de l'échantillon ont été établies. En outre, la gamme d'application de la méthode QuEChERS dans différentes matrices pour l'analyse de divers types de polluants est très large et a permis sa comparaison avec des méthodes de référence dans différentes applications

telles que la LLE, l'extraction solide-liquide, la SPE, l'ASE, la MAE et l'extraction assistée par ultrasons qui dépendent essentiellement de la nature des analytes à extraire et de la complexité de la matrice. Les méthodes mentionnées présentent toutes des inconvénients par rapport à la méthode QuEChERS tels que le temps d'extraction et le coût d'investissement élevé, la toxicité des solvants en fonction des molécules à extraire, la perte et la dégradation des composés thermolabiles / volatiles par la présence d'une température généralement élevée, présence de phénomène de saturation par l'application de grandes quantités de solvants et le problème de répétabilité et de reproductibilité lors de l'utilisation de sonde à ultrasons. En effet, la méthode QuEChERS est un choix raisonnable et performant capable de fournir des performances analytiques similaires ou meilleures sans les inconvénients des autres méthodes, ainsi que la nécessité de dispositifs spécifiques tels que les micro-ondes ou les ultrasons (Łozowicka et al., 2017 ; Ratola et al., 2011).

Une fois la méthode validée, nous l'avons appliquée à des échantillons réels d'escargots de type *Helix aspersa*. L'ensemble de l'escargot a été prélevé de la coquille et la même procédure d'extraction lui a été appliquée. L'analyse des échantillons d'escargot a été effectuée en utilisant la LC-MS/MS et la GC-MS/MS. 12 pesticides et 6 HAPs ont été détectés dans les échantillons avec des concentrations allant de 0.43 et 85.6 ng g⁻¹ pour les pesticides et 6.82 et 314.33 ng g⁻¹ pour les HAPs. Nos résultats montrent que les échantillons d'escargots préparés n'avaient aucun effet important sur les performances de la méthode rapportée. Les résultats obtenus ont montré que la méthode d'extraction et d'analyse proposée pouvait être employée comme technique appropriée pour l'analyse des composés représentatifs dans les échantillons des escargots. L'approche proposée pourrait être utile en tant que procédure analytique efficace pour une détection plus poussée des pesticides et des polluants organiques persistantes dans les échantillons des aiguilles de conifères.

3. Apports du développement d'une méthode multi-résidus pour la quantification de pesticides, d'hydrocarbures aromatiques polycycliques et de polychlorobiphényles par chromatographie en phase liquide et en phase gazeuse couplée à une spectrométrie de masse en tandem dans les aiguilles de conifères utilisées comme biomoniteurs environnementaux

Le choix des aiguilles de conifères dans cette étude a été basé sur le fait que ces matrices sont les plus utilisées comme biomoniteurs de la pollution de l'air et que la méthode d'extraction par QuEChERS n'a jamais été développée. En effet, les résultats de cette méthode surmontent les inconvénients présentés par les méthodes d'extraction traditionnelles utilisées. Par exemple, cette méthode s'est avérée rapide, efficace, respectueuse de l'environnement et fiable principalement en raison de la réduction du poids de l'échantillon et de la quantité de solvants utilisée (González-Curbelo et al., 2015). De plus, les récupérations obtenues semblent être encore plus élevées que celles obtenues par l'ASE ou la SPE notamment compte tenu de la quantité élevée de solvant utilisée dans ces dernières techniques (Garcia et Gotah, 2017). Les taux des récupérations obtenues avec notre méthode étaient plus élevés que celles fournies pour l'analyse des pesticides dans les aiguilles de conifères à l'aide de l'extraction sélective de liquide sous pression (Kim et al., 2019).

Sur la base des données présentées dans la littérature, il peut être conclu que la méthode développée introduit une nouvelle application dans le processus de détermination d'un large éventail de polluants (pesticides, PCBs et HAPs) dans les échantillons de conifères en comparaison avec d'autres méthodes déjà publiées tels que l'ASE suivie d'une extraction sélective de liquide sous pression pour l'analyse des pesticides et des PCBs (Kim et al., 2019), le Soxhlet pour les OCPs (Quan et al., 2004), les polychlorodibenzo-p-dioxines / furanes (Holt et al., 2016) et l'extraction assistée par ultrasons des HPAs (Yamaguchi et Lee, 2010). De plus, la méthode QuEChERS a montré de meilleures performances pour le dosage des pesticides dans le miel, les abeilles (Noth et al., 2013) et dans les racines des plantes médicinales (Tripathy et al., 2017) que la LLE et la SPE.

En fait, nos résultats obtenus à ce niveau ont montré que la combinaison de la chromatographie liquide et gazeuse avec la spectrométrie de masse résout la plupart des problèmes associés à l'évaluation des résidus de polluants tels que la petite quantité d'échantillon qui peut être détecté, le temps d'analyse, la précision et la large gamme d'échantillons (Liu et al., 2016). La chromatographie est utilisée dans une large gamme d'applications en raison de sa possibilité de séparer différents composants d'un mélange complexe en fonction de la polarité, du poids moléculaire et de la mobilité ionique (Yang et al., 2008). Cependant, les deux techniques de chromatographie sont nécessaires dans l'analyse des traces pour la détermination d'un large spectre de polluants

environnementaux tels que les PCBs, les OCPs, les HAPs et certains autres pesticides (Issa et al., 2020 ; Vaaland et al., 2020 ; Chamkasem et al., 2016). Les résultats ont prouvé que la combinaison QuEChERS-SPME avec les techniques chromatographiques peut être utilisée comme un outil de surveillance environnementale. Les méthodes développées ont été appliquées avec succès au suivi d'échantillons réels collectés dans 15 régions du Liban. Des polluants détectables (chloridazon, metalaxyl-M, pendimethalin, fluoranthène, boscalid, naphthalène, diflufenican, acénaphthylène, hexachlorobenzène, terbutryn, fenpropidin et sulcotrione) ont été trouvés dans la plupart des échantillons avec des niveaux résiduels variables allant de 2.66 jusqu'à 523.7 ng g⁻¹ selon la zone et différents facteurs tels que la température, l'humidité, l'altitude et les précipitations qui peuvent avoir un impact significatif sur la concentration de ces polluants.

4. Apports de l'utilisation d'aiguilles de conifères comme biomoniteur de la qualité de l'air au Liban

En raison des excellentes propriétés d'absorption de leur couche cireuse, la végétation, en particulier les aiguilles de conifères, se distingue en tant que biomoniteur efficace pour la quantification des composés organiques semi-volatils et volatils. Contrairement à l'échantillonnage passif ou actif de l'air, l'utilisation d'aiguilles de conifères en tant que biomoniteurs ne nécessite pas de mise en place préalable d'un site d'échantillonnage et puisque ces aiguilles restent sur l'arbre pendant plusieurs années, elles peuvent agir comme un «enregistreur de données biologiques» pour la qualité de l'air.

Après avoir vérifié au préalable la méthode fiable et originale d'analyse des polluants organiques persistants et des pesticides dans les aiguilles de conifères, nous nous sommes intéressés au cours d'un de nos travaux à déterminer les niveaux de 127 pesticides et 16 HAPs dans les aiguilles de *Pinus nigra* collectées dans 15 sites d'échantillonnage différents au Liban. Les informations disponibles dans la littérature sur les niveaux de ces polluants dans les aiguilles de conifères au Liban sont inexistantes. Néanmoins, il est intéressant de constater que les niveaux totaux de pesticides détectés répondent aux indicateurs d'utilisation des produits agricoles au Liban, où des zones comme Akkar ou Tripoli présentent un taux élevé et des villes comme Bcharre et Koura ont un faible taux de pesticides. Étant donné que ces études sont effectuées sur des sites différents, il est acceptable que les pesticides utilisés puissent différer, vu que les cultures peuvent

également être différentes. Le Liban se caractérise par un secteur du transport routier non durable, un secteur privé incontrôlé de générateurs diesel, une base de données insuffisante sur les pesticides utilisés et un mauvais contrôle de la qualité de l'air. Les émissions de diesel (Bcharre), les émissions d'essence (Tripoli), la combustion de combustibles fossiles (Tripoli et Koura) et la combustion du charbon, du bois et de l'herbe (Bcharre et Akkar), sont les principales sources des HAPs dans ces régions étudiées, tandis que l'absence de toutes les activités industrielles dans ces zones spécifiées pour la production agricole est la principale raison de la disparition des polychlorobiphényles et autres résidus de polluants issus des déchets industriels.

En essayant d'évaluer les aspects comportementaux de ces polluants, certaines corrélations avec des paramètres météorologiques, géographiques et économiques ont été tentées. Cela peut être le reflet d'une volatilisation importante de ces composés depuis les sols dans l'air, favorisant leur piégeage par la couche cireuse des aiguilles de conifères. Ces différences de concentrations des polluants pourraient être contrôlées par leurs propriétés physico-chimiques des composés ainsi que de la présence des matrices étudiées à proximité des sources d'émissions (Lavin et Hageman, 2012). D'autres études ont montré que la concentration de polluants augmente proportionnellement à l'âge de l'aiguille. Ainsi, le choix de la classe d'âge des aiguilles est nécessaire pour l'indication phytochimique de la pollution environnementale par les aiguilles de conifères (Chropeňová et al., 2016). L'abondance des polluants dans les aiguilles était le résultat d'une accumulation à long terme associée à la période d'exposition des aiguilles dans l'air. Plusieurs études ont montré que des concentrations plus élevées de polluants ont été identifiées dans les aiguilles plus anciennes que dans les aiguilles plus jeunes dans différentes espèces d'arbres, et ces concentrations indiquent une augmentation plus significative de l'âge des aiguilles (Kylin et al., 2017; Klánová et al., 2009).

L'un des aspects de l'étude des polluants dans différents sites est l'étude des différences de concentration le long des gradients altitudinaux. Par exemple, Davidson et al. ont constaté que les composés organiques plus volatils augmentaient à des altitudes plus élevées, alors que les composés organiques moins volatils n'étaient pas liés à l'altitude (Davidson et al., 2003). Ces incohérences sont causées par la proximité différente des sources des sites d'échantillonnage, la complexité des facteurs environnementaux régionaux et les différentes propriétés des composés (Pompa-García et al., 2017). Les

altitudes du site de Bcharre, comprises entre 1450 et 3088 m, étaient relativement plus élevées que les sites d'Akkar, Koura et Tripoli (600, 100 et 5 m respectivement). Les concentrations de certains polluants (pymétozine, sulcotrione et lufenuron) diminuent avec une augmentation de l'élévation. Des effets semblables indiquent que la proximité de la source du polluant peut être le facteur déterminant, que la source du polluant soit éloignée ou proche de la montagne (Bradford et al., 2010).

Plusieurs études ont décrit des variations importantes dans la distribution spatiale des HAPs et les résultats ont montré que les concentrations les plus élevées de HAPs étaient mesurées dans les zones à fort trafic (Tripoli et Akkar), suivies par les zones urbaines et rurales (Bcharre) (Lovinsky-Desir et al., 2016; Jaward et al., 2004). Ces concentrations de HAPs atmosphériques à l'extérieur peuvent également différer considérablement, et des concentrations plus élevées mesurées le long des routes principales (Tripoli et Akkar) peuvent être atteintes par rapport aux concentrations mesurées dans une rue privée (Koura et Bcharre), à plusieurs kilomètres de distance (Choi et al., 2007). Ces résultats peuvent être clarifiés par des variations de la proximité des routes, des automobiles et des émissions provenant des sources de trafic à une échelle relativement importante.

En revanche, la température et les précipitations peuvent avoir une importance significative sur la dégradation et sur la formation de résidus non extractibles qui affectent la persistance du sol et réduisent donc la volatilisation (Loos et al., 2012). Les basses températures n'ont pas un impact aussi fort que les températures extrêmement élevées, qui causent de grands dommages physiologiques. Yang et al. en 2008, ont montré que la différence de concentration est principalement causée par le gradient de température le long de l'altitude, et que la température peut également contrôler l'échange solide-gaz. Les zones les plus hautes (site de Bcharre) sont relativement plus froides et dominant ainsi l'effet de fractionnement, ce qui pourrait diminuer l'effet du vent sur la distribution des polluants.

La présence de HAPs dans l'air due à des sources anthropiques peut être affectée par la température qui joue un rôle important dans le taux d'échantillonnage des HAPs par échantillonnage passif de l'air. Des températures élevées peuvent entraîner une augmentation de la diffusion moléculaire, conduisant à une augmentation de la concentration des polluants. Ces procédures entraînent une plus grande variance dans les

niveaux d'échantillonnage lors de la comparaison de différentes régions ou climats (Melymuk et al., 2014; Armstrong et al., 2014). Des zones semblent être fortement contaminé par certains polluants en raison du fait que l'augmentation de la température provoquant également le transfert et l'accumulation de ces polluants dans les tissus végétaux, qui augmente rapidement et atteint l'accumulation maximale en quelques heures (Salehi- Lissar et al.2015; Shen et al.2019). En effet, il a été démontré que les plantes comme les conifères peuvent absorber certains composés par leurs aiguilles si elles sont en phase gazeuse et / ou particulaire, mais aussi via leurs racines lorsqu'elles sont dans le sol (Srogi 2007).

De nombreuses études ont indiqué que non seulement les caractéristiques environnementales et les propriétés physico-chimiques des polluants jouent un rôle important dans leur distribution dans la végétation à l'échelle mondiale, mais le développement économique élevé a également laissé sa marque sur leurs distributions environnementales. Des informations supplémentaires sur les niveaux de pesticides dans l'air sont nécessaires pour évaluer leur pertinence respective et pour renforcer encore le rôle des aiguilles de conifères en tant que contrôleurs biologiques de la pollution atmosphérique.

5. Apports de l'utilisation d'escargots et d'aiguilles de conifères comme biomoniteurs environnementaux pour l'étude de la variation temporelle de la pollution atmosphérique dans différentes régions géographiques du Liban

La pollution atmosphérique est devenue une préoccupation environnementale majeure en raison de ses effets néfastes sur l'écosystème et la santé humaine (Manisalidis et al., 2020 ; Ghorani-Azam et al., 2016). Il existe de nombreuses techniques utilisées pour obtenir des informations fiables sur les caractéristiques de l'environnement et la qualité de l'air. La biosurveillance, par rapport aux méthodes conventionnelles d'échantillonnage de l'air, est une méthode peu coûteuse et simple pour déterminer les composés organiques et leur répartition sur de vastes zones (Cen, 2015 ; Gallego-Cartagena et al., 2021).

Dans le cadre de la biosurveillance, des informations sur la qualité de l'air de certaines zones sont obtenues en utilisant les propriétés d'un organisme ou d'une partie de celui-ci

(Wang et al., 2019 ; Sturba et al., 2018). Dans cette étude, les escargots terrestres *Helix aspersa* et les aiguilles de conifères *Pinus nigra*, ont été choisis comme organismes de biosurveillance. La biosurveillance des escargots et des aiguilles de conifères a été appliquée pour déterminer la pollution de l'air dans de nombreuses études (Luo et al., 2020 ; Turkeyilmaz et al., 2018 ; Cossi et al., 2018 ; Krupnova et al., 2018). Il s'agit de la première étude au Liban qui détermine des résidus de pesticides, des PCBs, des OCPs et des HAPs dans ces matrices pour surveiller la qualité de l'air.

En outre, la méthodologie de biosurveillance a été appliquée pour la première fois dans la région de Liban nord et ainsi pourra servir de référence pour les futures études. Les intérêts de cette étude sont les suivants :

- la détermination de la teneur en polluants organiques ;
- la détermination des niveaux de pollution sur sept sites différents ;
- l'évaluation de la variabilité entre les mois d'échantillonnages ;
- l'identification des sources de pollution ;
- la comparaison des capacités d'accumulation de polluants dans des escargots et des aiguilles de conifères.

La présence de pesticides pourrait s'expliquer par les activités agricoles importantes basées sur la culture des olives et des fruits, tandis que la présence des HAPs pourrait s'expliquer par la position des sites d'échantillonnage à proximité d'une route avec les émissions des véhicules lourds (Mebdoua, 2019 ; Suman et al., 2016). Différentes caractéristiques telles qu'une couche cireuse, une teneur élevée en lipides, une large surface spécifique pour les aiguilles de conifères et une large distribution d'escargots simplement collectés pourraient conduire à l'accumulation de différents polluants organiques (Likus-Cieślik et al., 2020 ; Sturba et al., 2020). Le tableau 1 montre les limites maximales de résidus (LMR) pour tous les pesticides présents dans les escargots et les aiguilles de conifères selon les bases de données de l'Union européenne sur les pesticides.

Tableau 1 Résidus de pesticide et limites maximales de résidus (mg / kg)

Pesticide residue (mg/kg)	Maximum residue levels			
	(mg/kg)		<i>Pinus nigra</i>	<i>Helix aspersa</i>
	<i>Pinus nigra</i>	<i>Helix aspersa</i>		
Carbendazim	0.1	0.05	0.016	0.001688
Diflufenican	0.01	0.02	0.01436	0.001564
Diuron	0.02	0.05	0.002186	0.001484
Penconazole	0.01	0.01	0.005256	0.00141
Pendimethalin	0.05	0.01	0.003646	0.001498
Fenpropidin	0.01	0.02	0.016502	0.000806
Acetochlor	0.01	0.01	0.00609	0.000734
Chlorpropham	0.01	0.05	0.00694	0.001166
Clofentezine	0.05	0.05	0.009502	0.004346
Deltamethrin	0.02	0.02	0.018154	0.00196
Diclobenil	-	-	0.002366	0.000674
Diphenylamine	0.05	0.05	0.004554	0.00224
Ethofumesate	0.03	0.03	0.00596	0.003304
Kresoxim-methyl	0.01	0.05	0.008046	0.00274
Lambda-cyhalothrin	0.01	0.01	0.016684	0.00102
Pyraclostrobin	0.02	0.05	0.000568	0.000258
Heptachlor	0.01	0.01	0.005628	0.001064
Hexachlorobenzene	0.01	0.01	0.018724	0.002166
α-Endosulfan	0.1	0.01	0.019346	0.00152

Par la comparaison de ces deux matrices différentes, l'accumulation des polluants ne sera pas similaire, dans les mêmes conditions climatiques et les mêmes procédures d'échantillonnage et d'analyse, et a montré une différence de variation pour les pesticides et les HAPs pour chaque matrice. Afin de trouver une explication raisonnable à cette différence, nous avons étudié les données météorologiques pendant la période d'échantillonnage. Les quatre premiers mois de l'échantillonnage (juillet - octobre) sont connus par leur conditions climatiques favorables dans lesquelles aucune précipitation n'était mentionnée et la température était la plus élevée avant l'échantillonnage. Par contre, les deux derniers mois (novembre - décembre) ont connu des précipitations importantes, accompagnées de violentes tempêtes, avec une baisse des températures. En effet, les changements météorologiques et climatiques au cours de cette période de

l'année étaient des événements très normaux, ce qui peut être à l'origine de la diminution des polluants à la fin de l'étude.

Pour *Pinus nigra*, la concentration élevée de pesticides observée au cours des quatre premiers mois est probablement due à l'accumulation de ces polluants par les aiguilles, dont la forte concentration est généralement observée de la fin du printemps à l'été après pulvérisation des pesticides (Luo et al., 2020 ; Rybicki et Jungmann, 2018). Nos résultats obtenus dans ce cadre sont en corrélation avec de nombreuses études antérieures qui suggèrent une relation entre les changements climatiques et les concentrations de pesticides. Estellano et al. en 2015, ont montré que la plus forte concentration de pesticides en Italie a été identifiée dans des échantillons prélevés au printemps et en été. Li et al. en 2014 ont observé une variation au niveau des OCPs par rapport aux variations saisonnières dont la plus grande concentration a été observée pendant la saison sèche tandis que la plus faible concentration apparaisse en présence des pluies.

De plus, les phénomènes de photolyse, les précipitations et l'effet de lavage répété des polluants peuvent également avoir joué un rôle important dans la diminution des HAPs. Ce processus de photolyse peut être accéléré par la couche cireuse d'aiguilles et entraînent une réduction de la concentration des composés (Zheng et al., 2019 ; Li et al., 2014 ; Niu et al., 2004). Cependant, en raison de l'influence des conditions environnementales, des propriétés physicochimiques des polluants et des caractéristiques biologiques des plantes sur les comportements d'accumulation des POPs, les mécanismes d'absorption des ces polluants en suspension dans l'air par les plantes y compris les aiguilles de conifères sont compliqués (Klánová et al., 2009). Kylin et Sjödin ont démontré en 2003, que l'accumulation de POPs dans les aiguilles était influencée par la texture des feuilles et le temps d'exposition. Dans le cycle de vie des aiguilles de conifères, le comportement cumulatif est couvert par des modèles saisonniers, et des facteurs biologiques déterminent la façon dont les aiguilles absorbent les POPs dans l'air. En outre, Barber et al. en 2004 et Kylin et al. en 2003, ont montré que les différentes espèces de plantes accumulent différemment les POPs. Les fluctuations saisonnières affectent l'accumulation de polluants dans les aiguilles des conifères, en raison du cycle annuel de la teneur en terpène dans la cire dont le taux d'accumulation le plus élevée se présente en été (10%) et le plus faible en hiver (1%) (Kylin et Sjödin, 2003). La figure 1

montre la distribution des concentrations des pesticides et des HAPs (ng g⁻¹) trouvées dans la matrice *Pinus nigra*.

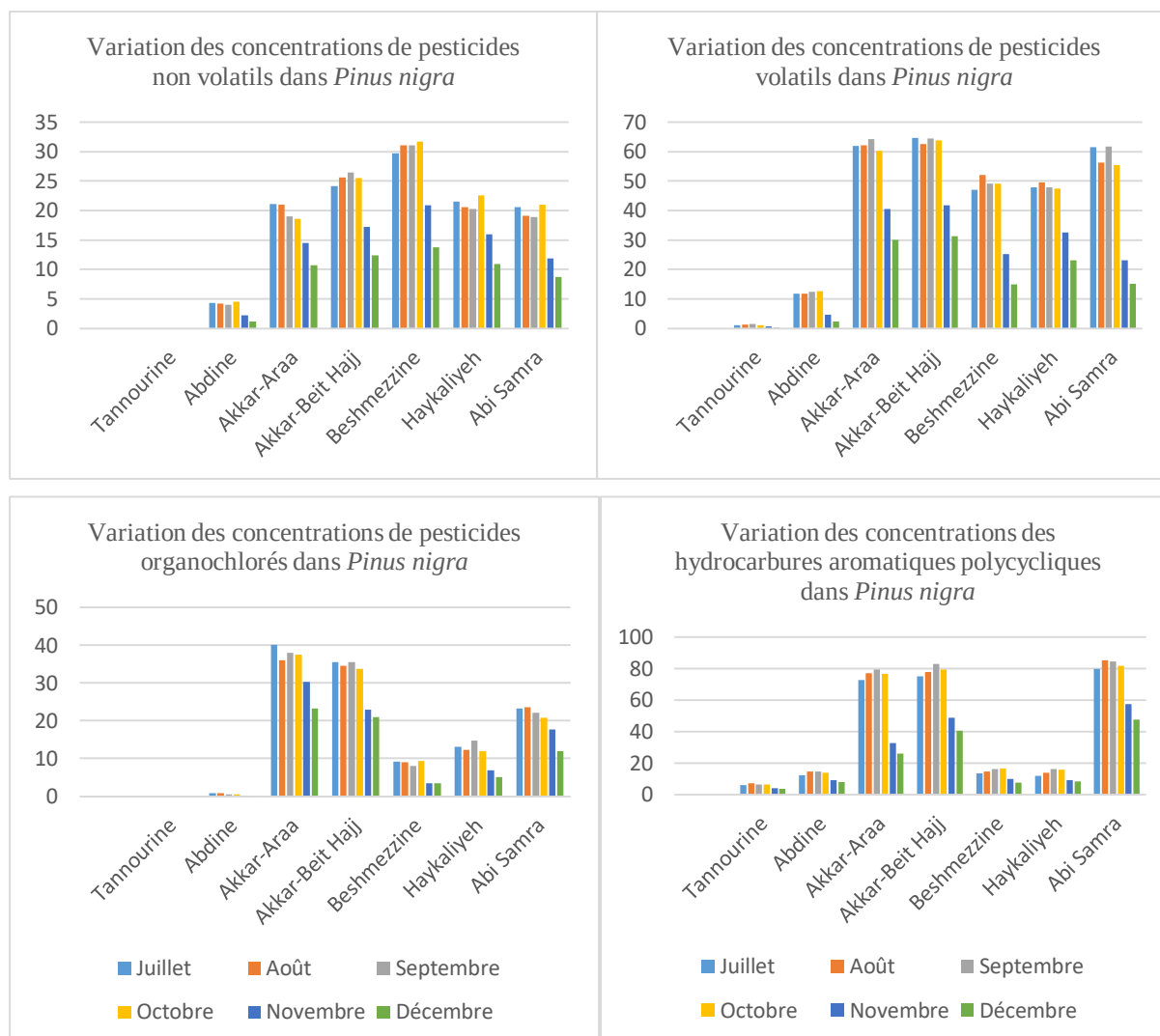


Figure 1. Concentrations mensuelles de pesticides et d'hydrocarbures aromatiques polycycliques dans les zones étudiées faisant état d'une baisse de ces polluants au cours des deux derniers mois de l'étude dans *Pinus nigra*

Les aiguilles de conifères peuvent être utilisés à des fins variées dans les études de la qualité de l'air et permettent aussi d'évaluer le transport des polluants à longue distance (Chropeňová et al., 2016), de détecter les sources de pollutions (Odabasi et al., 2016), de quantifier les niveaux de contamination (Ratola et al., 2014) ainsi que d'étudier l'effet de l'âge des aiguilles sur la capacité d'accumuler les polluants (Klánová et al., 2009).

Les escargots peuvent être contaminés par quatre sources : le sol, l'eau (ingestion et contact cutané), l'air (respiration et contact cutané) et les plantes (Girones et al., 2020). Au cours de la période d'échantillonnage, une accumulation de pesticides et des HAPs a

été observée chez les escargots, avec une augmentation de la concentration au cours de six mois pour atteindre un pic maximal au mois de décembre. Le transfert de pesticides dans les escargots peut-être dû à de nombreuses voies d'exposition différentes au moment de l'application puis par contact digestif avec les plantes et le sol, en particulier par les voies respiratoires et cutanées (Baroudi et al., 2020).

Les concentrations les plus élevées obtenues au cours des deux derniers mois dépendent de l'interaction des escargots avec le sol pollué par les pesticides (Al-Alam et al., 2017 ; Druart et al., 2011) présents en fortes concentrations en raison des effets des précipitations et du lavage continu des pesticides des plantes et des arbres par la pluie, en particulier les aiguilles de conifères et l'accumulation de ces pesticides dans le sol (Gill et Garg, 2014). Ces résultats correspondent à ceux rapportés par Zhao et al. en 2020, montrant que les résidus de pesticides dans les sols actuellement cultivés sont plus élevés que les sols non cultivés.

La concentration élevée des HAPs durant novembre et décembre est obtenue en raison de la diminution de la température de l'air, l'utilisation accrue du charbon et du phénomène de l'inversion du température en hiver (Wang et al., 2018 ; Zheng et al., 2019). Lin et al. en 2018, suggèrent que les conditions météorologiques et les sources d'émissions, ainsi que la distribution des particules de gaz, peuvent entraîner des différences de concentrations entre l'été et l'hiver. On constate que la teneur en HAPs en été est plus faible qu'en hiver, ce qui est cohérent avec des études précédentes (Kong et al., 2015 ; Sharma et al., 2007 ; Akyüz et Çabuk, 2009).

On suppose que durant les saisons de pluie, la concentration des HAPs est la plus élevée principalement en raison de l'augmentation de l'utilisation du charbon qui peut émettre une plus grande quantité de ces polluants (Wang et al., 2018), de l'apparition fréquente de conditions météorologiques à températures inverses en hiver qui conduit à la difficulté de diffusion des polluants, ce qui peut aggraver la pollution atmosphérique par les hydrocarbures, et finalement à cause de la baisse de la température ambiante, de la photolyse lente et de la réaction de dégradation radicale des HAPs en hiver pouvant entraîner une augmentation de leur niveau dans l'air (Cao et al., 2019). La figure 2 montre la distribution des concentrations des pesticides et des HAPs (ng g^{-1}) trouvées dans la matrice *Helix aspersa*.

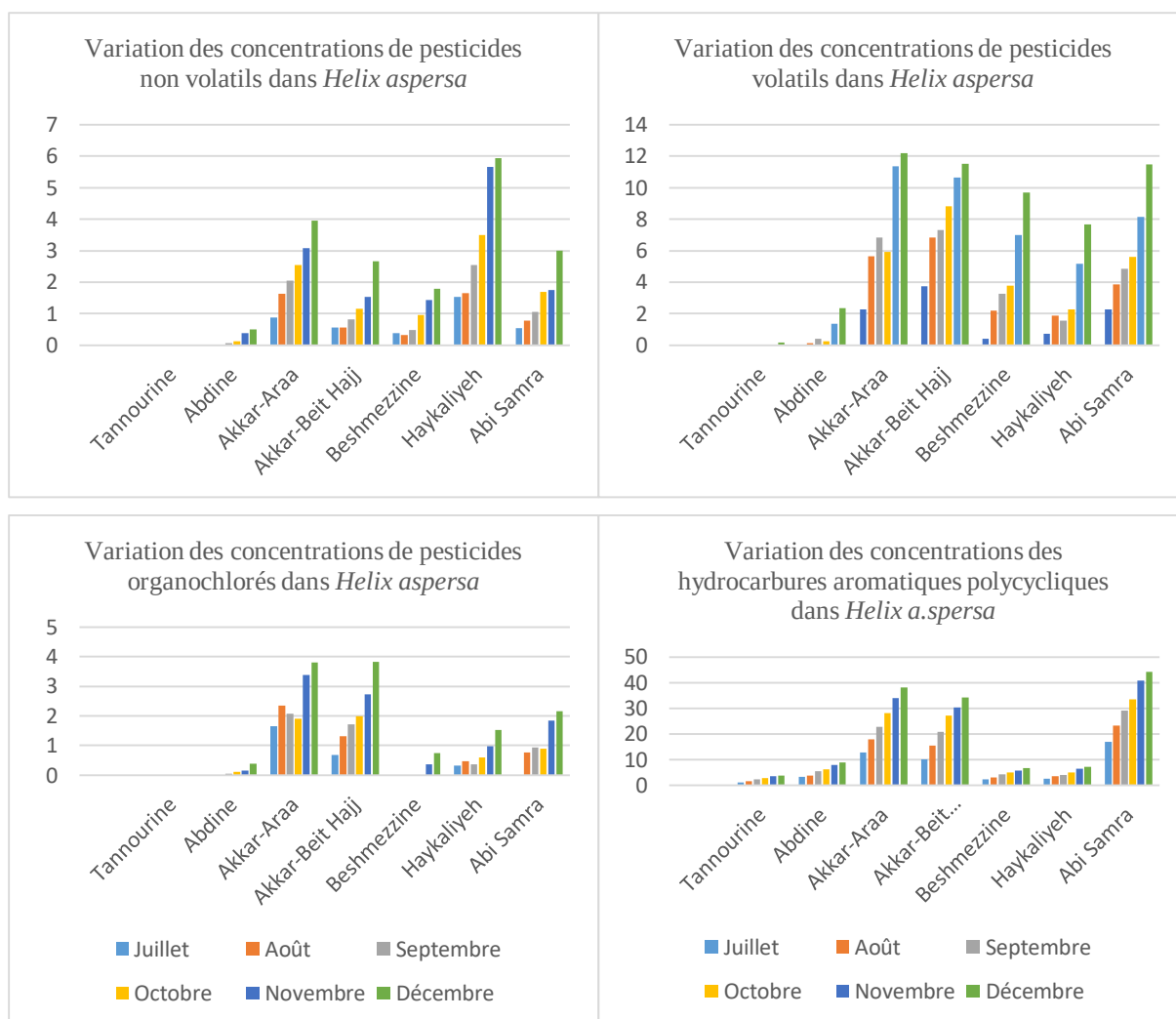


Figure 2. Concentrations mensuelles de pesticides et d'hydrocarbures aromatiques polycycliques dans les zones étudiées faisant état d'une augmentation des polluants évalués dans *Helix aspersa* pendant la durée totale de l'étude

Une régression linéaire multiple a été effectuée pour les concentrations de pesticides et des HAPs avec la température et la précipitation afin de classer les facteurs de contrôle pour l'agrégation des pesticides et des HAPs dans diverses matrices. Les résultats ont montré que les paramètres de la température et la précipitation étaient des facteurs significatifs pour décrire la variation des concentrations de pesticides et des HAPs dans les échantillons d'aiguilles de conifères et d'escargots avec des valeurs $< 0,05$. La figure 3 montre la valeur P pour les concentrations dans les échantillons avec la température et la précipitation des sites d'échantillonnage.

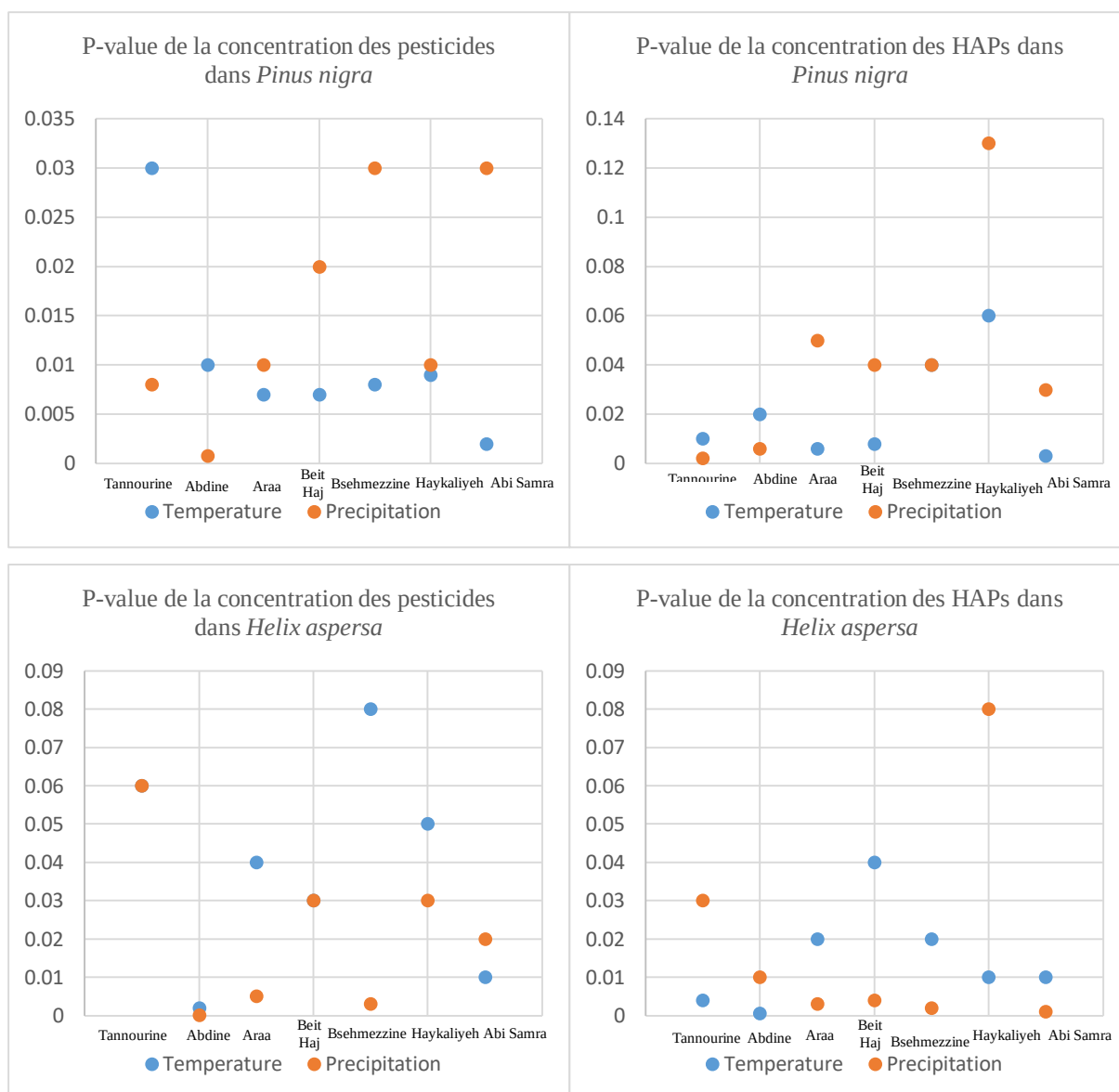


Figure 3. Valeur P pour les concentrations de pesticides et d'hydrocarbures aromatiques polycycliques dans les échantillons avec la température et la précipitation des sites d'échantillonnage

Cette étude montre que les escargots et les aiguilles de conifères sont bien adaptés à la détection des pesticides et des HAPs dans les différentes zones. Les aiguilles de conifères ont accumulé des concentrations significativement plus élevées pour tous les pesticides et les HAPs que pour les escargots. La meilleure accumulation des aiguilles se reflète sur sa persistance à long terme dans les zones d'étude par rapport aux escargots maintenus au moment de l'étude. Le comportement spécifique des deux matrices dans l'accumulation de pesticides et de HAPs dépend de leurs caractéristiques différentes, notamment de la forte teneur en lipides et de la grande surface spécifique des aiguilles qui ont favorisé le stockage des polluants. Le temps d'exposition semble être un facteur

crucial dans l'estimation des activités d'accumulation, du fait qu'au fur et à mesure que les escargots sont exposés sur le terrain, une accumulation de polluants a été observée avec une augmentation de leur concentration.

On constate que la connaissance de la structure et de la morphologie des espèces végétales et animales est une condition préalable essentielle au choix des organismes pour les contaminants cibles afin d'obtenir des résultats précis dans les études environnementales. Par conséquent, une modification de l'exposition des différents polluants aux matrices étudiées sont étroitement corrélées aux conditions climatiques et ainsi qu'à d'autres facteurs tels que les caractéristiques des sols et les propriétés physico-chimiques des polluants. Les variations météorologiques jouent un rôle important dans l'accumulation des polluants, et les données obtenues à partir des deux matrices confirment que les niveaux de pollution semblent varier différemment.

6. Apports du développement d'une méthode d'extraction liquide-liquide pour la détermination multirésidue des pesticides non volatils dans le miel en tant que biomoniteurs environnementaux

Compte tenu de l'insuffisance d'informations dans les publications sur la présence de pesticides et d'autres polluants organiques dans les produits apicoles biologiques au Liban, l'intérêt de cette dernière étude était le développement d'une méthode de préparation d'échantillons par extraction liquide-liquide suivie d'une analyse par LC-MS/MS. La LLE est la technique de purification et d'extraction la plus couramment utilisée pour la détermination des pesticides dans le miel (Pirard et al., 2007). Les solvants les plus largement utilisés sont l'ACN, le méthanol, l'AE (Lopez et al., 2014 ; Panseri et al., 2014). La LC a couramment été utilisée pour analyser les pesticides thermiquement labiles dans la matrice du miel, étant donné que la spectrométrie de masse est la technique la plus efficace et la plus appropriée pour cette forme d'analyse (Al Naggar et al., 2015 ; Bargańska et al., 2013). La MS / MS couplée à la LC permet la réduction des interférences de la matrice et la détection dans des matrices complexes de nombreuses familles de pesticides et d'autres polluants organiques à de faibles concentrations. D'autres types de détecteurs appliqués dans l'analyse de quelques pesticides ou quelques classes de pesticides ont également été utilisés, y compris le détecteur par spectrofluorimétrie (Amendola et al., 2010) et le détecteur à barrettes de diodes (Vichapong et al., 2015).

La technique développée dans cette étude a été utilisée pour l'extraction et l'analyse de 5 échantillons de miel collectés dans divers régions géographiques libanaises afin de détecter la présence de résidus de pesticides, confirmant également que le miel biologique est un indicateur approprié de pollution de l'air (Musarurwa et Tavengwa, 2020 ; El-Nahhal et al., 2020). Il est nécessaire de déterminer les résidus chimiques dans les aliments et l'environnement pour s'assurer que l'exposition du corps humain (en particulier par l'apport alimentaire) aux polluants ne dépasse pas les niveaux de santé tolérables. L'existence de nombreux résidus de pesticides dans les échantillons de miel indique que la colonie d'abeilles de la zone étudiée est susceptible d'être affectée par les pesticides à long terme (Sgargi et al., 2020 ; Ostiguy et al., 2019).

En outre, nos résultats de recherche ont montré que la présence des résidus de pesticides dans le miel biologique peut également être influencée par la zone géographique du contaminant confirmant que les abeilles mellifères et les matrices de ruche sont appropriées pour la surveillance de la pollution environnementale (Chiesa et al., 2016). Dans les zones agricoles où l'apiculture est développée, et en analysant les échantillons de miel collectés, des informations utiles sur les résidus de pesticides produits et distribués par le biais d'un traitement de protection des cultures peuvent être obtenues (Niell et al., 2017).

- Abdallah, C., Sartelet, K., Afif, C., 2016. Influence of boundary conditions and anthropogenic emission inventories on simulated O₃ and PM_{2.5} concentrations over Lebanon. *Atmospheric Pollution Research* 7, 971–979. <https://doi.org/10.1016/j.apr.2016.06.001>
- Abdel-Halim, K.Y., Abo El-Saad, A.M., Talha, M.M., Hussein, A.A., Bakry, N.M., 2013. Oxidative stress on land snail *Helix aspersa* as a sentinel organism for ecotoxicological effects of urban pollution with heavy metals. *Chemosphere* 93, 1131–1138. <https://doi.org/10.1016/j.chemosphere.2013.06.042>
- Abi Ghanem, D., 2018. Energy, the city and everyday life: Living with power outages in post-war Lebanon. *Energy Research & Social Science* 36, 36–43. <https://doi.org/10.1016/j.erss.2017.11.012>
- Akyüz, M., Çabuk, H., 2009. Meteorological variations of PM_{2.5}/PM₁₀ concentrations and particle-associated polycyclic aromatic hydrocarbons in the atmospheric environment of Zonguldak, Turkey. *Journal of Hazardous Materials* 170, 13–21. <https://doi.org/10.1016/j.jhazmat.2009.05.029>
- Al Naggar, Y., Codling, G., Vogt, A., Naiem, E., Mona, M., Seif, A., Giesy, J.P., 2015. Organophosphorus insecticides in honey, pollen and bees (*Apis mellifera* L.) and their potential hazard to bee colonies in Egypt. *Ecotoxicology and Environmental Safety* 114, 1–8. <https://doi.org/10.1016/j.ecoenv.2014.12.039>
- Al-Alam, J., Baroudi, F., Chbani, A., Fajloun, Z., Millet, M., 2020. A multiresidue method for the analysis of pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in snails used as environmental biomonitors. *Journal of Chromatography A* 1621, 461006. <https://doi.org/10.1016/j.chroma.2020.461006>
- Amendola, G., Pelosi, P., Dommarco, R., 2010. Solid-phase extraction for multi-residue analysis of pesticides in honey. *Journal of Environmental Science and Health, Part B* 46, 24–34. <https://doi.org/10.1080/03601234.2010.515170>
- Baayoun, A., Itani, W., El Helou, J., Halabi, L., Medlej, S., El Malki, M., Moukhadder, A., Aboujaoude, L.K., Kabakian, V., Mounajed, H., Mokalled, T., Shihadeh, A., Lakkis, I., Saliba, N.A., 2019. Emission inventory of key sources of air pollution in Lebanon. *Atmospheric Environment* 215, 116871. <https://doi.org/10.1016/j.atmosenv.2019.116871>
- Badaro-Saliba, N., Adjizian-Gerard, J., Zaarour, R., Abboud, M., Farah, W., Saliba, A.N., Shihadeh, A., 2014. A geostatistical approach for assessing population exposure to NO₂ in a complex urban area (Beirut, Lebanon). *Stoch Environ Res Risk Assess* 28, 467–474. <https://doi.org/10.1007/s00477-013-0765-3>
- Barber, J.L., Thomas, G.O., Kerstiens, G., Jones, K.C., 2004. Current issues and uncertainties in the measurement and modelling of air-vegetation exchange and within-plant processing of POPs. *Environmental Pollution* 128, 99–138. <https://doi.org/10.1016/j.envpol.2003.08.024>
- Bargańska, Ż., Ślebioda, M., Namieśnik, J., 2013. Pesticide residues levels in honey from apiaries located of Northern Poland. *Food Control* 31, 196–201. <https://doi.org/10.1016/j.foodcont.2012.09.049>

- Baroudi, F., Al Alam, J., Fajloun, Z., Millet, M., 2020a. Snail as sentinel organism for monitoring the environmental pollution; a review. *Ecological Indicators* 113, 106240. <https://doi.org/10.1016/j.ecolind.2020.106240>
- Baroudi, F., Al-Alam, J., Chimjarn, S., Delhomme, O., Fajloun, Z., Millet, M., 2020b. Conifers as environmental biomonitors: A multi-residue method for the concomitant quantification of pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls by LC-MS/MS and GC-MS/MS. *Microchemical Journal* 154, 104593. <https://doi.org/10.1016/j.microc.2019.104593>
- Cao, Z., Wang, M., Chen, Q., Zhu, C., Jie, J., Li, X., Dong, X., Miao, Z., Shen, M., Bu, Q., 2019. Spatial, seasonal and particle size dependent variations of PAH contamination in indoor dust and the corresponding human health risk. *Science of The Total Environment* 653, 423–430. <https://doi.org/10.1016/j.scitotenv.2018.10.413>
- Chamkasem, N., Lee, S., Harmon, T., 2016. Analysis of 19 PCB congeners in catfish tissue using a modified QuEChERS method with GC-MS/MS. *Food Chemistry* 192, 900–906. <https://doi.org/10.1016/j.foodchem.2015.07.088>
- Chen, J., 2007. Rapid urbanization in China: A real challenge to soil protection and food security. *CATENA, Influences of rapid urbanization and industrialization on soil resource and its quality in China* 69, 1–15. <https://doi.org/10.1016/j.catena.2006.04.019>
- Chiesa, L.M., Labella, G.F., Giorgi, A., Panseri, S., Pavlovic, R., Bonacci, S., Arioli, F., 2016. The occurrence of pesticides and persistent organic pollutants in Italian organic honeys from different productive areas in relation to potential environmental pollution. *Chemosphere* 154, 482–490. <https://doi.org/10.1016/j.chemosphere.2016.04.004>
- Chropeňová, M., Gregušková, E.K., Karásková, P., Příbylová, P., Kukučka, P., Baráková, D., Čupr, P., 2016. Pine needles and pollen grains of *Pinus mugo* Turra – A biomonitoring tool in high mountain habitats identifying environmental contamination. *Ecological Indicators* 66, 132–142. <https://doi.org/10.1016/j.ecolind.2016.01.004>
- Choi, S.-D., Baek, S.-Y., Chang, Y.-S., 2007. Influence of a large steel complex on the spatial distribution of volatile polycyclic aromatic hydrocarbons (PAHs) determined by passive air sampling using membrane-enclosed copolymer (MECOP). *Atmospheric Environment* 41, 6255–6264. <https://doi.org/10.1016/j.atmosenv.2007.03.058>
- Cossi, P.F., Herbert, L.T., Yusseppone, M.S., Pérez, A.F., Kristoff, G., 2018. Environmental concentrations of azinphos-methyl cause different toxic effects without affecting the main target (cholinesterases) in the freshwater gastropod *Biomphalaria straminea*. *Ecotoxicology and Environmental Safety* 162, 287–295. <https://doi.org/10.1016/j.ecoenv.2018.06.091>
- Davidson, D.A., Wilkinson, A.C., Blais, J.M., Kimpe, L.E., McDonald, K.M., Schindler, D.W., 2003. Orographic cold-trapping of persistent organic pollutants by vegetation in mountains of western Canada. *Environmental Science & Technology* 37, 209–215. <https://doi.org/10.1021/es020605q>
- DeArmond, P.D., Brittain, M.K., Platoff, G.E., Yeung, D.T., 2015. QuEChERS-based approach toward the analysis of two insecticides, methomyl and aldicarb, in blood and brain tissue. *Anal Methods* 7, 321–328. <https://doi.org/10.1039/C4AY02137A>
- Dimpe, K.M., Nomngongo, P.N., 2016. Current sample preparation methodologies for analysis of emerging pollutants in different environmental matrices. *TrAC Trends in Analytical Chemistry* 82, 199–207. <https://doi.org/10.1016/j.trac.2016.05.023>
- Druart, C., Millet, M., Scheifler, R., Delhomme, O., Raeppe, C., de Vaufléury, A., 2011. Snails as indicators of pesticide drift, deposit, transfer and effects in the vineyard. *Science of The Total Environment* 409, 4280–4288. <https://doi.org/10.1016/j.scitotenv.2011.07.006>
- El-Nahhal, Y., 2020. Pesticide residues in honey and their potential reproductive toxicity. *Science of The Total Environment* 741, 139953. <https://doi.org/10.1016/j.scitotenv.2020.139953>
- Estellano, V.H., Pozo, Karla, Efsthathiou, C., Pozo, Katherine, Corsolini, S., Focardi, S., 2015. Assessing levels and seasonal variations of current-use pesticides (CUPs) in the Tuscan atmosphere, Italy, using polyurethane foam disks (PUF) passive air samplers. *Environmental Pollution* 205, 52–59. <https://doi.org/10.1016/j.envpol.2015.05.002>
- Filippi, S., Meschini, R., Spognardi, S., Papetti, P., Angeletti, D., 2018. Genetic effects in *Helix aspersa* near a coal plant revealed by the micronucleus test. *Ecotoxicology* 27, 234–243. <https://doi.org/10.1007/s10646-018-1906-8>
- Gallego-Cartagena, E., Morillas, H., Carrero, J.A., Madariaga, J.M., Maguregui, M., 2021. Naturally growing grimmaceae family mosses as passive biomonitors of heavy metals pollution in urban-industrial atmospheres from the Bilbao Metropolitan area. *Chemosphere* 263, 128190. <https://doi.org/10.1016/j.chemosphere.2020.128190>
- Garcia, C.V., Gotah, A., 2017. Application of QuEChERS for Determining Xenobiotics in Foods of Animal Origin. *J Anal Methods Chem* 2017. <https://doi.org/10.1155/2017/2603067>
- Ghorani-Azam, A., Riahi-Zanjani, B., Balali-Mood, M., 2016. Effects of air pollution on human health and practical measures for prevention in Iran. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences* 21, 65. <https://doi.org/10.4103/1735-1995.189646>
- Gill, H.K., Garg, H., 2014. Pesticides: Environmental Impacts and Management Strategies. *Pesticides - Toxic Aspects*. <https://doi.org/10.5772/57399>
- Girones, L., Arias, A.H., Oliva, A.L., Recabarren-Villalon, T., Marcovecchio, J.E., 2020. Occurrence and spatial distribution of organochlorine pesticides in the southwest Buenos Aires using the freshwater snail *Chilina parchappii* as environmental biomonitor. *Regional Studies in Marine Science* 33, 100898. <https://doi.org/10.1016/j.rsma.2019.100898>
- González-Curbelo, M.Á., Socas-Rodríguez, B., Herrera-Herrera, A.V., González-Sálamo, J., Hernández-Borges, J., Rodríguez-Delgado, M.Á., 2015. Evolution and applications of the QuEChERS method. *TrAC Trends in Analytical Chemistry* 71, 169–185. <https://doi.org/10.1016/j.trac.2015.04.012>
- Helou, K., Harmouche-Karaki, M., Karake, S., Narbonne, J.-F., 2019. A review of organochlorine pesticides and polychlorinated biphenyls in Lebanon: Environmental and human contaminants. *Chemosphere* 231, 357–368. <https://doi.org/10.1016/j.chemosphere.2019.05.109>
- Holt, E., Kočan, A., Klánová, J., Assefa, A., Wiberg, K., 2016. Polychlorinated dibenzo- p -dioxins/furans (PCDD/Fs) and metals in scots pine (*Pinus sylvestris*) needles from Eastern and Northern Europe: Spatiotemporal patterns, and potential sources. *Chemosphere* 156, 30–36. <https://doi.org/10.1016/j.chemosphere.2016.04.111>
- Islam, A.K.M.M., Lee, H.-S., Ro, J.-H., Kim, D., Kwon, H., 2019. Application of high-surface-area graphitized carbon black with primary secondary amine as an alternative quick, easy, cheap, effective, rugged, and safe cleanup material for pesticide multi-residue analysis in spinach. *Journal of Separation Science* 42, 2379–2389. <https://doi.org/10.1002/jssc.201900066>
- Issa, M.M., M. Taha, S., El- Marsafy, A.M., Khalil, M.M.H., Ismail, E.H., 2020. Acetonitrile-Ethyl acetate based method for the residue analysis of 373 pesticides in beeswax using LC-MS/MS and GC-MS/MS. *Journal of Chromatography B* 1145, 122106. <https://doi.org/10.1016/j.jchromb.2020.122106>
- Jabali, Y., Millet, M., El-Hoz, M., 2019. Optimization of a DI-SPME-GC-MS/MS method for multi-residue analysis of pesticides in waters. *Microchemical Journal* 147, 83–92. <https://doi.org/10.1016/j.microc.2019.03.004>
- Jaward, F.M., Barber, J.L., Booij, K., Jones, K.C., 2004. Spatial distribution of atmospheric PAHs and PCNs along a north–south Atlantic transect. *Environmental Pollution* 132, 173–181. <https://doi.org/10.1016/j.envpol.2004.03.029>

- Kim, L., Lee, D., Cho, H.-K., Choi, S.-D., 2019. Review of the QuEChERS method for the analysis of organic pollutants: Persistent organic pollutants, polycyclic aromatic hydrocarbons, and pharmaceuticals. *Trends in Environmental Analytical Chemistry* 22, e00063. <https://doi.org/10.1016/j.teac.2019.e00063>
- Klánová, J., Čupr, P., Baráková, D., Šeda, Z., Anděl, P., Holoubek, I., 2009. Can pine needles indicate trends in the air pollution levels at remote sites? *Environmental Pollution* 157, 3248–3254. <https://doi.org/10.1016/j.envpol.2009.05.030>
- Kong, S., Li, X., Li, L., Yin, Y., Chen, K., Yuan, L., Zhang, Y., Shan, Y., Ji, Y., 2015. Variation of polycyclic aromatic hydrocarbons in atmospheric PM_{2.5} during winter haze period around 2014 Chinese Spring Festival at Nanjing: Insights of source changes, air mass direction and firework particle injection. *Science of The Total Environment* 520, 59–72. <https://doi.org/10.1016/j.scitotenv.2015.03.001>
- Krupnova, T.G., Mashkova, I.V., Kostyukova, A.M., Schelkanova, E.E., Gavrilkina, S.V., 2018. Gastropods as potential biomonitors of contamination caused by heavy metals in South Ural lakes, Russia. *Ecological Indicators* 95, 1001–1007. <https://doi.org/10.1016/j.ecolind.2017.12.005>
- Kylin, H., Hellström, A., Nordstrand, E., Zaid, A., 2003. Organochlorine pollutants in Scots pine needles—biological and site related variation within a forest stand. *Chemosphere* 51, 669–675. [https://doi.org/10.1016/S0045-6535\(03\)00101-2](https://doi.org/10.1016/S0045-6535(03)00101-2)
- Kylin, H., Sjödin, A., 2003. Accumulation of Airborne Hexachlorocyclohexanes and DDT in Pine Needles. *Environ. Sci. Technol.* 37, 2350–2355. <https://doi.org/10.1021/es0201395>
- Lavin, K.S., Hageman, K.J., 2012. Selective pressurised liquid extraction of halogenated pesticides and polychlorinated biphenyls from pine needles. *Journal of Chromatography A* 1258, 30–36. <https://doi.org/10.1016/j.chroma.2012.08.042>
- Li, W., Zhang, Z., Zhang, R., Jiao, H., Sun, A., Shi, X., Chen, J., 2020. Effective removal matrix interferences by a modified QuEChERS based on the molecularly imprinted polymers for determination of 84 polychlorinated biphenyls and organochlorine pesticides in shellfish samples. *Journal of Hazardous Materials* 384, 121241. <https://doi.org/10.1016/j.jhazmat.2019.121241>
- Li, Y., Lohmann, R., Zou, X., Wang, C., Zhang, L., 2020. Air-water exchange and distribution pattern of organochlorine pesticides in the atmosphere and surface water of the open Pacific ocean. *Environmental Pollution* 265, 114956. <https://doi.org/10.1016/j.envpol.2020.114956>
- Li, Y., Niu, J., Shen, Z., Zhang, C., Wang, Z., He, T., 2014. Spatial and seasonal distribution of organochlorine pesticides in the sediments of the Yangtze Estuary. *Chemosphere* 114, 233–240. <https://doi.org/10.1016/j.chemosphere.2014.04.112>
- Likus-Cieślak, J., Socha, J., Gruba, P., Pietrzykowski, M., 2020. The current state of environmental pollution with sulfur dioxide (SO₂) in Poland based on sulfur concentration in Scots pine needles. *Environmental Pollution* 258, 113559. <https://doi.org/10.1016/j.envpol.2019.113559>
- Lin, Y., Zou, J., Yang, W., Li, C.-Q., 2018. A Review of Recent Advances in Research on PM_{2.5} in China. *Int J Environ Res Public Health* 15. <https://doi.org/10.3390/ijerph15030438>
- Liu, H.-Y., Lin, S.-L., Fuh, M.-R., 2016. Determination of chloramphenicol, thiamphenicol and florfenicol in milk and honey using modified QuEChERS extraction coupled with polymeric monolith-based capillary liquid chromatography tandem mass spectrometry. *Talanta* 150, 233–239. <https://doi.org/10.1016/j.talanta.2015.12.045>
- Loos, M., Krauss, M., Fenner, K., 2012. Pesticide nonextractable residue formation in soil: insights from inverse modeling of degradation time series. *Environmental Science & Technology* 46, 9830–9837. <https://doi.org/10.1021/es300505r>
- Łozowicka, B., Rutkowska, E., Jankowska, M., 2017. Influence of QuEChERS modifications on recovery and matrix effect during the multi-residue pesticide analysis in soil by GC/MS/MS and GC/ECD/NPD. *Environ Sci Pollut Res Int* 24, 7124–7138. <https://doi.org/10.1007/s11356-016-8334-1>
- Lovinsky-Desir, S., Miller, R.L., Bautista, J., Gil, E.N., Chillrud, S.N., Yan, B., Camann, D., Perera, F.P., Jung, K.H., 2016. Differences in Ambient Polycyclic Aromatic Hydrocarbon Concentrations between Streets and Alleys in New York City: Open Space vs. Semi-Closed Space. *Int J Environ Res Public Health* 13. <https://doi.org/10.3390/ijerph13010127>
- Luo, Y., Sun, J., Wang, P., Li, Y., Li, H., Xiao, K., Yang, R., Zhang, Q., Jiang, G., 2020. Age dependence accumulation of organochlorine pesticides and PAHs in needles with different forest types, southeast Tibetan Plateau. *Science of The Total Environment* 716, 137176. <https://doi.org/10.1016/j.scitotenv.2020.137176>
- Manisalidis, I., Stavropoulou, E., Stavropoulos, A., Bezirtzoglou, E., 2020. Environmental and Health Impacts of Air Pollution: A Review. *Front. Public Health* 8. <https://doi.org/10.3389/fpubh.2020.00014>
- Massimi, L., Conti, M.E., Mele, G., Ristorini, M., Astolfi, M.L., Canepari, S., 2019. Lichen transplants as indicators of atmospheric element concentrations: a high spatial resolution comparison with PM₁₀ samples in a polluted area (Central Italy). *Ecological Indicators* 101, 759–769. <https://doi.org/10.1016/j.ecolind.2018.12.051>
- Mebdouda, S., 2019. Pesticide Residues in Fruits and Vegetables, in: Mérillon, J.-M., Ramawat, K.G. (Eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry. Springer International Publishing, Cham, pp. 1715–1753. https://doi.org/10.1007/978-3-319-78030-6_76
- Melymuk, L., Bohlin-Nizzetto, P., Prokeš, R., Kukučka, P., Příbylová, P., Vojta, Š., Kohoutek, J., Lammel, G., Klánová, J., 2017. Uncertainties in monitoring of SVOCs in air caused by within-sampler degradation during active and passive air sampling. *Atmospheric Environment* 167, 553–565. <https://doi.org/10.1016/j.atmosenv.2017.08.038>
- Merhaby, D., Ouddane, B., Net, S., Halwani, J., 2020. Assessment of persistent organic pollutants in surface sediments along Lebanese coastal zone. *Marine Pollution Bulletin* 153, 110947. <https://doi.org/10.1016/j.marpolbul.2020.110947>
- Mleiki, A., El Menif, N.T., Marigómez, I., 2020. Integrative assessment of the biological responses elicited by metal pollution in the green garden snail, *Cantareus apertus*: Laboratory and field studies. *Ecological Indicators* 117, 106589. <https://doi.org/10.1016/j.ecolind.2020.106589>
- Mokalled, T., Le Calvé, S., Badaro-Saliba, N., Abboud, M., Zaarour, R., Farah, W., Adjizian-Gérard, J., 2018. Identifying the impact of Beirut Airport's activities on local air quality - Part I: Emissions inventory of NO₂ and VOCs. *Atmospheric Environment* 187, 435–444. <https://doi.org/10.1016/j.atmosenv.2018.04.036>
- Mukhopadhyay, S., Dutta, R., Das, P., 2020. A critical review on plant biomonitors for determination of polycyclic aromatic hydrocarbons (PAHs) in air through solvent extraction techniques. *Chemosphere* 251, 126441. <https://doi.org/10.1016/j.chemosphere.2020.126441>
- Musarurwa, H., Tavengwa, N.T., 2020. Deep eutectic solvent-based dispersive liquid-liquid micro-extraction of pesticides in food samples. *Food Chemistry* 127943. <https://doi.org/10.1016/j.foodchem.2020.127943>
- Niell, S., Jesús, F., Pérez, N., Pérez, C., Pareja, L., Abbate, S., Carrasco-Letelier, L., Díaz, S., Mendoza, Y., Cesio, V., Heinzen, H., 2017. Neonicotinoids transference from the field to the hive by honey bees: Towards a pesticide residues biomonitor. *Science of The Total Environment* 581–582, 25–31. <https://doi.org/10.1016/j.scitotenv.2017.01.011>

- Niu, J., Chen, J., Martens, D., Henkelmann, B., Quan, X., Yang, F., Seidlitz, H.K., Schramm, K.-W., 2004. The role of UV-B on the degradation of PCDD/Fs and PAHs sorbed on surfaces of spruce (*Picea abies* (L.) Karst.) needles. *Science of The Total Environment* 322, 231–241. <https://doi.org/10.1016/j.scitotenv.2003.09.017>
- Noth, E.M., Katharine Hammond, S., Biging, G.S., Tager, I.B., 2013. Mapping and modeling airborne urban phenanthrene distribution using vegetation biomonitoring. *Atmospheric Environment* 77, 518–524. <https://doi.org/10.1016/j.atmosenv.2013.05.056>
- Odabasi, M., Dumanoglu, Y., Ozgunerge Falay, E., Tuna, G., Altioik, H., Kara, M., Bayram, A., Tolunay, D., Elbir, T., 2016. Investigation of spatial distributions and sources of persistent organic pollutants (POPs) in a heavily polluted industrial region using tree components. *Chemosphere* 160, 114–125. <https://doi.org/10.1016/j.chemosphere.2016.06.076>
- Ostiguy, N., Drummond, F.A., Aronstein, K., Eitzer, B., Ellis, J.D., Spivak, M., Sheppard, W.S., 2019. Honey Bee Exposure to Pesticides: A Four-Year Nationwide Study. *Insects* 10. <https://doi.org/10.3390/insects10010013>
- Panseri, S., Catalano, A., Giorgi, A., Arioli, F., Procopio, A., Britti, D., Chiesa, L.M., 2014. Occurrence of pesticide residues in Italian honey from different areas in relation to its potential contamination sources. *Food Control* 38, 150–156. <https://doi.org/10.1016/j.foodcont.2013.10.024>
- Pirard, C., Widart, J., Nguyen, B.K., Deleuze, C., Heudt, L., Haubruge, E., De Pauw, E., Focant, J.-F., 2007. Development and validation of a multi-residue method for pesticide determination in honey using on-column liquid–liquid extraction and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A* 1152, 116–123. <https://doi.org/10.1016/j.chroma.2007.03.035>
- Pompa-García, M., Sánchez-Salguero, R., Camarero, J.J., 2017. Observed and projected impacts of climate on radial growth of three endangered conifers in northern Mexico indicate high vulnerability of drought-sensitive species from mesic habitats. *Dendrochronologia* 45, 145–155. <https://doi.org/10.1016/j.dendro.2017.08.006>
- Quan, L., Li, S., Tian, S., Xu, H., Lin, A., Gu, L., 2004. Determination of Organochlorine Pesticides Residue in Ginseng Root by Orthogonal Array Design Soxhlet Extraction and Gas Chromatography. *Chromatographia* 59, 89–93. <https://doi.org/10.1365/s10337-003-0120-9>
- Ratola, N., Alves, A., Santos, L., Lacorte, S., 2011a. Pine needles as passive bio-samplers to determine polybrominated diphenyl ethers. *Chemosphere* 85, 247–252. <https://doi.org/10.1016/j.chemosphere.2011.06.005>
- Ratola, N., Amigo, J.M., Oliveira, M.S.N., Araújo, R., Silva, J.A., Alves, A., 2011b. Differences between *Pinus pinea* and *Pinus pinaster* as bioindicators of polycyclic aromatic hydrocarbons. *Environmental and Experimental Botany* 72, 339–347. <https://doi.org/10.1016/j.envexpbot.2011.04.012>
- Ratola, N., Homem, V., Silva, J.A., Araújo, R., Amigo, J.M., Santos, L., Alves, A., 2014. Biomonitoring of pesticides by pine needles — Chemical scoring, risk of exposure, levels and trends. *Science of The Total Environment* 476–477, 114–124. <https://doi.org/10.1016/j.scitotenv.2014.01.003>
- Rybicki, M., Jungmann, D., 2018. Direct and indirect effects of pesticides on a benthic grazer during its life cycle. *Environmental Sciences Europe* 30, 35. <https://doi.org/10.1186/s12302-018-0165-x>
- Saraiva, M., Cavalheiro, J., Lancelleur, L., Monperrus, M., 2016. Synthetic musk in seafood products from south Europe using a quick, easy, cheap, effective, rugged and safe extraction method. *Food Chemistry* 200, 330–335. <https://doi.org/10.1016/j.foodchem.2016.01.017>
- Schmielau, L., Dvorak, M., Niederwanger, M., Dobieszewski, N., Pedrini-Martha, V., Ladurner, P., Pedregal, J.R.-G., Maréchal, J.-D., Dallinger, R., 2019. Differential response to Cadmium exposure by expression of a two and a three-domain metallothionein isoform in the land snail *Pomatias elegans*: Valuating the marine heritage of a land snail. *Science of The Total Environment* 648, 561–571. <https://doi.org/10.1016/j.scitotenv.2018.07.426>
- SCHRLAU, J.E., GEISER, L., HAGEMAN, K.J., LANDERS, D.H., SIMONICH, S.M., 2011. Comparison of Lichen, Conifer Needles, Passive Air Sampling Devices, and Snowpack as Passive Sampling Media to Measure Semi-Volatile Organic Compounds in Remote Atmospheres. *Environ Sci Technol* 45, 10354–10361. <https://doi.org/10.1021/es202418f>
- Sgargi, D., Adam, B., Budnik, L.T., Dinelli, G., Moldovan, H.R., Perry, M.J., Scheepers, P.T.J., Schlünssen, V., Teixeira, J.P., Mandrioli, D., Belpoggi, F., 2020. Protocol for a systematic review and meta-analysis of human exposure to pesticide residues in honey and other bees' products. *Environmental Research* 186, 109470. <https://doi.org/10.1016/j.envres.2020.109470>
- Sharma, H., Jain, V.K., Khan, Z.H., 2007. Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in the urban environment of Delhi. *Chemosphere* 66, 302–310. <https://doi.org/10.1016/j.chemosphere.2006.05.003>
- Soukarié, B., El Hawari, K., El Hussein, M., Budzinski, H., Jaber, F., 2018. Impact of Lebanese practices in industry, agriculture and urbanization on soil toxicity. Evaluation of the Polycyclic Aromatic Hydrocarbons (PAHs) levels in soil. *Chemosphere* 210, 85–92. <https://doi.org/10.1016/j.chemosphere.2018.06.178>
- Srogi, K., 2007. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. *Environ Chem Lett* 5, 169–195. <https://doi.org/10.1007/s10311-007-0095-0>
- Sturba, L., Fattorini, N., Liberatori, G., Vannuccini, M.L., Nannoni, F., Protano, G., Tursi, A., Corsi, I., 2020. Multi-model inference analysis of toxicological responses and levels of heavy metals in soft tissue of land snail *Cornu aspersum* caged in proximity to an industrial setting. *Ecological Indicators* 117, 106688. <https://doi.org/10.1016/j.ecolind.2020.106688>
- Sturba, L., Liberatori, G., Vannuccini, M.L., Ancora, S., Corsi, I., 2018. Uptake and biological responses in land snail *Cornu aspersum* exposed to vaporized CdCl₂. *Ecotoxicology and Environmental Safety* 148, 377–383. <https://doi.org/10.1016/j.ecoenv.2017.10.050>
- Surma, M., Wiczowski, W., Cieślík, E., Zieliński, H., 2015. Method development for the determination of PFOA and PFOS in honey based on the dispersive Solid Phase Extraction (d-SPE) with micro-UHPLC–MS/MS system. *Microchemical Journal* 121, 150–156. <https://doi.org/10.1016/j.microc.2015.02.008>
- Tian, L., Yin, S., Ma, Y., Kang, H., Zhang, X., Tan, H., Meng, H., Liu, C., 2019. Impact factor assessment of the uptake and accumulation of polycyclic aromatic hydrocarbons by plant leaves: Morphological characteristics have the greatest impact. *Science of The Total Environment* 652, 1149–1155. <https://doi.org/10.1016/j.scitotenv.2018.10.357>
- Tripathy, V., Saha, A., Kumar, J., 2017. Detection of pesticides in popular medicinal herbs: a modified QuEChERS and gas chromatography–mass spectrometry based approach. *J Food Sci Technol* 54, 458–468. <https://doi.org/10.1007/s13197-017-2487-x>
- Turkylmaz, A., Sevik, H., Cetin, M., 2018. The use of perennial needles as biomonitors for recently accumulated heavy metals. *Landscape Ecol Eng* 14, 115–120. <https://doi.org/10.1007/s11355-017-0335-9>
- Vaaland, I.C., Pampanin, D.M., Sydnes, M.O., 2020. Synthesis of trans-dihydronaphthalene-diols and evaluation of their use as standards for PAH metabolite analysis in fish bile by GC-MS. *Chemosphere* 256, 126928. <https://doi.org/10.1016/j.chemosphere.2020.126928>

- Vichapong, J., Burakham, R., Srijaranai, S., 2015. In-coupled syringe assisted octanol–water partition microextraction coupled with high-performance liquid chromatography for simultaneous determination of neonicotinoid insecticide residues in honey. *Talanta* 139, 21–26. <https://doi.org/10.1016/j.talanta.2015.02.033>
- Voua Otomo, P., Owojori, O.J., Reinecke, S.A., Daniels, S., Reinecke, A.J., 2011. Using estimates of metal bioavailability in the soil and genetic variation of allozymes to investigate heavy metal tolerance in the earthworm *Eisenia fetida* (Oligochaeta). *Ecotoxicology and Environmental Safety* 74, 2070–2074. <https://doi.org/10.1016/j.ecoenv.2011.05.014>
- Waked, A., Seigneur, C., Couvidat, F., Kim, Y., Sartelet, K., Afif, C., Borbon, A., Formenti, P., Sauvage, S., 2012. Modeling air pollution in Lebanon: evaluation at a suburban site in Beirut. *Atmospheric Chemistry and Physics Discussions* 12. <https://doi.org/10.5194/acpd-12-29571-2012>
- Wang, R., Liu, G., Sun, R., Yousaf, B., Wang, J., Liu, R., Zhang, H., 2018. Emission characteristics for gaseous- and size-segregated particulate PAHs in coal combustion flue gas from circulating fluidized bed (CFB) boiler. *Environmental Pollution (Barking, Essex: 1987)* 238, 581–589. <https://doi.org/10.1016/j.envpol.2018.03.051>
- Wang, S., Qi, P., Di, S., Wang, J., Wu, S., Wang, Xiangyun, Wang, Z., Wang, Q., Wang, Xinquan, Zhao, C., Li, Q., 2019. Significant role of supercritical fluid chromatography - mass spectrometry in improving the matrix effect and analytical efficiency during multi-pesticides residue analysis of complex chrysanthemum samples. *Analytica Chimica Acta* 1074, 108–116. <https://doi.org/10.1016/j.aca.2019.04.063>
- Wang, X., Jia, R., Song, Y., Wang, M., Zhao, Q., Sun, S., 2019. Determination of pesticides and their degradation products in water samples by solid-phase extraction coupled with liquid chromatography-mass spectrometry. *Microchemical Journal* 149, 104013. <https://doi.org/10.1016/j.microc.2019.104013>
- Yang, R., Yao, T., Xu, B., Jiang, G., Zheng, X., 2008. Distribution of organochlorine pesticides (OCPs) in conifer needles in the southeast Tibetan Plateau. *Environmental Pollution, Chelating Agents in the Environment* 153, 92–100. <https://doi.org/10.1016/j.envpol.2007.07.033>
- Yavuz, H., Guler, G.O., Aktumsek, A., Cakmak, Y.S., Ozparlak, H., 2010. Determination of some organochlorine pesticide residues in honeys from Konya, Turkey. *Environ Monit Assess* 168, 277–283. <https://doi.org/10.1007/s10661-009-1111-6>
- Zhao, L., Li, Y., Ren, W., Huang, Y., Wang, X., Fu, Z., Ma, W., Teng, Y., Luo, Y., 2020. Pesticide residues in soils planted with *Panax notoginseng* in south China, and their relationships in *Panax notoginseng* and soil. *Ecotoxicology and Environmental Safety* 201, 110783. <https://doi.org/10.1016/j.ecoenv.2020.110783>
- Zhao, X., He, M., Shang, H., Yu, H., Wang, H., Li, H., Piao, J., Quinto, M., Li, D., 2018. Biomonitoring polycyclic aromatic hydrocarbons by *Salix matsudana* leaves: A comparison with the relevant air content and evaluation of environmental parameter effects. *Atmospheric Environment* 181, 47–53. <https://doi.org/10.1016/j.atmosenv.2018.03.004>
- Zheng, L., Ou, J., Liu, M., Chen, Y., Tang, Q., Hu, Y., 2019. Seasonal and spatial variations of PM10-bounded PAHs in a coal mining city, China: Distributions, sources, and health risks. *Ecotoxicology and Environmental Safety* 169, 470–478. <https://doi.org/10.1016/j.ecoenv.2018.11.063>

Conclusion et perspectives

À la suite de tous les travaux de recherche réalisés au cours de cette thèse de doctorat, les conclusions suivantes peuvent être tirées :

- La LC et GC couplée à la MS/MS est l'une des techniques les plus puissantes pour la détermination des résidus de pesticides et des POPs dans les échantillons alimentaires et environnementaux. L'excellente sélectivité et la sensibilité obtenues dans les méthodes développées permettent la surveillance et la quantification des composés à de très faibles niveaux de concentration ;
- Une méthode basée sur QuEChERS suivie d'une SPME a été développée pour la détermination de pesticides et des POPs et est adéquate pour surveiller leur présence dans les échantillons des aiguilles de conifères. Outre la détection des composés à de faibles concentrations, l'utilisation de la LC-MS/MS et GC-MS/MS a permis d'atteindre la sensibilité nécessaire pour valider cette nouvelle méthode ;
- Une méthode de détermination des résidus de pesticides et des POPs dans les échantillons d'escargots basée sur la LC-MS/MS et GC-MS/MS a été également développée. L'étape de préparation des échantillons a été critique, et ainsi, avec la procédure optimisée, les pertes de composés ont été minimisées, de sorte que des résultats acceptables ont été obtenus dans tous les échantillons étudiés ;
- Une méthode basée sur la technique de la LLE suivie de l'analyse par LC-MS/MS a été développée pour la détermination spécifique des résidus de pesticides dans des échantillons de miel. L'étape de préparation des échantillons, le choix du solvant ont été étudiés afin de minimiser les pertes de composés, et des résultats satisfaisants ont été obtenus pour tous les échantillons étudiés ;
- La détermination des résidus de pesticides et des POPs dans différents types de matrices ont été mis en œuvre. L'intérêt était de déterminer la teneur en ces composés dans les escargots terrestres *Helix aspersa* et les aiguilles de conifères *Pinus nigra*. Les résultats ont été évalués en fonction des changements saisonniers, de la source de pollution et des caractéristiques de chaque site d'échantillonnages. Les données indiquent qu'il y a un impact significatif de la variation saisonnière sur les concentrations pour tous les composés. Des concentrations croissantes ont été obtenues dans les escargots au cours des deux saisons. Les deux types de polluants

étudiés ont montré des concentrations élevées (pour l'été) et faibles (pour l'automne) pour les aiguilles de conifères. En conséquence, différentes sources peuvent altérer la concentration, à savoir les caractéristiques du site étudié, les sols contaminés, le trafic, le métabolisme des aiguilles, le développement de l'escargot et la combustion du charbon.

Les principaux avantages des études de qualité de l'air concernant la plupart des méthodologies d'échantillonnage de biosurveillance sont :

- Une meilleure couverture spatiale et temporelle ;
- De constituer un outil puissant pour la gestion de la qualité de l'air ;
- De fournir des informations sur les polluants dans l'air dans des endroits éloignés, où la surveillance de la qualité de l'air est impossible pour des raisons techniques et économiques ;
- De fournir des informations sur la concentration des polluants présentes dans l'air et à plusieurs niveaux ;
- D'être utilisés pour analyser les événements de pollution passés et présentes ;
- De fournir des informations détaillées sur les sources d'émission, les processus de transport, de dépôt et les transformations chimiques des polluants ;
- D'être utilisés pour évaluer l'exposition de la population à un polluant donné ;
- De donner un aperçu des propriétés des polluants et de leur impact sur la santé et l'environnement ;

Afin d'améliorer les études antérieures et de répondre à certaines des questions, plusieurs perspectives peuvent être pris en compte :

- Extension des études de biosurveillance à des échelles spatiales et temporelles plus étendus ;
- La mise au point d'une méthode d'extraction et d'analyse dans les sols permettant de déterminer les concentrations de polluants lors des campagnes d'études ;
- La mise au point d'une méthode d'extraction et d'analyse dans les aliments d'origine végétale est nécessaire afin de pouvoir détecter les concentrations dans les plantes ingérées par les escargots in situ ;

- La mise au point d'un développement analytique pour la détermination de la capacité de transfert du polluant à l'œuf puis à l'embryon d'escargots, la détermination de la quantité de polluants transférée par unité de temps, ainsi que les études de leurs effets sur la croissance ;
- Etude de la surveillance biologique humaine afin d'évaluer l'exposition et pour une meilleure gestion des risques que posent l'exposition aux substances chimiques sur la santé environnementale.

Étude comparative de trois biomoniteurs (conifère, escargot et miel) pour évaluer la variabilité spatio-temporelle de polluants organiques dans l'atmosphère au Liban

Résumé

La pollution de l'air a des impacts importants sur la santé humaine et l'environnement et représente aujourd'hui l'un des principaux sujets de préoccupation environnementale du monde. Ainsi, il est nécessaire d'améliorer la compréhension de la dynamique de la pollution afin de concevoir des stratégies efficaces pour contrôler la qualité de l'air et réduire l'impact de cette pollution. C'est dans ce contexte que se situe l'objectif principal de cette thèse consistant à caractériser la qualité de l'air en développant des méthodes d'extraction multi-résidus basées sur la biosurveillance qui utilise le vivant (organisme animal ou végétal) comme matrice pour surveiller l'évolution, les altérations ou les modifications de la qualité de l'air dans un environnement donné. En effet, certaines espèces vivantes sont sensibles à des polluants uniques spécifiques ou à des mélanges de polluants et pourraient donc probablement être utilisées pour surveiller les effets des polluants en tant que biomoniteurs.

Plusieurs méthodes analytiques ont été développées dans le cadre de ces travaux de thèse et ont permis l'analyse simultanée de 158 polluants environnementaux émergents du gastéropode terrestre *Helix aspersa* et 172 polluants du conifère *Pinus nigra*. La combinaison d'une méthode d'extraction simple telle que le QuEChERS avec un procédé de microextraction sur phase solide couplée à des techniques chromatographiques a été valorisée au cours de ces travaux et a permis l'extraction de ce grand nombre de polluants. Ces méthodes ont prouvé leur efficacité notamment en termes de rendement, de rapidité et de respect de l'environnement. De plus, la méthode QuEChERS a donné des bonnes performances en termes de linéarité, d'exactitude et de précision. Cette méthode d'extraction suivie par une analyse chromatographie en phase liquide et/ou gazeuse couplée à la spectrométrie de masse en tandem s'est révélée être un outil fiable pour l'analyse en routine d'une large gamme de composés, à l'état de traces, dans les escargots et les aiguilles de conifères. En outre, elle pourrait être appliquée dans d'autres études de biosurveillance environnementale sur d'autres matrices biologiques.

Les résultats obtenus ont montré que les conifères et les escargots peuvent refléter des émissions spécifiques de polluants et doivent être considérés comme des sentinelles appropriées pour la mise en évidence de la pollution de l'environnement, en particulier lorsque les échantillons sont prélevés dans plusieurs zones à différentes altitudes. De même, il a pu être montré que les différences de concentrations

des polluants observés pourraient être corrélées avec les propriétés physico-chimiques des composés, la proximité des sources et d'autres facteurs environnementaux tels que les conditions climatiques. Enfin, les résultats obtenus au Liban confirment la nécessité de créer une législation pour les pesticides et les polluants organiques persistants en incitant des recherches plus poussées pour identifier les principales sources de contamination, estimer leur contribution à la contamination globale et étudier les solutions possibles pour diminuer cette contamination.

Mots clés : Pollution de l'air - Biosurveillance environnementale - QuEChERS - *Helix aspersa* - *Pinus nigra* - Pesticides - Polluants organiques persistants.

Résumé en anglais

Air pollution has significant impacts on human health and the environment and currently is the one of the major environmental concern in the world. Thus, there is a need to improve understanding of the dynamics of pollution in order to plan effective strategies to control air quality and reduce the impact of this pollution. The main objective of this thesis is consisting in characterizing the air quality by developing multi-residue extraction methods based on biomonitoring which uses living organisms (animal or plant organism) as matrix to monitor the evolution, alterations or modifications of air quality in environment. Just as some living species are sensitive to specific pollutants or mixtures of pollutants and are therefore likely to be used to monitor the effects of pollutants as biomonitors.

Several analytical methods were developed as part of this thesis work and allowed the simultaneous analysis of 158 environmental pollutants from the terrestrial gastropod *Helix aspersa* and 172 pollutants from the *Pinus nigra* conifer. The combination of a simple extraction method such as QuEChERS with a solid phase microextraction process coupled with chromatographic techniques was also valued during this work and allowed the extraction of this large number of pollutants. These methods have proven their effectiveness, in particular in terms of efficiency and respect for the environment. The validation of the QuEChERS method shows also good performances in terms of linearity, accuracy and precision. This extraction method followed by liquid and / or gas chromatographic analysis coupled with tandem mass spectrometry has proven to be a reliable tool for the routine analysis of a wide range of compounds, at the trace, in snails and conifer needles and can be applied in other environmental biomonitoring studies.

In addition, obtained results also showed that conifers and snails can reflect specific environmental emissions and should be considered as appropriate sentinels for environmental pollution survey, especially when samples are taken from several areas at different altitudes. Likewise, it have shown that the differences in pollutant concentrations could be correlated with the physico-chemical properties of the compounds, the proximity of sources and other environmental factors such as climatic conditions. Finally, obtained results in Lebanon confirm the need to create a registration for pesticides and persistent organic pollutants through initiative research to identify the main sources of contamination, estimate their contribution to the overall contamination and study possible solutions during treatment.

Keywords: Air pollution - Environmental biomonitoring - QuEChERS - *Helix aspersa* - *Pinus nigra* - Pesticides - Persistent organic pollutants.