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Accumulation and colloidal mobilization of trace heavy metals in soil irrigated with treated wastewater

Ludovico Pontoni

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— PARIS-EST

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Spécialité: Science et Technique de l'Environnement



Dottore di Ricerca in Tecnologie Ambientali



UNESCO-IHE
Institute for Water Education

Degree of Doctor in Environmental Technology

Thèse – Tesi di Dottorato – PhD thesis

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**Accumulation and colloidal mobilization of trace heavy metals in soil
irrigated with treated wastewater**

To be defended December 15th, 2016

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Abstract

Reuse of treated wastewater for irrigation purposes is worldwide accepted and implemented to face water scarcity and save high quality water resources. Although such practice has undoubtable advantages and is certainly more sustainable in respect to the use of fresh water, it is not exempt from severe concerns. Potentially harmful pollutants contained at trace levels in the reused water might indeed impact the receiving soil and crops. Among these pollutants, trace heavy metals (HMs) play a primary role due to their widespread presence in the used water and to their persistence once released into the environment. The fate of HMs in soils can be hardly predicted as mobility mechanisms through soils are extremely diverse and related to highly complex simultaneous phenomena and chemical equilibria. HMs, in fact, as many other contaminants, are not only partitioned between the solid immobile and the water mobile phases, but also colloids and nanoparticles act as a further mobile phase, with their own rheological properties and velocity. This latter component has been one of the main focus of the thesis.

In details this thesis describes the results of several experimental tests conducted by irrigating the Organization for Economic Cooperation and Development (OECD) standard soil with real and/or synthetic wastewater, containing HMs in trace level concentrations. For each test a specific soil (e.g. with varying organic matter content) and wastewater composition (e.g. synthetic wastewater with varying trace metals concentrations, salinity, dissolved organic matter content, or real treated wastewaters) has been chosen in order to evaluate the effects of different conditions on the overall HMs fate. The increase of soil organic matter from 2.5 to 10% linearly enhanced the mobility of Cd, Cu and Ni. The maximum mobility increase obtained by comparing soils with 2.5 and 10 % of organic matter was of 35.6, 43.7 and 49.19 % for Cd, Cu and Ni, respectively. In most experiments, metals accumulated in the top soil layer (0 - 0.5 cm). Nevertheless, peaks of contamination were detected at different depths in the soil deeper layers and at different leaching time in the soil leachates depending on the metal and on the soil and wastewater characteristics. Peaks of metals in the leachate appeared simultaneously with release of organic matter and/or release of silicates, demonstrating outstanding involvement of colloids in metals transport. Sodium concentration (20 mM) was demonstrated to highly reduce colloidal mobilization whereas more than 95 % of the influent metal was detected in the soil top layer despite the soil organic matter content. Conversely, low sodium concentrations (1-5 mM) enhanced colloidal and hence metals mobilization. Salinity displayed different effects. The irrigation with real treated wastewater with quite high content of Ca and Mg (111 and 134 mg/L, respectively) resulted in higher average release of silicon from the soil inorganic matrix (8.2 mg/L) compared to the low salinity artificial wastewater (1.9 mg/L). Consequently higher mobilization of

Cd, Cu, Ni and Zn was observed when the soil was irrigated with real treated wastewater. An advanced spectroscopic characterization of the soil leachates was performed to identify such colloidal aggregates with the aim of clarifying their nature, chemical properties and aggregation state. The observation of 3D excitation-emission matrix demonstrated in all the leachates samples the presence of fulvic (230-450 nm ex-em fluorescence area) and humic (330-445 nm ex-em) substances. Proton nuclear magnetic resonance ($^1\text{H NMR}$) spectra of the leachates resulted poorly resolved in the aromatic region (6 ppm $<\delta<$ 8 ppm) due to hydrophobic interactions in the core of the supramolecular structure of humic substances.

In this context, a novel analytical method was developed to quantify phenolic substances in soil matrices allowing the monitoring of humic matter migration in soil profiles. The novel method was more accurate in respect to the traditional one, allowing to obtain higher recovery of total phenols in peat soil (15.5 % increase) with a decrease of the coefficient of variation (30.1% decrease).

A novel migration physical-chemical model was qualitatively developed on the basis of the experimental results. The model considers a multiphase transport mechanism through the soil layers that includes : i) the effect of soil composition, in terms of organic and inorganic matter; ii) the generation of colloidal aggregates, responsible for the transport of a part of the metals at a velocity different from the one of the water flow; iii) the effect of different physical-chemical properties of the generated colloids, that determines their selectivity in binding metals; iv) the disaggregation of the complex aggregates composing soil organic matter, which results in the release of small molecular weight ligands which coordinate HMs accelerating the transport rate; v) the effect of wastewater characteristics, including salinity, sodium content, and presence of organic matter, which influences the mobilization of colloidal aggregates through soil.

The proposed model represents a powerful tool to depict the fate of trace metals in the soil, and could be extended to describe the fate of other contaminants. In that sense, it could also become an important tool in the risk assessment of irrigation with reclaimed wastewater.

Keywords: Trace heavy metals, Colloidal mobilization, Soil humic matter, Mineral organic association, Contaminants fate in the environment, Wastewater reuse

Résumé

La réutilisation des eaux usées traitées pour l'irrigation est globalement acceptée et pratiquée pour faire face à la pénurie d'eau et économiser les ressources en eau de haute qualité. Bien que cette pratique présente des avantages indéniables et contribue à un usage plus durable de l'eau douce, elle n'est pas exempt de problèmes liés à l'impact potentiel des micropolluants contenus dans l'eau réutilisée sur la qualité des sols récepteurs et sur les cultures. Parmi ces polluants, les métaux lourds (ML) en concentrations traces jouent un rôle primordial en raison de leur présence systématique dans l'eau utilisée et de leur persistance une fois libéré dans l'environnement. Le devenir des ML dans les sols peut difficilement être prédit parce que les mécanismes de mobilité à travers les sols sont extrêmement variés et liés à des phénomènes simultanés et très complexes impliquant différents équilibres chimiques. Les ML, comme beaucoup d'autres contaminants, ne sont pas seulement partagés entre la phase immobile (le sol) et la phase mobile que représente l'eau du sol. En effet, les colloïdes et les nanoparticules agissent comme une troisième phase mobile, avec leurs propres propriétés rhéologiques et des vitesses de migrations qui leur sont propres. Ce dernier aspect a été l'un des principaux objectifs d'étude de la thèse.

Plusieurs essais expérimentaux ont été menés en irriguant un sol standard selon l'Organisation de coopération et de développement économiques (OCDE) avec une eau usée traitée réelle et / ou synthétiques, contenant des ML en concentrations traces. Pour chaque test, un sol spécifique (avec différentes teneurs en matière organique) et des eaux usées traitées de composition différente (avec différentes concentrations en métaux traces, de salinité, de teneur en matière organique pour les eaux usées synthétiques, ou des eaux usées traitées réelles) ont été choisis afin d'évaluer les effets des conditions différentes sur le devenir global des ML. L'augmentation de la matière organique du sol de 2,5 à 10% a linéairement amélioré la mobilité des Cd, Cu et Ni avec une augmentation de la mobilité maximum de 35,6, 43,7 et 49,19% pour le Cd, Cu et Ni, respectivement. Pour la plupart des expériences, les ML ont été capturés dans la couche superficielle du sol (0 à 0,5 cm). Néanmoins, des pics de contamination ont été détectés à des profondeurs différentes dans les couches plus profondes du sol. L'étude de la composition des lixiviats montre des variations de concentrations fonction du métal étudié et des caractéristiques du sol et des eaux usées. Des pics de métaux dans le lixiviat sont apparus en même temps que la libération de la matière organique et / ou la libération de silicates, ce qui démontre l'implication significative des colloïdes dans le transport des métaux. La concentration en sodium (20 mM) a été démontrée comme ayant un impact fort sur la réduction de la mobilisation colloïdale et que plus de 95% du métal apporté par irrigation a été détecté dans la couche superficielle du sol en dépit de sa teneur en matière organique. La salinité présente donc des effets significatifs.

L'irrigation avec des eaux usées traitées présentant une très haute teneur en Ca et Mg (111 et 134 mg / L, respectivement) a abouti à une libération moyenne plus élevée de silicium à partir de la matrice inorganique du sol (8,2 mg / L) au contraire des eaux usées artificielle peu saline (1,9 mg / L). Par conséquent, la mobilisation ultérieure de Cd, Cu, Ni et Zn a été observée lorsque le sol a été irrigué avec des eaux usées traitées réelles. Une caractérisation spectroscopique avancée des lixiviats a été réalisée pour identifier les agrégats colloïdaux libérés par le sol dans le but d'en déterminer leur nature, leurs propriétés chimiques et leur état d'agrégation. L'analyse des lixiviats à l'aide des matrices d'excitation et d'émission de fluorescence a démontré dans tous les échantillons la présence d'acides fulviques (230-450 nm ex-em zone de fluorescence) et humiques (ex-em 330-445 nm). Les spectres RMN des lixiviats sont mal résolus dans la région aromatique ($6 \text{ ppm} < \delta < 8 \text{ ppm}$) en raison d'interactions hydrophobes dans le noyau de la structure supramoléculaire des substances humiques.

Dans ce contexte, une nouvelle méthode analytique a été développée pour quantifier les substances phénoliques dans les matrices de sol permettant le suivi de la migration des matières humiques dans le profil du sol. La nouvelle méthode proposée est plus précise que la méthode traditionnelle, ce qui permet d'obtenir une meilleure quantification des phénols totaux dans le sol de tourbe (augmentation de 15,5%) avec une diminution du coefficient de variation (30,1% de baisse).

Un nouveau modèle physico-chimique de migration des ML a été proposé sur la base des résultats expérimentaux. Le modèle considère un mécanisme de transport polyphasique à travers les couches de sol qui comprend: i) l'effet de la composition du sol, en termes de matière organique et inorganique; ii) la génération d'agrégats colloïdaux, responsable du transport d'une partie des métaux à une vitesse différente de celle de l'écoulement d'eau de vitesse; iii) l'effet des différentes propriétés physico-chimiques des colloïdes générés, qui détermine leur sélectivité des métaux transportés; iv) la désagrégation des agrégats complexes qui composent la matière organique, ce qui conduit à la libération de ligands de faible poids moléculaire qui se lient aux ML et accélèrent la vitesse de transport; v) l'effet des caractéristiques des eaux usées, y compris la salinité, la teneur en sodium, et la présence de la matière organique, qui influence la mobilisation des agrégats colloïdaux dans le sol.

Le modèle conceptuel proposé constitue un outil puissant pour représenter le devenir des métaux traces dans le sol, et pourrait être étendu pour décrire le sort des autres contaminants. En ce sens, il pourrait aussi devenir un outil important dans l'évaluation des risques liés à l'irrigation avec des eaux usées traitées.

Sommario

Il riutilizzo di acque reflue trattate per scopi irrigui è diffusamente praticato al fine affrontare la scarsità d'acqua e risparmiare risorse idriche di alta qualità. Sebbene tale pratica presenti indubbi vantaggi e sia più sostenibile rispetto all'utilizzo di acqua potabile, non è esente da gravi preoccupazioni relative al potenziale impatto di inquinanti potenzialmente nocivi contenuti nell'acqua riutilizzata sul suolo ricevente e sulle colture. Tra tali inquinanti, i metalli pesanti in tracce, (HMs) assurgono ad un ruolo di primaria importanza, legato alla loro ampia diffusione negli effluenti depurati e alla persistenza una volta rilasciati nell'ambiente. Il destino dei HMs nei terreni è decisamente arduo da prevedere. I meccanismi di mobilità attraverso i terreni sono infatti estremamente vari e collegati a fenomeni ed equilibri chimici simultanei altamente complessi. I HMs, infatti, come molti altri contaminanti, non si ripartiscono semplicemente tra le fasi solida immobile del suolo e mobile dell'acqua. Colloidi e nanoparticelle agiscono, infatti, come una terza fase mobile, con sue proprie caratteristiche reologiche e di velocità. Quest'ultimo aspetto è stato uno degli obiettivi principali della tesi.

In particolare la tesi descrive i risultati di diverse prove sperimentali condotte irrigando il terreno standard dell'Organizzazione per la cooperazione e lo sviluppo economico (OCSE) con acque reflue sintetiche e / o reali contenenti HMs in tracce. Per ogni prova sono stati scelti specifici terreni (ad esempio variando il contenuto di sostanza organica) e composizioni delle acque reflue (ad esempio variando la concentrazione dei metalli, la salinità, il contenuto di materia organica, o utilizzando effluente da un impianto reale) al fine di valutare gli effetti di differenti condizioni sul destino dei HMs nel suolo. L'aumento di sostanza organica nel suolo dal 2,5 al 10% ha migliorato linearmente la mobilità dei Cd, Cu e Ni fino ad un aumento massimo del 35.6, 43.7 e del 49.2% per Cd, Cu e Ni, rispettivamente. Nella maggior parte degli esperimenti condotti, i metalli si sono accumulati nello strato di terreno superiore (0 – 0,5 cm). A seconda del metallo e delle caratteristiche del suolo e delle acque di irrigazione utilizzati, sono stati tuttavia rilevati picchi di contaminazione al variare della profondità nel terreno e dei tempi di rilascio nei lisciviati. I picchi di concentrazione riscontrati nei lisciviati sono apparsi in contemporanea con il rilascio di sostanza organica e / o di silicati, dimostrando un notevole coinvolgimento dei colloidi nel trasporto dei metalli. L' aumento di concentrazione di sodio (20 mM) ha altamente ridotto la mobilizzazione colloidale, tanto che più del 95% del metallo influente è stato rilevato nello strato superiore indipendentemente dal tenore di sostanza organica del suolo. L'aumento di salinità ha invece causato l'effetto opposto. L'irrigazione con acque reflue trattate reali con non trascurabile contenuto di Ca e Mg (111 e 134 mg / L, rispettivamente) ha provocato un maggiore rilascio medio di silicio dalla matrice inorganica terreno

(8,2 mg / L) rispetto alle acque reflue artificiali a bassa salinità (1,9 mg / L). Parallelamente, quando il terreno è stato irrigato con acque reflue trattate reali si è osservata una maggiore mobilitazione di Cd, Cu, Ni e Zn. Una avanzata caratterizzazione spettroscopica dei lisciviati è stata effettuata per identificare tali aggregati colloidali rilasciati dal suolo con l'obiettivo di chiarirne la natura, le proprietà chimiche e lo stato di aggregazione. L'osservazione delle matrici di eccitazione-emissione 3D ha evidenziato in tutti i campioni la presenza di sostanze fulviche (zona di fluorescenza ex-em 230-450 nm) e umiche (ex-em 330-445 nm). Gli spettri NMR dei lisciviati sono apparsi mal risolti nella regione aromatica ($6 \text{ ppm} < \delta < 8 \text{ ppm}$) a causa di forti interazioni idrofobiche nel core della struttura supramolecolare.

In tale contesto, è stato sviluppato un metodo analitico in grado di quantificare sostanze fenoliche in matrici solide consentendo il controllo della migrazione delle sostanze umiche nei profili di suolo. Il nuovo metodo è risultato più accurato e preciso rispetto a quello tradizionale, permettendo di ottenere una maggiore recupero dei fenoli totali analizzando un suolo torboso (aumento del 15,5%), con una diminuzione del coefficiente di variazione (diminuzione del 30,1%).

Sulla base dei risultati sperimentali, è stato qualitativamente sviluppato un nuovo modello chimico-fisico di migrazione. Il modello considera un meccanismo di trasporto multifase attraverso gli strati di terreno che comprende: i) l'effetto della composizione del suolo, in termini di materia organica e inorganica; ii) la produzione di aggregati colloidali, responsabili del trasporto dei metalli ad una velocità diversa da quella del flusso dell'acqua; iii) l'effetto delle differenti proprietà chimico-fisiche dei colloidali generati, che ne determina la selettività nel coordinare i metalli; iv) la disaggregazione degli aggregati complessi che compongono la sostanza organica, che provoca un continuo rilascio di molecole a basso peso molecolare che coordinano i HMs accelerandone il tasso di trasporto; v) l'effetto delle caratteristiche delle acque reflue, tra cui la salinità, il contenuto di sodio, e la presenza di sostanza organica, che governano la mobilitazione degli aggregati colloidali attraverso il suolo.

Il modello proposto rappresenta un utile strumento per predire il destino di metalli in tracce nel terreno, e potrebbe essere esteso per descrivere il destino di altri contaminanti. In tal senso, potrebbe anche diventare uno strumento importante applicabile alla valutazione del rischio legato al riutilizzo di acque reflue a fini irrigui.

Samenvatting

Hergebruik van gezuiverd afvalwater voor irrigatie wordt wereldwijd geaccepteerd en geïmplementeerd om waterschaarste geconfronteerd en opslaan van hoge kwaliteit middelen. Hoewel dergelijke praktijken heeft undoubtable voordelen en is zeker meer duurzaam ten opzichte van het gebruik van zoet water, het is niet vrijgesteld van ernstige bezorgdheid. Potentieel schadelijke stoffen bevatte op sporen niveaus in het water hergebruikt zou inderdaad invloed op de ontvangende bodem en op de gewassen. Onder deze stoffen, sporen zware metalen (HMS) een primaire rol spelen als gevolg van hun wijdverspreide aanwezigheid in het gebruikte water en hun doorzettingsvermogen eenmaal in het milieu terecht. Het lot van HMS in de bodem kan nauwelijks worden voorspeld als mechanismen mobiliteit door bodems zijn zeer divers en gerelateerd aan zeer complexe gelijktijdige verschijnselen en chemische evenwichten. HMS, in feite, vele andere verontreinigingen, niet alleen verdeeld tussen de vaste immobiele en mobiele fasen water. Inderdaad, colloïden en nanodeeltjes fungeren als derde mobiele fase, met hun eigen rheologische eigenschappen en snelheid. Dit laatste aspect is een van de belangrijkste focus van het proefschrift.

In Dit proefschrift beschrijft de resultaten van verschillende experimentele tests uitgevoerd door de irrigatie van de Organisatie voor Economische Samenwerking en Ontwikkeling (OESO) standaard bodem met echte en / of synthetische afvalwater, met HMS in traceringsniveau concentraties. Voor elke test een bepaalde grond (het variëren van de organische stofgehalte) en afvalwater samenstelling (bijvoorbeeld variëren van de metalen concentratie, het zoutgehalte, de organische stof, of testen real behandeld afvalwater) is gekozen om de effecten van verschillende te evalueren op de totale HMS lot. De toename van de bodem organische stof 2,5-10% lineair versterkt de mobiliteit van Cd, Cu en Ni. De maximale verhoging mobiliteit verkregen vergelijking bodems op 2,5 en 10% van de organische stof was 35,6, 43,7 en 49,19% voor Cd, Cu en Ni, respectievelijk. In de meeste experimenten metalen opgebouwd in de bovenste bodemlaag (0 – 0,5 cm). Niettemin pieken besmetting werden gedetecteerd op verschillende dieptes in de grond diepere lagen en op verschillende uitloging weer in de bodem percolaat afhankelijk van het metaal en de bodem en afvalwater kenmerken. Pieken van metalen in het percolaat verscheen gelijktijdig met de release van organische stof en / of het vrijkomen van silicaten, waaruit blijkt opmerkelijke betrokkenheid van colloïden in metalen transport. Natriumconcentratie (20 mM) werd aangetoond colloïdale mobilisatie sterk verminderen terwijl meer dan 95% van het influent metaal in de toplaag ondanks het gehalte aan organisch bodemmateriaal gedetecteerd. Omgekeerd, weinig natrium concentraties (1-5 mM) verbeterde colloïdale en dus metalen mobilisatie. Zoutgehalte weergegeven verschillende effecten. De irrigatie met echte behandeld afvalwater met een vrij hoog gehalte aan Ca en Mg (111 en 134 mg

/ l respectievelijk) resulteerde in hogere gemiddelde afgifte van silicium uit de bodem anorganische matrix (8,2 mg / L) in vergelijking met het lage zoutgehalte kunstmatige afvalwater (1,9 mg / l). Bijgevolg hogere mobilisatie van Cd, Cu, Ni en Zn werd waargenomen als de grond werd geïrrigeerd met echte gezuiverd afvalwater. Een geavanceerde spectroscopische karakterisering van de bodem percolaat uitgevoerd om deze colloïdale aggregaten identificeren teneinde hun aard en chemische eigenschappen aggregatietoestand verduidelijken. De waarneming van 3D excitatie-emissie matrix aangetoond in alle percolaat monsters de aanwezigheid van fulvic (230-450 nm ex-em fluorescentiegebied) en humus (330-445 nm ex-em) stoffen. NMR spectra van het percolaat resulteerde slechte resolutie in het aromatische gebied (6 ppm δ <math><8</math> ppm) door hydrofobe interacties in de kern van de supramoleculaire structuur van humusstoffen.

In dit verband werd een nieuwe analytische methode ontwikkeld waarbij fenolische stoffen kwantificeren bodem matrices waarmee kan worden gecontroleerd humus materie migratie bodemprofielen. De nieuwe werkwijze is nauwkeuriger ten opzichte van de traditionele, waardoor hogere terugwinning van totale fenolen in veengrond (15,5% toename) met een afname van de variatiecoëfficiënt (30,1% afname) te verkrijgen.

Een nieuwe migratie gysicochemische model kwalitatief ontwikkeld op basis van de experimentele resultaten. Het model beschouwt een uit meerdere transportmechanisme door de grond lagen die bestaat uit: i) het effect van de samenstelling van de bodem in termen van organische en anorganische stof; ii) het genereren van colloïdale aggregaten, voor het transport van een deel van de metalen bij een doorvoersnelheid verschilt van die van de waterstroom; iii) het effect van verschillende fysisch-chemische eigenschappen van de gegenereerde colloïden, bepaalt selectiever bij binding metalen; iv) de opsplitsing van het complex aggregaten samenstellen van organische stof, wat resulteert in het vrijkomen van kleine molecuulair gewicht liganden die HMS het versnellen van de transportsnelheid te coördineren; v) het effect van afvalwater kenmerken, waaronder zoutgehalte, natrium, en de aanwezigheid van organische stof, die de mobilisatie van colloïdale aggregaten beïnvloedt in de bodem.

Het voorgestelde model is een krachtig instrument om het lot van spoormetalen portretteren in de bodem, en kan worden uitgebreid tot het lot van de andere verontreinigende stoffen te beschrijven. In die zin kan het ook een belangrijk instrument bij de risicobeoordeling van irrigatie met afvalwater teruggewonnen worden.

Chapter 1.

1. Introduction

1.1 Background

1.1.1 Wastewater reuse in agriculture

Water has been considered for decades as an inexhaustible good free for everybody. This concept is currently being overcome and water is more and more considered as a limited resource that requires to be properly managed because of the world population increase and the consequent growth of water demand. In the last decades, indeed, many water conservation and saving programs have been promoted, accompanied by relevant sensitization campaigns. A large part of these programs have been addressed to the correct use of water for agricultural purposes, as irrigation is certainly the most intense water-demanding sector (Seckler, 1998). In this scenario, wastewater recycling plays a prominent role. The economic advantage of such practice consists in providing alternative source for water supply, which otherwise would be completely wasted. Every year, new projects promoting wastewater recycling are being practiced in the Middle Eastern and Mediterranean countries, Southern American countries, United States, China and various islands in the world (Friedler, 2001). Historically the reuse of wastewater for irrigation purposes has been a very common practice dating ancient times back to the Greek and Roman history. With the growth of environmental sensitivity and in response to the pressing needs to increase the amount of water availability, the concept of water reuse has been gaining important focus also in countries with considerable resources of primary water (Jiménez & Asano, 2008). Municipal wastewater reuse is the most promising reuse prospect, since it improves the security of supply, reduces the resource constraints due to variable rainfalls, and the need for expensive water storage construction and freshwater supply infrastructure. Wastewaters often contain significant concentration of organic and inorganic nutrients, i.e. nitrogen and phosphorus (Toze, 2006) and therefore supply to the scarcity of these latter. Finally wastewater reuse, simultaneously solves water shortage and wastewater discharge problem (Oron et al., 1999). Despite the described advantages, several risks are attested worldwide concerning soil and groundwater pollution caused by the presence in the reused source of organic and inorganic pollutants (Lapworth et al., 2012). Moreover, raw wastewater reuse for crop production may cause health risks due the presence of bacteria, viruses and parasites (Navarro et al., 2015). Hence, as stated by the Hyderabad declaration on Wastewater use in Agriculture: “without proper management, wastewater use poses serious risks to human health and the environment”. In the last decades primary attention has been dedicated by the scientific community in measuring the concentration, monitoring the fate and finally removing the harmful chemicals brought to the soil (Lu et al., 2015) by irrigation with reclaimed wastewater. Even well treated wastewater represents a potential hazard. Many compounds of

anthropogenic origin, difficult to remove, in fact could be present in the wastewater below the allowed threshold values, but can increase in concentration in the environment as consequence of accumulation and biomagnification effect.

1.1.2 Emerging contaminants from water discharged in soil and the case of heavy metals

Although research conducted during the 1980s and early 1990s demonstrated that recycled water can elicit adverse responses in bioassays, such as the Ames test, the causative agents have not been identified (Sedlak et al., 2000). Quantification of chemical contaminants has been limited to priority pollutants and several well-studied compounds, such as disinfection byproducts and certain industrial products, including alkylphenol and polyethoxylate detergents. Concerns have been raised periodically about the possible presence of contaminants that are not routinely monitored, but little information about their occurrence has been available. Previously undetected effluent-derived micro-contaminants have been identified in numerous wastewater effluents and in surface waters subjected to wastewater discharges.

A great diversity of trace pollutants can be found in treated effluents from urban wastewater treatment plants (WWTPs). Several examples of “new” or “emerging” contaminants are identified and classified yearly. Emerging contaminants are unregulated contaminants, which may be candidates for future regulations depending on research on their potential health effects and monitoring data regarding their occurrence and fate (Petrovic et al., 2004). The properly called emerging contaminants include pharmaceuticals, UV filters, surfactants and fragrances, and are sometimes classified as endocrine disruptors contaminants (EDCs) or as pharmaceuticals and personal care products (PPCPs). EDCs are compounds which can impact the structure and function of an organism’s endocrine system causing effects on the organism itself or its progeny. Known EDCs that can be found in wastewaters and in the environment include the oestradiol compounds commonly present in the contraceptive pill, phytoestrogens, pesticides, industrial chemicals such as bisphenol A or nonyl phenol, and some heavy metals. The majority of PPCPs detected in environmental waters and waters are drugs used for a variety of therapeutic uses for both humans and animals. Examples include analgesics such as Ibuprofen, caffeine, antiepileptics, cholesterol reducing drugs, antibiotics and antidepressants as well as products of general daily use like surfactants, UV filters, synthetic fragrances, antiseptics, flame retardants. This group is mainly composed of products used in everyday life and for most of them ecotoxicological data are not yet available, and is therefore difficult to predict which health effects they may have on humans and aquatic organisms. Moreover treated

wastewaters are often disinfected in order to remove pathogens prior to agricultural re-use and in this case another class of contaminants is emerging, embodied by the disinfection by-products (DBPs). This class of pollutants was widely studied since the early 1970s and found present in drinking waters subjected to disinfection treatment (Richardson, 2003). It is anyway attested how the formation of these by-products is strictly related to the concentration of dissolved organic matter in the influent water to the disinfection system. Clearly disinfecting the WWTPs effluents gives place to higher risk of DBPs formation. Disinfection by-products are in first analysis divided in chlorination by-products and ozonation by-products, depending by the used disinfection technology. Together with these classes there also other emerging contaminants also indicated as priority pollutants, which means that is already known their behaviour against human health at high concentrations. Risks related to this class of compounds are well attested and the hazard of having a bioaccumulation in crops can be very high even when the concentrations in the reclaimed water are below the law limits. Priority pollutants include HMs, pesticides and organohalogenated solvents. Although well known and widely regulated from most countries, these compounds are emerging in the sense that is not known what could be their evolution even at trace levels concentrations once discharged in the environment.

A synthetic scheme of the most frequent classes of emerging contaminants (Muñoz et al., 2009; Richardson, 2003; Richardson & Kimura, 2016) in WWTPs effluents is reported below:

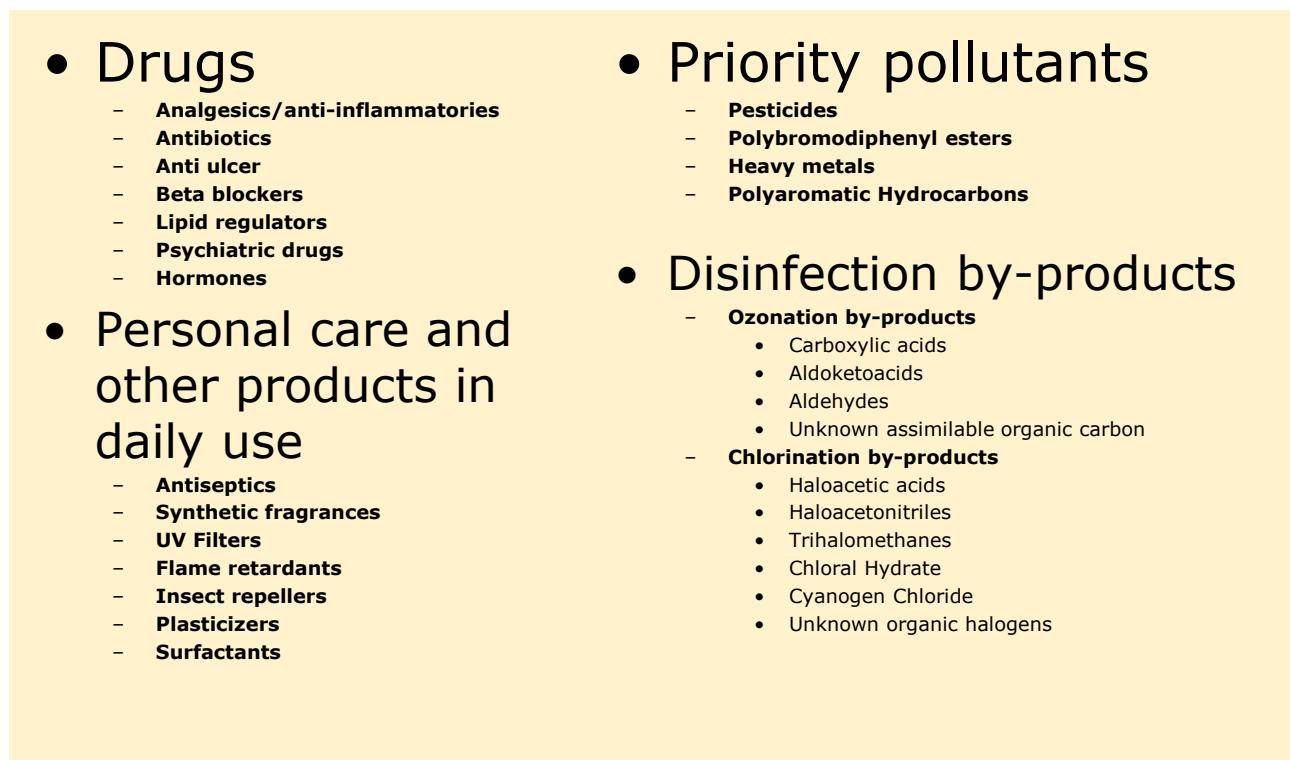


Figure 1.1 Classes of emerging contaminants from WWTPs effluents

All these compounds have completely different molecular structures as well as physical-chemical properties. It is thus impossible to generalize their behaviour in the environment and their affinity to soils and roots. Moreover, the concentration level would certainly vary from one plant to the other due to the obvious differences of loads and operating conditions. The wide variability showed by different soils concerning their affinity with pollutants, makes the evaluating potential risks related to the release of such compounds in the environment and above all, to the use of wastewater in irrigation a challenging issue that requires a deep knowledge of the soil/water adsorption/release equilibria. On the other side the simple monitoring of this kind of contaminants requires advanced and sometime expensive analytical techniques being their concentration generally very low, down to trace and ultra-trace level. Heavy metals (HMs), as said, are of primary concern since they are able to accumulate in soil and crops and to mobilize and pollute groundwater. This class of elements displays a range of properties in soil including differences in mobility and bioavailability, leaching losses and plant uptake. These potentially toxic elements slowly accumulate in the soil profile over the period of time they are subjected to irrigation. Micro concentrations of heavy metals (HMs) are permitted by existing legislations all over the world, because they do not represent a short-term hazard for the environmental quality. Nonetheless, HMs can be accumulated in the soil and in the food chain (biomagnification), because they cannot be naturally degraded.

Generally, the removal of specific heavy metals is scarcely considered in the reclamation of treated urban effluents. The major of the treatment plants, and even more those treating municipal wastewater, are not specifically designed to enhance the HMs removal. Research on the removal of HMs generally has its focus on specific industrial wastewater (Fu & Wang, 2011). The fate of heavy metals in the conventional biological wastewater treatment is described in several research studies. Karvelas et al. (2003), closed the balance for metal fractionation during the different phases of a conventional activated sludge plant in Thessaloniki, Greece. Ni and Manganese were found primarily in the dissolved phase of the wastewater Cu, Cr, Pb, Cd and Zn were mostly associated with the particulate phase and were removed already in the primary sedimentation. The relative distribution of individual heavy metals in the treated effluent and the sludge streams indicated that Mn and Cu were primarily (>70%) accumulated in the sludge, while 47-63% of Cd, Cr, Pb, Fe, Ni and Zn remained in the secondary treatment effluent. Chipasa (2003) studied the accumulation and removal of Cd, Cu, Pb, Zn in the conventional activated sludge plant located in Gdansk, Poland. The author found that conventional biological treatment was not able to remove low metals concentrations from the influent while the removal rate was not negligible in case of high influent concentration values. Corresponding to the influent contents, in increasing order, the reduction in heavy metals content was Cd<Pb<Cu<Zn. The order of increase in metal accumulation in the digested sludge (Zn<Pb<Cu<Cd)

was opposite to that of the removed amounts from the influent wastewater. HMs, are very likely to be present in WWTP effluent, usually below the legislation threshold. A complete removal of such compounds would require very advanced and expensive tertiary treatments, scarcely applicable at large scale and even less in developing countries. For these reasons, trace HMs are included among the emerging contaminants (Bolong et al., 2009). At low concentration levels, indeed, is not known neither their fate in the environment nor their effects to human and animal life. A deep comprehension of their fate in the soil is hence imperative to foresee their behaviour and to setup the opportune strategies to avoid eventual dramatic consequences.

1.2 Fate of emerging contaminants in soil – The colloidal mobilization mechanism

The organic compounds most likely to cause environmental and health problems are the synthetic chemicals that are difficult to be mineralized or adsorbed in common WWTPs and have a toxic impact on humans or other environmental indices. These xenobiotic compounds, although found in trace concentrations in wastewater, may have bioaccumulating properties when accumulated in crops through irrigation and may end up in humans through the food chain. In addition they may contaminate groundwater and thus indirectly, drinking water (Díaz-Cruz & Barceló, 2008). It follows that the study of contaminant mobility through soil is of relevant importance to assess the risk of reclaimed water use for irrigation. High mobility create risks for pollutants to leach in the groundwater or to be fast adsorbed by plant roots, at the same time, high affinity with soils can create persistent pollution and bioaccumulation in crops. Kinney et al found accumulation of pharmaceuticals in golf court and city hall landscapings irrigated with treated wastewater in a Colorado medium-size city. The accumulation appeared to be regulated by soil organic matter contained in the soil (Kinney et al., 2006). Singh et al. (2004) reported that the use of treated wastewaters in agriculture in a specific program in India determined higher levels of metals and pesticides in each of the environmental media receiving them, respect to the adjoining areas not included in the reuse project. Metals and pesticides residue levels in plants/crops, vegetables and food grains consumed by the cattle and humans being, were also much higher in the wastewater disposal areas than in those not receiving wastewater. It is also reported that several organic (xenobiotics) can be entered to the food chain when they are adsorbed by root system of plants from soil solution (Mc Farlane & Trapp, 1994). During irrigation on agricultural lands with effluents from WWTPs, xenobiotics are adsorbed onto the soil colloids and are in equilibrium with the soil solution. Micro-pollutants could interfere with the growth of crops, limiting the germination rate of seeds and

retarding the first phases of seedling growth, thus facilitating the negative competition of weeds. Moreover they will be passively taken up by the root system and trans located through the xylem and the phloem to the aerial parts of the plants (stems, leaves, fruits) with different physiological mechanisms (Korte et al., 2000; Trapp & Matthies, 1995). In the plant tissues, xenobiotics accumulate in cell vacuoles or in other subcellular system after conjugation with biological molecules. It becomes apparent that bioaccumulation of micro-pollutants in crop can play a critical role in the assessment of reusability of wastewater.

Another effect of water reuse is related to the salinity of the recycled water, mostly linked to the concentrations of sodium. Sodium and other forms of salinity are the most persistent in recycled water and are among the most difficult to remove from water, usually requiring the use of expensive cation exchange resins or reverse osmosis membranes. The salinity of recycled water can impact both on the soil itself, as well as influencing the growth of the crops being irrigated. Moreover, sodium cations interact with the negatively charged layers of clay particles. As sodium concentration increases, the electrophoretic mobility of the clay anionic layers (platelets) increases resulting in swelling dispersion of the clay particles which impacts on soil permeability. The final effect is the difficulty of water to infiltrate into the soil profile with subsequent surface ponding problems (Toze, 2006). The over mentioned effect is of primary importance, being one of the factors that regulates the mobility of water through soil and thus the adsorption/release equilibria of contaminants moving through the soil layers. It increases the contact time between the contaminants and the solid matrix and consequently with the plants roots, thus influencing the processes of contamination and accumulation.

Sorption to soils is probably the most influential factor on the transport and fate of organic contaminants in the environment. The organic matter and mineral matter in soil and sediments are known to exhibit distinct contribution to the sorption of non-ionic compounds. The soil organic matter (SOM) functions in first approximation as a partition medium and the mineral matter as an adsorbent. For relatively non-polar solutes in soil–water systems where a significant SOM content is present, the solute partition in SOM predominates over adsorption on mineral matter because of strong suppression by water of solute adsorption on polar mineral surfaces. In such systems the solute sorption isotherms exhibits a high linearity over a wide range of relative concentrations. For polar solutes, where mineral adsorption may be less effectively suppressed by water, the partition to SOM may not dominate at very low concentrations because the possibility of significant unsuppressed mineral adsorption cannot be excluded. Several polar organic contaminants are in fact found to be strongly retained by clay minerals and the effect of SOM is the reduction to the access to the clays' binding sites. Whenever the mineral sorption is negligible, because of the predominance in sorption

of relatively non-polar compounds, the partition coefficient between SOM and water (K_{om}) depend on SOM composition and particularly on its polarity. Partition coefficient can also be expressed as K_{oc} in terms of soils carbon content instead of SOM content. In light of the expected dependence of K_{oc} on SOM composition it is essential to determine the variability in SOM medium properties between soils that affect the partition coefficient (Kile et al., 1995). Humic substances (humic acids [HA], fulvic acids [FA] and humin) play critical roles in the retention and transportation of contaminants. Sorption processes with humic acids are believed to reduce bioavailability, hinder remediation, encourage environmental persistence and limit the mobility of hydrophobic compounds. Sorption processes with fulvic acids, instead, facilitate the transport and the environmental toxicity. Great attention has to be paid to structural differences in HA or FA when discussing the sorption mechanism of organic pollutants. Aromaticity is believed to be one of the factors controlling the binding interaction. Nonetheless, despite many efforts dedicated to this aspect, (Ahmad et al., 2001), a clear understanding of the interaction between SOM and pollutants remain obscure. One reason for the lack of clarity is the structural heterogeneity of HA, FA and dissolved organic matter. The arrangement and formation of functional groups, such as aromatic and aliphatic moieties, may vary significantly. Thereto, to correctly asses the behaviour of organic contaminants, a deeper knowledge of the composition of the SOM is strongly needed to define a degree of the interaction strength that finally influences the mobility and bioavailability of organic pollutants in soils. The same conclusions affect the behaviour of polar and inorganic contaminants with the only difference that it is necessary, in this, case to consider also the adsorption on the mineral fraction of the soils.

Mobility in soil of most hazardous inorganic pollutants such as HMs as well as the availability to plants can be evaluated following the BCR three step sequential extraction (Tokalioglu et al., 2006). This procedure allows determining the type of bond linking a metal to the soil matrix. Total HMs present in a soil are ideally divided in four fractions. The first three are extracted in the three step of the BCR procedure and are defined as Exchangeable, Reducible and Oxidizable fractions. The fourth is the residual fraction and represents the Residual fraction of the HMs. This latter is so strongly linked to the soil matrix that is not extractable during the three steps. Also for HMs, the mobility could be related to the quality of humic and fulvic acids composing the soil organic fractions in terms of aromaticity and aliphaticity. More specific studies are taking in consideration the influence of the organic fraction of soils, determining by ^{13}C NMR the percentage of aliphatic and aromatic carbons present in humic acids, relating their quality to sorption and mobility of metals (Barančíková & Makovníková, 2003). Concerning organic pollutants, while for heavy metals contamination a standard procedure is well defined as described above, is less clear how to evaluate their mobility and binding fractions through soils.

Another variable that plays primary role is the presence in the wastewater of dissolved carbon. Dissolved organic matter (DOM) interacts with organic pollutants and thus affects their behaviour in soil and the aquatic environment. DOM may reduce the sorption of organic pollutants through stable DOM-pollutant interaction in solution or by competing with the pollutant molecules for the sorption sites on the soil surface. In addition to the association of chemical to DOM, DOM applied in soils can sorb to soil surfaces resulting in an overall enhancement in sorption and retardation of mobility. Furthermore, DOM and many contaminants move through soil in particulate or colloidal forms, either because they are inherently particulate due to low solubility or because are adsorbed onto otherwise harmless mobile particles, which are present in the soil. In some way is possible to assess that contaminants are not only partitioned between an immobile solid phase and a mobile aqueous phase. Colloids and nanoparticles in water can act as a third mobile solid phase, that can migrate at rates similar or even greater than the mobile aqueous phase (Hofmann & Von der Kammer, 2009; McGechan & Lewis, 2002). Although colloidal mobilization might be considered negligible when contaminants are present in high concentration, this is not the case of micro-pollutants. Their concentration in the pore water, is indeed always comparable to that of the colloids which are continuously generated at soil water interface, and colloids mobilization becomes the key factor that determines the overall mobility in soil. A multiphase approach is therefore necessary to simulate the migration of trace contaminants. Such mobile solid phase consists of colloidal particles in the size range from 1nm to 1 μ m. This kind of transport mechanism is of course correlated with the experimental observation about DOM effects on K_{oc} partition coefficients.

Several sorption and desorption studies are carried out on real soils by mean of column tests for several pharmaceutical compounds. Carbamazepine and diclofenac are found to be slow-mobile compounds in SOM-rich soil layers. On the contrary in SOM poor soils their mobility increases significantly (Chefetz et al., 2008). In presence of DOM, imidacloprid has a reduced sorption due the competition of DOM for soil binding sites. While for two aromatic aniline derivatives studied with higher log K_{ow} (less polar) the sorption is found enhanced by the presence of DOM, especially with addition of tannic acid (Flores-Céspedes et al., 2006; Kile et al., 1995). The binding behaviour between organic matter and pollutants is doubtlessly influenced also by pH. Generally pH exerts a negative effect on the binding process, because the charge on HA or FA increases at increasing pH, thus reducing the binding affinity with non-polar compounds (De Paolis & Kukkonen, 1997) while the affinity with ionic compounds (HM_S) increases (Benedetti et al., 1996). Anyway, when the pollutant concentration is at micro or semi-micro level this effect should be negligible being the number of binding sites much higher than the ligands, of course in presence of high ionic strength the effect of competition between the cations can become significant.

1.2.1 Nature of colloidal nanoparticles and their involvement in contaminants mobilization

Colloidal enhanced mobilization of HMs definitely depends on the colloids nature and generation rate. Clearly either wastewater or soil composition have a fundamental role in the complex dynamics that regulate HM mobility through the different layers. Particularly, natural organic matter is considered to have a crucial effect, able to greatly influence the retention and the transportation of HMs and other micro-pollutants. In general terms, the transport of inorganic and organic pollutants in water and soil can be strongly influenced by the mobility of natural dissolved organic matter.

Organic molecules are present both in the wastewater and in the soil matrix. These molecules may belong to different classes of compounds and may have completely different chemical structure and biogenesis. Generally natural organic matter contains bio-polymers such as polysaccharides, proteins, lignin and their derivatives and finally humic substances (HS). HS are a very complex class of organic compounds, of which the biogenesis, the biological function and even the chemical structure were not completely elucidated and defined. The partitioning and the mobility of organic matter plays a fundamental role in transport of contaminants. Organic matter can either enhance, when dissolved (DOM), or retard, when bound to the soil (SOM), the transport of inorganic pollutants (Weng et al., 2002). HMs and contaminants in general are not only partitioned between the solid immobile and the water liquid phases. Colloids and nanoparticles act as a third mobile phase which is characterised by its own rheological properties and velocity (McGechan & Lewis, 2002). The third mobile phase could be organic, inorganic or composed by mineral organic associations (MOAs). Minerals and organic matter (OM) can form a huge number of different types of associations (Kleber et al., 2014).

In soils, these associations are mainly investigated because of their role in determining the long-term retention of OM (Janzen, 2006). The processes and mechanisms that retain OM in soil are a central concern to very different branches of environmental research, associations of OM with pedogenic minerals (MOAs) are known to be key controls in these and many other processes (Kleber et al., 2014). On their own, HS are in some way still an unknown on the organic chemistry point of view. A suggestive and stimulating scientific debate is instituted in the last decades about the HS structure (Piccolo, 2016). The classical idea of a class of unknown polymeric macromolecules - namely Humic acids (HA), Fulvic Acids (FA) and humin - is going to be passed by the new concept according to which HS are supramolecular aggregates of small organic compounds held together by weak dispersive forces (Piccolo, 2002). In any case, the determination of the real structure of such ubiquitous class of compounds is hardly challenging for several reasons. HS are rich in very polar or

charged substituents (e.g. carboxylic acid, hydroxyl, amino) that make them highly hydrophilic. Conversely, they are also rich in aromatic and aliphatic residues that are decidedly hydrophobic. It follows that HS in water are present in colloidal form exposing the hydrophilic moieties in the external part of the colloid and hiding the non-polar domain in the inner part (Kelleher & Simpson, 2006). Within the hydrophobic core, organic residues are bound together by hydrophobic interactions. Although such dispersive forces are weak on the energy point of view, the supramolecular structure of HS is so stable that is very difficult to extract hydrophobic molecules from the inner core of the colloids which, due the very high amount of polar groups are practically non soluble in non-polar organic solvents. A scheme of such micellar structure is reported in Figure 1.2 as described by von Wandruszka (2000).

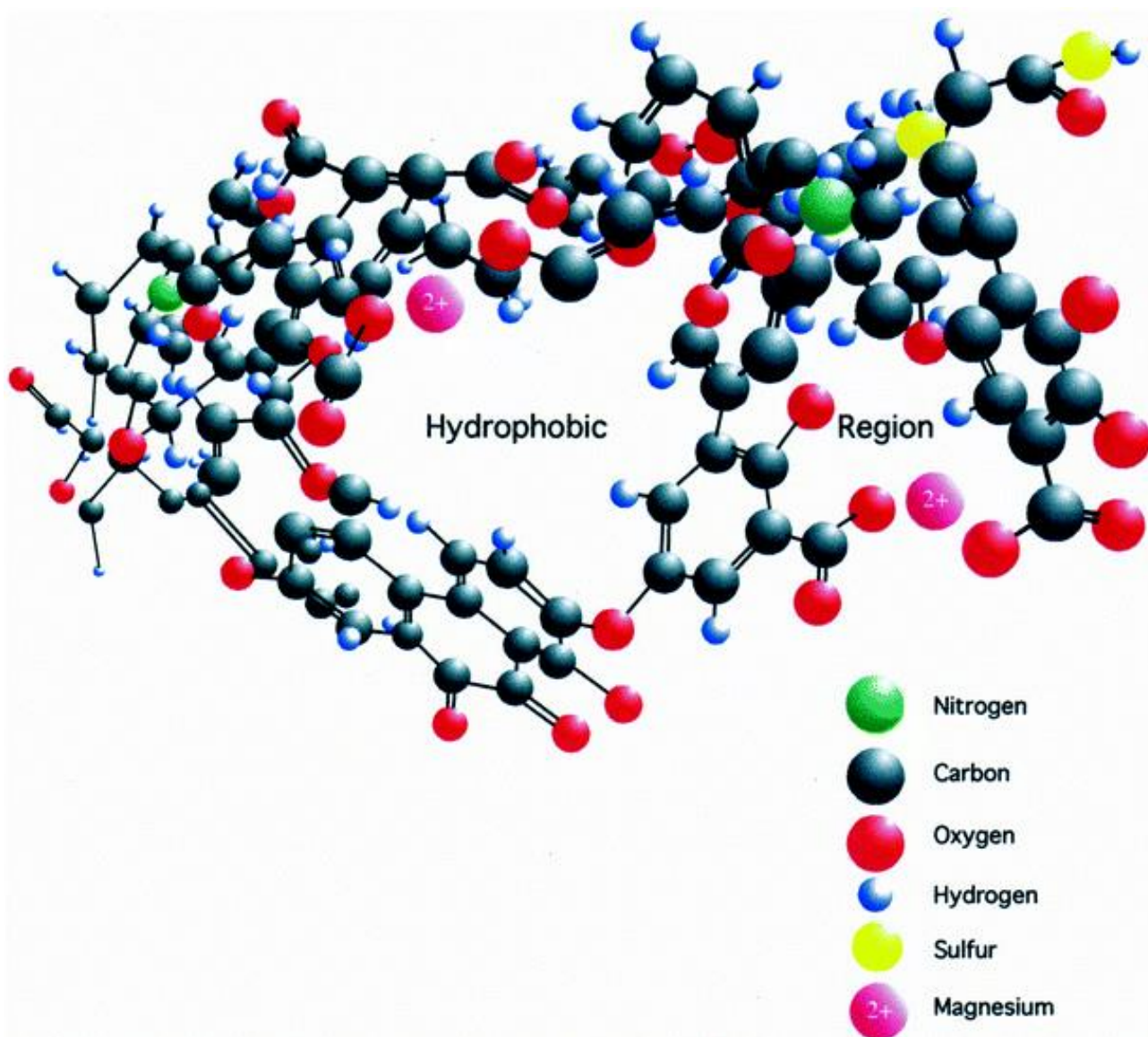


Figure 1.2 Micellar structure of Humic supramolecular aggregates (von Wandruszka, 2000), © The Royal Society of Chemistry, 2000

Due their specific properties, HS colloids are the perfect transport medium for any kind of contaminant in any environmental compartment (soil, air, water) where they are ubiquitous. Carboxylic and polar groups bind effectively heavy metals while the hydrophobic core can potentially host perfectly any kind of organic contaminant despite the lipophilicity degree. Understanding such colloidal aggregates mobility through soils is hence of primary importance to assess the fate of contaminants in the environment, to prevent their diffusion through the food chain and their bioaccumulation. At the same time, this unique class of compounds were successfully exploited to remediate contaminated soils due their high affinity with HMs and their enhanced mobility through soils.

1.3 Scope and structure of the thesis

1.3.1 Thesis scope

The aim of the present thesis is to give an overview of the HMs fate once they are released in the environment through wastewater irrigation, in order to better understand the complex mechanisms that influence the contamination process. Such information might be useful to choose the best solution for the remediation of such kind of soils and is crucial to assess the potential risk of wastewater reuse in agriculture.

Colloidal mobilization of HMs is considered as key factor of the contamination process. Thereto the goal of the study is to relate the information obtained about the colloidal aggregates isolated from soil with their ability to selectively mobilize HMs.

The main idea is to clarify as much as possible the quality and the strength of the interactions that regulate the transport of contaminants through soils in aqueous mediums. As explained before the overall process is affected by several variables and the wide variety of properties while changing from soil to soil is not of help in this direction. It was thus decided to simplify the problem studying the behaviour of an artificial soil irrigated with real and / or synthetic wastewater containing trace HMs. From literature investigation it was decided to follow the approach standardized by the Organization for the Economic Co-operation and Development (OECD) that developed a standard protocol for preparation of artificial soils (Wilhelm & Maibach, 2008).

Artificial soil was first introduced as substrate for the earthworm acute toxicity test and has been used as a medium for other bioassays. It has also been used as a reference soil when testing complex solid samples like wastes or contaminated soils. Figure 1.3 shows the composition of OECD reference soil.



Figure 1.3 Composition of standard OECD artificial soil.

The artificial soil composition (peat and clay content, pH, water content) was optimized to achieve a standardized soil-like medium, less variable than real soils. The artificial soil composition was defined as: 70% fine quartz sand (above 50% particles 0.05 – 0.2 mm), 20% kaolin clay (kaolinite content preferably above 30%) and finely ground Sphagnum peat. Although OECD soil was probably quite far to properly mimic the behaviour of a real soil, it was chosen as “simple model”. The relatively low metal background, and the absence of aggregates, inhomogeneity, or other factors influencing the metal fate in soil (i.e. Fe and Mn hydroxides,) allowed studying the “sole” effect of SOM (described in chapter 2), excluding other major effects. As the OECD itself indicates, the capacity to retain or release contaminants is related to the amount of organic matter (peat) contained in the soil (Wilhelm & Maibach, 2008). It was hence possible to vary the peat amount, keeping constant the content of kaolinite clay. Although the use of this kind of soil should grant stability and repeatability of the experiments, the composition of the soil has still some source of variability (Bielská et al., 2012), probably related to the Sphagnum peat used to grant the required content of organic matter. Sphagnum peat is known to be rich in humic and fulvic acids and is normally sold for gardening purposes. No information is thus given about the properties of the organic matter, so that a detailed characterization was required.

1.3.1 Thesis structure

In order to achieve the above reported aims, the entire work was divided in several parts that constitute the various chapters of the present thesis. Fig. 1.4 schemes out chapters and structure of the present work.

Chapter 1 briefly summarizes the characteristics of trace HMs as emerging contaminants, and briefly describes the mechanism of colloidal mobilization, focusing on the humic substances structure and aggregation state.

Chapter 2 treats the effect of organic matter content on colloidal mobilization. The developed mobilization model is introduced and extrapolated from the experimental results. The observation of sodium content effect on colloidal mobilization is also reported and discussed. This latter will be deeper investigated in chapter 5.

Chapter 3 is a methodological chapter. It contains the description and the statistical validation of a novel analytical method, which is used in the subsequent chapter 4 to monitor the migration pattern of humic substances through soil profiles.

Chapter 4 reports the results of experiments comparing irrigation of OECD soil with real and artificial wastewaters. The composition of colloidal metal carriers were better defined as mineral organic associations. The hypothesis of supramolecular structure of organic aggregates is confirmed by spectroscopic data.

Chapter 5 deepens the effect of sodium content as inhibitor of colloidal mobilization. Several sodium concentrations are tested in the irrigation water to define a threshold sodium value for colloidal mobilization inhibition in OECD soil.

Finally general discussion and conclusions are reported in Chapter 6 to highlight the results achieved in the present work and suggest possible future perspective, improvements and investigations on trace HMs colloidal mobilization mechanisms.

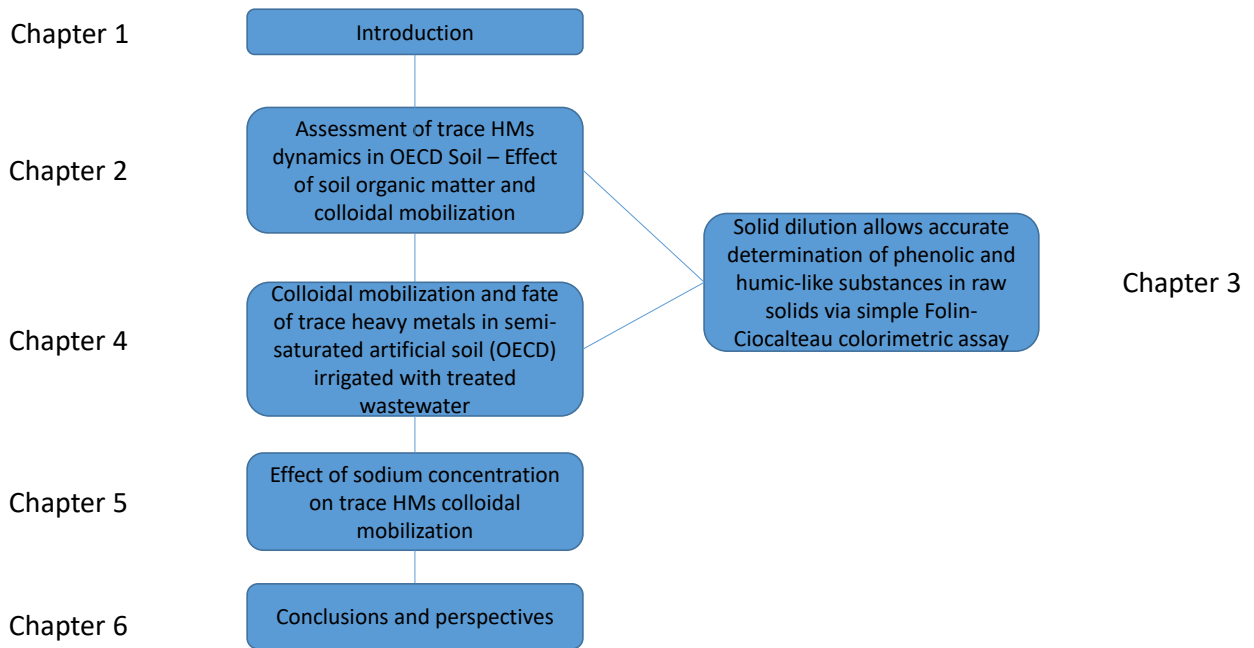


Figure 1.4 Scheme of chapters subdivision and structure of the thesis.

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Chapter 2.

Assessment of trace heavy metals dynamics during the interaction of aqueous solutions with the artificial OECD soil: evaluation of the effect of soil organic matter content and colloidal mobilization

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2.1 Introduction

In the last few decades, all over the world and especially in arid and semi-arid regions (Avnimelech, 1993), several strategies have been developed to front the water scarcity caused by climate changes and population growth. These strategies are mainly addressed by wastewater recovery and reuse, which is often the most convenient solution, especially for agriculture purposes. Reuse of wastewater for irrigation has several advantages, as it reduces the resource constraints due to variable rainfalls, and the need of expensive water storage and freshwater supply network. Moreover it allows saving high quality water for drinking water supply. At the same time it also has some drawbacks, mainly related to the potential impact on the receiving soil and on the crop itself caused by the low quality of the reused water. Real or perceived problems are generally attributed to salt and nutrients presence, as well as to heavy metals, pathogens, pharmaceuticals and endocrine disruptors (Toze, 2006).

The existing regulations, which strictly rule the characteristics of treated wastewater to be reused for irrigation purposes, together with the advances in the techniques applied for wastewater treatment, which allow attaining very high efficiency of the adopted process, have drastically reduced the risk related to traditional contaminants. Nonetheless, new and more cogent concerns are now addressed to an emerging class of organic and inorganic contaminants, which are not ruled by precise water reuse guidelines, have a not completely known behaviour in the environment, and could represent a serious hazard for human health. These contaminants are therefore indicated as Contaminants of Emerging Concerns (CECs). Among the CECs, heavy metals (HMs) in concentrations below the discharge limits play a prominent role. They are ubiquitous in the wastewater treatment plant effluent (Bolong et al., 2009), and even in trace or ultra-trace concentration are able to be retained by the soil-plants system (Singh et al., 2010). Consequently their possible risks for human health are not related to their concentration in the irrigation water, but to the capacity of soil and plants to retain and accumulate them.

The fate of HMs in the environment is really hard to predict. Mechanisms of mobility through the soil are, in fact, very heterogeneous and strongly related to: i) soil physical-chemical nature; ii) soil-water retention capacity; and iii) fluid transmission characteristics (Mingorance et al., 2007). Soil water sorption-release equilibria are hence really complex, and uneasy to model or generalize (McGechan & Lewis, 2002).

Clearly soil composition has a fundamental role in the complex dynamics that regulate HM mobility through the different layers. Particularly soil organic matter content is considered a crucial

factor, able to greatly influence the retention and the transportation of HMs and other micro-pollutants (Guillon et al., 2003). Sorption processes with humic acids (HAs) are believed to reduce pollutants bioavailability, hinder remediation, encourage environmental persistence and limit the mobility of hydrophobic compounds (Shaker & albishri, 2014). At the same time fulvic acids (FAs) might facilitate the migration and the toxicity of bound pollutants (Shahid et al., 2012; Sun et al., 2012).

In addition to that it is possible to assess that contaminants are not only partitioned between an immobile solid phase and a mobile aqueous phase. Colloids and nanoparticles in water act as a third mobile solid phase which migrates at a lower, similar or even greater rate than the mobile aqueous phase (Hofmann & Von der Kammer, 2009). A three-phase approach is therefore necessary to simulate the migration of sorbed contaminants.

Although several studies have investigated metal migration and adsorption into soils, little (Grybos et al., 2007; Linde et al., 2007; Moreno-Jiménez et al., 2011; Yu & Li, 2012) or no attention has been paid, so far, to the behaviour of HMs at trace level concentration, i.e., below the discharge limits established by the existing regulation for wastewater reuse in agriculture.

Starting from these considerations the present work aims to investigate the accumulation of trace HMs in soil caused by irrigation with treated wastewater, focusing on the metals retention and mobility related to the different content of soil organic matter. In order to have generalizable data and repeatable experiments, the research is conducted on an artificial model soil (Wilhelm and Maibach 2008), prepared according to the Organization for Economic Cooperation and Development (OECD). The effect of soil organic matter content, and the effect of irrigation water characteristics are also investigated, varying the amount of peat contained in the soil, and the sodium content of the synthetic water used for soil irrigation.

2.2 Materials and methods

2.2.1 Experimental setup

The experimental study was carried out at laboratory scale, using an artificial soil of known composition, prepared according to the OECD guidelines. The soil, developed to test the eco toxicity of organic and inorganic chemicals and widely used as reference soil in the testing of complex solid samples (Joško & Oleszczuk, 2013), was composed as follows: kaolinite clay 20%, quartz sand 74%,

CaCO₃ 1%, Sphagnum peat 5%. Although OECD soil was probably quite far to properly mimic the behaviour of a real soil, it was chosen as “simple model”. The low metal background, and the absence of aggregates, inhomogeneity, or other factors influencing the metal fate in soil (i.e.: Fe and Mn hydroxides) allowed studying the “sole” effect of SOM, excluding other major effects. As the OECD itself indicates that the capacity to retain or release contaminants is related to the amount of organic matter (peat) contained in the soil (Wilhelm & Maibach, 2008), the peat content was varied, keeping constant the content of kaolinite clay. At this aim the amount of sand was reduced, having ascertained, during preliminary adsorption tests, carried out separately on the three components of the soil, that the contribution of sand to the overall capacity of adsorption was negligible (data not shown). All the components of the soil were analysed to quantify the background HM concentration. The soil was irrigated using an artificial irrigation water (IW) containing a mixture of three HMs frequently found, at trace level, in reused wastewater: Cd (0.0025 mg/L), Cu (0.5 mg/L) and Ni (0.15 mg/L). The solution was prepared starting from analytical grade CuCl₂·2H₂O (Carlo Erba Reagenti, Italy), NiCl₂·6H₂O (Sigma-Aldrich, USA), and from 1000 mg/L Cd analytical standard (Carlo Erba Reagenti, Italy) and made up to the final concentration by means of subsequent dilutions with milli-q water (Elga, USA). The IW solution was adjusted to pH 6.5 by means of addition of few drops of KOH 0.05 M. The concentration of metals were well below the threshold values suggested by FAO in the wastewater quality guidelines for agricultural use (Pescod, 1992). More in details it was used, as a reference, one half of the threshold concentration established by the Italian regulation (DM 185/2003). Speciation diagrams (Figure 2.1) at the tested concentration were calculated by means of the software Visual Minteq. pH was made to variate from 1 to 14 by increments of 0.1. According to the obtained data the prevalent species in the artificial IW were the free divalent ions. The only other specie occurring at non negligible concentration resulted CuOH⁺.

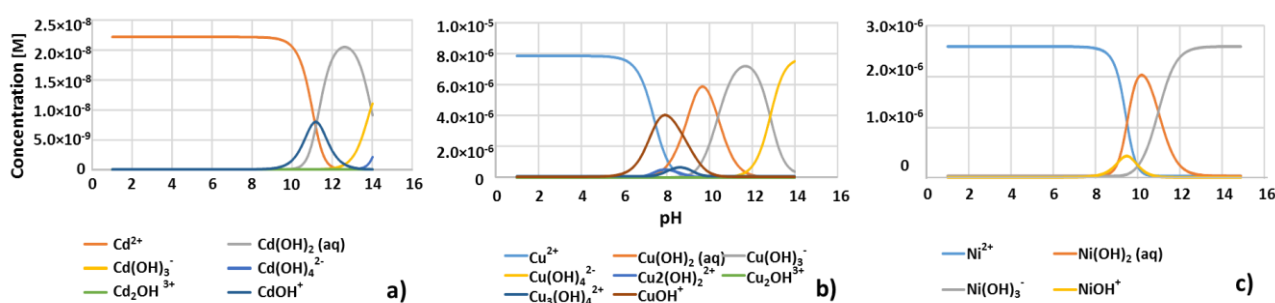


Figure 2.1 Speciation diagrams as function of pH for Cd (a), Cu (b), Ni (c)

To assess the effect of HM transportation due to colloids mobilization (Plassard et al., 2000), the sodicity of the IW was kept very low (<0.05mM), and then adjusted to 20 mmol/L for the final part of the study, adding the opportune amount of analytical grade NaClO₄ (Sigma-Aldrich, USA).

The solution containing the trace contaminants was led to interact with the soil in a modular column system built with soil layers of variable depth. Each module of the column was composed by a 50 mL polypropylene conical tube ($\Phi = 2.5$ cm), holed in the bottom and filled with a lower layer of glass wool and glass beads, as indicated in Figure 2.2. Soil was packed in the columns by tapping the tubes on the bench until no reduction of volume due the soil packing was observed. Once reached the desired depth (7 cm in experiment A; 5 cm in experiments B,C,D) the columns were irrigated with the synthetic solution.

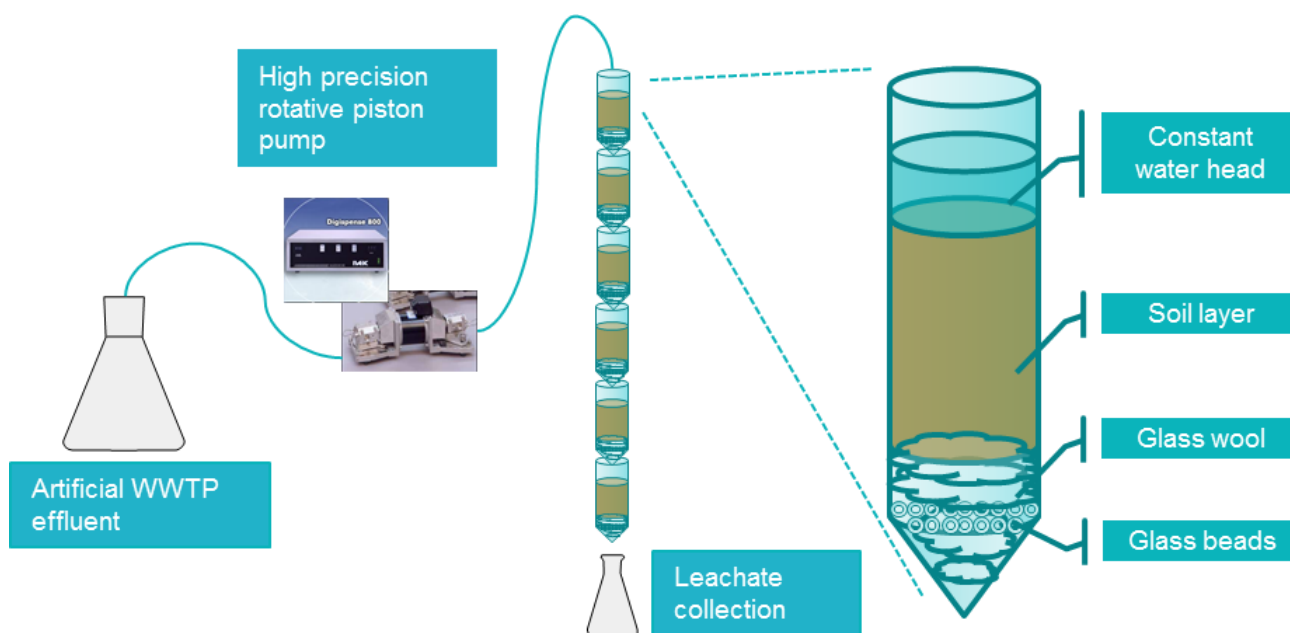


Figure 2.2 Leaching column scheme.

The IW was pumped throughout the columns using a digispense 800 (IVEK, USA) high precision, dual channel, rotative ceramic piston pump. The flow rate was adjusted according to the soil water retention, leaving the same constant water head (2 cm) on the top of each column. The effluent of the columns (leachate) was recorded daily, and analysed, for the entire duration of the test, to obtain the amount of HMs and the amount of soluble organic matter (in terms of total organic carbon, TOC) leached from the soil.

Overall four sets of experiments were performed, varying: i) the amount of soil organic matter (SOM); ii) the column depth; iii) the sodicity of the IW. The experimental set-up is summarized in Table 2.1.

Table 2.1 Experimental setup.

Experiment	SOM content [%]	Column depth [cm]	Sodium in IW [mM]	pH of IW	Exp. Time (d)
A	5 (OECD)	49	<0.05	6.5	22
B	2.5; 5; 7.5; 10	5	<0.05	6.5	36
C	5 (OECD)	5	<0.05	6.5	17
D	2.5; 5; 7.5; 10	5	20	6.5	55

At the end of experiment A the soil was collected, dried at room temperature in vacuum conditions using a desiccator, and subjected to acid digestion to evaluate the content of HMs of each module of the column. At the end of experiment B, instead, the soil contained in the polypropylene tube was frozen at -20°C and cut in slices of 1 cm each by means of an electrical saw (Bosch, Germany), to better define HM spatial distribution. A blank soil sample was moisten with ultrapure water, frozen and finally cut and analysed to exclude any metal contamination from the saw blade. In order to achieve the metals mass partitioning between the soil and the liquid leachate, the daily leachates of each column were collected together, and, at the end of the test, concentrated by evaporation at mild temperature (40°C) in a thermostatic oven (Binder, Germany) to a final volume of 50 mL prior to analysis. The leachates collected from experiment C were instead analysed daily as described in the next sub-section. To define the quality of the interactions between the solid matrix and the contaminant, a sequential extraction was performed too (Pueyo et al., 2008). Finally at the end of experiment D, it was applied the same procedure described for the experiment B.

All tests were carried out in triplicate and the variance among the triples was reported as standard deviation by means of error bars.

2.2.2 Analytical methods

HMs analyses in soil were performed by Atomic Absorption Spectroscopy (AAS). In details, Ni, Cu and Pb contents in the soil were measured by flame atomization (F-AAS), while trace concentrations (Cd content in the soil, Ni, Cu, Cd, content in the leachates of experiment B) were measured by graphite furnace atomization (GF-AAS). A fully equipped AVANTA (GBC, Australia) and a SpectrAA (Varian, USA) atomic absorption spectroscope were used for these purposes. Quantification limits (LOQ) for GF-AAS were determined as 0.05; 0.5 and 0.75 ppb for Cd, Cu and Ni respectively. Soils were dissolved by means of microwave assisted digestion using a Start D (Milestone, USA) microwave digester. The acid digestion was performed using a 9:1 HNO₃:H₂O₂ reagent mixture. The fractionation of contaminants in the soil was determined following the modified BCR three steps sequential extraction as described by Rauret et al. (2000). TOC was measured using an OI Analytical AURORA 1030W TOC Analyzer set in TC-TIC mode, and evaluated as difference of total carbon (TC), detected after acidic oxidative degradation with 10% sodium persulfate (Carlo Erba Reagenti, Italy), and total inorganic carbon (TIC), detected after the sole acidification by the addition of 5% concentrated phosphoric acid (Carlo Erba Reagenti, Italy). The leachates collected from the columns at 5% SOM (OECD soil) in experiment C were further investigated. In detail the concentration of Cd, Cu, Ni, and the absorbance at 245, 285, 350, 650 nm (A_{245}) were measured daily. Moreover the 3DEEM matrices were recorded. The E_4/E_6 ratio was calculated from the measured absorbance according to Chen et al. (1977). HM determination in the leachates was carried out by means of a Nexion 300 ICP-MS (Perkin Elmer, USA) operating in collision mode. LOQs were determined as to be 0.021; 0.06; 0.03 ppb for Cd, Cu and Ni, respectively. The UV measurements were carried out using a UV-VIS Lambda3 spectrophotometer (Perkin Elmer, USA). The 3DEEM matrices were acquired through a LS 45 spectrofluorimeter (Perkin Elmer, USA) using the following excitation-emission range: ex. 210-455nm - em. 370-585 nm.

2.3 Results and discussion

2.3.1 Mobility of Cd, Cu, Ni, in the standard OECD artificial soil

Results of metal concentrations in the different soil layers at the end of the experiment A are reported in Figure 2.3.

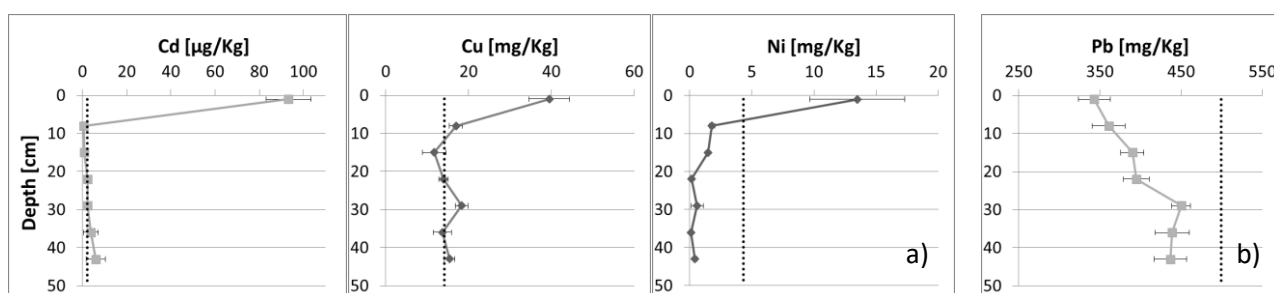


Figure 2.3 a) Mobility profiles of trace Cd, Cu, Ni through OECD soil. b) Mobility profile of kaolinite bound Pb after the leaching experiment. Vertical dotted lines indicate initial background levels in the artificial soil.

As it can be easily deduced from the obtained metal profiles, the OECD soil showed high affinity with the metals that were totally immobilized in the first centimetre of the soil layer. The metals concentration, which was monitored daily within the leachates, was very low and during only few days copper and nickel were barely above the quantification limits, while cadmium was always below during the whole duration of the test. The metals, being present in very low concentration in the IW, interacted strongly with the soil binding sites that were more abundant than the pollutants. The major part of HMs was hence quickly and irreversibly trapped in the first centimetres and created a very thin contamination front, which reasonably would have expanded once the binding sites in the topsoil had been saturated. Of course, at the tested concentrations, this was not likely to occur within the established experimental time, and therefore no evident mobility of the contaminants appeared.

Together with the accumulation of HMs occurring in the first centimetres of the soil, a different phenomenon was also observed. Copper concentration, in fact, showed a broad peak (18.3 mg/kg) at 29 cm depth. This peak could not be explained as a consequence of the sorption-desorption equilibria, because the column was fed continuously with the IW, and the effect of sorption-desorption would have caused a monotonic decrease of concentration at increasing depth, instead of causing an

accumulation of metal at two third of the column. Figure 2.3b reports the concentration trend of Pb that was found to be present in the initial Kaolinite at quite high concentration (499 ± 5 mg/Kg). It is clear that lead was washed from the soil by the IW, since its concentration was lower than the initial background throughout the soil profile. On the other side a considerable fraction of the Pb moved at very low rate through the soil layers. Finally such slow mobilization generated, after the leaching time, a sudden increase (around 80 mg/Kg) of lead concentration at the same depth (29 cm) where Cu also accumulated.

To explain this behaviour it was assumed a mobilization mechanism based on a three-component physical model of the soil, described in Figure 2.4, including: i) an immobile phase; ii) a slowly mobile phase; and iii) a mobilizable phase.

Movement inside the soil is driven by the water flow, but it does not always take place, necessarily, at the same velocity of the water. Once the leaching starts, the slowly mobile phase moves with it, even if at a slower pace, determining a sort of front descending through the soil. The slowly mobile phase is mainly composed by colloids, both organic and inorganic, as well as by macromolecular aggregates of kaolinite clay platelets with humic substances. The descending front divides the soil into two parts, one below the front, in which colloids maintain the same initial concentration, and one above the front in which their concentration is decreasing during time and is always lower than the initial one (Figure 2.4a). At the same time water leaching brings HMs to the soil. The metals interact mainly with the immobile phase, and are trapped in the first layers of the soil. The interactions are quite strong, and not easily reversible, since the continuous water flow is not able to mobilize the metals and distribute them in the deeper layers of the soil. Nonetheless part of the immobile phase is subject to transformation during the leaching process, and becomes mobile (mobilizable phase). It is well attested, in fact, how organic matter undergoes to oxidative processes in the soil, which may be both biotic and abiotic (Kleber et al., 2014; Sunda & Kieber, 1994; ten Have & Teunissen, 2001). This leads to a production of a wide variety of low molecular weight molecules and oligomer humic fragments, water soluble and thus highly mobile, which interact with the contaminants. Ligands generation take places constantly during the leaching, and is promoted by a series of reactions also called “oxidative depolymerisation” (Figure 2.4b). Because of their mobility, formed ligands act as chaperones molecules through the soil column, carrying downward the metals. The migration of the chaperones molecules is quite fast, but at least some of them are somehow slowed and even blocked by the colloidal front, producing a peak of contamination at a certain depth of the column (Figure 2.4c-2.4d). It is reasonable to suppose that such interaction is higher for high molecular weight compounds than for small molecules.

As metal mobility is related to the presence and quality of the chaperones molecules, it will be completely different if: i) the metals are dissolved; ii) the metals are adsorbed in their charged free form; iii) the metals are coordinated to the ligands that are present in the system. In other words the mobility will be strongly affected by metal speciation.

The described mechanism can find confirmation in some of the study described in the available literature. Many researches (Khodadoust et al., 2005; Renella et al., 2004; Schwab et al., 2008) observed that organic acids may affect heavy metals desorption, solubility and thus mobility. Moreover, Cu and Cd are known to mobilize according to different mechanisms (Ahumada et al., 2001; Lafuente et al., 2008; Li & Zhou, 2010; Zhao et al., 2007a; Zhao et al., 2007b), Cd being more mobile than Cu (Beesley et al., 2010) and more subjected to be mobilized by interaction with small organic acids (Gerritse, 1996; Li & Zhou, 2010; Weng et al., 2002). In contrast, Cu in soil is frequently mobilized also in presence of high MW molecules as metal-organic complex (Flogeac et al., 2004; Pérez-Novo et al., 2009; Schwab et al., 2008; Temminghoff et al., 1997). In accordance to this result, no contamination front was observed for Cd in the present study (Figure 2.3a). Likely Cadmium was transported by small molecules and was affected by the phase change in presence of the colloidal front. It is possible to observe that the Cd-Ligand complexes penetrated within the colloidal phase front and the concentration, although very low, is arising in the last 10 cm of the column.

Finally the formulated hypothesis is in agreement with the presence of contamination fronts within the soils taking place in correspondence of soil composition changes, usually at the interfaces between different horizons. The slowly mobile phase described so far, might be more strongly affine with the new type of soil respect to the one from which was released, and therefore could be retained at the interface, creating thus a barrier that may continue to trap even trace contaminants and increase their concentration during time. This behaviour is very well described, for example, by the experiments conducted by Zhao et al. (2007a) and Zhao et al. (2009).

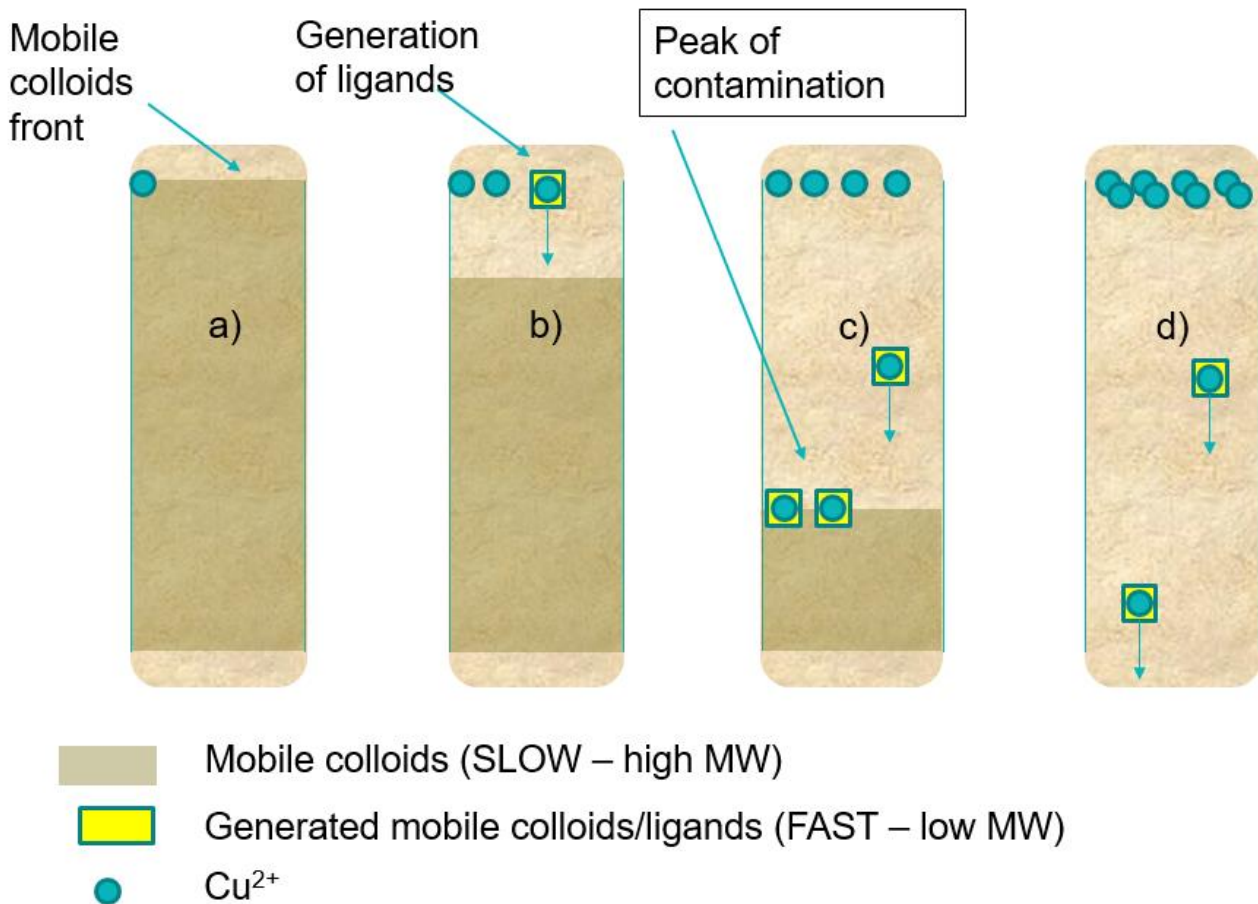


Figure 2.4 Proposed mechanism for copper mobility in OECD soil.

2.3.2 Effect of SOM content

To further confirm the supposed mechanism of HM mobilisation it was run a second set of tests (experiment B) by varying the SOM content. This time the column was only 5 cm deep to avoid the presence of a colloidal front inside the soil. As expected the modification of the soil composition caused a variation of its permeability, testified by the volume of leachate collected at the end of the experiment (Figure 2.5c).

Evidently, the higher amount of peat made the columns more permeable to the water solution. These results are often encountered in the literature, since water infiltration may be improved by the presence of organic matter and microbiological activity (Davis & Wilson, 2000; MacRae & Mehuys, 1985). This meant that the column containing the highest amount of organic matter (10%) was loaded with the highest amount of HMs, since the total amount of IW passing through this column was about 30% higher than the total amount passing through the column containing only 2.5% of peat. Moreover, as expected, preliminary batch test with Ni and Cu confirmed that peat has a higher

maximum adsorption capacity (14.6 mgCu/g and 13.44 mgNi/g) compared to kaolinite (4.3 mgCu/g and 6.5 mgNi/g) in accordance with Covelo et al. (2007), therefore it would have been reasonable to expect a higher contamination of the soil containing higher percentage of organic matter. Obtained data, displayed in Figure 2.5a, contradicted such an expectation, showing that the columns had an opposite behaviour for all the tested metals.

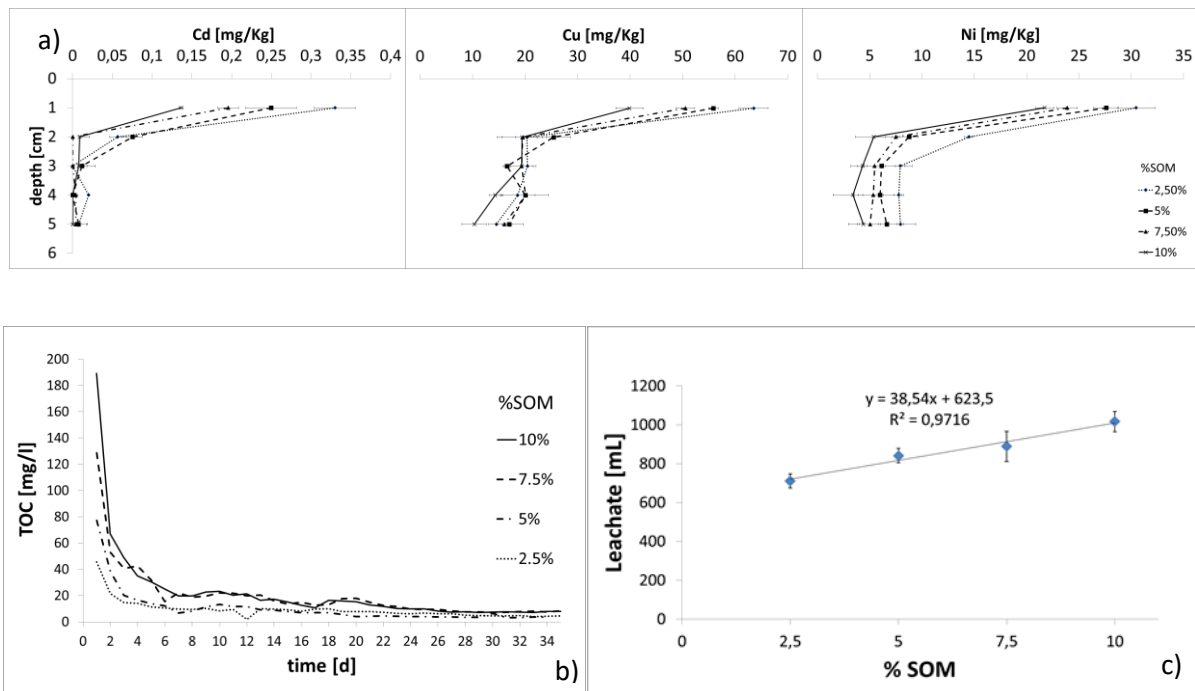


Figure 2.5 Results of experiment B. a) Concentration of Ni, Cd, Cu in the soil increasing SOM content. b) Concentration of TOC in the leachates. c) Total volume of leachate collected at the end of the experiments at increasing SOM % in the soil.

The accumulation of contaminants, which happened, once more, in the top layer of the soil, was in inverse proportion with the SOM content. The highest contamination was reached for the soil prepared with 2.5% of peat (30.4 mg/kg of Ni, 0.33 mg/kg of Cd, and 63.5 mg/kg of Cu) while the lowest was observed at 10% SOM (21.7, 0.14, 39.9 mg/Kg for Ni, Cd, Cu respectively). Such an apparently unexpected result was coherent with the mechanism described in the previous section. The generation of mobilizing ligands was more probable at higher SOM content. Because of the limited depth of the column, the slow colloidal fraction washed out of the column in the first days of leaching, and the ligands were not trapped by it, exiting from the column with the leachate. This was also confirmed by the TOC concentration in the leachate (Figure 2.5b). The peak of TOC, in fact, ranged from 45 mg/L for the soil having the lowest SOM content, to 180 mg/L for soil having the highest

SOM content. Moreover, after the first 7 days it was observed a continuous release of organic matter, which lasted until the end of the experiment.

Clearly the TOC did not account directly for the ligands concentration, since these latter were only a small fraction of the total organic carbon measured in the leachates. Nonetheless it assessed that soil was continuously releasing organic material in the leachate. It is worth noting that the mobilization mechanism performed a sort of washing of the soil, removing even part of the background HM concentration. Measured metal concentration below the first 2 centimeters, in fact, were always below the initial background level, the more at higher SOM content.

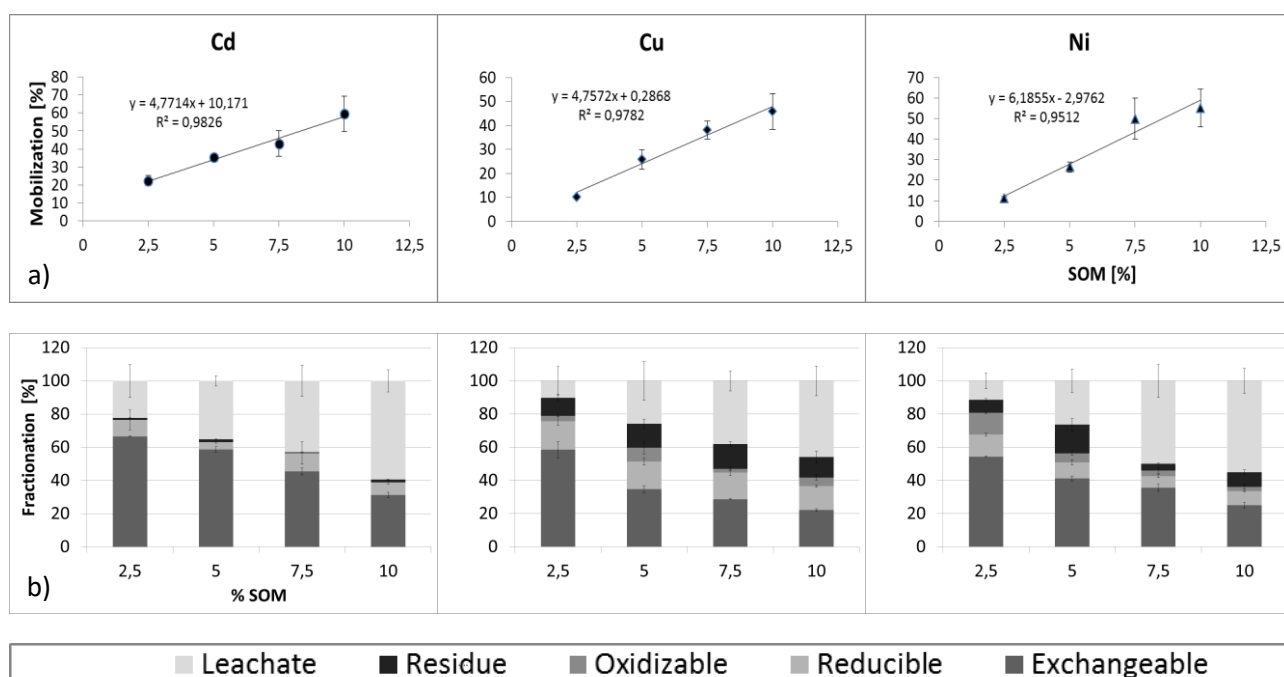


Figure 2.6 a) Linear correlation between metals mobilization and SOM content. b) Fractionation of heavy metals after the irrigation simulation (error bars at the top of the columns refer to mass balance discrepancy between adsorbed and leached metals).

Figure 2.6a plots the percentage of metals that was mobilized through the columns at increasing SOM content. As it can be easily observed there is an excellent correlation between the two series of data for all considered metals, indicating that SOM content played a primary role in defining the trace metals mobilization coefficients. Furthermore, considering that the intercepts of the lines are quite close to the zero value, it is reasonable to suppose that the interaction with the organic matter was the only parameter affecting the metals dynamics in the tested conditions. The similar values of slopes obtained also suggest that the mobilization of at least Cd and Cu, happened according to the same transport mechanism. No evidence of selectivity nor competition between the ions were observed.

Figure 2.6b shows also metal distribution among different fractions of the soil, together with metal amounts detected in the leachate at the end of the experiment. The sum of these amounts equals the sum of HMs added to the system plus the background values, allowing to close the mass balance with a maximum discrepancy of 12.5%.

Several interesting information can be deduced by the obtained distribution. First of all, data referred to HMs detected in the leachate confirm the previous observation concerning metal mobilisation. Moreover, it can be noted that the majority of metals bound to the soil was distributed in the acid-labile fraction.

This exchangeable fraction was the only one involved in the observed mobility. The SOM increase did not cause any variation of the percentage of metals bound to the other fractions of the soil, even if the percentage found in the leachate increased. It follows that both the contamination of the soil, and the mobilization of the metal into the soil were mainly associate to ionic exchange phenomena, and therefore the metals were easily bioavailable for crops or microorganisms. We also observed a decrease at increasing of SOM of the nickel bound to the oxidizable fraction that is likely related to the mobilization of Ni adsorbed on complex organic ligands (likely FA) from the peat and that was already present in the soil background. No Cd at all was found in the oxidizable fraction. Moreover Cadmium is confirmed to be more mobile respect to the other metals and in all the configurations tested a higher percentage of the added metal was released in the leachate respect to Cu and Ni.

2.3.3 Characterization of released soil colloids

Results referring to the characterization of the leachates collected in experiment C are reported in Figure 2.7. From Figures 2.7a-b it is possible to notice the presence of several peaks of HMs concentration at different leaching times (e.g.: 6, 9, 11, 14, 15 d). Cu and Ni followed a very similar mobility pattern with peaks often coincident (6, 9, 11, 14 d). Conversely, Cd showed a constant release with concentration oscillating around 1 $\mu\text{g/L}$ during the first days of the experiment (1-11 d). Then the concentration decreased and finally a peak was recorded on day 15 (Figure 2.7b). Figure 2.7a also shows the measured trend of A_{245} in comparison with the metals release. Except for one sample (14 d), there is a notable correspondence between the peaks of Cu and Ni and the A_{245} values. Such correspondence confirms the hypothesis that the organic colloidal matter is involved in metal transport through soil. The OM release from the soil is hence controlling metals mobility. The reported trend (Figure 2.7a) of Pb concentration accounts instead for inorganic colloids mobilisation.

Indeed, as previously described Pb is intimately related to the Kaolinite composition, that is the only Pb source in the studied system. The correspondence of peaks of Pb and A_{245} (9, 12, 15 d) led to hypothesize the presence of colloidal mineral organic associations (MOAs) that were strongly related to the transport of Cu and Ni (Kleber et al., 2014). This confirms the hypothesis that the slow colloidal fraction is in the first days regulating the metal release, where the mineral organic aggregates are those hypothesized in Figure 2.4c and are responsible for the peak of Cu in soil recorded in Figure 2.3a. The initial release of Cd is clearly related to this inorganic colloids release. Such dispersed release of Cd confirms that Cd complexes are penetrating within the slow colloids front and hence no accumulation peak is recorded, in accordance with what previously discussed concerning Figure 2.3a. The E_4/E_6 ratio displayed in Figure 2.7b confirms that Cd is preferentially complexed by small molecular weight ligands. E_4/E_6 ratio is in fact related to the degree of humification and in general is increasing at decreasing the humification degree (Chen et al., 1977; Yang et al., 2016).

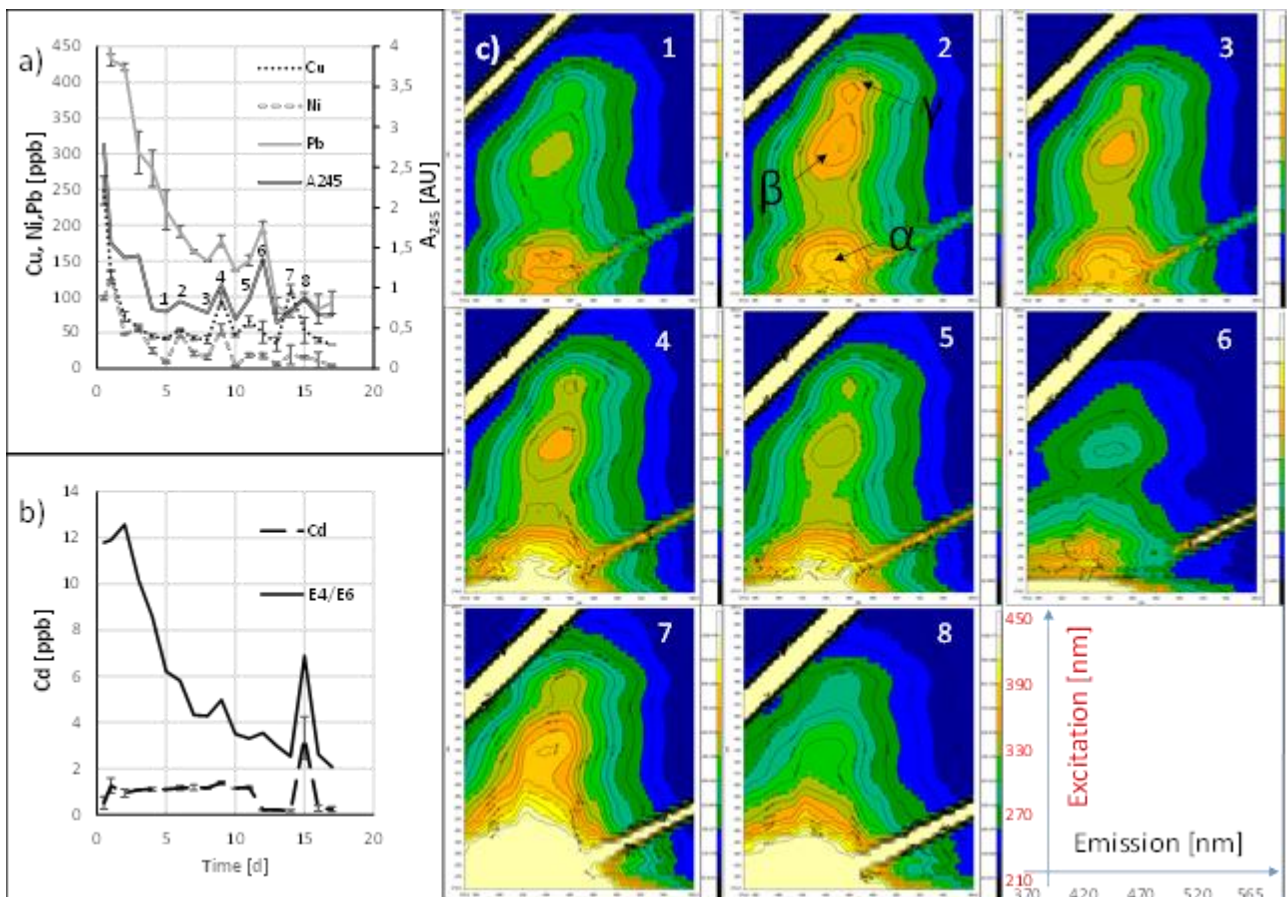


Figure 2.7 Characterization of the colloidal fractions mobilised from the columns in experiment C; a) trends of Cu, Ni, Pb and A_{245} versus time; b) trends of Cd and E_4/E_6 versus time; c) selected 3DEE matrices of the leachate solutions. Up-right numbers in Figure c correspond to the experiment time indicated in Figure a.

A selection of the 3DEEM acquired at specific leaching time was reported in Figure 2.7c. More in detail the 3DEEM were chosen according to the HMs trends observed in Figure 2.7a-b. During the whole experiment (Figure 2.7c, graphs 1-8) it was detected the constant presence of two peaks of excitation- emission. The first one (ex 230 – em 420-460 nm), namely α in graph 2, is in a zone of the matrix that is usually associated to FA-like fluorophore (Boguta et al., 2016). FA has indeed higher amounts of aliphatic domains and generally lower molecular weight distributions compared to HA. Hence less chromophores conjugation is possible and the excitation wavelength (WL) becomes lower. The second peak, which was present in all the recorded graphs, was identified as β in the zone associated to HA (ex 330- em 445 nm). The higher MW and the higher content in aromatic moieties makes the excitation WL to become higher. Both these substances were always present within the collected leachates, and were connected to the background constant release of metals (Figure 2.7a,b), which were continuously released from the column, even at very low concentration. It was pretty much evident that, at least qualitatively, the relative intensities within the two described excitation-emission regions were changing during the experimental time. At the beginning (Figure 2.7c, graphs 1-3) Humic substances were more abundant. Conversely, at the end of the experiment, the peak α showed very high excitation-emission intensity. This meant that a constant release of organic molecules happened from the soil to the leachate, but their quality and quantity was decidedly changing over time. This assumption was in good agreement with Figures 2.7a,b regarding the trends of A_{245} and the E_4/E_6 ratio. It is worth noting that in correspondence of the simultaneous Cu and Ni release (Figure 2.7a) a further neat peak, γ in graph 2, appeared at high excitation WL (Figure 2.7c, graphs 2, 4, 5, 7). The higher excitation WL could be related not only to higher MW and to enhanced conjugation of the chromophores, but also to higher presence of auxochromes heterogroups (carboxyl, hydroxyl, etc.) capable to establish very stable coordination bonds with the metals. In other words, the higher ex-em WL explained also the higher metal affinity deducible from Figure 2.7a. A further confirmation of the interaction of the fluorophore with Cu and Ni came from the effect of quenching that Cu and in minor amount Ni have on fluorescence. Several studies have evidenced this effect of Cu binding to HA-like fluorophores (Boguta et al., 2016; Wu et al., 2004). In the collected leachates, Cu concentration ranged between 1 and 2 μM and the quenching due to Cu could not be higher than 10% of the total fluorescence intensity (Yamashita & Jaffé, 2008). Nevertheless it was possible to notice that the intensity of the peak γ was respectively decreasing in graphs 2,4,5,7. Such decrease was consistent with the Cu concentration in the leachate. It is finally clear that Cu and Ni interacted with the humic fraction of OM and the peak γ was identified as the binding site of the chaperon molecule. Conversely, in accordance to what observed about the relationship between E_4/E_6

ratio and the release in the leachate, Cd was evidently more affine to FA-like fraction. Such fraction was in fact much higher in graph 8 and corresponded to the peak observable in Figure 2.7b. In the same sample, no accumulation was encountered of Cu and Ni. Neither Cu nor Ni were detected in days 6 and 8, when A_{245} expressed relative maximums. This meant that the binding of these metals with the organic colloids detected in Figure 2.7c graphs 2, 4, 5, 7 is highly specific and selective. In the same way Cd was mobilized only by FA and poor or no interactions were possible with the HA fraction responsible for Cu and Ni transport. Deeper in detail, graph 7 had emission intensities in the HA domain, and consequently Ni and Cu were detected in Figure 2.7a. Although very high signals were present in the FA domain, no Cd accumulation was same time recorded. The day after (graph 8), almost no signal was counted for HA, indeed no peak of Cu and Ni was present in Figure 2.7a. On the contrary Cd was released in the same sample. It is to underline that humic substances are supramolecular aggregates of organic fragments (Conte et al., 2007; Šmejkalová & Piccolo, 2008). Identifying an excitation emission peak does not give information about the supramolecular structure of the released colloids. Such supramolecular structure binds together HA and FA by means of weak dispersive forces, changing their affinity with metals. In this case, the chaperone molecules identified by signal α that are more aliphatic would be hidden in the hydrophobic core of the supramolecular colloid, while β and γ fluorophores that are more rich in hydrophilic residues would be in the outer sphere of the macromolecular aggregate. This could explain why Cd was transported by FA only in presence of low HA signals (graph 8) and high E_4/E_6 ratio (Figure 2.7b).

2.3.4 Trace metal mobility in absence of colloidal mobilisation

A final set of experiments (experiment D) was run in order to evaluate the mobility of metals in absence of colloidal mobilisation. In this case the IW was characterized by a high concentration of sodium, as described in the methods section. Obtained results (data described only) showed substantially the following main points. A severe clogging of the column was observed almost immediately, and the flow was particularly slower than in the experiments described so far. It was quite variable without any correlation with the content of organic matter. After two months of leaching, in all the studied cases, it was not possible to collect more than 450 mL of leachate (maximum) while the analysis of this latter showed very low concentration values, often below the instrumental quantification limits. In all the columns, despite the SOM content, the recovery of metals was above 95% of the influent metal always in the top layer of the column (≤ 1 cm). This implies that the mechanisms of mobilization, although related to the organic matter, depend strongly from the colloidal mobilization that may affect soil permeability. This could be related to the detachment

mechanism of the ligands and to the mobility of the high molecular weight slow colloidal phase. The ionic strength did not allow the solubilisation of the colloids that remained in their native solid configuration and thus inhibited any mobilization of these macromolecular aggregates. Sodium cation is known to interact with the negatively charged layers of clay particles. As sodium concentrations increase, the electrophoretic mobility of the clay anionic layers (platelets) increases resulting in swelling dispersion of the clay particles thus impacting on soil permeability (Toze, 2006). The detachment of the organic ligands, which are aggregated in the supramolecular structures of HA (Piccolo, 2001; Piccolo, 2002) by several types of interactions, may need the opportune kinetic energy to overcome the binding strength of ligands. Once the slow colloidal phase starts to move within the column, it is reasonable to suppose that several preferential pathways are created in the soil texture, thus allowing higher permeability through the soil, as observed in Figure 2.4. If water has not enough kinetic energy, this detachment is at least slowed or even highly improbable. The effect of sodium ions is different from that of general “salinity”. Ca and Mg, i.e. being smaller than sodium do not increase the clay dispersion, but in contrast they facilitate the aggregation of clay particles, keeping soil flocculated and competing with sodium for binding sites (Duan et al., 2010; Warrence et al., 2002). On the other side the mobility of colloids and particles through soil usually happens in condition of non-homogeneous packing of the soil, creating preferential paths and hence preferential flows, that determine the ability of organic matter macromolecules to bypass the soil “chromatographic column” (Kleber et al., 2014). It is hence to keep in mind that the behaviour of the OECD soil columns with the salinity of a real wastewater will be different. Anyway behaviour observed during experiment C excludes the possibility that the phenomena observed during experiment A may be related to protonic exchange equilibria. The peat, in fact, is known to be quite acid (pH 4-4.5) and the trends observed in Figures 2.5, 2.6 and 2.7 may be erroneously attributed to the pH lowering due the increase of acid peat. This was not the mobilization mechanism since, although being theoretically possible in the low permeability experiments, it did not happen, confirming that the supposed transport dynamics are the more likely to occur. Moreover, no mobilization due to any thermodynamic or kinetic ions competition for binding sites was observed among sodium and the trace metals in the IW, suggesting that the studied metals are retained in the soil solid phase by interactions that are more stable and faster than with Na.

2.4 Conclusions

- The accumulation and mobility of trace amount of Ni, Cd and Cu was evaluated through an artificial soil of known composition on which the organic content was tuned in standard and repeatable conditions. The effect of SOM enhanced HMs mobility, since the highest SOM percentage corresponded to the lowest metals accumulation, and the highest concentration in the effluent leachate.
- The HMs were mobilized according to a mechanism that implies the interaction with colloidal phases present in the soil, and a slow and constant release of small molecular weight ligands that, detaching from the soil immobile matrix, act as chaperones transporting the metals through the soil medium and finally in the groundwater leachate. This mobility pattern was found to be directly related to the SOM content, since higher amount of SOM generate a higher number of chaperon molecules.
- A linear correlation was found between the metals mobilization and the organic matter content in the column. The effect of organic matter is depending on metals concentration and experiments configuration. At high concentration and in batch mode organic matter increased adsorption capacity. At trace levels and in continuous flow mode the effect is exactly opposite due the colloidal mobilization. Such colloidal mobilization is substantially negligible at high concentration and the higher adsorption capacity prevails resulting in higher contamination at higher SOM content
- The released chaperon molecules were chemically different and moved with different velocities over time. Cu and Ni were preferentially transported by HA-like fragments, while Cd is preferentially mobilized by FA-like. The formation of colloidal MOAs was also hypothesized on the basis of the obtained data to be involved in the observed mobility.
- The soil behaves as a metals accumulator, increasing their concentration during time and generating contamination fronts that may remain fixed to the immobile matrix or move with variable velocities throughout the soil layers. The water/soil transfer of pollutant was not only related to the contaminant trace concentration in the irrigation water but strongly depends on the soils physical-chemical properties (i.e. SOM content, structure of the released transport molecules) as well as on the properties (i.e sodicity) of the aqueous medium.
- Further studies are needed to evaluate other effects that might sum to the sole effect of SOM (i.e.: Fe and Mn oxides, presence of DOM already in the IW, bacterial activity) and to assess

if and how the behaviour observed in the model OECD soil deviates with different types of real soils.

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Chapter 3

Solid dilution allows accurate determination of phenolic and humic-like substances in raw solids via simple Folin-Ciocalteu colorimetric assay

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3.1 Introduction

The determination of total phenolic (TP) content in liquid and solid organic matrices has received increasing attention in the last few decades. The fields of application have extended from simple environmental monitoring, agri-food characterization (Stratil et al., 2006), and soil quality assessment (Ohno & First, 1998), to recently developed field of bio-resource technologies, which includes lignocellulosic materials widely studied as renewable energy sources (Ghimire et al., 2015).

Lignocellulosic materials are rich in lignin, a complex polymer of three so-called monolignols (p-coumaryl, conyferil, synapyl alcohols). Monolignols have been well chemically characterized as polyphenols, due the presence of more than one phenolic hydroxyl. It is well known that lignin rich wastes are not readily biodegradable (Ariunbaatar et al., 2014a). Thus, when improperly discarded in the environment, these wastes give rise to slow prolonged release of phenolic compounds in the environment, which can lead to groundwater's pollution and loss of soil fertility. Moreover polymeric phenolic compounds have been scrutinised in the last few decades due the detrimental effects incurred when lignocellulosic substances are subjected to anaerobic digestion with excessively high loading rates (Liotta et al., 2016; Pontoni et al., 2015). On the other hand there is good evidence to show that polyphenols possess very good anti-oxidant properties, and thus, more and more studies have been characterizing foods and beverages in terms of polyphenols content (Croft, 2016; Francesca et al., 2016).

Noticeable amounts of phenolic moieties contribute to the structure of humic substances. Humic substances are strongly involved in many processes related to soil chemistry and biochemistry, such as contaminants transport through soil, microbial distribution, soil water retention and consequently soil fertility (Paramashivam et al., 2016; Roosta et al., 2016). On the other hand, phenol-rich biosolids are often spread onto agricultural soils to increase their fertility. The presence of aromatic moieties in the polymers relates to the capacity of soil to adsorb or coordinate micro-pollutants (organic and inorganic). Thus aromatic moieties of bio-solids spread on natural soils have serious consequences on micro-pollutant mobility and bioavailability in the environment (Komprdová et al., 2016; Tang et al., 2016).

The analytical challenges in achieving precise and effective quantification of the TP in all these matrices are mostly related to the high solid content. Indeed phenolic groups are heterogeneously speciated; they can occur as small hydrophilic molecules, often described as “free phenols”; they can be dissolved; or they can form colloidal or suspended macromolecules (Pontoni et al., 2015). Thus

the target analytes might distribute into different phases of a solid or semi-solid matrix; moreover they may be more or less water-soluble.

Although the Folin-Ciocalteu (F-C) method has been validated and standardized for determining TP and lignin contents in water and wastewater (APHA, 1998), to the best of our knowledge, there is no standard F-C procedure for determinations in solid or semi-solid samples. Estimating TP in these heterogeneous matrices, requires a method for it is dealing with water suspensions of samples that often contain insoluble particles that interfere with photometric determinations. Hence, sample preparation plays an essential role in determination related to this kind of complex matrix. It follows that, depending on sample preparation and analysis method, different TP estimations in given substrate type might range over several orders of magnitude. Most methods available in the literature involve TP quantification after extraction with various solvents from the analytical matrix (Ainsworth & Gillespie, 2007; Blainski et al., 2013; Cicco et al., 2009; Francesca et al., 2016). This leads to underestimation or absence of the non-extractable compounds, which in many cases, may display major biological activities. Analytical data are highly dependent on the recovery after the extraction and when high amounts of non-extractable phenols are present, the data does not provide an accurate snapshot of the system. Hence, a method is needed that allows a rapid, accurate quantification of TP by directly analysing the solid sample, regardless of the speciation of phenols in the system.

The aim of this study was to demonstrate the applicability of solid dilution (SD) preparative method to this analytical method. SD consists of mixing and homogenizing a sample with a known amount of a salt that will remain inert during the analytical reactions. The SD method was previously successfully applied to the determinations of the chemical oxygen demand (COD) in solid and semisolid samples with very good results in terms of recoveries and repeatability (Noguerol-Arias et al., 2012).

Here, we optimized and validated this method on three matrices: rice straw (RS), peat soil (PS) and food waste (FW). The results, expressed as mg equivalents of phenol (C_6H_5OH), were compared to those obtained when the homogenized sample was suspended in water (traditional method). We analysed the performances of these two methods with Grubbs' test and Student-T test. Grubb's test was used to exclude anomalous data that could have distorted the results (e.g. outliers related to experimental errors). The Student-T test was used to verify that the two methods generated two different sample populations.

3.2 Materials and methods

RS was harvested from rice fields in Pavia (Italy). FW was prepared in the laboratory according to previous Valorgas report (Valorgas, 2012) as described in details by Ariunbataar et al., (2014b). PS was purchased from a local gardening store.

F-C reagent was purchased from Carlo Erba Reagenti (Italy). Sodium tartrate, sodium carbonate and sodium sulfate anhydrous were purchased from Sigma Aldrich (USA). A sodium carbonate and tartrate solution was prepared by dissolving 200 g Na_2CO_3 and 12 g $\text{Na}_2\text{C}_4\text{H}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$ in 750 mL of hot ultrapure (Elga Option-q, USA) water. The solution was cooled down to 20 °C, then adjusted to 1 L with ultrapure water. The calibration curve was constructed with pure phenol in crystals from Carlo Erba Reagenti (Italy). Spectrophotometric measures were acquired with a Photolab 6600 UV-Vis spectrophotometer from WTW (Germany).

3.2.1 Analytical Procedure

Raw samples were accurately weighed and mixed with diluting agent at a dilution ratio of 1:20. This ratio was previously reported to be the most effective for COD analysis (Noguerol-Arias et al., 2012). The salt-sample mixture was finely crushed in a ceramic mortar until no particles were visible anymore. The overall dilution procedure was similar to that described to be effective for COD by Noguerol-Arias et al. (2012), except for the choice of diluting agent. Instead of magnesium sulfate, which formed a precipitate of magnesium carbonate after adding sodium carbonate and tartrate solution, we used sodium sulfate anhydrous, and no precipitation was observed. The homogenized samples were divided into aliquots expected to have TP concentrations within the linear range of the method (i.e. 0.25-2.5 mg/L). These aliquots were suspended in water for a final volume of 50 mL. For the traditional method, separate raw samples, which had not been subjected to SD were finely homogenized in the ceramic mortar. For testing, aliquots expected to have TP concentrations similar to the ones previously prepared were suspended in water. All the suspensions were maintained in perfect mixing conditions and continuously stirred with magnetic stir-bars during sample withdrawal for the F-C testing procedure. In a 2.5 mL disposable spectrophotometry cuvette, we added in sequence 2 mL of the described suspension and 15 μL of F-C reagent. The mixture was shaken upside-down by hand to ensure the reagent dissolved uniformly. Finally, 600 μL of the tartrate and carbonate solution was added, and the sample was mixed again. A bright blue colour developed as a function of the TP present in the sample.

When the 2 mL sample was withdrawn from the flask, the mixture was continuously stirred with magnetic stir-bar to ensure that the suspended particles did not settle. However, immediately after mixing in the cuvette, a residue was visible due to incomplete solubilisation of the compound. Therefore, samples were filtered before absorbance reading ($\lambda=700$ nm). We selected a filter that would minimize loss in the absorbance read. We tested various filtering pore sizes and materials (laboratory filter paper, glass fibers, glass wool, polypropylene, cellulose acetate, regenerated cellulose, TFE). Among these, we found that the mixed cellulose ester with a pore size of 0.8 μm provided the least reduction absorbance; absorbance measured after filtering was less than 0.01 compared to the unfiltered sample in case of phenol standards. The overall procedure is summarized in Figure 3.1.

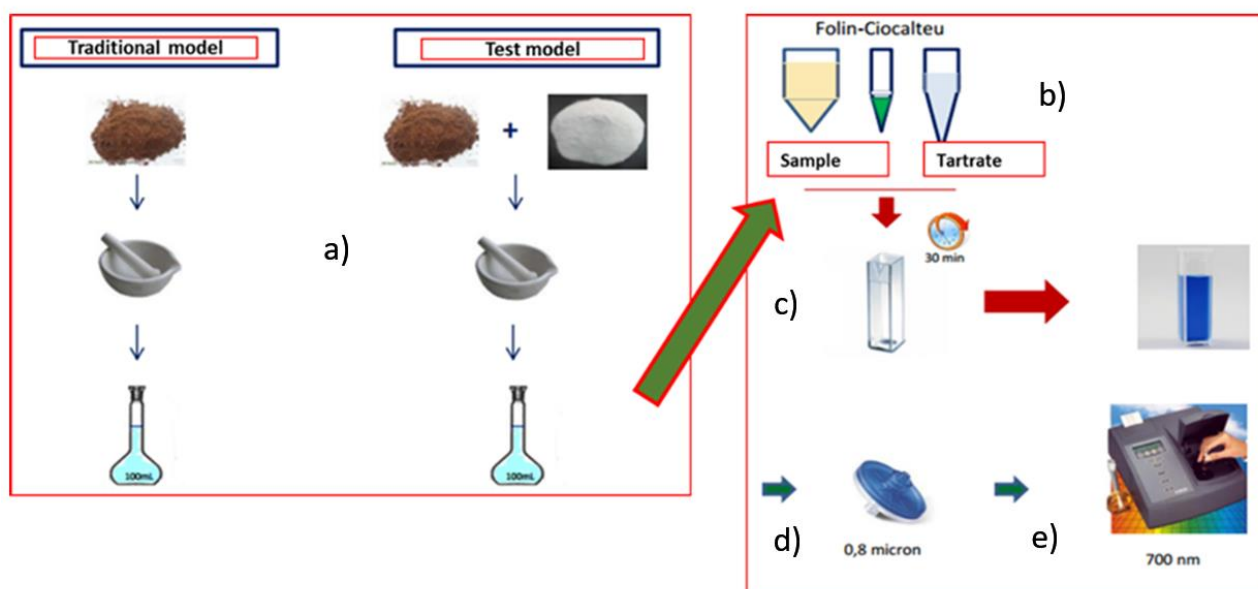


Figure 3.1 General scheme of the proposed procedure: a) sample homogenisation with (Test model) and without SD (Traditional model); b) addition of the F-C reagents to the water suspension; c) reaction time; d) 0.8 μm filtration; e) absorbance reading at 700 nm.

We also evaluated the time required to complete the reactions. After 15 min triplicate measurements of a single cuvette showed a high variation, and the absorbance continued to increase, which indicated that the reaction was incomplete. After 30 min, the measurements showed consistency. Hence we filtered the samples and measured the absorbance after a 30 min reaction time. We also verified absorbance to remain stable for at least 2 hours after the reading. Potential interference from the added sodium sulfate was excluded by comparing the absorbance of solutions

prepared with and without added salt to the absorbances of pure phenol and a commercial sample of soluble lignin (Carlo Erba Reagenti, Italy). We found that the linearity interval of the method was between 0.25 to 2.5 mg/L of phenol. We constructed a seven-point calibration curve within this linear interval with increasing concentrations of phenol standards (Figure 3.2).

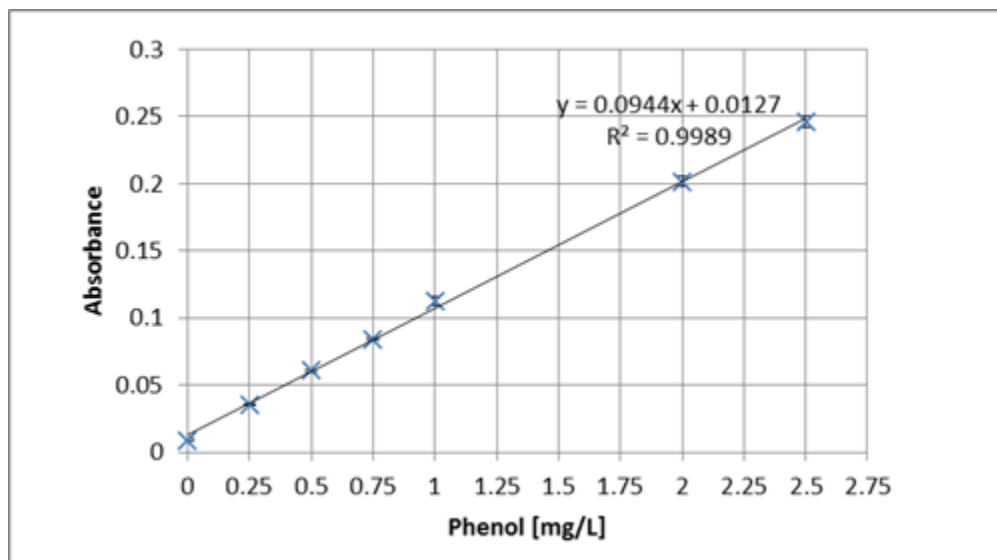


Figure 3.2 Phenol calibration curve. Phenol standards of known concentrations were measured on a spectrophotometer to determine the absorbance at 700 nm after 30 min reaction time and 0.8 μ m filtration. Each point represents the average of three measurements.

We prepared eight replicates of the suspensions of the raw homogenized sample (traditional method) and eight replicate suspensions of the SD sample (test method). Each suspension was analysed in triplicate by the described F-C assay, for total 48 analyses per each of the tested matrices. Results were compared between the two methods (traditional and SD).

3.2.2 Statistical analysis

To compare phenol concentration measurements between the two different methods (i.e. the traditional method with water dilution and the new method with SD), we performed a statistical analysis of experimentally collected data as follows:

A normal distribution was plotted for each sets of data; a Grubbs' test was performed to delete data affected by experimental errors; a Student's T-test was performed to verify that the data sets obtained with the two methods were significantly different.

3.2.2.1 Normal distribution

For each series of values measured with both methods, the means and standard deviations were calculated with the following expressions:

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i \quad (1)$$

$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}} \quad (2)$$

Where \bar{x} the average of the data set and s is the related standard deviation.

Assuming that averages and standard deviations measured from samples (\bar{x} and s , respectively) corresponded to the averages and standard deviations of the populations (μ and σ , respectively) from which the samples were taken (i.e. $\mu = \bar{x}$ and $\sigma = s$), we plotted a normal distribution for each series of data with the following expression:

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left[-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2\right] \quad (3)$$

Where x ranged from $\mu-3\sigma$ and $\mu+3\sigma$.

3.2.2.2 Grubbs' test

Once we verified that the data sets could be reasonably approximated with a normal distribution, the Grubbs' test was performed. This test detects outliers from the normal distributions. The candidate outliers are the minimum and maximum values in each set of data. The candidates were tested one at

time. The Grubbs's test evaluated if for a fixed confidence level (i.e. 95%) the candidate belonged to the population. This procedure comprised the following steps:

1. A confidence level of $1-\alpha$ was fixed
2. The value of parameter T^* (dimensionless) was calculated with the following expression:

$$T^* = \frac{|\bar{x} - x^*|}{s}$$

(4)

Where \bar{x} is the average of the data set, s the standard deviation and x^* is the candidate value.

3. T^* was compared to the tabled T relative to α and n degrees of freedom, where n is the number of values in the data set. When $T^* \leq T$, then x^* belonged to the data set; when $T^* > T$, then x^* did not belong to data set with a probability of $(1-\alpha)$ %. In the latter case was excluded from the data set.

Grubbs' test was iterated until no outliers were detected. The minimum number of values required to perform Grubbs' test was six.

3.3.2.3 Students' T-test

We used Students' T-test to verify that two sets of data were significantly different.

This test can be performed when the sample size is less than 30 and the population standard deviation is unknown, otherwise it is preferable to perform the Z-test.

This test evaluates the probability that the null hypothesis can be rejected. The null hypothesis holds that the two data sets belong to the same population, and that differences in terms of means and standard deviations between the two data sets are due exclusively to accidental causes.

The procedure comprised the following steps:

1. A confidence level α was fixed
2. The value of parameter T_{obs}^* (dimensionless) was calculated with the following expression:

$$T_{obs}^* = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{x_1-x_2}} \quad (5)$$

where \bar{x}_1 and \bar{x}_2 are the averages of the two data sets respectively and $s_{x_1-x_2}$ is the pooled standard deviation obtained from the two data sets.

3. T_{obs}^* was compared with the tabled T , relative to α and $n+m-2$ degrees of freedom, where n and m are the number of values in the two sets of data. When $T_{obs} > T$, the null hypothesis could be rejected with a probability higher than $(1-\alpha)$ %. When $T_{obs}^* \leq T$, the null hypothesis could not be rejected and the two data sets were judged to belong to the same population with a probability higher than $\alpha\%$.

3.3 Results and discussion

The proposed SD method was successfully used to quantify the TP content in all tested solid matrices. However, we encountered several differences in precision and accuracy among samples prepared according the SD method.

None of the experimentally collected values were excluded with Grubbs' test, because none were statistically out of range. The results of comparing the two preparation methods are shown in Figure 3.3a-c and summarized in Table 3.1.

The normal distributions of phenol values measured with the two methods were clearly different (Figure 3.3 a-c). The difference was statistically significant, based on the T-test, (Table 3.1). Thus the two methods generated values that belonged to different populations.

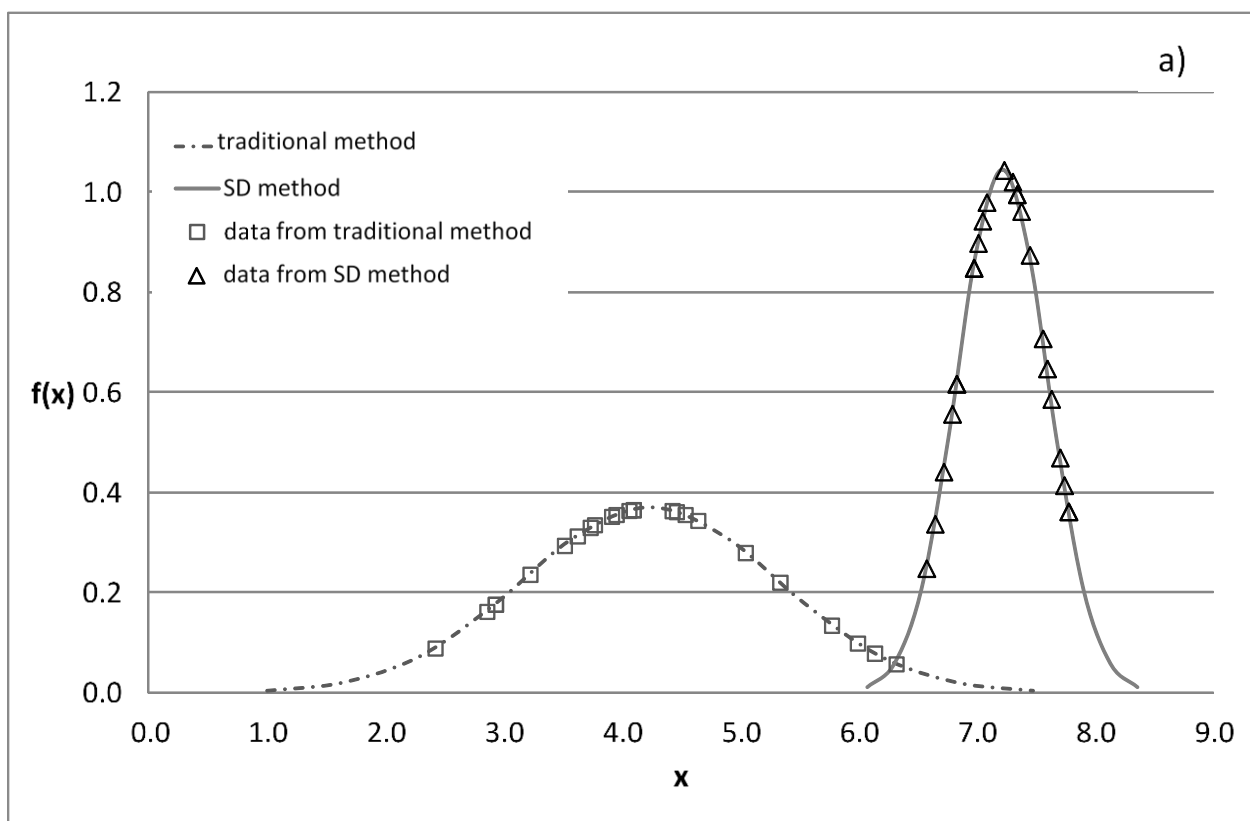
Table 3.1 Main values of TP values for the investigated matrices compared between two preparation methods.

Sample	Sample preparation	Number of tests	TP g/Kg	σ^2	s	σ^*	T-test
RS	Traditional	24	4.239	1.167	1.080	0.25	2.51×10^{-13}
	Test (SD)	24	7.219	0.146	0.382	0.05	
PS	Traditional	24	5.961	0.624	0.790	0.13	1.47×10^{-5}
	Test (SD)	24	7.053	0.390	0.625	0.09	
FW	Traditional	24	2.422	1.379	1.174	0.48	1.66×10^{-11}
	Test (SD)	24	6.016	1.839	1.356	0.23	

TP = Total phenols; RS = rice straw; PS = peat rich soil; FW = food waste; SD = solid dilution
 σ^2 = variance
s= standard deviation
 σ^* = coefficient of variation

Our results clearly showed that diluting the sample with salt drastically increased the recovery of phenols for the F-C assay. A comparison of the average TPs (Table 3.1) showed that for all substrates, the concentration recovered with the SD process was substantially greater than that obtained with the traditional method (41.4, 15.5, 59.4 % recovery increases for RS, PS and FW, respectively). These findings indicated that the traditional method, where the solids were only suspended in water tended to underestimate the actual phenolic content. With the SD method, more accurate estimate was achieved. This may be due to an improvement in the homogenization of the substance. The added salt may increase the efficiency of breaking up macroscopic particles, because during homogenization, the salt crystals act as micro-blades, finely crumbling even high compressive strength substrates, such as lignocellulosic materials. The smaller particles could disperse better when suspended in water and allow more efficient contact between the phenolic components and the reagents. Another possible explanation may be related to the very high salinity, which can denature the hydrophobic core of organic matter. This denaturation could expose the core end the phenols within, which would otherwise be sequestered away from the water phase and thus inaccessible to the reagent. The standard deviation of the RS substrate measurement was much lower in the SD method than in the traditional method. In fact, the standard deviation value for the SD method was nearly half the value

obtained with the traditional method. In the PS sample, the difference between methods was slightly less, but the SD method (Figure 3.3b) displayed less dispersion in the data (higher precision). Conversely, for the FW substrate (Figure 3.3c), the standard deviations value obtained with the SD method showed slightly greater data dispersion (less precise) than that obtained with the traditional method, although the values were comparable. On the other hand, when compared the coefficients of variation (σ^* in Table 3.1), the SD method displayed more precision than the traditional method. As expected a higher variance was observed in the FW values compared to the other tested matrices, due the greater heterogeneity in FW (both in composition and in macroscopic shape) than the other tested matrices. The simultaneous presence of different organic macromolecules (Ariunbaatar et al., 2014b) (i.e. carbohydrates, proteins, lipids) which could give rise to higher uncertainties in the sampling phase (Noguerol-Arias et al., 2012).



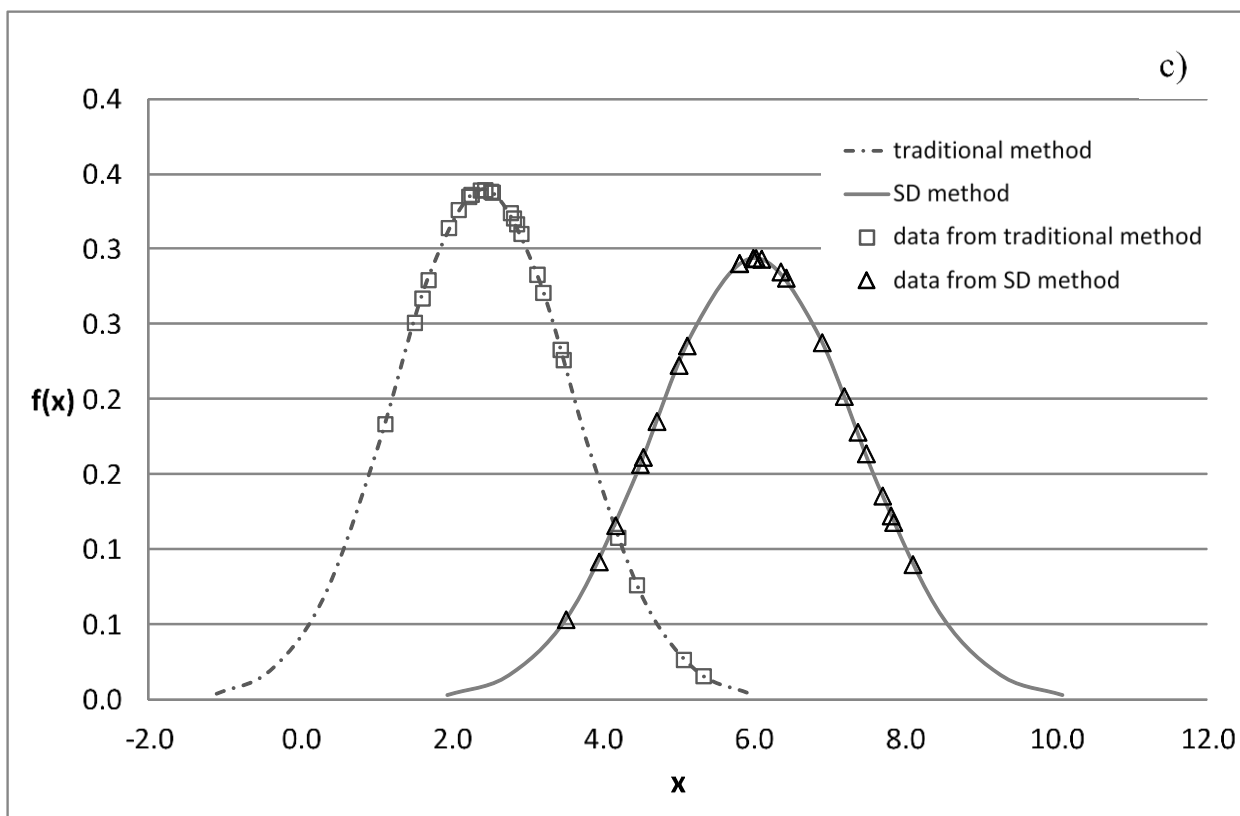
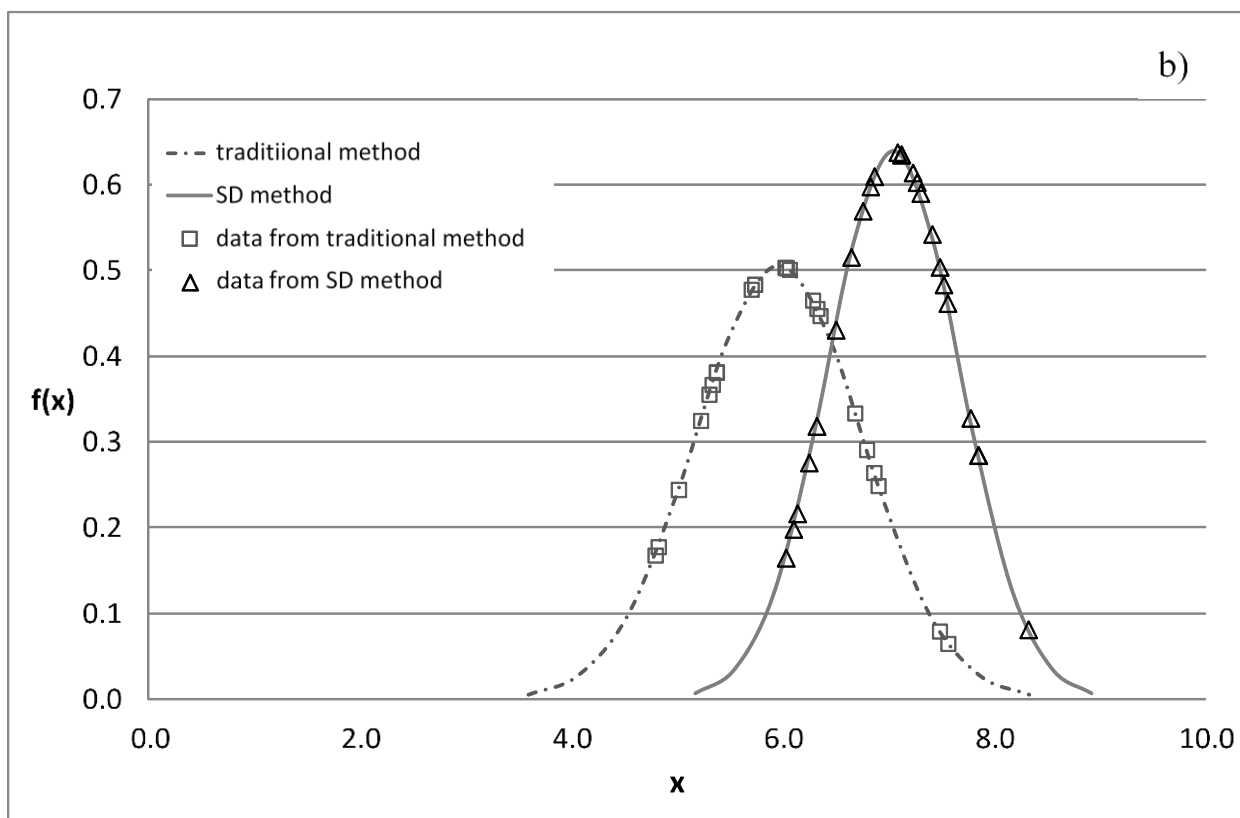


Figure 3.3 Normal distribution of phenol measured in: RS (a); PS (b); FW (c). The two different methods were compared. We represented Traditional method with triangles and dotted lines and SD method with squares and continue lines. $f(x)$ values were calculated with equation (3), where x ranged from $\mu - 3\sigma$ and $\mu + 3\sigma$.

Concerning the reported improvement in data distribution, the obtained results were comparable with those reported by Noguerol-Arias et al. (2012), who applied the SD method to COD analysis. The authors applied the SD method to a certified reference material, and obtained a reduction of σ^* ranging from 0.104 to 0.014. When the SD method was applied to “real” pig slaughterhouse waste samples, the authors obtained an average reduction of σ^* from 0.27 to 0.08. Here we verified that the SD method applied to TP measures reduced the relative standard deviation in all tested samples. The reduction ranged: i) from 0.25 to 0.05 for RS; ii) from 0.13 to 0.09 for PS; and iii) from 0.48 to 0.23 for FW (Table 3.1).

It is worth noting that COD analysis does not depend on oxidizable matter speciation in the matrix, therefore the SD method applied to COD simply allows to withdraw a greater amount of sample, increasing its representativeness and improving the precision and the accuracy of the estimate. On the contrary TP analysis has intrinsically a high variance and is subject to several interferences (Ainsworth & Gillespie, 2007). The aim of the present work was to obtain a quantification of TP in very complex matrices, regardless their speciation. This meant that the F-C reagent had to interact with phenols bound to suspended particles or sequestered in hydrophobic moieties and micelles. Although several published studies and protocols suggest to perform F-C assay on sample extracts to increase precision (Ainsworth & Gillespie, 2007; Blainski et al., 2013; Cicco et al., 2009; Stratil et al., 2006; Wieder & Starr, 1998), our results ascertain that a complete analysis of the matrix “as is”, except for the only SD preparation, gives back more accurate and “real” results. These results showed good reproducibility and acceptable relative standard deviation even for very heterogeneous samples.

3.4 Conclusions

The study work has highlighted some aspects of new SD preparative method for measuring phenolic compounds in solid and semisolid matrices, which make this method preferable to the traditional one. The SD sample preparation method reduced time for performing analyses, because it did not require the extra time needed to solubilize or extract in water and other solvents phenolic compounds. Moreover, for all the substrates, (i.e. RS, PS and FW), the SD method yielded higher concentrations of phenolic compounds than the traditional method. This was probably due to the effect of salt addition that improved sample homogenisation and disrupted hydrophobic aggregates, favouring the contact of sequestered phenols with the F-C reagent. This result proved that the proposed method was more accurate than the traditional method. Finally, the proposed method was

also more precise than the traditional method, because exhibited less relative data dispersion for all the substrates tested. Although for FW the absolute standard deviation was higher than the traditional method, this result was not discordant with our other findings, because the coefficient of variation was smaller with the SD method than with the traditional method. Furthermore, the wide dispersion in FW data could be reasonably attributed to the heterogeneity of the matrix investigated (i.e. FW) rather than to the method used.

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Chapter 4.

Colloidal mobilization and fate of trace heavy metals in semi-saturated artificial soil (OECD) irrigated with treated wastewater

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4.1 Introduction

The recovery and reuse of wastewater in agriculture is a promising strategy to face water scarcity issues (Toze, 2006). Treated wastewater is a “new” water resource, which can positively contribute to the agriculture sustainability. The advantage consists in restoring the organic and inorganic nutrient pools of soils, solving, at the same time, water shortage and wastewater discharge problems (Chen et al., 2015a), especially in arid and semi-arid regions (Ilias et al., 2014). However, wastewater reuse for irrigation purposes can represent a serious threat for the environmental quality (Lu et al., 2015). The presence of trace level concentrations of potentially harmful pollutants is in fact permitted in irrigation water by existing regulations all over the world, since it does not represent a short-term hazard for the environmental quality. Nonetheless some compounds, such as heavy metals (HMs), can accumulate in the soil through the years, finally reaching the crops and eventually entering in the food chain (Nicholson et al., 2003).

The fate of HMs in the soils can be hardly predicted. Mechanisms of mobility through the different soils horizons are, in fact, extremely diverse, being related to: i) the soil physical-chemical nature; ii) the soil-water retention capacity; and iii) the fluid transmission characteristics. Clearly both wastewater and soil composition have a fundamental role in the complex dynamics that regulate HMs mobility through the different layers. Particularly, organic matter is considered to have a crucial effect, by greatly influencing the retention and the transportation of HMs and other micro-pollutants. Organic molecules are present both in the wastewater and the soil matrix. The presence of organic matter can either enhance, when dissolved (DOM), or retard, when bound to the soil (SOM), the transport of inorganic pollutants (Weng et al., 2002). HMs and contaminants in general are not only partitioned between the solid immobile and the water mobile phases. Colloids and nanoparticles act as a third mobile phase, with their own rheological properties and velocity (McGechan & Lewis, 2002). This third mobile phase can be organic, inorganic or composed by mineral organic associations (MOAs) (Kleber et al., 2014). Three criteria must be met for colloid-facilitated contaminant transport: i) colloids must be present; ii) contaminants must interact with colloids; and iii) colloids and associated contaminants must be transported through the aquifer (Ryan & Elimelech, 1996).

Studying the interaction of HMs in “artificial” water solutions with the standard OECD soil, a novel HMs transport pattern was developed in Chapter 2. A multi-component mechanism was assumed including: i) the interaction of HMs with the colloidal phase of the soil; ii) the slow and constant release of small molecular weight ligands detaching from the soil immobile matrix; iii) the transportation of HMs through the soil by these low molecular weight chaperon molecules. To further

improve the knowledge of the proposed mechanism, a deeper understanding of the structural composition of soil organic matter would be required. Soil organic matter, in fact, is constantly under study, and its structural composition is subject of debates among the scientific community (Piccolo, 2002). The classical idea of a class of unknown polymeric macromolecules - namely Humic acids (HA), Fulvic Acids (FA) and humin - is going to be passed by the new concept which considers humic substances as supramolecular aggregates of small organic compounds, held together by weak dispersive forces (Piccolo, 2001). In the light of this theory, soil released chaperone molecules or their aggregates, can have different characteristics in terms of chemical structure and molecular weights distribution. According to these characteristics, they can have different moving rate through the porous media, as well as different affinity and selectivity with metals. As consequence of the existing heterogeneity among colloids and chaperon molecules generated in soil, the “three phase model” becomes a “multiphase model”. Each metal is preferentially transported by one or more phases and moves through soil with a velocity that is governed by the phase interaction with the porous media and hence by its own physical chemical properties (de Jonge et al., 2004; Pédrot et al., 2008). In other words, assessing the mobility of such colloidal aggregates equals to achieve the fate of HMs in soil. Therefore, a structural characterization of the released phases as well as the definition of structural relations with metals affinity and with migration patterns in soil are needed. Starting from these premises, the present paper aims to investigate the effect of irrigation wastewater characteristics on the colloidal mediated transport of Cd, Cu, Ni and Zn in soil. The research is conducted on a standard soil, irrigated with a “real” wastewater effluent. The soil is packed in columns, and semi-saturation conditions are always maintained. To better understand the HMs transport mechanism according to the over-mentioned multi-phase model, several spectroscopic methods (UV-VIS, 3DEEM, ¹H-NMR) are used, to achieve a deeper characterization of the organic metal binding phases.

4.2 Materials and methods

4.2.1 Leaching experiments

A micro-contamination phenomenon was reproduced and studied at laboratory scale, simulating the irrigation of an artificial soil with either the effluent collected from a wastewater treatment plant (WWTP) or a synthetic water containing four trace HMs in the same concentrations as in the real treated wastewater. To evaluate the dynamics of accumulation of micropollutants as well as their

migration in the leachate, HMs concentration was determined daily in the leachate and, at the end of the experiment in the soil column matrices. The mobilization of humic substances in soil was monitored by determining the concentration of total phenols. The release of organic substances in the leachates was measured as chemical oxygen demand (COD) and Ultra-Violet (UV) absorbance. Leached organic matter was further characterized through 3DEEM spectrofluorimetry and NMR spectroscopy.

The used standard soil was prepared according to the "recipe" described in the guidelines of the Organization for Economic Cooperation and Development (OECD) (Wilhelm & Maibach, 2008). These guidelines were developed in order to test the eco-toxicity of inorganic and organic chemicals in soil, therefore the OECD soil is widely used to test complex solid samples (Joško & Oleszczuk, 2013). The reference soil was composed as follows: kaolinite, 20%; quartz sand, 74%; CaCO₃, 1%; sphagnum peat, 5%. Kaolinite, quartz sand and CaCO₃ were purchased from Sigma-Aldrich (USA). Sphagnum peat was obtained from a local gardening store. As the OECD itself indicates, the ability to retain or release contaminants is related to the amount of organic matter (peat) contained in the soil (Wilhelm & Maibach, 2008). Before use the soil was analyzed in the single components to define the background levels of the tested metals. Results of analysis are reported in Table 4.1. A very high concentration of Pb was found in the kaolinite.

The experimental tests were conducted at laboratory scale using a treated wastewater (W1), sampled from a conventional activated sludge WWTP located near Paris (France). W1 was characterized in terms of metals content. The HMs concentrations were well below the threshold values suggested by Food and Agriculture Organization of the United Nations (FAO) in the wastewater quality guidelines for agricultural use (Pescod, 1992). On the basis of the obtained results, an artificial effluent (W2) containing the same concentrations of Cd, Cu, Ni and Zn but no dissolved organic matter (DOM) nor other major elements was prepared. In details W2 was obtained dissolving analytical grade CuCl₂·2H₂O (Carlo Erba Reagenti, Italy), NiCl₂·6H₂O (Sigma-Aldrich, USA), and ZnCl₂ (Applichem, Germany) and analytical standard Cd (Carlo Erba Reagenti, Italy), in analytical grade HNO₃ 1% (J.T. Baker, USA) solutions. The obtained stock solutions, containing 1 g/L of the selected metal, were successively diluted with ultrapure water (Elga, USA) to obtain the required concentrations. Final pH was adjusted to 6.8, adding a few drops of KOH 0.05 M. HMs concentrations in W1 and W2 are reported in Table 4.1.

Table 4.1 Characteristics of W1, W2 and OECD soil

Parameter	OECD soil (mg/L)	W1 ($\mu\text{g/L}$)	W2 ($\mu\text{g/L}$)
Al		118	-
Ca		1.11×10^5	-
Cd	0.0363	0.232	0.25
Co		<0.5	-
Cr		ND	-
Cu	23.827	8.73	10
K		8.62×10^4	-
Mg		1.34×10^5	-
Mn		153	-
Na		3.5×10^5	-
Ni	1.525	1.31	1.5
Pb	499.3	ND	-
Si	2530		
Sr		928	-
Zn	89.0	24.82	25
pH	7.6	6.8	6.8
DOM (as TOC)		6.71×10^3	-

The described solutions were used to irrigate the soil packed in a polypropylene conical tube, holed in the bottom and filled in the lower part with two layers of glass wool and a layer of glass beads, to avoid soil loss in the leachate. The scheme of the columns was the same described in chapter 2 (Figure 2.2). The columns (5 cm depth) were fed with 20 mL of wastewater every 12 hours. All tests were performed in triplicate.

The test lasted for 17 days. After this time both the soils, divided into 5 layers of 1 cm each, and the daily leachates were characterized in terms of metals and organic matter content. Since very high concentration of Pb was detected in the kaolinite, Pb was monitored in the soil and in the leachate as a tracer of the clay silicates. Kaolinite was indeed the only source of Pb in the experiment (Table 4.1). Clay silicate migration in the leachate was also assessed by monitoring Si concentration in the leachates.

4.2.2 Analytical procedures

Each soil layer and the “blank” soil were dried at 35°C until constant weight and homogenized. Aliquots of 1g were mineralized in 15 mL of aqua regia using a Milestone (USA) Start D microwave digester. Leachates were collected daily and divided into two aliquots. The first aliquot was acidified with 3% HNO₃ for metals analysis. The concentrations of Cd, Cu, Ni, Pb, Si and Zn, were measured by ICP-OES spectroscopy (Perkin Elmer Optima 8300, USA) and ICP-MS (Perkin Elmer Nexion 300, USA) operating in dual detector mode. The second aliquot was stored at -20°C for spectroscopic characterization. Once defrost, the UV-VIS absorbance at 245nm and the COD were determined. Moreover the three dimensional excitation emission matrices (3DEEM) and for some selected leachate samples the proton nuclear magnetic resonance (¹H-NMR) spectra were recorded. The UV measurements were carried out using a Lambda3 UV-VIS spectrophotometer from Perkin Elmer (USA). COD was determined according to APHA (1998) standard method 5220D. Total Phenols were determined by Folin-Ciocalteu colorimetric assay. COD and total phenols absorbance readings were performed by means of a Photolab 6600 UV-VIS spectrophotometer (WTW, Germany). The 3DEEM matrices were acquired through the spectrofluorimeter Perkin Elmer LS 45 (USA). Excitation wavelength was varied between 210-450 nm; emission wavelength was recorded between 370-585 nm. NMR spectra were acquired on an AVANCE 400 NMR spectroscope (Bruker, Germany), equipped with Prodigy cryo-probe. Solvent suppression was achieved using standard pre-saturation sequence.

4.3 Results

4.3.1 Mobility profiles in soil

The results of metals analysis in the soil and in the leachate are summarized in Figure 4.1. Figure 4.1 also displays phenols concentration in the different soil layers to have an indication of organic materials distribution and mobility.

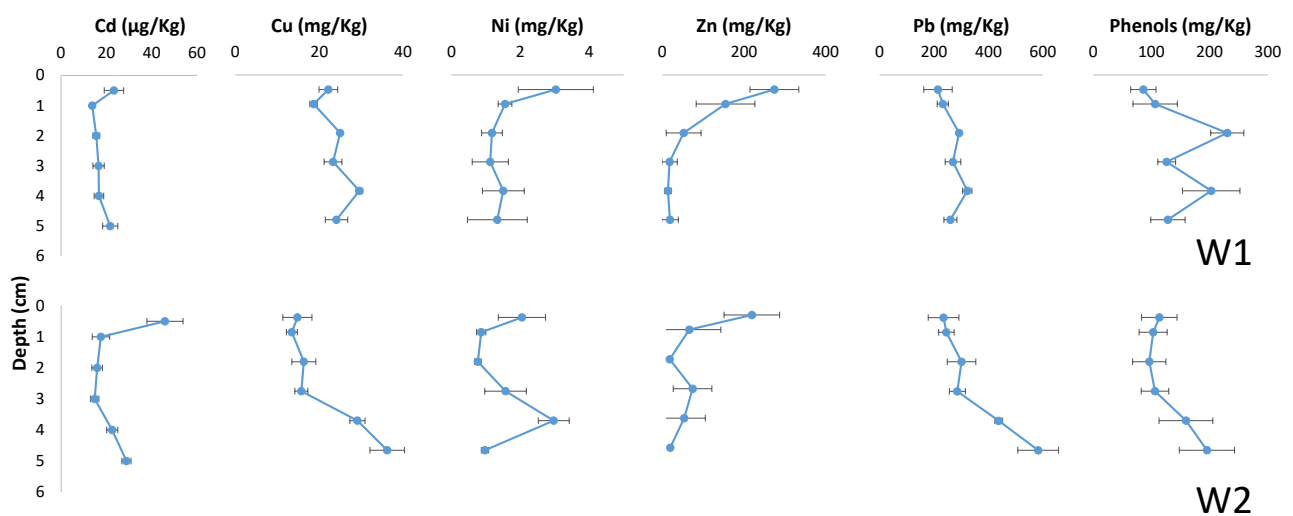


Figure 4.1 Metals and total phenols concentration in soil columns irrigated with W1 and W2. Irrigation lasted 17d and a total leachate of 650 mL was collected at the end of the experiment. Soil background HMs concentrations are reported in Table 4.1.

The distributions of Pb and total phenols were very similar. Peaks of both concentrations were observable at the same depth. Pb and phenols together showed different trends in soil irrigated with W1 and W2. In the experiments conducted using W1 as irrigation water (W1 tests) total phenols and Pb concentrations showed two peaks, located at 2 and 4 cm depth. Overall Pb concentration was quite uniform, and accounted for almost one half of the initial background value. In the experiments conducted using W2 as irrigation water (W2 tests) there was a clear increase of the phenols and Pb concentrations which started below 3 cm of depth. At 6 cm depth, Pb concentration was very similar to the initial background value (509 mg/Kg). This is an illustration of a slow colloidal front that was still present at 4 cm depth at the end of the W2 tests. A different migration rate of the soil colloids,

which moved faster in the soil irrigated with W1, was evidenced. According to the observed mobilization, HMs behaved differently in the two experiments. Cu distribution was very similar to Pb and phenols. In W2 tests, in fact, there was an evident accumulation below 3 cm depth, while in W1 tests the concentration presented two small peaks at 2 and 4 cm depth. Very small Cu contamination was present in the top layer. Conversely Cd, Ni and Zn always displayed a concentration peak in the top of the column. Accumulation of Cd at 0.5 cm depth was higher in W2 tests (49.51 $\mu\text{g/Kg}$) than in W1 tests (23.35 $\mu\text{g/Kg}$). Ni concentration in the top layer was higher in W1 tests (3.03 mg/Kg) than in W2 tests (1.92 mg/Kg). The same behavior was observed for Zn (274 and 189 mg/Kg in W1 and W2 respectively). However, quite high standard deviations were observed among the replicates in the case of Ni and Zn. In the deeper layers practically no peaks were observed in the soil irrigated with W1. In contrast, in the soil irrigated with W2, the concentration of Cd, Cu, Ni and Zn greatly increased below 3 cm, in accordance with Pb and phenols mobilization. Differently from the other metals, Ni and Zn concentrations markedly decreased at 5 cm depth where were comparable to the initial background levels. Due to the accumulation in the lower layers, higher HMs retention was generally observable in W2 tests compared to W1 tests.

4.3.2 Leachates characterization

HMs mobilization information obtained from metal concentration in the different soil layers was confirmed by the concentration values of HMs detected in the leachates (Figure 4.2a). Obtained data showed that the irrigation with W1 led to higher mobilization of the kaolinite silicates (Figure 4.2b). The release of Si was indeed around three times higher in W1 than with W2. This is reflected by higher metals release into the leachate respect to which observed during the irrigation with W2.

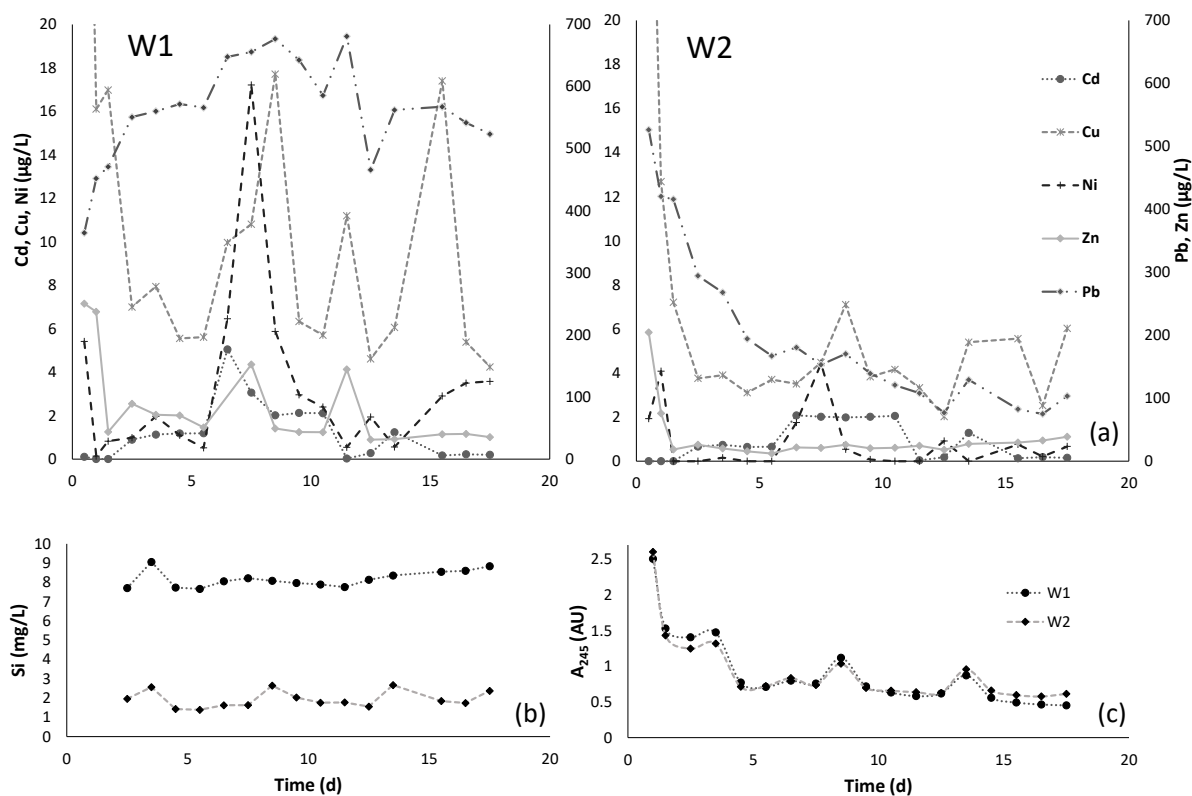


Figure 4.2 Evolution of HMs (a), Si (b) concentrations and A₂₄₅ (c) in the leachate produced by soil irrigation with W1 and W2

The release of inorganic colloids and HMs in the leachate was indeed much higher during W1 tests (W1 leachate) than during W2 tests (W2 leachate). A remarkable difference between the two experiments was represented by the concentration of Pb. In accordance with metal data in the soil columns (Figure 4.1) and Si trends (Figure 4.2b), a higher release was observed in W1 leachate where Pb concentration never went below 350 µg/L. Conversely, W2 leachate displayed a decreasing trend of Pb, which, at the end of the experiment, was stabilized near 100 µg/L. Generally, HMs did not show a constant concentration in the leachates and several peaks were observable at given experimental times. In detail, peaks of metals were observed at the same time (days 8-10 and days 14-16) in both W1 leachate and W2 leachate. In W2 leachate, differently from W1 leachate, Zn concentration peaks were not observed, and Zn concentration remained constant in time, ranging between 20 and 40 µg/L. High concentration of Zn and Cu was also recorded together in W1 leachate (day 11). In the same day, Pb released in the leachate also showed a marked increase. These peaks were not observable in W2 leachate. Ni mainly eluted with the other metals during days 8-10 in both the experiments.

Contemporarily with metal release, a constant release of organic matter in the leachate was observed, as indicated by UV A₂₄₅ trend, reported in Figure 4.2c. A₂₄₅ peaks were popping up at the

same time (days 8-10 and days 14-16) as the major of HMs peaks (Figure 4.2a). At the same experimental time, peaks of Si in W2 leachate were noticeable in Figure 4.2b.

COD in the leachates displayed a trend very similar to A_{245} (data not shown). Also in the case of COD relative maximum peaks (up to 68.5 mgO₂/L) appeared at the same experimental time as A_{245} and metals peaks. A constant background release of COD was also recorded ($\approx 30 \pm 4$ mgO₂/L).

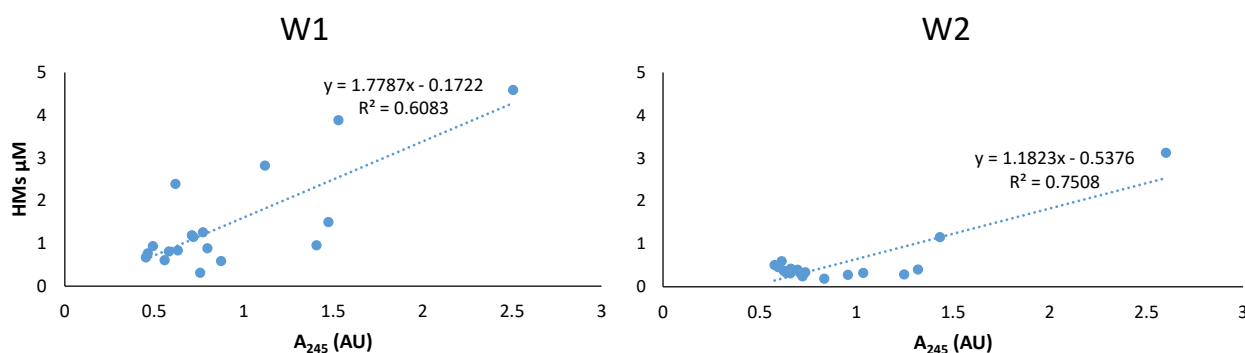


Figure 4.3 Correlation diagrams between HMs and A_{245} in the leachates

Correlation diagrams between total HMs (i.e. Cu, Cd, Ni, Zn) and A_{245} are plotted in Figure 4.3 for both experiments. A better correlation was achieved for W2 tests respect to W1 tests. The slope coefficient instead was higher in W1 tests. This latter result indicated that the interaction between the organic matter and the metals was more effective in W1 experimental condition, meaning that other factors played significant role in determining overall metal mobilization by DOM.

4.3.3 Spectroscopic characterization of released soil colloids

A qualitative characterization of the organic substances was achieved by the excitation emission matrices of the leachates, presented in Figure 4.4. Figure 4.4 reports the contour plots related to leaching time from day 7.5 to day 9.5, corresponding to the previously described peaks of HMs.

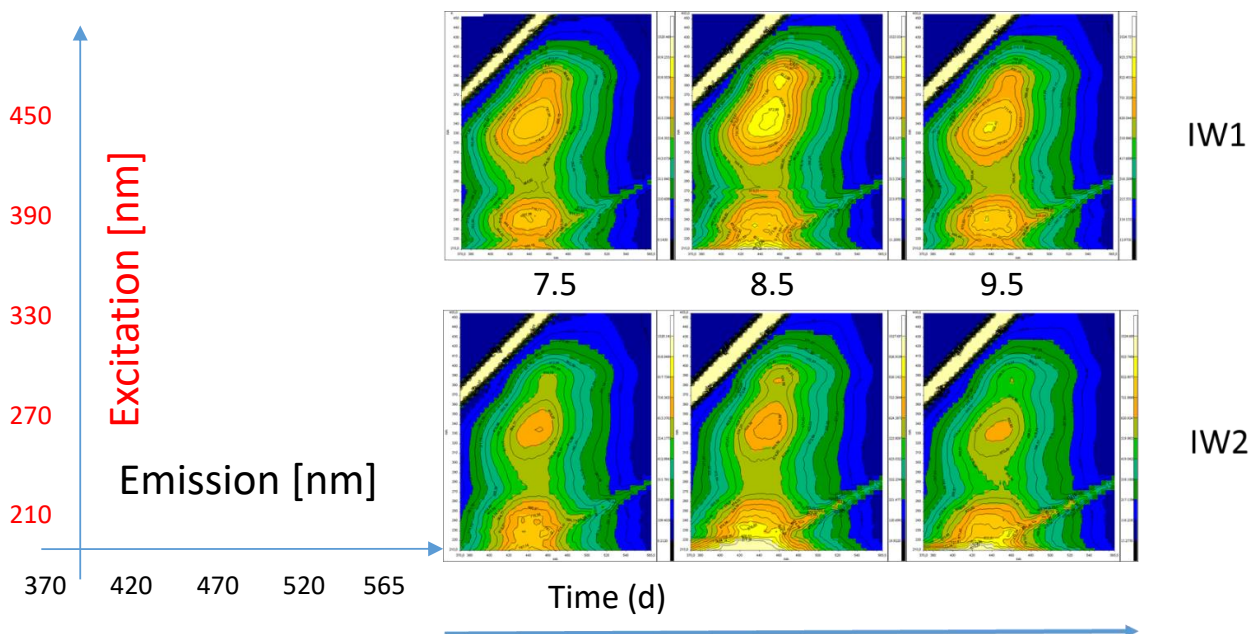


Figure 4.4 Selection of 3DEE matrices recorded in W1 and W2 leachates at time between 7-10 d

In most of the collected samples, the leachate presented a fluorescence matrix very similar to the one shown for the day 7.5. It was possible to observe two notable fluorescence peaks, corresponding, respectively to fulvic acids (ex230 - em440) and humic acids (ex330 - em445) (Yamashita & Jaffé, 2008). Such a result confirmed that a continuous and constant release of organic substances from the soil to the liquid phase (background) took place during the experiments. A fluorescence peak (ex385 - em460) appeared again at day 8.5 in both W1 and W2 tests. This peak identified a net change in the quality and quantity of DOM released into the leachate. The variation led to an increase of the absorption wavelength and consequently a slight increase of the emission wavelength compared to the previously mentioned background signal. The displacement was attributable to the presence of higher conjugation in the aromatic system as well as to the presence of auxochromes functional groups. The increase in ex-em wavelengths was also associated with higher molecular weight distribution and “humic” character of the fluorophores (Boguta et al., 2016; Boguta & Sokołowska, 2016).

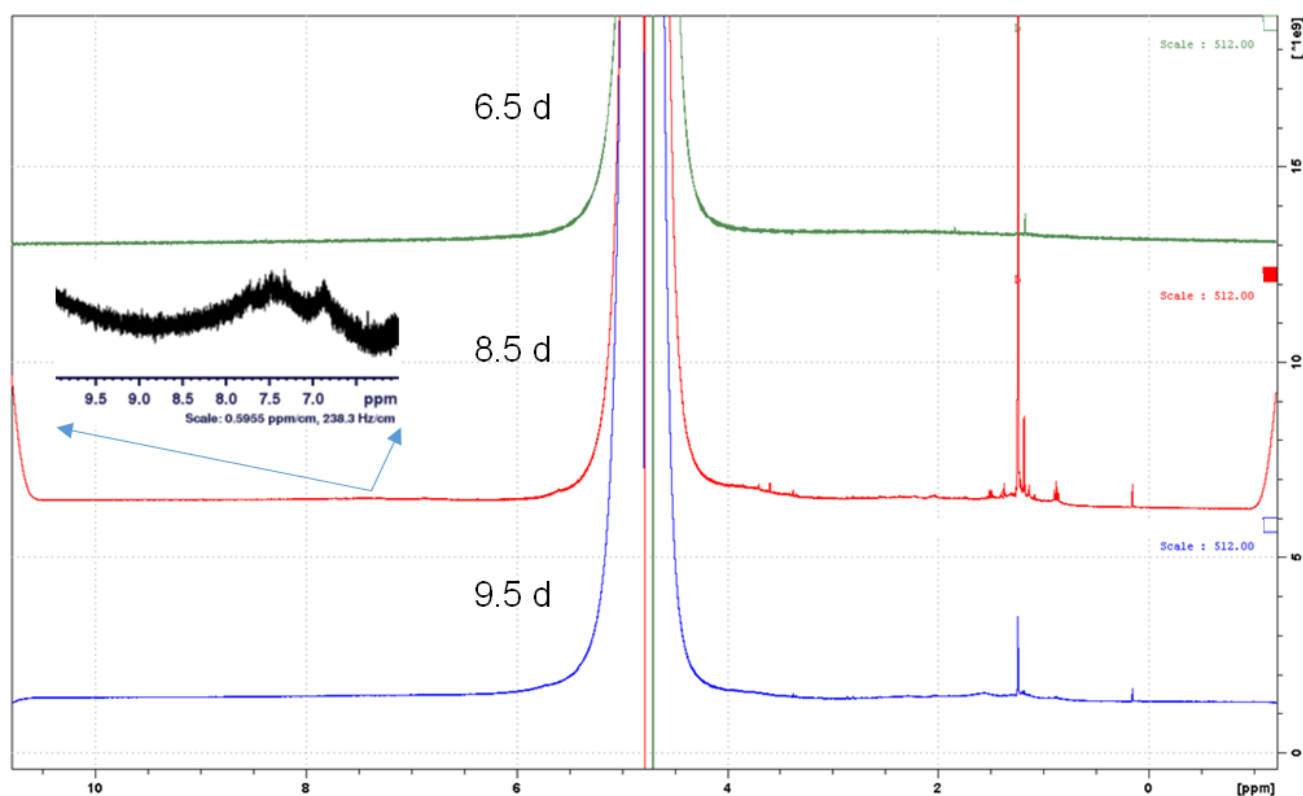


Figure 4.5 400MHz ^1H NMR spectra of collected leachates in correspondence of metals accumulation. Spectra were acquired with 20480 scans, probe temperature of 298 K, solvent suppression with pre-saturation field of 50Hz.

Further information was obtained by the ^1H -NMR spectra, plotted in Figure 4.5. It was possible to notice the higher intensity of the signals in the sample relative to day 8.5, in agreement with the peak observed in the fluorescence matrix (Figure 4.4), confirming the peak of organic matter release in the leachate (Figure 4.3). The spectrum recorded on day 8.5 displayed high intensity signals in the aliphatic region ($1 < \delta < 1.8$ ppm). A singlet signal was noticeable in the region of organo-silanes ($\delta = 0.150$ ppm). Several signals were observable (8.5d) within the carbohydrates region ($3 < \delta < 6$ ppm). Two signals of anomeric carbons were barely visible ($5.5 < \delta < 6$ ppm) suggesting the presence of polysaccharides. These signals absent or much less intense in the other days samples confirmed that the quality of the organic matter release was changing over time. It is noticeable that the presence of carbohydrates was recorded in significant concentration at experimental time corresponding to metal accumulation. At first glance no peak was present in the aromatic region ($6 < \delta < 8$ ppm) since the spectrum was not opportunely resolved (see magnification in Figure 4.5). Signals in such chemical shift region resulted in broadened line shapes and of very low intensities.

4.4 Discussion

4.4.1 Colloidal mobilization of HMs

By comparing Figures 4.1-4.5, it was evident that colloids containing organic molecules of humic nature and/or their association with inorganic kaolinite platelets were involved in the transport of metals. These aggregates, generated in the soil, migrated through the column layers with a different moving rate compared to the water flow. Colloidal fronts were generated through the soil depth profile as indicated by the migration profiles of Pb and total phenols in soil and by the simultaneous release in the leachate of organic and inorganic matter, forming stable complexes with the metals (Figures 4.1-4.2). The similar distribution of the two parameters inside the soil strongly suggested the formation of colloidal MOAs moving at a rate influenced by: i) the wastewater different salinity; ii) the presence of DOM; and iii) their own characteristics (i.e. amount of organic matter, humification degree, hydrophobicity, amount of polar / charged groups, molecular weight distribution, stability of the supramolecular structure). In subsection 2.3.4 it was reported a decreasing colloidal mobilization speed at increasing sodium content. Here, although W1 had a consistent concentration of Na (350 mg/L) while W2 had none, the colloids migration rate was higher for W1 than W2. The effect of sodium ions was therefore different from that of general “salinity”. Ca and Mg (111 and 134 mg/L in W1 respectively), having lower ionic radius, divalent charge and hence more concentrated charge distribution than sodium, did not increase the clay dispersion. In contrast they facilitated the aggregation of clay particles, keeping soil flocculated and competing with sodium for binding sites (Duan et al., 2010; Kretzschmar & Sticher, 1997; Warrence et al., 2002). This effect overrode the sodicity influence, resulting in higher colloidal mobilization in soil irrigated with W1 as ascertained by the Si trend reported in Figure 4.2b

Also the presence (W1 test)/absence (W2 test) of DOM caused different mobility. During W2 tests, zinc concentration peaks, characteristics of W1 tests, were not observed (Figures 4.1 and 4.2). Two different explanations were proposed: i) Zinc from the real wastewater was already present as bound to different transport molecules (DOM) upstream to the interaction with the organic substance of the soil; ii) Zinc had high affinity to immobile matrix as confirmed by the high concentration in the first 0.5 cm (Figure 4.1). The lower colloids release allowed by W2 irrigation made the mobilizable fraction of Zn to reach only 3 cm depth at the end of the experiment. The same Zn fraction was mobilized earlier within the leachate (Figure 4.2a) during the irrigation with W1, which was characterized by an enhanced colloidal mobilization. Such a result highlighted how the mobility of

metals was not only dependent on the characteristics of the soil, but also strongly correlated to the speciation of the metals themselves within both the irrigation and the pore water (Moreno-Jiménez et al., 2011).

4.4.2 Role and nature of colloidal aggregates

Under the tested experimental conditions, DOM seemed to act as metal carrier through the layers of soil. This assumption was confirmed by the monitoring of UV absorbance of the leaching solution. The presence of both A_{245} (Figure 4.2c) and COD backgrounds was due to the release of organic molecules, already described in subsections 2.3.2 and 2.3.3. The origin of the observed organic matter was attributed to soil release phenomena. Absorbance and COD backgrounds were present even in the W2 solution that was devoid of any source of DOM (Table 4.1). The presence in W2 of peaks of Si simultaneously to A_{245} (Figure 4.2b-c) reinforced the hypothesis of aggregation of the detected organic matter with kaolinite platelets. Clearly, the presence of DOM is not the sole parameter to influence metal release in the leachate, and coefficients of correlation in Figure 4.3 were quite far from 1. Nevertheless, the trend was clearly observable and it was possible to assess that metals were released together with organic matter into the leachate. Salinity played major role in enhancing the dispersion of data in W1 test, which were characterized by a lower value of R^2 (Figure 4.3). On the other side the higher value of the slope in W1 meant that in presence of salinity, DOM mobilization of metals was “more effective”. Obviously A_{245} is an indirect measure of DOM and does not give information about the quality of organic matter and this is another reason for the observed data dispersion. As discussed below, speciation of DOM, and in detail its structure, aggregation state, humification degree, molecular weight distribution, presence of metal chelating functional groups are all factors that influence the interaction with HMs (Boguta & Sokołowska, 2016; Chen et al., 2015b). From the observation of 3DEEM (Figure 4.4) it was clear that at the experimental time corresponding to the metals accumulation, the features of the organic substance in the leachate were significantly different from the background. This means that the quality of the released humic and fulvic substances were changing over time. Several studies have evidenced quenching of fluorescence related to metals and mainly Cu binding to HA-like fluorophores (Boguta et al., 2016; Wu et al., 2004). In the collected leachates, Cu concentration was never above 1 μM and the quenching due to Cu could not be higher than 10% of the total fluorescence intensity (Yamashita & Jaffé, 2008). The lower fluorescence intensity in W2 leachate was hence attributable to the mobilization of total phenols, clearly indicated in Figure 4.1: less humic matter mobilized in W2 test. $^1\text{H-NMR}$ spectra further confirmed the heterogeneity over time of the released organic matter. The hypothesis made about the formation of

MOAs was emphasized by the silanic signal (Figure 4.5, 8.5 d) at high fields, suggesting also the formation of covalently bound aggregates. The presence of aliphatic signals was in accordance with the fluorescence observed at low excitation wavelengths (fulvic acids area). Although very high fluorescence is observable in the humic area (Figure 4.4), aromatic signals displayed very low intensity and poor resolution in all the acquired NMR spectra. This was attributable to the supramolecular structure of the colloidal humic substances detected. The aggregate was hence stabilized by the aromatic moieties, which strongly interacted forming a very stable hydrophobic core of the colloidal micelle. The polar residues (i.e. carbohydrates, O-Alkyl groups, amines) were instead exposed to the water phase and resulted to be easier NMR detectable. This type of structure made, indeed, the protons contained within the micelles to have different relaxation times, which resulted in broad unresolved peaks. Such behavior was already reported in literature (Lam & Simpson, 2008), suggesting that the broadening of line shapes might be due mainly two factors: aggregation state of the molecules and interaction with paramagnetic metals or a combination of both these effects. Both hypotheses are in good agreement with data obtained in this work. This indicates that the released organic matter was in form of colloidal supramolecular aggregates, with a very stable micellar structure and very high heterogeneity.

4.4.3 Results comparison with literature

The main observation of this study is that trace HMs mobility in soil was driven by colloidal mobilization. Such behavior was already qualitatively described in literature (McCarthy & Zachara, 1989), and several studies reported that metal mobility was severely affected by colloidal mobilization in the case of HMs at μ moles level (de Jonge et al., 2004; Klitzke & Lang, 2007; Kretzschmar & Sticher, 1997). Generally it is very difficult to compare data, since experiments were carried out at different concentrations and soil colloids are in general poorly characterized and potentially very heterogeneous. For instance, Pédrot et al. (2008) divided trace metals into three groups according to their interaction with colloids. Cu, Ni, and Zn were catalogued within the same group of metals influenced by organic rich colloids. From Figure 4.1 it was possible to conclude that Cu, Ni and Zn behaved differently from each other. Cu showed a strong affinity with the slow colloidal phases that were moving within the soil, since its profile in Figure 4.1 was very similar to that displayed by Pb and phenols. No accumulation of Cu was detected in the top layer. Ni and Zn, conversely, accumulated in the top layer (depth 0.5 cm). On one hand, all metals interacted with the slow colloidal front at depth below 3 cm. On the other hand, in the W1 test they eluted together (Figure 4.2) at day 8.5 into the leachate. After 10.5 days, only Ni and Zn eluted, and after 15 day,

only Cu. As mentioned before, no Zn peak was detected in W2 and again, only Cu eluted after 15 days. Even in the simplified model of the OECD artificial soil, different kinds of colloids were generated in different boundary conditions (Figures 4.5 and 4.6), each with its own moving rate and affinity to specific metals. This did not exclude in general the interaction with organic rich colloids as reported by the cited literature, but highlighted the huge heterogeneity of mobility patterns that are possible for metal-colloid complexes. Several studies are available in the literature concerning interaction of metals with organic and inorganic matter (Boguta et al., 2016; Covelo et al., 2007; Flogeac et al., 2004; Grybos et al., 2007; Temminghoff et al., 1997). Although the majority of them deal with case studies on real scale or with higher concentration ranges, some comparison is still possible with the obtained results. It was reported that low molecular weight organic acids in soils were released after the partial degradation of soil organic matter (Sposito, 2008). Simple organic acids, dissolved or aggregated in humic supramolecular structures (Kelleher & Simpson, 2006; Piccolo, 2002), enhanced metal mobility in soil profiles by reducing soil pH and forming complexes with heavy metals (Renella et al., 2004). The investigation by thin layer chromatography (TLC) of Zn, Cd and Pb mobility in a sandy loam soil in presence of aqueous solutions of several organic acids, enhanced the transport of heavy metals, especially Zn and Cd. Although Pb was slightly mobilized by citric and tartaric acid, it was found that this metal formed stable complexes with organic acids which strongly interacted with inorganic soil matrix resulting in little mobility (Schwab et al., 2008). It was observed that Cd mobility decreased with increasing of soil organic matter, while it increased with the increasing of salinity and dissolved organic matter (Gerritse, 1996) in accordance to the results of the present study. Cd, Pb and Zn were completely immobilized by the soil matrix, mostly to the acid-soluble fraction, in the first few centimetres of the soil, while in the leachates Cd behaved differently from other metals, due to the marked difference in adsorption kinetics (Plassard et al., 2000). According to another study, depth penetration of Cd into the soil profile took place due to the preferential paths through macropores. It was reported that colloids might have blocked the soil matrix capillary, which led to the final accumulation of Cd in the first 5 mm. In contrast, the application of DOM increased the diffusion of Cd, as observed in this study (Li & Zhou, 2010). Metals mobility was hence affected by very complex equilibria in which the interaction with colloids and nanoparticles played a prominent role in determining their fate in the soil. Further studies are of course needed to achieve a deeper knowledge of the humic supramolecular structures and their aggregation with inorganic colloids as well as the interaction that this complex transport media display with contaminants, even at trace concentrations.

4.5 Conclusions

Trace HMs mobilization in soil is controlled by colloidal mobilization. Colloids, that might be organic, inorganic or MOAs, are released from the soil matrix during time and act as a metal carrier, moving through the soil column with lower velocity than the water flow (multiphase medium), selectively interacting with contaminants contained in the irrigation water. The result of this interaction is the generation of contamination fronts that move in the soil matrix at the pace of the colloidal phase and generate accumulation peaks of HMs in the leachate and in the soil layers. A direct correlation was found between the abundance of HMs in the leachate and the contemporary release of DOM, expressed as UV A245. The organic molecules responsible for the mobilization, detected by UV-VIS measurements, were shown to belong to the class of humic and fulvic acids, as indicated by 3DEEM spectrofluorometric data. A very stable and strongly hydrophilic micellar structure was hypothesized for these molecules on the basis of ¹H-NMR data. Colloidal aggregates seemed to be generated from the soil organic matter, since they were also detected in the leachate produced by irrigation with an artificial solution deprived of any dissolved organic matter. It was concluded that the transfer of pollutants in the complex water / soil system was not only related to the concentration of contaminants in the irrigation water, but also to the physical-chemical properties of the soil as well as the generated colloidal aggregates.

4.6 References

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Chapter 5.

Effect of sodium concentration on colloidal mobilization of trace heavy metals in standard OECD soil.

This chapter is to be submitted as:

“Pontoni, L., van Hullebusch, E.D., Fabbicino, M., Esposito, G., Pirozzi, F. 2016. Effect of sodium concentration on colloidal mobilization of trace heavy metals in standard OECD soil”

5.1 Introduction

Irrigation with low quality water, often reclaimed from wastewater treatment plants, is practiced all over the world, resulting in constant release of under-threshold levels of contaminants (Angelakis et al., 1999). These contaminants might accumulate over years with consequent environmental and health concerns. Among the wide variety of contaminants usually released in soil by irrigation practices, heavy metals (HMs) play a primary role due to their persistence and their tendency to bioaccumulate. It is therefore extremely important to understand their fate into the soil and their possible migration to the groundwater.

Colloidal mobilization is one of the key phenomena determining the fate of contaminants present at trace level in the water discharged into soils (Chapters 2 and 4). Soil organic matter is indeed able to release colloidal aggregates which sorb efficiently all type of contaminants including HMs. Such a mechanism, which is negligible for pollutants present at high concentration, becomes instead crucial for pollutants at trace level, i.e. whenever the colloids concentration generated into the soil is comparable or higher than the concentration of the pollutants (Kretzschmar et al., 1999; Pédrot et al., 2008). Since colloidal phases move into the soil column at a pace which is different from those of the water flow, the migration pattern of trace pollutants is governed by colloids mobility properties through the porous medium.

The sodium concentration in the irrigation water is reported to be one of the crucial factor affecting the mobilization of inorganic colloids (Toze, 2006). Sodium and other forms of salinity (i.e. Ca, Mg, K) are the most persistent in recycled water and are among the most difficult to remove from water, usually requiring the use of expensive cation exchange resins or reverse osmosis membranes. The salinity of recycled water has well-known impact on the infiltration rate with the extent of crust formation on the soil itself. This is due to chemical dispersion which depends on the soil exchangeable sodium percentage and the electrolyte concentration of the applied water (Agassi et al., 1981). Such impact influences as well the growth of the crops (Halliwell et al., 2001). More in detail, sodium cations interact with the negatively charged layers of clay particles. As sodium concentrations increase, the electrophoretic mobility of the clay anionic layers (platelets) increases resulting in swelling dispersion of the clay particles impacting soil permeability (McNeal et al., 1966) with subsequent surface ponding problems (Toze, 2006). This effect is of primary importance being one of the factors that regulates the mobility of water through soil and thus the sorption/desorption equilibria of contaminants moving through the soil layers, increasing the contact time with the solid matrix and consequently with plants roots, thus influencing the processes of contamination and

accumulation. The described phenomenon also implicates a lower mobilization of colloidal clays which at trace levels could mean lower HMs mobilization. Some authors reported threshold value of 20 mM sodium concentration able to inhibit colloidal mobilization (Plassard et al., 2000). How this effectively influences the fate of trace HMs from wastewater irrigation is not described in the literature.

This work aims to clarify the effect of increasing sodium concentration on the trace Cd, Cu, Ni and Zn mobility and the effect of sodicity on the release of organic matter from soil to the pore water and finally to the leachate.

5.2 Material and methods

5.2.1 Experimental setup

A micro-contamination phenomenon was reproduced and studied at laboratory scale, simulating the irrigation of an artificial soil with a synthetic water containing four trace HMs (Cd, Cu, Ni and Zn) in the concentrations reported in Table 5.1 at various sodicity levels (0, 1, 5, 10 and 50 mM).

To evaluate the dynamics of accumulation of micropollutants as well as their migration in the soil column at varying sodium content, HMs concentration was determined daily in the leachate and, at the end of the experiment in the soil column matrices. The mobilization of humic substances in soil was monitored by determining the concentration of total phenols. The release of organic substances in the leachates was measured as chemical oxygen demand (COD) and Ultra-Violet (UV) absorbance. Leached organic matter was further characterized through three dimensional excitation-emission matrix (3DEEM) spectrofluorimetry.

The used standard soil was prepared according to the "recipe" described in the guidelines of the Organization for Economic Cooperation and Development (OECD) (Wilhelm & Maibach, 2008). These guidelines were developed in order to test the eco-toxicity of inorganic and organic chemicals in soil, therefore the OECD soil is widely used to test complex solid samples (Joško & Oleszczuk, 2013). The reference soil was composed as follows: kaolinite, 20%; quartz sand, 74%; CaCO₃, 1%; sphagnum peat, 5%. Kaolinite, quartz sand and CaCO₃ were purchased from Sigma-Aldrich (USA). Sphagnum peat was obtained from a local gardening store. Before use the soil was analyzed to define

the background levels of the tested metals. Results of analysis are reported in Table 5.1. A very high concentration of Pb was found in the kaolinite.

HMs concentrations were chosen to be well below the threshold values suggested by the Food and Agriculture Organization of the United Nations (FAO) in the wastewater quality guidelines for agricultural use (Pescod, 1992). In details the different wastewater solutions were obtained dissolving analytical grade $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (Carlo Erba Reagenti, Italy), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (Sigma-Aldrich, USA), and ZnCl_2 (Applichem, Germany) and analytical standard Cd (Carlo Erba Reagenti, Italy), in analytical grade HNO_3 1% (J.T. Baker, USA) solutions. The obtained stock solutions, containing 1 g/L of the selected metal, were successively diluted with ultrapure water (Elga, USA) to obtain the required concentrations. The opportune amount of analytical grade NaClO_4 (Sigma-Aldrich, USA) was added to reach the desired sodicity. ClO_4^- was chosen to minimize counterion effects. Final pH was adjusted to 6.8, adding a few drops of KOH 0.05 M.

Table 5.1 Concentration of HMs in OECD soil and in the synthetic wastewater

	Cd [ppb]	Cu [ppb]	Ni [ppb]	Pb [ppb]	Zn [ppb]
<i>Kaolinite</i>	14.2	62565	748	4031350	10879
<i>Sphagnum Peat</i>	96.7	2407	1800	9499	19308
<i>Quartz Sand</i>	5.0	1910	787	4964	8683
<i>Wastewater</i>	5.00	100	100	/	500
<i>FAO guidelines</i>	10.0	200	200	/	2000

5.2.2 Analytical procedures

Each soil layer and the “blank” soil components were dried at 35°C until constant weight and homogenized. Aliquots of 1 g were mineralized in 15 mL of aqua regia using a Milestone (USA) Start D microwave digester. Leachates were collected daily and divided into two aliquots. The first aliquot

was acidified with 3% HNO₃ for metals analysis. The concentrations of Cd, Cu, Ni, Pb, Si and Zn, were measured by ICP-OES spectroscopy (Perkin Elmer Optima 8300, USA) and ICP-MS (Perkin Elmer Nexion 300, USA) operating in dual detector mode. The second aliquot was stored at -20°C for spectroscopic characterization. Once defrost, the UV-VIS absorbance at 245, 285, 445, 645 nm and the COD were determined. The ratio between absorbance read at 445 and 645 nm (E₄/E₆ ratio) was calculated according to Chen et al. (1977). Moreover 3DEEM of the daily leachates was recorded. The UV-VIS measurements were carried out using a V-530 UV-VIS spectrophotometer from Jasco (Japan). COD was determined according to APHA (1998) standard method 5220D. COD absorbance readings were performed by means of a Photolab 6600 UV-VIS spectrophotometer (WTW, Germany). The 3DEEM matrices were acquired through the spectrofluorimeter Jasco FP 750 (Japan) and elaborated through the software SPEKWIN 32. Excitation wavelength was varied between 220-450 nm; emission wavelength was recorded between 370-585 nm.

5.3 Results and discussion

5.3.1 HMs accumulation in soil – effect of sodium content

From the data reported in Figure 5.1 it is possible to confirm what was observed in Chapter 2. Most of the metals contained into the solution fed to the columns were retained within the 0.5 cm soil top layer. Very low oscillations in the deeper layers were observed for all the tested metals concentrations.

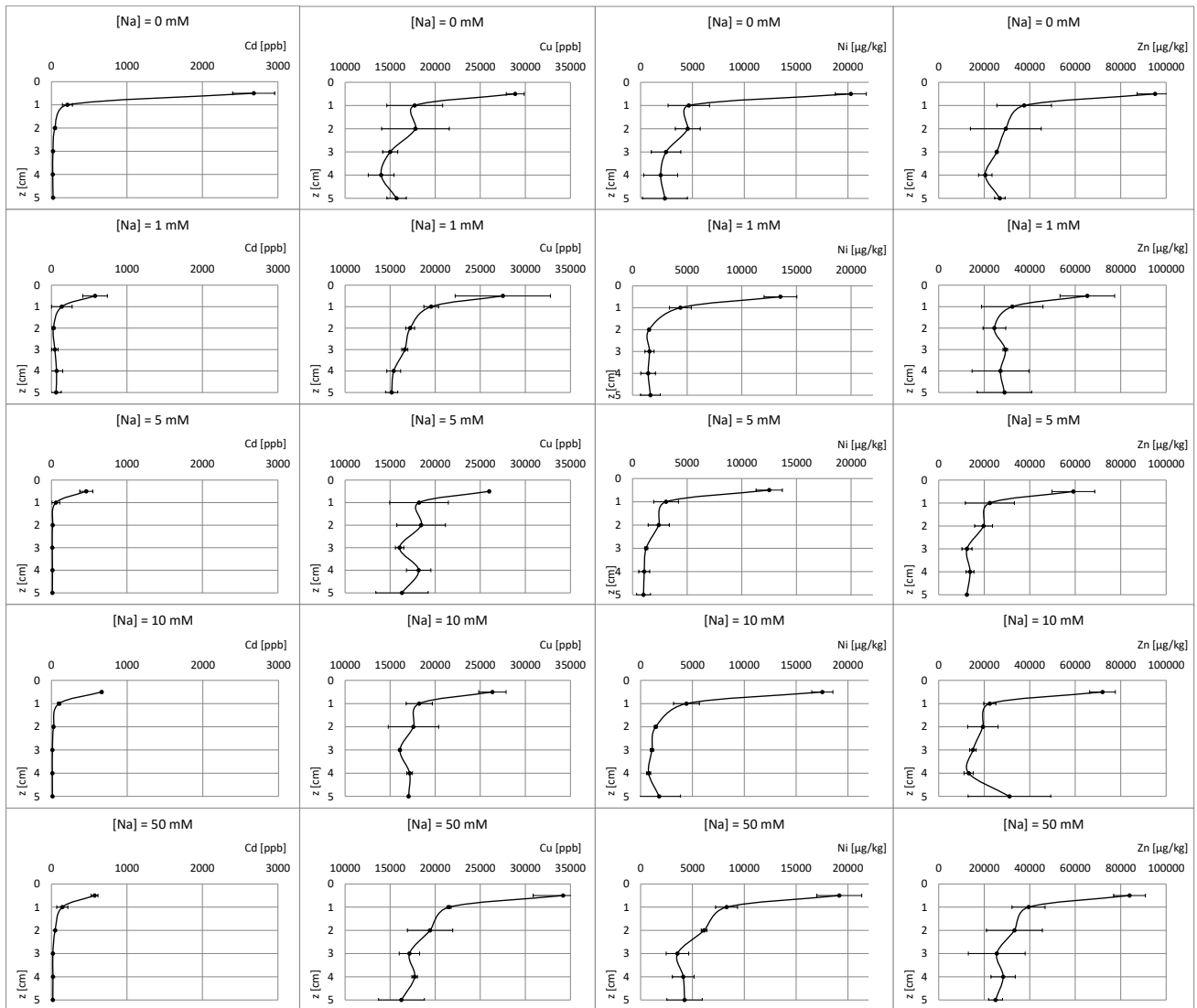


Figure 5.1 Accumulation of Cd, Cu, Ni and Zn in OECD soil after irrigation with artificial wastewater. Sodium concentration was increased from 0 to 50 mM.

The observed trends were generally very similar among the different conditions tested, with the higher metal concentration in the top layer and concentrations decreasing with depth. Nevertheless the change of Na concentration in the water fed to the column highlighted different behavior of the tested metals. In first analysis it was clearly evidenced a different effect of sodicity on the accumulations of Cd respect to Cu, Ni and Zn. The highest cadmium concentration in the top layer was achieved in the experiment at 0 mM [Na]. The increase of sodicity at 1 mM concentration caused a lower accumulation at the top of the column. No significant differences were observed while increasing the Na concentration up to 50 mM. This means that the presence of sodium increased the mobility of Cd, but without registering any effect related to its concentration. This behavior is consistent with results available from literature. Amrhein et al. (1992) attributed the increase of Cd

mobilization to the concomitance of two factors: competitive cation exchange with sodium as well as complexation by Cl^- and acetate. Ghallab and Usman (2007) reported an increase of cadmium mobility and plant availability at increasing Na concentration. Authors attributed higher Cd mobility to the counterion effect of Cl^- . Speciation modeling in the case gave back the formation of CdCl^+ specie to be dominant at increasing concentration and, indeed, they found linear increase of Cd dissolved species as a function of NaCl concentration. The results displayed on Figure 5.1 are instead probably ascribed to effects of competition of Na for some of the binding sites on the immobile soil matrix. Harter and Naidu (2001) reviewed the effect of Na on Cd sorption to soil, hypothesizing that, since even at very high sodium levels Cd sorption was always recorded, Cd has onto soil specific binding sites, which are not affected by sodium exchange. This was consistent with the obtained results and explains why at increasing sodicity the sorption of Cd on the top layer remained substantially constant. At 1 mM, sodium concentration was indeed much higher than the trace Cd and sodicity was already in excess to compete with cadmium ions for sodium exchangeable binding sites. In contrast, Cu, Ni and Zn displayed different behavior against sodicity. Some competition was still observable in Figure 5.1 at sodicities equal to 1 and 5 mM. The concentrations in topsoil are in these cases lower than the experiment carried without Na. Differently to what observed for Cd, the concentrations in the top layer were increasing at increasing sodicity. To better highlight such behavior, the concentrations in the top layer (0.5 cm depth) are plotted in Figure 5.2 as a function of the wastewater sodicity.

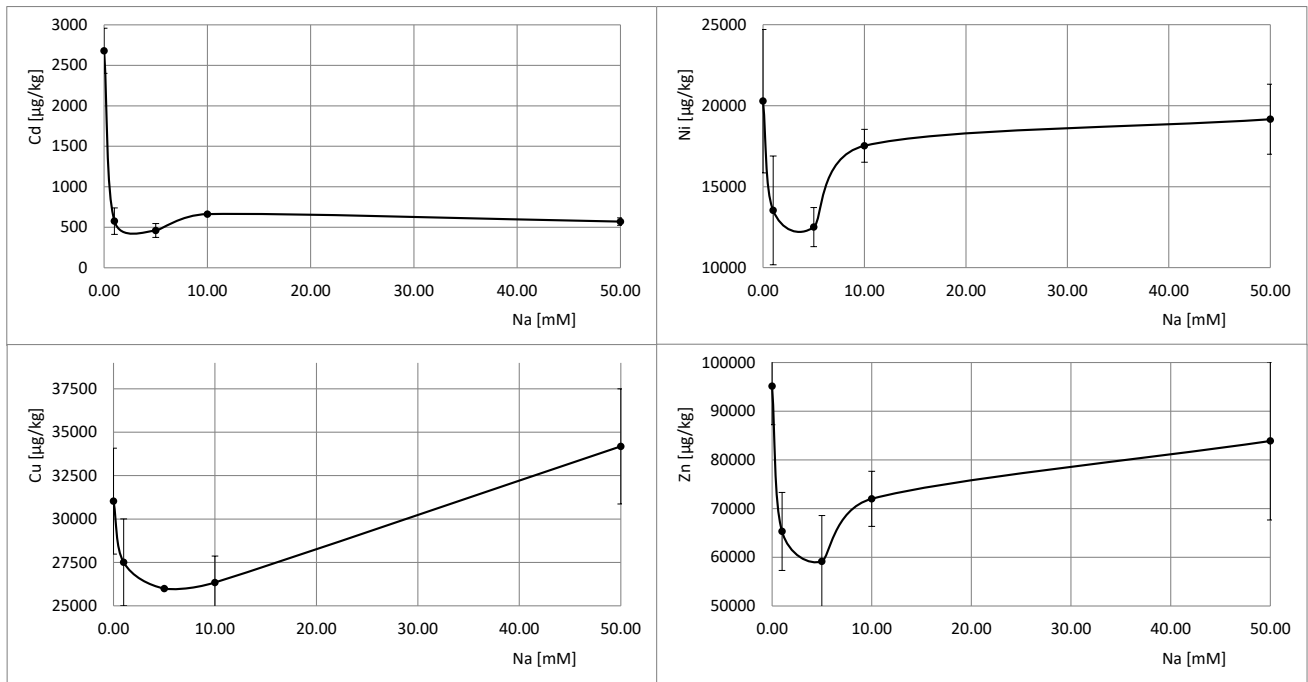


Figure 5.2 Accumulation of Cd, Cu, Ni and Zn at 0.5 depth at increasing wastewater sodicity.

The trend of Cd highlights what was previously discussed: no effect of increasing sodicity was observed. At sodicity higher than 1 mM, Cd values were practically distributed on a straight line. The accumulation of Cu, Ni and Zn was decreasing when sodium ranged from 0 to 5 mM, and increasing when Na concentration range was between 5 and 50 mM. This means that sodium displayed opposite effect in influencing metal mobilization depending on its concentration in the wastewater solution. At low Na concentration, Cu, Ni and Zn were less accumulated on the top layer where the concentrations decreased from 31.0, 20.3 and 95.1 mg/Kg at 0 mM (Na) to 25.1, 12.5 and 59.2 mg/Kg at 5mM (Na) of Cu, Ni and Zn, respectively. Such decrease could be ascribed to competition with sodium ions as described in the case of Cd. Such explanation, although convincing in the case of Cd, could not justify the observed increase of accumulation at sodicity higher than 5 mM. The main known effect of sodium concentration is in fact to swell the dispersion of clay platelets (McNeal et al., 1966). This means that increasing Na concentration changes the hydraulic conductivity of the medium. Dispersed clay platelets clog the pores of the solid medium and slower the water flow through soil. During the experiments was indeed qualitatively observed that the increase of sodicity from 5 to 10 and 50 mM caused a decrease in the leachate flow and hence of the soil desaturation time. This reflects in two main consequences: i) being the water flow slower contaminants have longer time to interact with soil matrix enhancing the adsorption and or the ionic exchange; ii) pores clogging slows the mobilization of colloids through the soil layers. This explains why at sodicity higher than 5 mM metal retention in the top layer of the soil was enhanced. It is noticeable that the effect of sodium concentration increase is to enhance the generation of inorganic colloids which are,

due to their high concentration, less mobile. At intermediate sodicity (1-5 mM) colloids dispersion was still enhanced, but their concentration into the pore water was reasonably insufficient to generate clogging. As a consequence top layer accumulation substantially decreased (Figure 5.2).

5.3.2 Leachates characterization

Figure 5.3 displays the concentration trends of HMs released from the columns into the collected leachates. In first approximation, it was easy to observe that the concentrations of metals were generally low in accordance with observation that most of the metals added were retained in the soil top layer (Figure 5.1). Nevertheless several oscillations were visible and it was possible to relate metal behavior to the results described in previous subsection. The obtained trends were appreciably different from each other, meaning that sodium concentration strongly affected the mobilization rate of the metals. Concerning Cd, it was possible to notice that except for the leachate at 0 mM [Na] where the majority of the metals was retained into the top 0.5 cm, the trends are quite disturbed by the presence of several concentration peaks. It was possible to observe that the release of Cd was in some way delayed by the sodicity increase since highest peaks of Cd were detected in the last five days of the experiments carried on at 10 and 50 mM [Na]. Cu release was more consistent in terms of average concentration and this was mainly due the higher background in the soil. For the same reason, the concentration trends were very similar since most of Cu detected into the leachate was probably originating from the soil background. Nevertheless several differences were observable. A peak was observable only with 1 mM [Na] at day 11. With 5, 10 and 50 mM [Na] peaks were detected at day 9, 14 and 19, respectively. Conversely, only a small peak at day 14 was observed with 1 mM [Na].

Ni trends into the leachates were less affected by releases from the soil background, Ni background in soil was indeed around one order of magnitude lower than Cu (Table 5.1). Hence, several differences were observable at increasing of sodicity. Figure 5.3 shows that trends at 0, 10 and 50 mM [Na] were less disturbed respect to 1 and 5 mM [Na]. In these latter experiments a high peak was recorded day 14 while peaks at day 9 and 14 were detected only at 5 mM [Na]. A similar behavior was displayed also by Zn. At low sodium concentration peaks of Zn popped up between 8 and 10 d while at 10 and 50 mM [Na] the release resulted to be almost constant. The described results were consistent with the results reported in Figure 5.2. Higher peaks (especially of Ni and Zn) were indeed observed at 1 and 5 mM [Na] where the accumulation into the soil top layer was lower than

0, 10 and 50mM [Na]. This means that higher mobilization of colloids was allowed in such conditions. This observation was further confirmed by the spectroscopic characterization of the leachates.

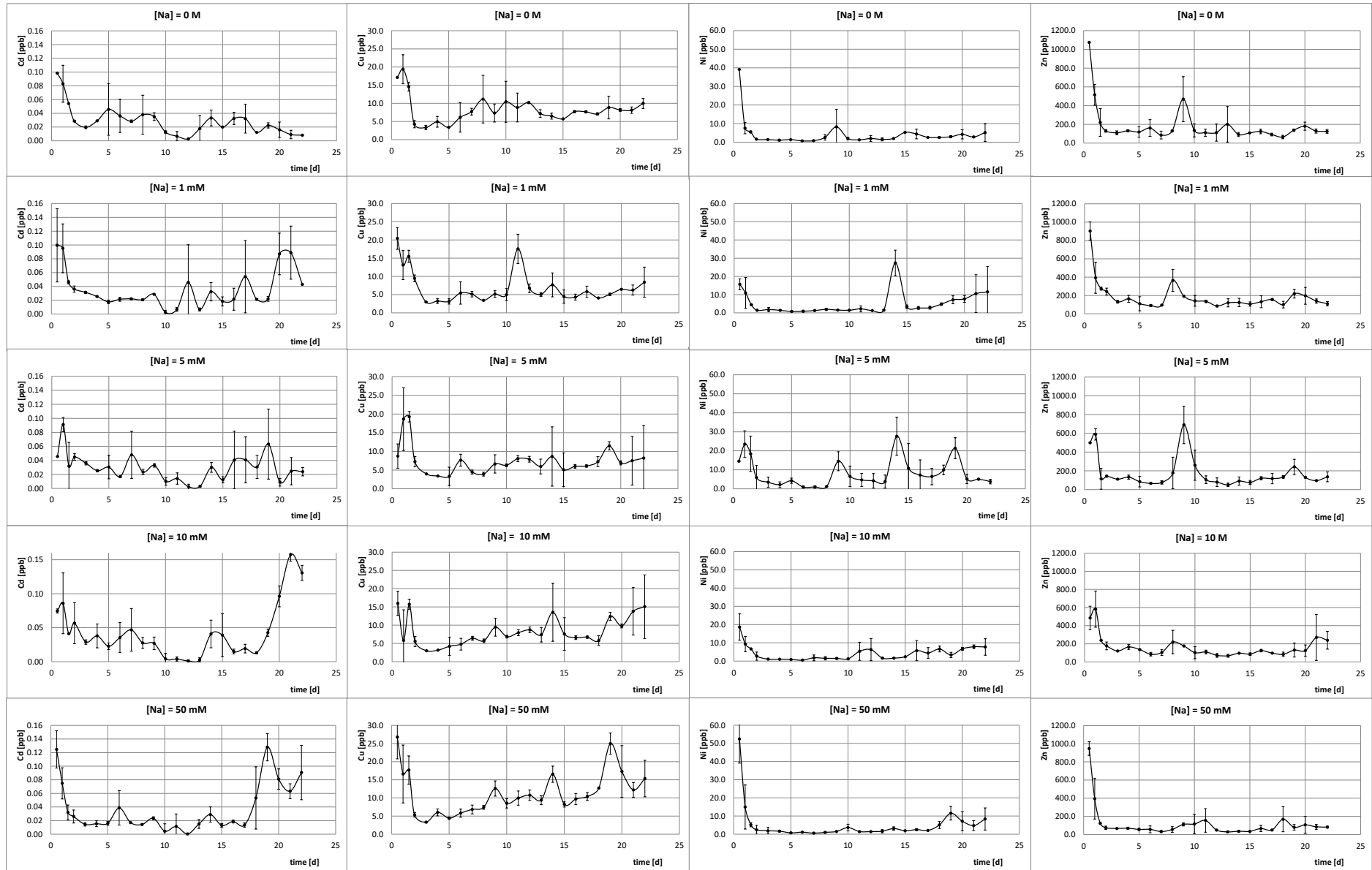


Figure 5.3 Trends of Cd, Cu, Ni and Zn concentration into the leachates.

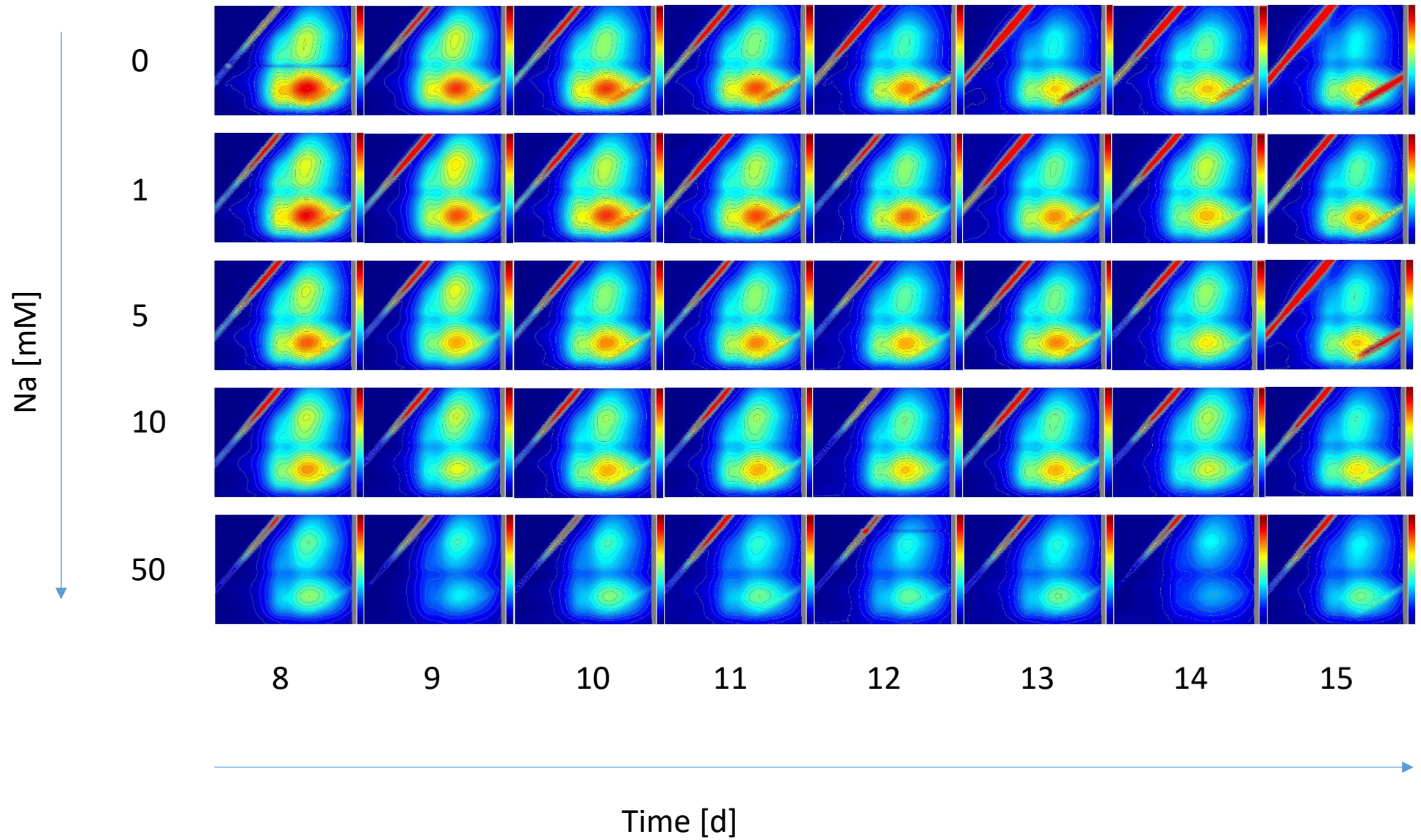


Figure 5.4 3DEEM of the leachates collected between day 8 and 15.

Figure 5.4 displays the 3DEEM of the leachates recorded in the time interval between 8 and 15 d. In most of the collected samples, the leachate presented a very similar fluorescence matrix. It was possible to observe two notable fluorescence peaks, corresponding, respectively to fulvic acids (ex230 - em440) and humic acids (ex330 - em445) (Yamashita & Jaffé, 2008). Such a result confirmed that a continuous and constant release of organic substances from the soil to the liquid phase (background) took place during the experiments. A decrease in fluorescence occurred at high sodium content. This was, at least in part, due a well-known effect of ionic strength in quenching the fluorescence signal. Ghosh and Schnitzer (1980) reported a reduction of 10 % of both humic and fulvic signals fluorescence changing the concentration of NaCl from 1 to 100 mM. The observed signals were at 50 mM [Na] less than 50 % intense compared to the samples at 0 mM [Na]. This implicates that less fluorophores were released at high ionic strength. Several differences were displayed among the matrices acquired at different experimental times, mostly related to the relative intensities of the two described fluorescence areas. This indicates noticeable variations over time of the organic matter quality in terms of humic and fulvic substances released into the leachates (Boguta et al., 2016). Higher variability was observed at 0 mM [Na], whereas the fluorescence strongly decreased at day 13 and 15 mostly in the humic region. Reduction of fluorescence was also observed, primarily of the fulvic signal, at 50 mM [Na] at day 9 and 14 of leaching time. By comparing Figures 5.3 and 5.4, several relations were observable between the metals release and the detected fluorescence. Changes in humic fluorescence areas were observed contemporarily to most of the peaks of metal reported in Figure 5.3. In detail, matrices acquired at day 9 and 14, when several peaks of Ni and Zn were observable from Figure 5.3, showed, respect to previous and later days, marked increase of fluorescence in the humic area. The signal also resulted slightly shifted at higher ex-em wavelengths indicating higher conjugation and humification degree (Yamashita & Jaffé, 2008). Conversely, very low signal was recorded at 50 mM [Na] in the same days and same time no peaks of Ni and Zn were recorded in Figure 5.3. It is worth noting that in the same experiment, peaks of Cu were observed at day 9 and 14. Complexation with Cu could be the reason of the observed fluorescence lower intensity in the fulvic area compared to the other days. The same was observable at 10 mM [Na] where again at 9 and 14 d the fulvic signal was lower in respect to other days.

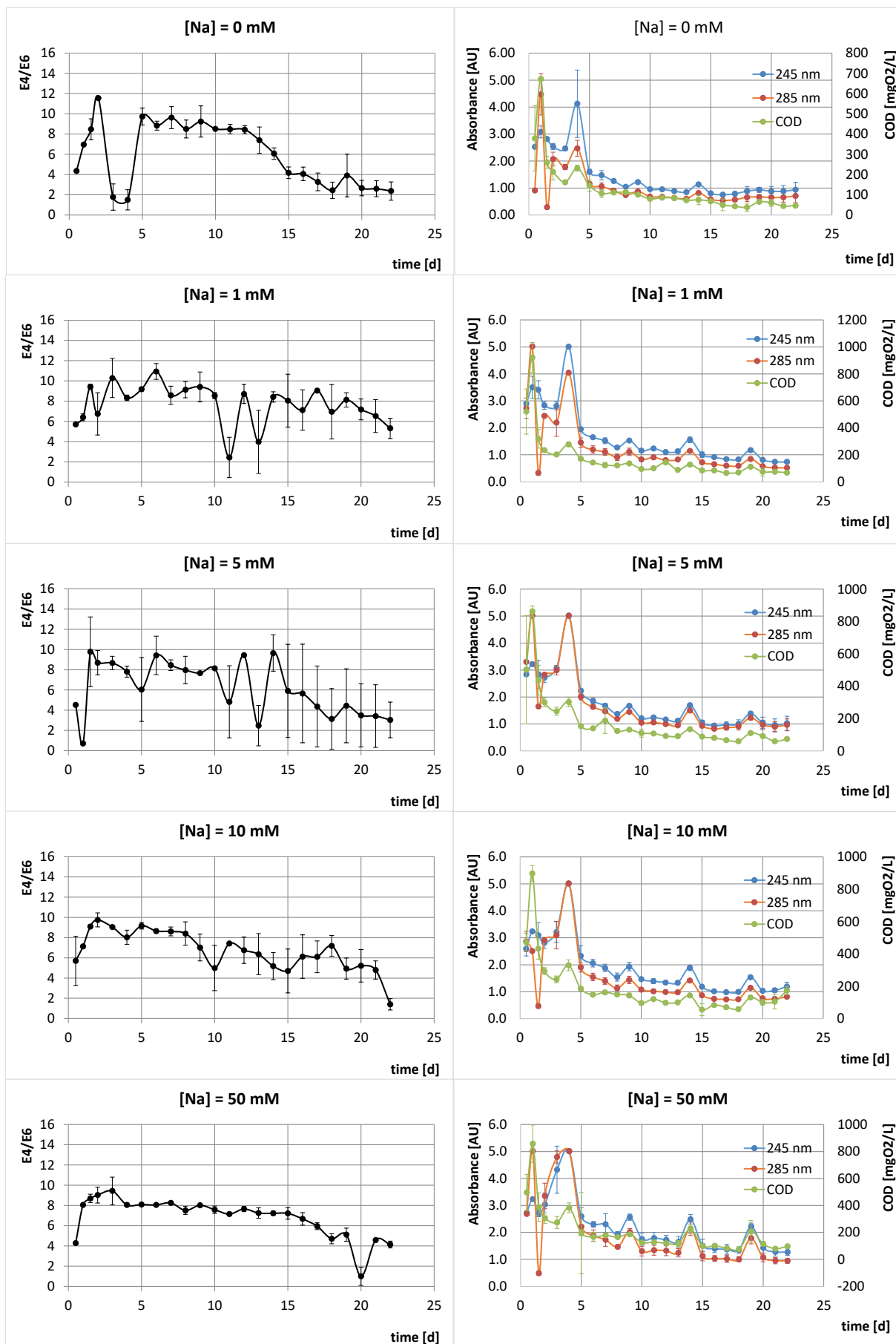


Figure 5.5 Trends over time of E4/E6 ratio, A₂₄₅, A₂₈₅ and COD at varying of sodium concentration.

From the observation of Figure 5.5, despite the described differences in behavior of both metal concentration and fluorescence, trends of measured absorbances (right side of the figure) and COD were very similar. Peaks popped up again at day 9, 14 and 19, and rose at increasing sodicity. The release of organic matter was indeed enhanced by high sodium concentration. The trend of E_4/E_6 ratio was influenced much more by the sodium concentration. E_4/E_6 ratio is in fact related to the degree of humification and in general is increasing at decreasing the humification degree (Chen et al., 1977). The highest oscillations of such ratio were observed at 1 and 5 mM [Na], while the tendencies were more stable in the other experiments. This means that in such sodicity interval, where higher metal mobilization was deducible from Figure 5.2, the quality of the released organic matter was continuously changing over time due the mobilization of colloids moving with different humification degrees and moving rates. At higher sodicity where colloids were slowed down by the described effect of pore clogging, the release of organic matter happened more regularly in quality. A possible explanation of the described effects, is that, being humic substances, supramolecular aggregates of small organic fragments (Piccolo, 2001), the increase of Na concentration operated a denaturation of such aggregates. The increase of ionic strength, in fact reduces the entropic advantage gained through hydrophobic interactions by supramolecular aggregates, making more probable the detachment of fragments and small molecules from the hydrophobic core. This would also explain the increasing release of COD at increasing of Na concentration. Moreover, such denaturation effect would be in good agreement with the observed lower fluorescence intensity displayed in Figure 5.4. The aggregation of fluorophores in the hydrophobic core of the aggregate would in fact reduce the energy transfer with the sodium quencher (Stork et al., 2002). The conclusion is that probably the fluorophores released at 50 mM Na were more quenched because less aggregated.

5.4 Conclusions

- The colloidal mobilization of Cu, Ni and Zn was strongly affected by the concentration of sodium. Cd was less sensitive to Na concentration and no differences were highlighted in its mobility at increasing sodium concentration.
- In all the experiments the majority of metals were retained into the top soil layer (0.5 cm).
- At low concentrations (1-5 mM) sodium enhanced Cu, Ni and Zn mobilization and less metals were accumulated into the soil top layer of the columns.

- The release of metals into the leachate happened contemporarily with variations in the excitation emission matrix.
- At low sodium concentration, higher variations were observed of the E_4/E_6 ratio.
- Sodicity enhanced the release of organic matter into the leachate due to an effect of denaturation/disaggregation of humic substances supramolecular structure.

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Chapter 6.

General discussion, conclusions and future perspective

6.1 General discussion

6.1.1 Main parameters affecting trace metals retention-mobilization in soil

The fate of trace metals is the result of a very complex system of simultaneous physical chemical equilibria and phenomena. The approach chosen in the experimental plan of the present work was to try to isolate each of the parameters to evaluate the single effect of each of the components on the overall fate of trace HMs in soil. This was partially possible thanks to the study of the Organization for the Economic Cooperation and Development (OECD) artificial soil (Wilhelm & Maibach, 2008) and in most of the cases artificial wastewaters which simplified enormously the research questions. Although OECD soil was probably quite far to properly mimic the behaviour of a real soil, it was chosen as “simple model”. The generally low metal background, and the absence of aggregates, inhomogeneity, or other factors influencing the metal fate in soil (i.e.: Fe and Mn hydroxides, presence of soil aggregates, soil disturbance status) allowed studying singularly the effect of each of parameters investigated, excluding other major effects. On the other side, since the OECD soil is widely used to assess the eco-toxicological risk of chemicals, the mobilization of contaminants in such medium has very high relevance in determining their bioavailability and hence the risk assessment itself. The main drawback was that many of the conclusions extrapolated from the experimental data still need to be tested and scaled up to different undisturbed real systems. Obtained data allowed anyway to clarify the main mechanisms and consequently the parameters affecting the overall fate in soil of trace metals from wastewater irrigation. In chapter 2 it was proposed a novel mechanism (Figure 2.4) which considers the presence and the mobilization through the soil matrix of colloidal phases which strongly interact with metals influencing strongly the mobilization kinetics. The same mechanism considered also the disaggregation of the soil organic matter which includes also the organic colloidal phases and constitute a source of small organic molecules which are continuously released in the pore water and after complexation act as carrier enhancing the metals mobilization. In chapter 4 the presence of MOAs among the metals carriers to the leachate resulted in describing the metal migration as a multiphase model, in which colloidal phases are different in composition and aggregation state, influencing the migration rate. Moreover each phase displayed specific affinity with selected metals. Each of these phases is on its own differently affected by the system condition as highlighted in chapter 5. The effect of sodium concentration in the wastewater affected differently the mobilization of each of the phases present in the soil reflected in different migration patterns at each sodium concentration tested. Definitely the main parameters affecting HMs fate in soil and investigated throughout the thesis are summarized hereby:

- the soil composition, in terms of organic and inorganic matter;
- the generation rate of colloidal aggregates, responsible for the transport of part of the metals at a migration rate different from the one of the water flow;
- the different physical-chemical properties of the generated colloids, which determines their selectivity in binding metals;
- the disaggregation yield of the complex aggregates composing soil organic matter, which results in the release of small molecular weight ligands which coordinate HMs accelerating the transport rate;
- the wastewater characteristics, including salinity, sodium content, and presence of organic matter, which influences the mobilization of colloidal aggregates through soil.

6.1.2 Soil composition

The effect of SOM content was mainly studied in chapter 2. The use of the artificial OECD soil allowed to range the SOM content from 2.5% to 10 % and compare the results in terms of metal retention – mobilization. Preliminary batch test with Ni and Cu - conditions of pH and ionic strength (pH= 6.8, $I \leq 0.05$ mM) were the same of the column experiments - confirmed that peat had a higher maximum adsorption capacity (14.6 mg Cu/g and 13.44 mg Ni/g) compared to kaolinite (4.3 mg Cu/g and 6.5 mg Ni/g).

The increase of SOM in the soil, contrarily to what expected, enhanced HMs mobility, since the highest SOM percentage corresponded to the lowest metals accumulation, and the highest concentration in the effluent leachate. The accumulation of contaminants, which happened, mainly, in the top layer of the soil, was in inverse proportion with the SOM content. The highest contamination (Figure 2.5) was reached for the soil prepared with 2.5% of peat (30.4 mg/kg of Ni, 0.33 mg/kg of Cd, and 63.5 mg/kg of Cu) while the lowest was observed at 10% SOM (21.7, 0.14, 39.9 mg/Kg for Ni, Cd, Cu, respectively). A continuous release of TOC was detected in the leachates collected (Figure 2.5). The concentration was proportional to the initial soil organic matter contained into the soil. This meant that organic molecules were continuously detached from the immobile soil matrix and transported into the water phase. Such organic molecules were involved in metal complexation acting as metal carriers through the soil layers. Several studies (Beesley et al., 2010; Flogeac et al., 2004; Gerritse, 1996) report the influence on metal mobility of small organic ligands

and the obtained results were found to be consistent with literature data. The generation of mobilizing ligands was more probable at higher SOM content. A linear correlation was found between the metals mobilization and the organic matter content in the column (Figure 2.6). The effect of organic matter is depending on metals concentration and experiments configuration. At high concentration and in batch mode organic matter increased adsorption capacity (Covelo et al., 2007). At trace levels and in continuous flow mode the effect is exactly opposite due the colloidal mobilization. Such colloidal mobilization is substantially negligible at high concentration and the higher adsorption capacity prevails resulting in higher contamination at higher SOM content (Clemente & Bernal, 2006).

6.1.3 Colloidal mobilization of trace HMs

Colloidal mobilization has been highlighted in chapters 2 and 4 as one of the key phenomena in determining the fate of trace contaminants from water discharged in soil. Soil organic matter is indeed able to release colloidal aggregates constituted of both organic and inorganic matter as described in chapter 4. Such colloidal particles adsorb many contaminants and specifically HMs (Mingorance et al., 2007). Such mechanism could be considered negligible when high concentrations of HMs are present in the irrigation water and the mobilization of metals is governed by sorption-desorption equilibria between the soil immobile and the aqueous mobile phases (McGechan & Lewis, 2002). In contrast, at HMs trace level, the colloids concentration generated in soil is comparable or higher than HMs concentration. In such condition, the adsorption/aggregation of HMs to the colloidal phase is not negligible (Kretzschmar et al., 1999; Pédrot et al., 2008). Since colloidal phases move in soil with different migration rates in respect to the water flow as described in chapter 4, the result is that the migration pattern of HMs is finally governed by colloids mobility properties through the porous medium. The development of the analytical method described in chapter 3 helped to monitor the migration of slow organic colloidal phases directly into the soil as described in chapter 4, while organic colloids released in the leachate were monitored through the use of spectroscopic methods. The novel method, applied to total phenols determination in solid and semi-solid matrices, was more accurate and more precise respect to the traditional one (Table 3.1), allowing to obtain higher recovery of total phenols in peat soil (15.5 % increase) with a decrease of the coefficient of variation (30.1% decrease). Inorganic colloids were monitored by following the concentration trends of Pb (Chapter 2) and Si (Chapter 4). Several correlations were found between the metal concentration and the presence of colloidal phases in both soil and leachate samples. In chapter 2 it was reported that Cu and Ni were preferentially transported by HA-like fragments, while Cd is preferentially mobilized by FA-like. The formation of colloidal MOAs was also hypothesized on the basis of the appearance of

peaks of Pb in concomitance with absorbance measured at 245 nm and of course of metal release. The relationship between Cd transport and FA-like colloids was not found in presence of high sodicity as discussed in chapter 5. The transport of Cu and Ni by Humic like colloids was instead confirmed by the experiments in chapters 4 and 5. A direct correlation was found in chapter 4 (Figure 4.3) between the abundance of HMs in the leachate and the contemporary release of DOM, expressed as UV A_{245} . The same correspondence was observed for the release in the leachate of silicon, directly related to inorganic colloids migration. The organic molecules responsible for the mobilization, detected by UV-VIS measurements, were further confirmed to belong to the class of humic and fulvic acids, as indicated by 3DEEM spectrofluorometric data and its comparison with literature (Boguta et al., 2016; Boguta & Sokołowska, 2016; Chen et al., 2015). A very stable and strongly hydrophilic micellar structure of the detected colloids was hypothesized for these molecules on the basis of ^1H -NMR data.

6.1.4 Influence of wastewater composition on colloidal mobilization

The colloidal mobilization was found to be strongly affected by the characteristics of the irrigation wastewater. In experiment D of Chapter 2 conducted by irrigating soil with artificial wastewater containing 20 mM sodium, it was observed a decrease of metal mobility and inhibition of colloidal mobilization. Such aspect was further investigated in the experiments described in chapter 5. The concentration of sodium was made to range in the artificial irrigation water from 0 to 1, 5, 10 and 50 mM to evaluate the effect of increasing sodicity on metal migration in soil. In all the experiments the majority of metals originating from treated wastewater were retained into the top soil layer (0.5 cm), confirming the results already described in chapter 2. The colloidal mobilization of Cu, Ni and Zn was severely affected by the concentration of sodium. Conversely, Cd was less sensitive to sodium concentration and no differences were highlighted in its mobility at increasing sodium concentration. In detail, mobilization of Ni, Cu and Zn was strongly enhanced at low sodicity values (e.g. 1-5 mM [Na]). At higher sodicity values metals were less mobile and higher accumulation happened (Figure 5.2) in the top soil layer (0.5 cm depth). This means that higher colloids mobilization was achieved in such sodicity range. This observation was further confirmed by the variations displayed during time by the ratio between absorbance read at 445 and 465 nm (namely E_4/E_6 ratio) (Chen et al., 1977). E_4/E_6 ratio is in fact related to the degree of humification and in general is increasing at decreasing the humification degree (Boguta et al., 2016). The highest oscillations of such ratio were observed at 1 and 5 mM [Na], while the tendencies were more stable in the other experiments (Figure 5.5). This means that in such sodicity interval, the quality of the

released organic matter was continuously changing over time due to the mobilization of colloids characterized by different humification degrees and moving rates. At higher sodicity where colloids were slowed by effect of pore clogging, the release of organic matter happened more regularly in quality. In Chapter 4 irrigation of OECD soil with artificial low salinity solution and with real treatment plant effluent was compared. Data showed that, although the real effluent had a consistent concentration of sodium (350 mg/L) while the artificial had none, the colloids migration rate was higher irrigating with the real wastewater. The effect of sodium ions was therefore different from that of general “salinity”. Ca and Mg contained in the real effluent (111 and 134 mg/L respectively), having lower ionic radius, divalent charge and hence more concentrated charge distribution than sodium, did not increase the clay dispersion. In contrast they facilitated the aggregation of clay particles, keeping soil flocculated and competing with sodium for binding sites (Duan et al., 2010; Kretzschmar & Sticher, 1997; Warrence et al., 2002). This effect overrode the sodicity influence described in chapters 2 and 5, finally resulting in higher colloidal mobilization in soil irrigated with real effluent as ascertained by the Si trend reported in Figure 4.2b. From the results described in chapters 2, 4 and 5, it is possible to conclude that even in the simplified model of the OECD artificial soil, different kinds of colloids were generated in different boundary conditions (Figures 4.5 and 4.6), each with its own moving rate and affinity to specific metals.

6.2 Conclusions and future perspective

The entire study carried out in the present work focused on the factors affecting the fate of trace heavy metals in soil. The results showed a very complex system of parameters which simultaneously affect metals retention-mobilization behavior once released in soil. The system behaves as a multiphase model in which each phase is characterized by its own properties and affinity with specific metals. Consequently, boundary conditions (i.e. pH, ionic strength, redox potential, sodium concentration, metals speciation, bacterial activity) influence in different way each of the metal binding phase present in the soil. The conclusion is that the fate of trace metals in soil, at the actual knowledge, remains obscure and still very difficult to foresee. The experimental approach applied in this thesis, despite the mentioned drawbacks, allowed to isolate many of these simultaneous effects. The results, although far to be exhaustive and complete, may help in understanding such effects.

The final aim should be to have a model that, known the boundary conditions, could be applied to predict the fate of trace contaminants released in the environment. Foreseeing the fate of

contaminants in the environment will contribute to the correct risk assessment of irrigation reuse of treated wastewater. A lot of work is still to be performed in this direction.

The majority of the results obtained in this thesis refer to laboratory scale experiments with artificial soil. The behavior of the disturbed artificial soil prepared in the lab and packed in small columns is probably quite different from real undisturbed soil. The latter might present, indeed, differences in porosity and other major factors influencing the HMs mobility (e.g. Fe and Mn hydroxides, presence of soil aggregates, presence of bacterial activity). This limitation should be overroded by scaling the experiments and extending the study to real undisturbed soils. Moreover the conducted experimental studies only regarded the fate of trace metals, while in literature it is reported that colloidal mobilization also affects the fate of other emerging contaminants (i.e. pharmaceuticals and personal care products, organic priority pollutants) (Chen et al., 2011; de Jonge et al., 2004; Kanti Sen & Khilar, 2006; Zou & Zheng, 2013). Hence, the experimental approach hereby described could be successfully extended to other micro-pollutants. A deeper characterization of colloidal humic nanoparticles involved in the trace metals mobilization still needs to be performed. In this research was indeed highlighted that soil humic substances properties and their aggregation state are primarily involved in determining the overall fate of trace HMs. A better definition of their composition and of the forces involved in the stabilization of the three dimensional structure of the supramolecular aggregates, will doubtlessly help to further clarify many of the phenomena observed in this thesis.

6.3 References

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