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Colloidal Mobilization and Fate of Trace Heavy Metals in Semi-Saturated Artificial Soil (OECD) Irrigated with Treated Wastewater

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Abstract: The mobility of selected heavy metals in trace concentrations was investigated in a standard OECD soil irrigated with the effluent of a real municipal wastewater treatment plant. While Cd, Cu and Ni accumulation-migration patterns were mainly influenced by the mobility of colloids generated from soil organic and inorganic matter, Zn mobility was more influenced by the wastewater content of dissolved organic matter and by its salinity. Metal accumulation caused by interaction with colloids resulted in contamination peaks both in different zones of the soil column and in the leaching solution. The release of metals in the leachate was correlated to the contemporary release of silicates from kaolinite and dissolved organic matter, identified through UV absorbance and chemical oxygen demand monitoring. The hypothesized colloidal mobilization was confirmed by spectroscopic studies. The highly heterogeneous complexes of organic and inorganic molecules responsible for metal transport through soil appeared to be structured in highly stable micellar aggregates.

Keywords: colloidal mobilization of HMs; fate of trace metals; humic substances; mineral organic associations; soil organic matter

1. Introduction

The recovery and reuse of wastewater in agriculture is a promising strategy to face water scarcity issues [1]. 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 [2], especially in arid and semi-arid regions [3]. However, wastewater reuse for irrigation purposes can represent a serious threat to environmental quality [4]. 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 environmental quality. Nonetheless some compounds, such as heavy metals (HMs), can accumulate in the soil through the years [5], finally reaching the crops and eventually entering the food chain [6].

The fate of HMs in soils is difficult to predict. 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 [7]. 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 [8]. This third mobile phase can be organic, inorganic or composed by mineral organic associations (MOAs) [9]. 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 [10].

Studying the interaction of HMs in "artificial" water solutions with the standard OECD soil, a novel HMs transport pattern was recently proposed [11]. 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 [12]. The classical idea of a class of unknown polymeric macromolecules—namely Humic Acids (HA), Fulvic Acids (FA) and humin—is going to be surpassed by the new concept which considers humic substances to be supramolecular aggregates of small organic compounds, held together by weak dispersive forces [12]. 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 rates through the porous media, as well as different affinity and selectivity with metals. As a 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 [13,14]. In other words, assessing the mobility of such colloidal aggregates allows us to determine the fate of HMs in soil. Therefore, a structural characterization of the released phases and a definition of structural relations with metal 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, such as ultraviolet-visible spectroscopy (UV-VIS), three dimensional excitation emission matrix spectrofluorimetry (3DEEM), proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), are used to reach a deeper characterization of the organic metal binding phases.

2. Materials and Methods

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 concentrations were 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) [15]. 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. The reference soil was composed of the following: kaolinite, 20%; quartz sand, 74%; $CaCO_3$, 1%; sphagnum peat, 5%. Kaolinite, quartz sand and $CaCO_3$ 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 [15]. 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 1. A very high concentration of Pb was found in the kaolinite.

The experimental tests were conducted at laboratory scale using treated wastewater (W1), sampled from a conventional activated sludge WWTP located near Paris (France). W1 was characterized in terms of metal 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 [16]. 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) or other major elements was prepared. In details W2 was obtained dissolving analytical grade $CuCl_2 \cdot 2H_2O$ (Carlo Erba Reagenti, Cornaredo, Italy), $NiCl_2 \cdot 6H_2O$ (Sigma-Aldrich, Saint Louis, MO, USA), and $ZnCl_2$ (Applichem, Gatersleben, Germany) and analytical standard Cd (Carlo Erba Reagenti, Italy), in analytical grade HNO_3 1% (J.T. Baker, Center Valley, PA, USA) solutions. The obtained stock solutions, containing 1 g/L of the selected metal, were successively diluted with ultrapure water (Elgalab Option Q, Elga-Veolia, Saint Maurice, France) 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 1.

Table 1. Characteristics of W1, W2 and OECD soil.

Parameter	OECD Soil (mg/Kg)	W1 (µg/L)	W2 (µg/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 is described elsewhere [11]. The columns (5 cm depth) were fed with 20 mL of wastewater every 12 h. 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 a 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 migration. Kaolinite was indeed the only source of Pb in the experiment (Table 1). Clay silicate migration in the leachate was also assessed by monitoring Si concentration in the leachates.

2.2. Analytical Procedures

Each soil layer and the "blank" soil 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 (Shelton, CT, USA) Start D microwave digester. Leachates were collected daily and divided into two aliquots. The first aliquot was acidified with 3% HNO_3 for metals analysis. The concentrations of Cd, Cu, Ni, Pb, Si and Zn, were measured by ICP-OES spectroscopy (Perkin Elmer Optima 8300, Waltham, MA, 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 nm and the COD were determined. Moreover 3DEEM and for some selected leachate samples the ^{1}H-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 [17] standard method 5220D. Total Phenols were determined by Folin-Ciocalteau colorimetric assay. COD and total phenols absorbance readings were performed by means of a Photolab 6600 UV-VIS spectrophotometer (WTW, Weilheim, Germany). The 3DEEM matrices were acquired through the spectrofluorimeter Perkin Elmer LS 45 (USA). Excitation wavelength was varied between 210 and 450 nm; emission wavelength was recorded between 370 and 585 nm. NMR spectra were acquired on an AVANCE 400 NMR spectroscope (Bruker-BioSpin, Billerica, MA, USA), equipped with Prodigy cryo-probe. Solvent suppression was achieved using a standard pre-saturation sequence.

3. Results

3.1. Mobility Profiles in Soil

The results of metals analysis in the soil and in the leachate are summarized in Figure 1. Figure 1 also displays phenol concentration in the different soil layers to give an indication of organic material distribution and mobility.

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 little 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 μg/Kg) than in W1 tests (23.35 μg/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. In contrast to other metals, Ni and Zn concentrations markedly decreased at 5 cm depth. At such depth, Ni and Zn concentration was indeed 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.
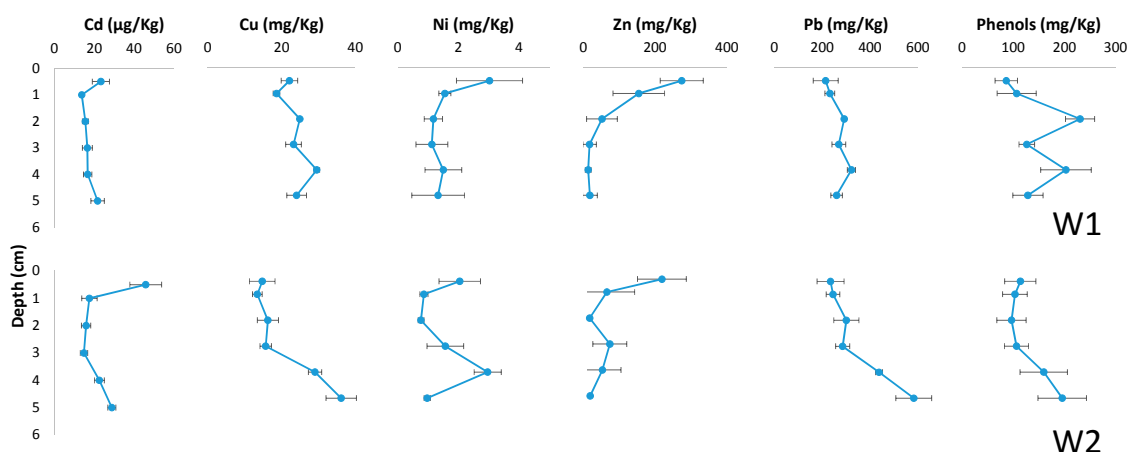
Figure 1. Metals and total phenols concentration in soil columns irrigated with W1 and W2. Irrigation lasted 17 d and a total leachate of 650 mL was collected at the end of the experiment. Soil background HMs concentrations are reported in Table 1.

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 2a). Obtained data showed that the irrigation with W1 led to higher mobilization of the colloidal silicates originating from kaolinite (Figure 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 W1 leachate respect to one observed during the irrigation with 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 1) and Si trends (Figure 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 A_{245} trend, reported in Figure 2c. A_{245} peaks were popping up at the same time (days 8–10 and days 14–16) as the major of HMs peaks (Figure 2a). At the same experimental time, peaks of Si in W2 leachate were noticeable in Figure 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 mgO$_2$/L) appeared at the same experimental time as A_{245} and metals peaks. A constant background release of COD was also recorded ($\approx 30 \pm 4$ mgO$_2$/L).
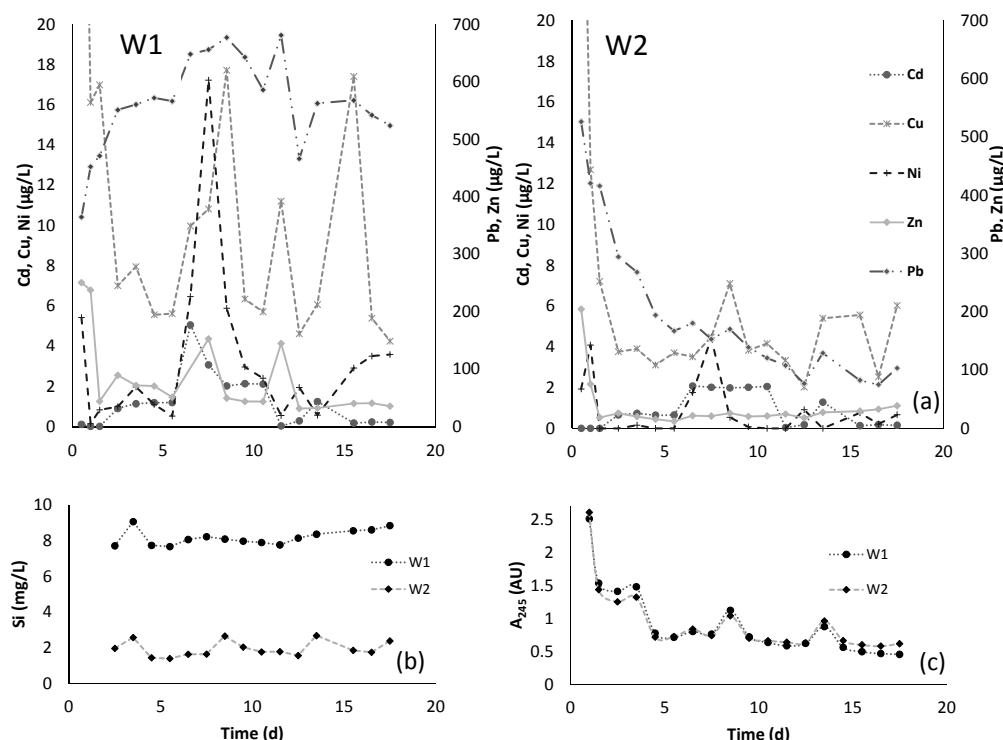

Figure 2. Evolution versus time of HMs (**a**); Si (**b**) concentrations and A_{245} (**c**) in the leachate produced by soil irrigation with W1 and W2. Time is expressed in days (**d**).

Correlation diagrams between total HMs (i.e., Cu, Cd, Ni, Zn) and A_{245} are plotted in Figure 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 a significant role in determining overall metal mobilization by DOM.
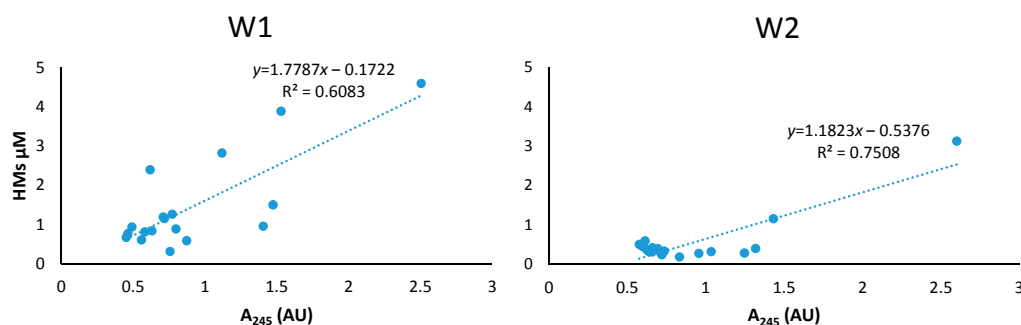

Figure 3. Correlation diagrams between HMs and A_{245} in the leachates.

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. Figure 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.

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) [18]. 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 [19,20].
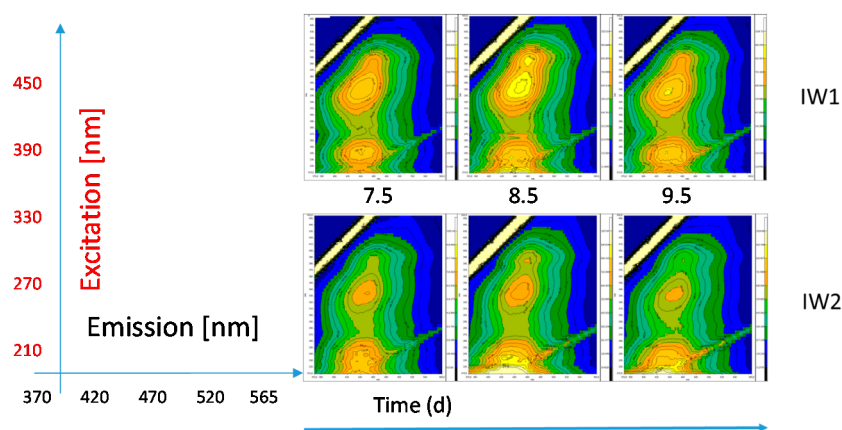

Figure 4. Selection of 3DEE matrices recorded in W1 and W2 leachates at time between 7.5 and 9.5 days.

Further information was obtained via the ^{1}H-NMR spectra of the leachates collected at days 6.5, 8.5, 9.5, plotted in Figure 5. It was possible to observe 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), confirming the peak of organic matter release in the leachate (Figure 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 (8.5 days) 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 concentrations 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 5). Signals in this chemical shift region resulted in broadened line shapes with very low intensities.
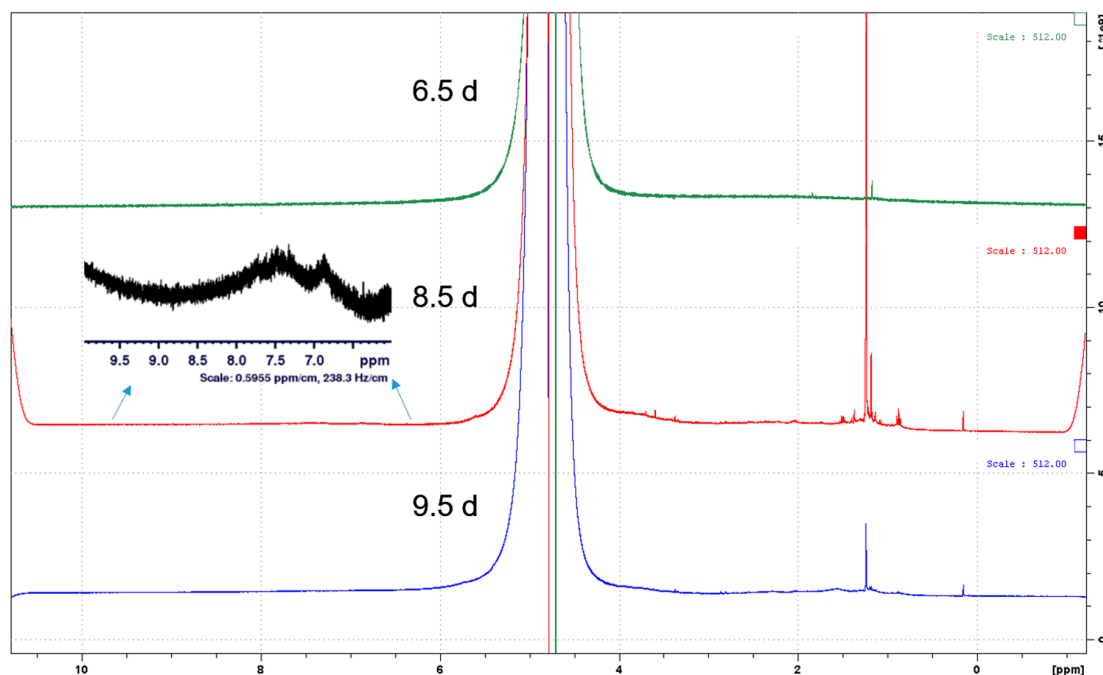
Figure 5. 400 MHz ^{1}H-NMR spectra of collected leachates in correspondence of metals accumulation (days 6.5, 8.5, 9.5). Spectra were acquired with 20,480 scans, probe temperature of 298 K, and solvent suppression with pre-saturation field of 50 Hz.

4. Discussion

4.1. Colloidal Mobilization of HMs

By comparing Figures 1–5, it was evident that colloids containing organic molecules of a 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 1 and 2). The similar distribution of the two parameters inside the soil strongly suggests 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). A previous study [11] reported a decreasing colloidal mobilization speed at increasing sodium content. In this study, 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), with a lower ionic radius and 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 [21–23]. 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 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 1 and 2). Two different explanations are 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 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 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 [24].

4.2. Role and Nature of Colloidal Aggregates

Under the tested experimental conditions, DOM seemed to act as metal carrier through the soil column. This assumption was confirmed by the monitoring of UV absorbance of the leaching solution. The presence of both A_{245} (Figure 2c) and COD backgrounds was due to the release of organic molecules, as already described in a previous study [11]. 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 1). The presence in W2 of peaks of Si simultaneously to A_{245} (Figure 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 influencing metal release in the leachate, and coefficients of correlation in Figure 3 were quite far from 1. Nevertheless, the trend was clearly observable and it was possible that metals were released together with organic matter into the leachate. Salinity played a major role in enhancing the dispersion of data in the W1 test, which were characterized by a lower value of R^2 (Figure 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, molecular characterization 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 [20,25]. From the observation of 3DEEM (Figure 4) it was clear that at the experimental time corresponding to the metal 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 was changing over time. Several studies have shown quenching of fluorescence related to metals and mainly Cu binding to HA-like fluorophores [19,26]. 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 [18]. The lower fluorescence intensity in W2 leachate was hence attributable to the mobilization of total phenols, clearly indicated in Figure 1: less humic matter mobilized in the W2 test. ^{1}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 5, 8.5 days) 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), aromatic signals displayed very low intensity and poor resolution in all the acquired NMR spectra. This was ascribed 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 as a result were easily detectable by NMR. This type of structure indeed caused the protons contained within the micelles to have different relaxation times, which resulted in broad unresolved peaks. Such behavior has already been reported in literature [27], 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 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.

4.3. Comparison of Results with the 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 [28], and several studies reported that metal mobility was severely affected by colloidal mobilization in the case of HMs at μmoles level [13,23,29]. 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. [14] 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 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 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 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 and 5), 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 [19,30–33]. Although the majority of them deal with case studies on real scale or with higher concentration ranges, some comparisons are 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 [34]. Simple organic acids, dissolved or aggregated in humic supramolecular structures [12,35], enhanced metal mobility in soil profiles by reducing soil pH and forming complexes with heavy metals [36]. 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 acids, it was found that this metal formed stable complexes with organic acids which strongly interacted with inorganic soil matrix resulting in little mobility [37]. 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 [38] 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 [39]. 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 0.5 cm. In contrast, the application of DOM increased the diffusion of Cd, as observed in this study [40]. Metal 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 has with contaminants, even at trace concentrations.

5. Conclusions

Trace HMs mobilization in soil is controlled by colloidal mobilization. Colloids, which might be organic, inorganic or MOAs, are released from the soil matrix over time and act as a metal carrier, moving through the soil column with a 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 A_{245}. 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 ^{1}H-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.

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