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Effect of organic amendments on the mobility of trace elements in phytoremediated techno-soils: role of the humic substances

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Abstract

The efficiency of aided phytostabilization using organic amendments such as ramial chipped wood (RCW) and composted sewage sludge (CSS) was studied on contaminated techno-soils, on nine experimental plots. The objective was to characterize the role of fulvic (FA) and humic acids (HA) on the mobilization of trace elements, specifically As, Cu, Mo, Pb and Zn.

Results showed that the addition of CSS increased the total organic carbon and nitrogen content more than with RCW and as a result, the C/N ratio in the CSS soil was higher than in the RCW and non-amended (NE) soil, reflecting the high decomposition of soil organic matter in the CSS soil compared with the other soils. The RCW and CSS amendments increased the hydrogen index values and the oxygen index values compared with the NE soil, especially for the soil treated with CSS which contained more aliphatic than aromatic compounds. The addition of CSS to the techno-soil significantly increased the percentage of C_{org} associated with the HA fractions compared with the RCW and NE soils. The soil amended with CSS showed the highest E₄/E₆ ratio and the lowest E₂/E₃ ratio of FA. Zn and As were more abundant in the FA fraction than in the HA fraction, whereas Pb, Cu and Mo were more associated to HA than to FA in the treated and untreated soils, which may explain the difference in their mobility and availability.

Key words: Contaminated techno-soil, Trace element, Humic acid, Fulvic acid, Mobility.

30 1. Introduction

1 31
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3 32 Techno-soil is a soil “whose properties and function are dominated by technical human activity as
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5 33 evidenced by either a substantial presence of artifact” (WRB, 2006). Moreover, all techno-soils are not
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7 34 necessary contaminated by inorganic or organic contaminants. The pollution of anthropogenic soils with a
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10 35 high concentration of trace elements is more problematic than for other types of soils as the properties of
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12 36 these techno-soils change over time, making handling more difficult (Kelly et al. 1996). Moreover they
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14 37 present a real risk for human health and especially for children's health (De Burbure et al. 2006).

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16 38 The contamination of a techno-soil with trace elements requires special soil-management techniques to
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19 39 limit the trace element solubility and thus plant availability. Among remediation techniques,
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21 40 phytoremediation has attracted attention as it is a low-cost and ecologically sustainable alternative to
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23 41 physicochemical methods, can be applied to large areas, and is accepted by local populations (Mench et al.
24
25 42 2010). Aided phytostabilization is a phytoremediation technique that is recognized as a potentially cost-
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27 43 effective and ecologically sound approach to the containment of trace element-polluted soils and mine
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29 44 tailings. Its principal objective is to reduce the mobility, ecotoxicity, and dispersion of metals/metalloids
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32 45 through the environment (Mendez and Maier, 2008; Ginocchio et al. 2007; Mench et al. 2010). Aided
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34 46 phytostabilisation requires the incorporation of both plant species and organic or inorganic amendments that
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36 47 are appropriate for the type of contamination in order to immobilize trace elements such as metals/metalloids
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38
39 48 and reduce their availability in these soils (Mendez and Maier 2008; Berti and Cunningham 2000) and to
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41 49 restore the physical, chemical and biological properties of contaminated soils (Mench et al. 2000; Bolan et al.
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43 50 2003; Pérez de Mora et al. 2005; Raicevic et al. 2005; Kumpiene et al. 2006, 2008). Several mineral and
44
45 51 organic amendments such as lime, coal fly ashes, phosphates, red muds, compost, biosolids, iron grit and
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47
48 52 Fe/Mn/Al oxides can improve phytostabilization and the production of plant-based feedstock through the
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50 53 decrease in the solubility, leaching and bioavailability of trace elements (Lombi et al. 2002; Bolan et al.
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52 54 2003; Brown et al. 2004; Geebelen et al. 2003; Basta et al. 2004; Kumpiene et al. 2008; Mench et al. 2010).
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54 55 This reduction is achieved via various complex processes, e.g. adsorption onto mineral surfaces, formation of
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56 56 stable compounds with organic ligands, surface precipitation and ion exchange (Kumpiene et al. 2008;
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59 57 Ahmad et al. 2011).

58 The addition of organic matter amendments such as compost, manure or various organic wastes with
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2 59 the plants is a common practice for the remediation of contaminated soils. The effect of organic matter
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4 60 amendments on trace element bioavailability depends on the nature of the organic matter, its microbial
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6 61 degradability, salt content and effects on soil pH and redox potential as well as on the particular soil type and
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8 62 metals concerned (Walker et al. 2003, 2004). Organic amendments may contain a high proportion of
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11 63 humified organic matter, which has a large capacity to interact with metal ions and mobilize or immobilize
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13 64 trace elements through the formation of more or less stable complexes. The formation of these insoluble
14
15 65 metal compounds reduces their mobility through the soil profile and the pool available for biota (Geebelen et
16
17 66 al. 2003). The characteristics of humus fractions have proven useful in the interpretation of organic matter
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19 67 dynamics in soil. There is an extensive literature on the characterization of humic substances (Abbt-Braun
20
21 68 and Frimmel, 1999; Gondar et al. 2005; Shirshova et al. 2006) and on the binding of metals to HS, mainly
22
23 69 concerning laboratory experiments with humic or fulvic fractions purified from water or soil (Vaca-Paulín et
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25 70 al. 2006; Gondar et al. 2006; Doig and Liber 2007; Evangelou et al. 2007). The ability of HS to react with
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27 71 trace elements has long been recognized (Wu et al. 2002; Clemente and Bernal, 2006; Evangelou et al.
28
29 72 2007). Humic substances have the capacity to control the behavior of trace elements in the environment.
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31 73 However the reactions between trace elements and humic substances are highly complex and depend on the
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33 74 trace element itself, the humic material and pH. There are two possible outcomes of humic-metal binding: (i)
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35 75 metal ions may form soluble organic complexes with the potential to contaminate groundwater and to retain
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37 76 the metal in soil solution; and (ii) insoluble complexes can form and result in a reduction in bioavailability
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39 77 and consequently, in the ecotoxicity of the metals (Logan et al. 1997).
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44 78 In the present work we assess the role of humic substances (Humic acid and Fulvic acid) extracted
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46 79 from two different organic soil amendments - composted sewage sludge and fresh ramial chipped wood - and
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48 80 from a untreated soil on the mobility of several trace elements in metallurgical techno-soils treated by aided
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50 81 phytostabilisation.
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52 82 **2. Materials and methods**

53 83 **2.1. Site description and experimental design**

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56 84 The studied site is a metallurgical landfill (Industeel-Loire; 45°32' N; 4°38'E) located near Lyon.
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85 France. It is located behind a steel and iron factory which is still in operating. The site was used from about
1 1850 to 2001 to dispose of foundry waste, slag, fire-bricks, and other by-products from the industrial
2 86 process, such as more or less hydrated lime. It extends over nearly 15 ha and is 8 to 10 m thick. The soil
3 87 (“foundry technosol” type) is highly contaminated with iron (Fe) and different metals used in various alloys:
4 88 (mainly molybdenum (Mo), arsenic (As), copper (Cu), lead (Pb) and zinc (Zn). This site is part of a French
5 89 network of contaminated sites (“SAFIR” network. <http://www.safir-network.com>) and is partly dedicated to
6 90 research into contaminant transfer, the biological impact of pollution and remediation technologies.
7 91

15 92 In this context, the efficiency of aided phytostabilization using organic amendments was studied on
16 93 nine 50 m² (5 m X 10 m) experimental plots. Plots were prepared in December 2009 by shallow ploughing
17 94 (10 to 15 cm depth) and they were enriched using the following materials: (1) ramial chipped wood (RCW)
18 95 applied on approx. 500 m³/ha (3 plots), (2) composted sewage sludge (CSS) applied on approx. 120t DM/ha
19 96 (3 plots) and (3) no organic enrichment (NE) (3 plots). An extensive pedo-geochemical characterization of
20 97 the different plots was performed in March 2010, i.e. four months after preparation of the plots.
21 98

29 98 The soils were air-dried and sieved to < 2mm. The main pedological characteristics of the soils studied
30 99 (taken at 0-20 cm depth), and their chemical composition are given in table 1. The soil pH was measured in
31 100 1:2.5 soil: water suspension using a glass electrode pH meter (NF ISO 10390 procedure (AFNOR, 1994)).
32 101 Total organic carbon (TOC) was determined in the soil samples by Rock-Eval pyrolysis. Total N contents
33 102 were determined using a Fisons Instruments (Crawley. UK) elemental analyzer model EA 1108. The cation
34 103 exchange capacity (CEC) was determined by the Metson method at pH=7. Particle size distribution of the <2
35 104 mm fraction was determined by sieving and using the pipette method. Extraction of the major and metallic
36 105 elements was performed using hydrofluoric (HF) and perchloric (HClO₄) acids, according to the NF X 31-
37 106 147 procedure. The contents of major and metallic elements in various extracts were measured by
38 107 inductively coupled plasma optical emission spectrometry (ICP-OES) using a Horiba Jobin-Yvon apparatus.
39 108

51 108 **2.2. Characterization of soil organic matter by Rock-Eval pyrolysis**

54 109 Rock-Eval pyrolysis (Vinci Technologies, Rock-Eval 6 Turbo, France) is used to identify the type and
55 110 maturity of organic matter (Béhar et al. 2001; Disnar et al. 2003; Sebag et al. 2006). This technique can be
56 111 applied to soils (Di Giovanni et al. 1998). The analysis comprised 2 steps. First, pyrolysis under nitrogen
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112 adsorption of hydrocarbon is maintained at 200 °C for 5 min. Then cracking occurs between 200 and 650 °C
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2113 at 30 °C min⁻¹.

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5114 In summary, the four basic parameters obtained by pyrolysis are as follows: S₁ = the amount of free
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7115 hydrocarbons in the sample (in milligrams of hydrocarbon per gram of sample); S₂ = the amount of
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10116 hydrocarbons generated through thermal cracking of nonvolatile organic matter; S₃ = the amount of CO₂ (in
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12117 milligrams CO₂ per gram of sample) produced during pyrolysis of kerogen. S₃ is an indication of the amount
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14118 of oxygen in the kerogen and is used to calculate the oxygen index; T_{max} = the temperature at which the
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16119 maximum release of hydrocarbons from cracking of kerogen occurs during pyrolysis (T_{max} is an indication of
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18120 the stage of maturation of the organic matter).

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21121 The type and maturity of organic matter in our soils was characterized from Rock Eval pyrolysis data
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23122 using the following parameters: HI = hydrogen index (HI = [100 x S₂]/TOC). This parameter is used to
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25123 characterize the origin of organic matter. Marine organisms and algae, in general, are composed of lipid- and
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27124 protein-rich organic matter, where the ratio of H to C is higher than in the carbohydrate-rich constituents of
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29
30125 land plants. OI = oxygen index (OI = [100 x S₃]/TOC) is a parameter that correlates with the ratio of O to C,
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32126 which is high for polysaccharide-rich remains of land plants and inert organic material (residual organic
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34127 matter) encountered as background in marine sediments. OI values range from near 0 to ~150.

36128 Total organic carbon (TOC) was determined in the soil samples by Rock-Eval pyrolysis. This
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38
39129 instrument uses a ramped temperature pyrolysis technique in which a small amount of sample (70 -100 mg)
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41130 is heated in an inert atmosphere (helium or nitrogen) and also combusted with air to obtain several key
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43131 geochemical parameters such as the total organic carbon (TOC).

45132 In the present work a small amount of sample (70 -100 mg) is heated with a precision over 0.5 mg in
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47
48133 an inert atmosphere (helium or nitrogen) and also combusted with air to obtain several key geochemical
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50134 parameters. Only the TOC and HI vs OI were considered as the main parameters, following the original work
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52135 by Van Krevelen (1950).

54136 2.3. Extraction and characterization of humic substances (HS)

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57137 One hundred grams of each soil sample was extracted with 500 ml of 0.5 N NaOH (Schnitzer and
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60138 Schuppli 1989). The humic and fulvic acids used were extracted following the procedure given in Holtzclaw

139 et al. (1976). The mixture was stirred for 17 h under N₂ gas. Humic acids were precipitated with 6 M HCl
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2140 (pH= 1) and separated by centrifugation (3000 rpm, 15 min). The TOC of each solution was determined by
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4141 hot catalytic combustion (Teckmar – Dohrman Phoenix 8000).
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7142 Total metal contents in FA and in the mixture of FA+HA were determined by HR-ICP-MS (Element
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9143 2. Thermofischer. Waltham. USA) using a Twinna bar cyclonic spray chamber with a Micromist nebulizer
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11144 (Glass Expansion, Melbourne, Australia) for As, Cu, Mo, Pb and Zn.
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13145 The ultraviolet-visible (UV-vis) is an instrumental technique used to identify the functional groups
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15146 present in organic compounds by measuring their absorption of infrared radiation over a range of
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17147 frequencies. The absorption pattern is then compared to the infrared spectra of known substances for
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19148 identification. The ultraviolet-visible (UV-vis) absorption spectra of separated FA and HA were recorded by
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21149 a Jasco V-530 spectrophotometer. The E₄/E₆ ratios were calculated as the ratio of absorbance at 465 and at
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23150 665 nm according to Chen et al. (1977). The E₂/E₃ ratios were calculated as the ratio of absorbance at 280
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25151 and at 365 nm. The SUVA index was calculated by dividing the UV absorbance measured at λ= 254 nm by
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27152 the TOC concentration (Weishaar et al. 2003).
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32153 Infrared characterizations in the region of 400 - 4000 cm⁻¹ were undertaken in transmission mode for
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34154 several samples in classical pellet form. The preparation of the pellets was as follows: the sample was finely
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36155 ground in an agate mortar and 1 to 1.5 mg of the resulting powder was mixed with KBr, previously dried at
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38156 120 °C for 24 h. to make a 150 mg pellet. The mixture was homogenized and pressed in an evaluable die to
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41157 prepare a 13 mm diameter pellet. The operating conditions were 256 scans, 2 cm⁻¹ resolution over the 400 –
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43158 4000 cm⁻¹ range without ambient H₂O and CO₂ correction.
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45159 **2.4. Statistical analyses** 46

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48160 All statistical analyses were performed using StatSoft Statistica (version 6). All analytical
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51161 determinations were performed in three replicates for each plot. Differences were considered statistically
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53162 significant at p < 0.05.
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165 **3. Results and discussion**

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2166 **3.1. Physico-chemical properties**
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5167 The physico-chemical properties of these samples are presented in Table 1. The studied soils are
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7168 alkaline (pH between 9.08 to 9.34), sandy and with CEC ranging from 5.63 to 6.77 cmol/kg. As expected,
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9169 application of the organic amendments slightly modified the physico-chemical properties of the soil (Larney
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12170 and Angers, 2012). A significant decrease in pH and an increase in the CEC are observed. In the case of
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14171 carbonated soils, Séré et al. (2010) showed that the application of organic amendments as compost can lead
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16172 to a decrease in pH by soil decarbonization. The increase in CEC can be explained by the increase in the
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18173 TOC measured in treated soils ($p=0.00037$) compared with untreated soil (NE). Soil amended with RCW and
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20
21174 CSS contained 2.2 and 3.5 fold more total organic carbon respectively than the NE soil. TOC in the soil
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23175 treated with RCW was less than that measured in the CSS soil. This could be due to microbial decomposition
24
25176 of carbon and its subsequent release as CO_2 (Basiramakenga and Simard, 1998). Moreover, Soumare et al.
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27177 (2003) showed that an increase in the concentration of organic matter is related to the decomposition of
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29
30178 organic amendments in soil, and ramial chipped wood decomposes more slowly than compost sewage
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32179 sludge. RCW and CSS added to the soil significantly increased the total nitrogen by 1.6 and 7 fold
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34180 respectively compared with the NE soil ($p=0.0047$) (Table 1). This result agrees well with that found by
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36181 Vaca-Paulin et al. (2006), who reported that the addition of compost and sewage sludge increased the total
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38
39182 nitrogen in the soil threefold compared with the control soil. Our results are also in good agreement with
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41183 those of Kaschl et al. (2002) who investigated the effect of compost added to a calcareous soil on the
42
43184 physicochemical characteristics of the soil. They found a positive correlation between the addition of
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45185 compost and soil organic carbon contents. Crecchio et al. (2001) also reported that amendment with
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47
48186 municipal compost increased the total C and N in the soil significantly compared with the other treated and
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50187 control soils. RCW added to the soil increased the C/N ratio by 1.4 fold, whereas the addition of CSS
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52188 reduced the C/N ratio by 1.9 fold compared with the NE soil. The decrease in the C/N ratio in the CSS soil
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54189 reflects the high microbial activity, which leads to a high decomposition of organic matter (Hsu and Lo,
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56190 2001; Amir et al. 2005) and a greater degree of humification.
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Table 1: The main pedological characteristics and chemical composition of the studied soils. For each treatment, data are the mean of 30 measures (10 samples per plot).

		Treatment		
		RCW	CSS	NE
physico-chemical parameters	Texture	sandy	sandy	sandy
	pH	9.08±0.07	9.07±0.12	9.34±0.18
	C _{org} (g.kg ⁻¹)	19.7±0.34	31.4±0.34	8.9±0.24
	N (g.kg ⁻¹)	0.9±0.03	3.9±0.13	0.6±0.01
	C/N	22.67±4.31	8.45±2.01	15.76± 4.73
	CEC (Cmol/kg)	5.63±1.10	6.77±0.15	5.77±0.55
Major elements	P ₂ O ₅ (g.kg ⁻¹)	0.03±0.00	0.25±0.04	0.02±0.00
	K ₂ O (g.kg ⁻¹)	0.29±0.05	0.59±0.07	0.09±0.07
	CaO (g.kg ⁻¹)	18.88±6.71	20.70±1.13	24.67±2.17
	MgO (g.kg ⁻¹)	2.66±0.44	1.29±0.09	2.79±0.44
	Na ₂ O (g.kg ⁻¹)	0.08±0.07	0.14±0.02	0.14±0.03
trace elements	Zn (mg.kg ⁻¹)	1719.00±777.78	1348.33±560.02	1326.00±739.13
	Pb (mg.kg ⁻¹)	688.33±453.8	623.00±246.43	839.00±390.20
	Mo (mg.kg ⁻¹)	523.66±142.30	652.66±69.28	682.33±124.44
	Cu (mg.kg ⁻¹)	399.00±84.01	410.33±78.23	523.00±160.36
	As (mg.kg ⁻¹)	76.66±22.47	73.00±11.78	91.33±15.54

Ramial chipped wood (RCW), composted sewage sludge (CSS), non-amended soil (NE)

3.2. Organic geochemistry

Rock Eval analyses of the treated (RCW and CSS) and untreated soil (NE) was conducted in order to recognize the origin and the evolution of the MO contained in each soil. Examination of the results obtained leads to the following conclusions: (1) the TOC content in treated soils was significantly higher than that measured in the untreated one (Table. 1); (2) the same behavior was marked for the hydrogen index (HI) where RCW and CSS presented 63% and 75% of HI more than the NE soil. However, addition of organic amendment to the soil increased slightly soil Oxygen Index (OI) by 23% and 13 % in soil amended with RCW and CSS respectively (Table 2). The HI values presented by the soil amended with CSS were nearly 2 fold higher than that presented by RCW, whereas RCW presented 1.8 fold of OI higher than that present by the soil amended with the CSS (Table. 2). This result is in agreement with the low organic content in the untreated soil (low TOC) and thereafter the expression of the nature of the two organic amendments in the

RCW and CSS respective values: their addition in the soil increases the TOC and the IH index (RCW). Thereafter a very strong correlation was observed between TOC and HI measured in the 3 types of soils ($r=0.94$). Our results are in agreement with that reported by Walter et al. (1982) who found very good correlation between TOC content and the kerogen hydrogen index.

Table 2: Rock-Eval Pyrolysis. Total Organic Carbon (TOC, %). Hydrogen Index (HI, mg.g^{-1}). Oxygen Index (OI, mg.g^{-1})

Soil	TOC (%)	HI (mg.g^{-1})	OI (mg.g^{-1})	HI/OI
RCW	1.97ab \pm 0.34***	181.41ab \pm 15.08***	188.86a \pm 23.84 NS	0.965
CSS	3.14a \pm 0.34***	264.54a \pm 11.50***	165.52a \pm 7.58 NS	1.600
NE	0.89b \pm 0.24***	66.01b \pm 13.43***	143.67a \pm 28.99 NS	0.459

0.01<(*)P <0.05, 0.01>(**) p >0.001, (***) p < 0.0001, (NS) insignificant difference, ramial chipped wood (RCW), composted sewage sludge (CSS), non-amended soil (NE)

The relation between the hydrogen and oxygen index values is shown on Fig.1, which plots the origin of the organic matter in the three types of soil studied here on the well-known Van Krevelen diagram. This diagram consists of three lines highlighting the natural origin of the organic matter in the soil (Tissot and Welte 1984; Espitalié et al. 1977; Hassen et al. 2009). Lines I and II correspond to organic material of microbial or planktonic origin, line III corresponds to the OM of higher continental plants.

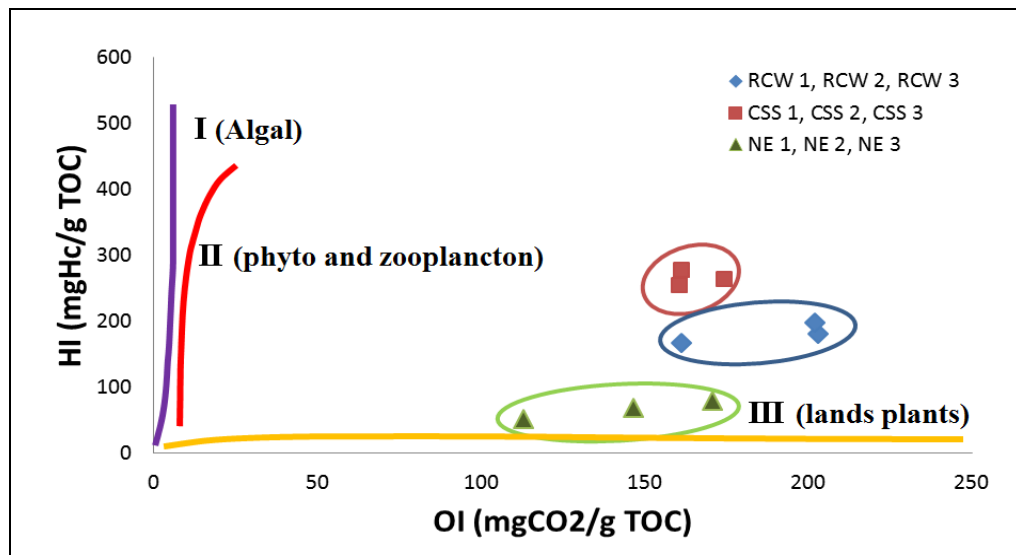


Figure 1: Origin of the organic matter in the three types of soils: RCW (blue diamond), CSS (red square) and NE (green triangle) as a function of the hydrogen and oxygen indices.

It can be clearly seen from Fig. 1 that the points representing the treated and untreated soils studied here plot along lineage III, indicating the terrestrial origin of the organic matter in the untreated soil. It also shows that the values obtained in the amended soils are highly dependent on that of the amendment itself.

Ramial-chipped wood (RCW) has a higher OI because of the presence of wood cellulose. As the original kerogen of the non-treated soil is not abundant and also because its OI is low, RCW soil has the highest OI, close to that of wood. Due to its origin, composted sewage sludge (CSS) has a higher HI because it is richer in aliphatic groups than wood. The soil amended with CSS therefore has a higher HI value than the others soils.

3.3. Characterization of the humic fraction

Humic substances (HS) (fulvic and humic acids) extracted from the control and amended soils accounted for 7.0 to 12.9 % of the soil organic C ($C_{\text{HS}}/C_{\text{org}}$). The addition of composted sewage sludge significantly increased the proportion of C_{org} associated with HS compared to control soil. Usman et al. (2004) explained this result by the high proportion of easily biodegradable organic components in sewage sludge. In contrast, ramial chipped wood residues have a high lignin concentration, inducing a slower rate of decomposition of these residues compared to sludge. Amendment with RCW had no effect on the proportion of HS (N'dayegamiye and Angers 1993). The proportion of FA extracted to soil organic carbon ($C_{\text{FA}}/C_{\text{org}}$) ranged from 3.7 to 4.4 % (Figure 2.a) and the organic amendments (RCW and CSS) added to the soil had no significant effect on the percentage of organic carbon associated with the FA fractions. The HA fractions represent from 2.7 % to 9.1 % of the SOC ($C_{\text{HA}}/C_{\text{org}}$). Moreover, the addition of CSS to the soil significantly increased ($p < 0.001$) the percentage of C_{org} associated with the HA fractions compared with the RCW and NE soils. The increase in the organic carbon fraction in the form of humic acid despite the drastic extraction procedures used suggests that part of the organic carbon from organic amendments (CSS) has been incorporated into the humic acid fraction. The maturity and stability of organic matter are expressed by the $C_{\text{HA}}/C_{\text{FA}}$ ratio and shown in figure 2.b. A significant increase in the $C_{\text{HA}}/C_{\text{FA}}$ ratio for CSS soil ($p < 0.0001$), by 3.9 fold compared to RCW and NE soils was observed which is not the case for the RCW-type amendment. These results indicate a higher degree of humification of organic matter in the CSS soil compared to RCW and NE soils (Hsu and Lo 2001).

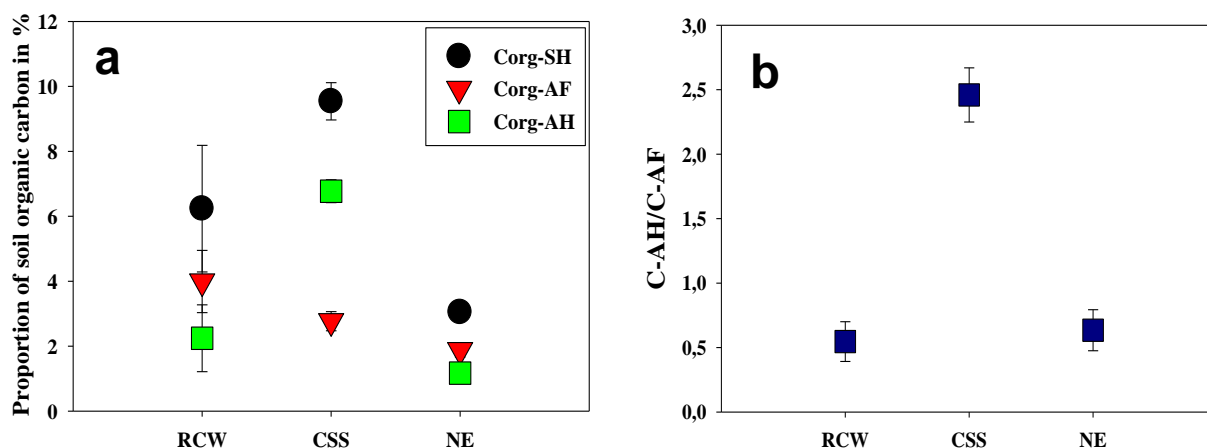


Figure 2: a) proportion in % of total organic carbon between HS, HA and FA; b) C-HA/C-FA ratio measured in the three soils. Values are: Box: Median; Whisker: Error bars (n=9)

3.4. UV-visible spectroscopy results

The absorbance ratio E_2/E_3 is considered in the literature as an indicator of the size of the organic molecules and their hydrophilicity (Thomsen et al. 2002). The addition of CSS significantly decreased the E_2/E_3 ratio ($P < 0.0001$), whereas in soil amended with RCW the E_2/E_3 ratio of FA increased weakly compared with NE soil (Table 3). This means that the organic particle size of the RCW and NE soils was larger than that of CSS, and that the hydrophobic properties in the RCW and NE soils were lower than that in CSS soil (Guo and Chorover 2003).

Typically, the E_4/E_6 ratio is considered to be inversely related to: the particle size (Chen et al. 1977), the molecular weight (Christl et al. 2000), and the degree of condensation of the aromatic network in HS macromolecules (Senesi and Loffredo 1999). Table 3 shows that the addition of CSS to the soil increased the E_4/E_6 ratio by 30% in comparison with the NE soil whereas in soil amended with RCW, the E_4/E_6 ratio decreased by 19% compared with the NE soil. Our results show that CSS soil has a low degree of aromatic condensation, indicating the presence of low-molecular-weight organic acids (Senesi and Loffredo 1999; Chen et al. 1977; Christl et al. 2000; Thomsen et al. 2002). Finally, it can be seen that the highest E_4/E_6 ratio and the lowest E_2/E_3 ratio of fulvic acids were extracted from the soil amended with CSS, indicating that this soil contains more hydrophilic and aliphatic compounds and fewer condensed and aromatic structures than RCW and NE soils.

Table 3: E_2/E_3 , E_4/E_6 and $SUVA_{254}$ Values calculated for fulvic acid (FA) fraction by UV-Visible spectroscopy

	E_2/E_3	E_4/E_6
RCW (FA)	5.44 a \pm 0.41***	5.12 NS \pm 1.56
CSS (FA)	2.40 ab \pm 0.21***	9.06 NS \pm 1.53
NE (FA)	4.41 b \pm 0.67***	6.33 NS \pm 3.18

0.01<(*P <0.05, 0.01>(**) p >0.001, (***) p < 0.0001, (NS) insignificant difference, fulvic acid (FA), ramial chipped wood (RCW), composted sewage sludge (CSS), non-amended soil (NE)

3.5. FTIR

FTIR has been widely used for the characterization of HS extracted from soils. Even if the interpretation of the analysis may be difficult due to the overlapping of spectral features of the two fractions, the assignment of the absorption bands, determined by several studies, highlights certain differences once soils have been amended with RCW and CSS. **The FTIR spectra of HAs and FAs show typical absorption bands relative to the organic groups of the polyfunctionality of humic substances** (Piccolo and Stevenson 1982; Baes and Bloom 1989; Vaca Paulin et al. 2005). The most important features are: (i) the two peaks of variable intensity at 2918–2926 and 2850–2855 cm^{-1} due to aliphatic C–H stretching; (ii) the strong peak centered between 1615 and 1624 cm^{-1} mostly attributed to structural vibrations of aromatic C=C and antisymmetrical stretching of COO- groups; (iii) the medium intensity absorption centered between 1398 and 1450 cm^{-1} , mostly due to aliphatic C-H bending and symmetrical COO- stretching; and (iv) absorption at 1030 and 1090 cm^{-1} associated with C-O stretching of alcohols. The aliphatic group absorptions are often associated with weak peaks of aliphatic CH chains centered at about 770–778 cm^{-1} as both spectra show. Moreover, the weak absorption bands at 1220 and 1260 cm^{-1} , due to the C-O stretching of carboxylic groups, phenols and/or aromatic ethers appear more intense for HS and more particularly in a soil treated by RCW. The sharp bands at 1375 cm^{-1} present on all the spectra are attributed to residual inorganic salts resulting from the extraction of organic matter. The wide bands centered around 1100 cm^{-1} are attributed to inorganic Si-O vibrations of clay impurities co-precipitated with both FAs and HAs and not eliminated by the purification process (Figure. 3)

The most significant differences in absorption bands were found for the humic fractions. The FTIR spectra of soils treated with RCW and CSS show a more pronounced absorption band at 1720 cm^{-1} than the

raw or standard one, indicating a larger content in carboxylic groups. The pH values of the untreated and treated soils confirm that this band (shoulder around 1720 cm^{-1}) results from COOH groups. The introduction of amendments decreases soil pH and consequently increases such bands. This is further confirmed by the concomitant appearance of two new bands in the 1620 and 1380 cm^{-1} regions ascribed to COO^- ions resulting from the conversion of HAs to their salts. Another difference in the spectra of HA fractions is the appearance of weak absorption bands at 1520 cm^{-1} for amended soils, relative to N-H vibration of amide groups. The identification of these bands suggests the presence of complex structural components with a high molecular weight and high humification.

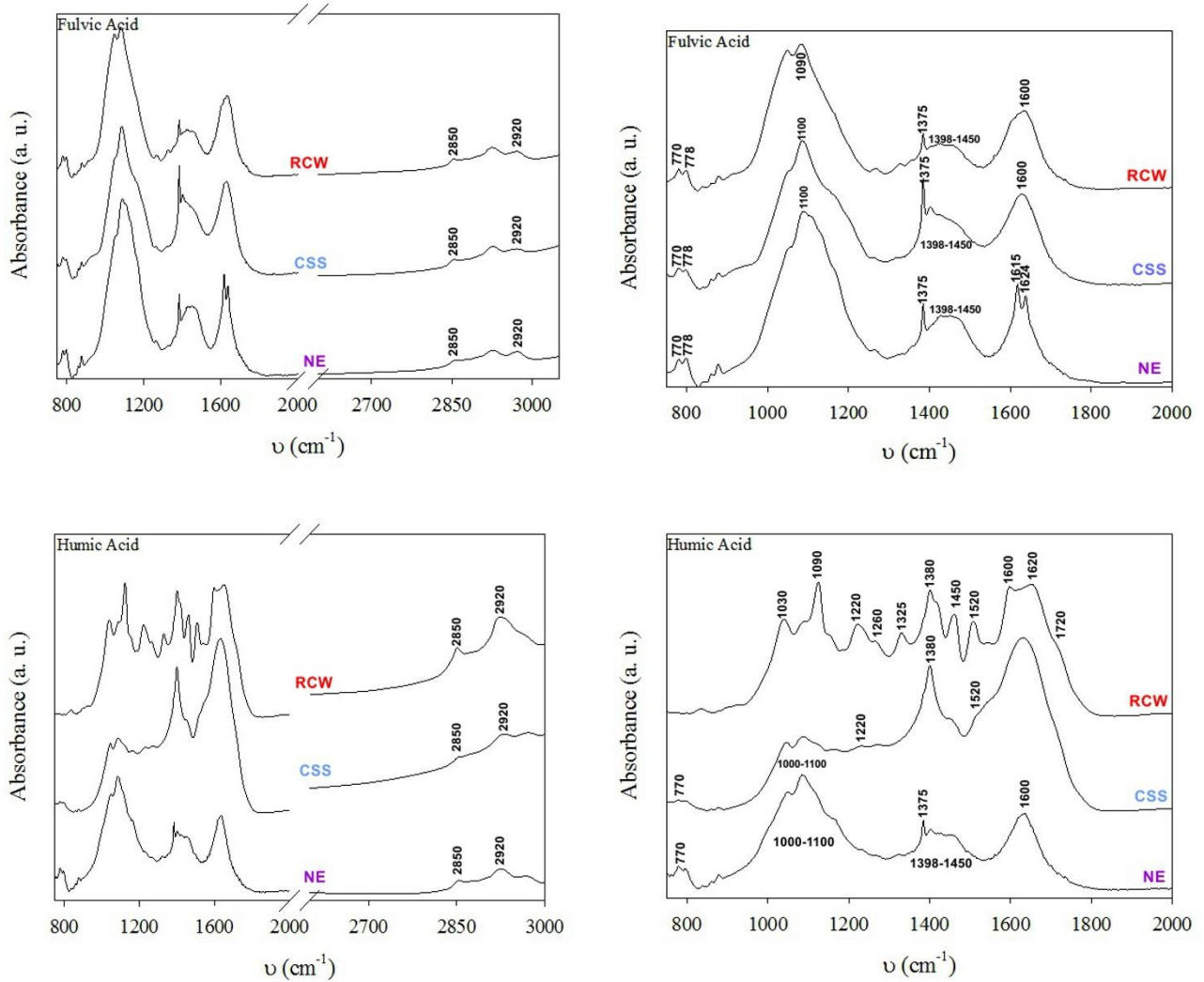
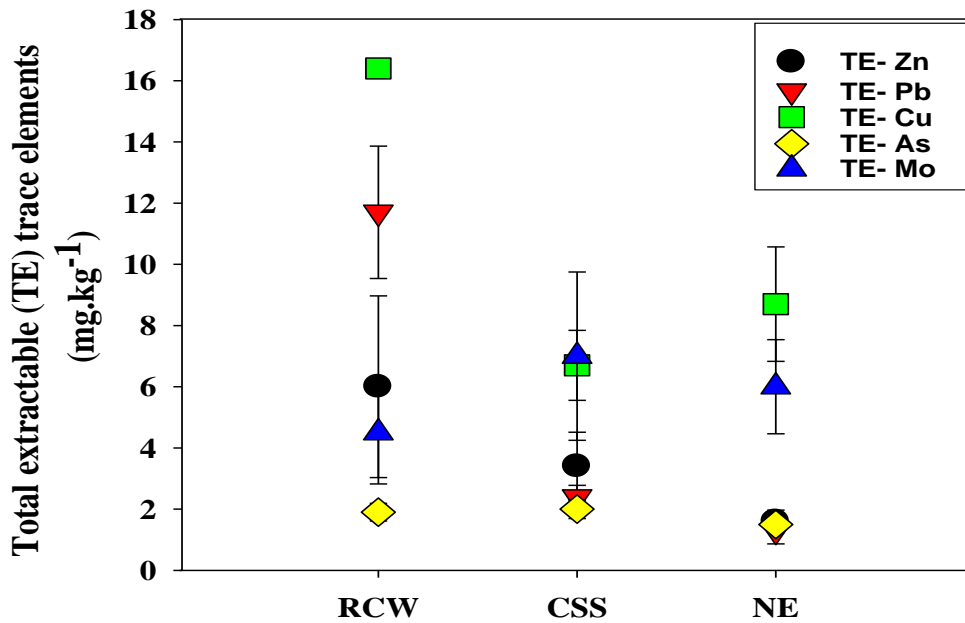


Figure 3: FTIR analysis of the treated (RCW and CSS) and untreated soils (NE). Typical absorption vibration bands of both humic and fulvic substances were labeled and assigned in the main text. The two amendments contribute to the formation of high molecular weight and humification substances which are highlighted with additional bands for the extracted humic acids.

3.6. Effect of organic amendments on trace element dynamics

The ecological effects of trace elements (TE), metals and metalloids, in soils are closely related to the concentration and speciation of the elements in the soil (Bruemmer et al. 1986). Whatever the elements, their contents are considerably higher than natural background (Baize, 2000) (Table 1), which confirms the high level of polymetallic contamination in the soils. Moreover, the addition of CSS and RCW to the soil has no significant effect on the total metal concentrations compared with the NE soil.

The contents of As, Cu, Mo, Pb and Zn associated to HS extracted from all soils are presented in Figure 4. Cu, Pb and Zn were extracted from the fractions bound to organic constituents by NaOH in higher amounts than Mo and As. Our results show that RCW added to the soil significantly increased the concentration of extractable Zn, Pb and Cu by 4.9 and 2 fold and slightly increased the extractable arsenic by 1.3 fold in the solution, whereas it decreased the extractable fraction of Mo by 0.8 fold compared with the NE soil. The effect of CSS added to the soil on the concentration of extractable trace elements was less than that shown by RCW: the addition of CSS increased the extractable Zn, Pb, Mo and As by 2.1, 1.8, 1.2 and 1.3 fold and decreased Cu by 1.3 fold compared with the NE soil. In general we can note that organic amendments (RCW and CSS) added to the soil increased the extractable trace elements especially in the case of RCW amendment in comparison with the NE soil.



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Figure 4: Total extractable Zn, Pb, Cu, As and Mo (from the NaOH extract) in mg.kg⁻¹

Humic substances constitute a large fraction of soil organic matter (SOM) and their binding capacities affect the mobility of TE in soils. Fulvic acids are the most mobile fraction and a major component of dissolved organic matter (DOM) in soil (Stevenson, 1994). Therefore, the presence of soluble organic matter (as fulvic acids) may be responsible for the solubilization of TE in soils whereas insoluble organic matter (such as humic acids) may contribute to TE immobilization in soils. Indeed, fulvic acid is characterized by smaller molecular size fractions and a high ability to form complexes with trace metals that are more mobile than those formed with humic acid (Stevenson, 1982). As Hsu and Lo point out (2001), knowledge about the distribution of TE between HA and FA fractions is essential for understanding and predicting the mobility of TE in contaminated soils after organic amendments. The distribution of TE in FA and HA fractions show a large variability depending on the chemical elements and the treatment (Figure 5).

Figure 5.a shows that Zn in the amended soils is more abundant in the fulvic acid fraction than in the humic acid fraction. The proportion of Zn bound to FA was about 23 % and 32 % higher than that bound to HA in the soils treated with RCW and CSS respectively. Almas et al. (2000) and Usman et al. (2004) found that the addition of sewage sludge increased the solubility of Zn due to the formation of soluble organo-

363 metallic complexes. Our results concerning Zn agree with Donisa et al. (2003), who studied the distribution
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2364 of trace metals between humic and fulvic fractions in natural soils. They found that Zn associated more with
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4365 fulvic acid than with humic acid. Boruvka and Drabek (2004) also found that 95.7% of Zn bound to Fulvic
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6366 acid compared to humic acid, whereas over 75% of Pb and Cu fractions bound to HA in treated and
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8367 untreated soils, e.g. in soil amended with CSS, 80 % of Cu associated with HA compared with 20 %
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11368 associated with FA (Fig. 5.b.c). He et al (1995) also found that more Cu in the compost was recovered in HA
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13369 than in FA. In a study of the irrigation of Mediterranean sandy soil by treated wastewater, Tarchouna Gharbi
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15370 et al. (2010) demonstrated that copper had a high affinity for the HA fraction. Concerning Pb and Cu, our
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17371 results do not agree with those reported by Donisa et al. (2003) or Boruvka and Drabek (2004), who
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20372 investigated the distribution of trace elements between the dissolved organic fraction and found that Pb and
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22373 Cu were more abundant in fulvic acid than in humic acid. Hsu and Lo (2001) isolated the HS from several
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24374 separated swine manures (SSW) to investigate the effect of HS on the leachability and availability of trace
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26375 elements. They found that more than 98% of Cu and Zn complexed with FA.

28 Arsenic is more abundant in the fulvic fraction than in the humic fraction for all soils. The proportion
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31377 of arsenic associated with fulvic acid was 13%, 8% and 35% higher than that associated with HA in the soils
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33378 treated with RCW, CSS and NE respectively (Fig. 5.d). Mo is about 60% more abundant in humic acid than
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35379 in fulvic acid in the soil treated with RCW and about 25% and 60% less abundant in HA than in FA in the
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38380 soil treated with CSS and NE respectively (Fig. 5.e). This result can be explained by the capacity of RCW
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40381 soil to provide a greater quantity of organic carbon in the form of fulvic acid than CSS soil.
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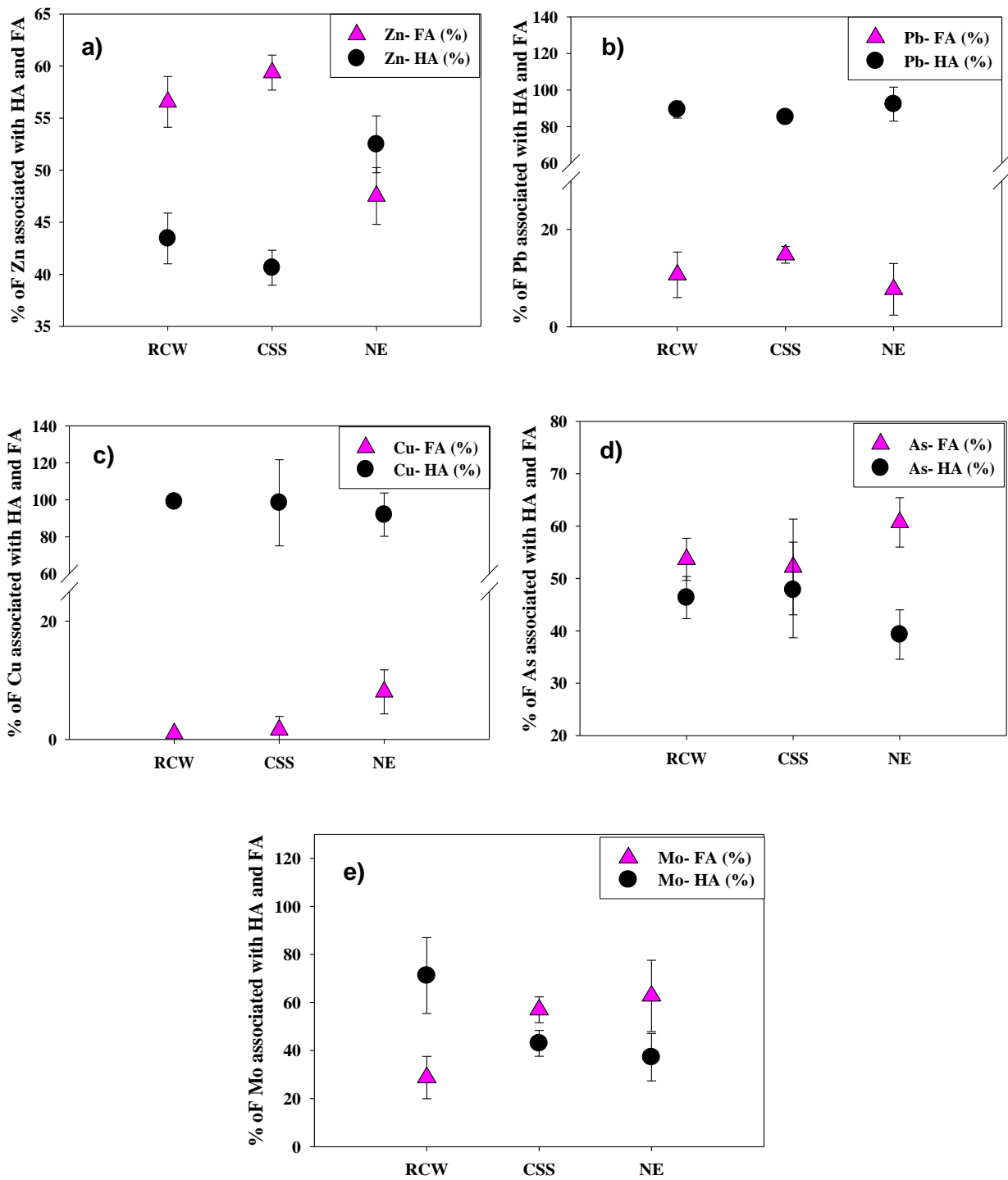


Figure 5: % of Zn, Pb, Cu, As and Mo associated with HA and FA.

392 **4. Conclusion**

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3393 In aided phytostabilization, the additions of organic amendments of various natures (ramial chipped
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5394 wood and and composted sewage sludge) were utilized: (i) for improve the physical, chemical and biological
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7395 properties of contaminated soils and (ii) to immobilize the trace elements and reduce their availability in
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10396 contaminated soils. This study showed that the quantity and quality of soil organic matter were modified
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12397 according to the type of organic amendments. Although the two amendments significantly increase the
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14398 content of SOM, only composted sewage sludge (CSS) increased the proportions of humic acids in soil
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16399 highlighting that added organic matter was more mature and stable in the time. In treated and untreated soils,
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18400 humid acids are the main organic fraction associated to Pb, Cu and Mo and contribute to their
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21401 immobilization in soil. However, formation of a large amount of soluble Zn-and As-fulvic acids complexes
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23402 can result in their mobilization in the soil profile, and then play an important role in term of contamination
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25403 risks. Moreover, whatever the type of organic amendments, an increase in the proportion of zinc associated
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27404 with fulvic acid is highlighted indicating a high risk of Zn leaching at short and long term.
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33
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