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Influence of organic matter transformations on the bioavailability of heavy metals in a sludge based compost

Florencio Ingelmo^{a,b,*}, Maria José Molina^a, Maria Desamparados Soriano^c, Antonio Gallardo^d, Leonor Lapeña^d

^a Centro de Investigaciones sobre Desertificación, CIDE (CSIC-UVEG-GV), Apartado Oficial, 46113 Moncada, Valencia, Spain

^b Instituto Valenciano de Investigaciones Agrarias (IVIA), Apartado Oficial, 46113 Moncada, Valencia, Spain

^c Escuela Técnica Superior del Medio Rural y Enología, Avda. Blasco Ibáñez 21, 46010 Valencia, Spain

^d Universitat Jaume I de Castelló, Campus de Riu Sec, 12071 Castellón, Spain

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ABSTRACT

The agricultural use of anaerobically digested sewage sludge (ADSS) as stable, mature compost implies knowing its total content in heavy metals and their bioavailability. This depends not only on the initial characteristics of the composted substrates but also on the organic matter transformations during composting which may influence the chemical form of the metals and their bioavailability.

The objective of this work was to examine the relationships between the changes in the organic matter content and humus fractions, and the bioavailability of heavy metals.

A detailed sampling at 0, 14, 84, and 140 days of the composting process was performed to measure C contents in humic acids (HAs), fulvic acids, (FAs) and humin, the total content of Zn, Pb, Cu, Ni, and Cd, and also their distribution into mobile and mobilisable (MB), and low bioavailability (LB) forms.

Significant changes of C contents in HA, FA, and Humin, and in the FA/HA, HA/Humin and $C_{\text{humus}}/\text{TOC}$ ratios were observed during composting. The MB and LB fractions of each metal also varied significantly during composting. The MB fraction increased for Zn, Cu, Ni, and Cd, and the LB fraction increased for Pb. Stepwise linear regressions and quadratic curve estimation conducted on the MB and LB fractions of each metal as dependent on the measured organic variables suggested that Zn bioavailability was mainly associated to percentage of C in FAs. Bioavailability of Cu, Ni and Cd during composting was associated to humin and HAs. Pb concentration increased in the LB form, and its variations followed a quadratic function with the $C_{\text{humus}}/\text{TOC}$ ratio. Our results suggest that the composting process renders the metals in more available forms. The main forms of metal binding in the sludge and their availability in the final compost may be better described when metal fractionation obtained in sequential extraction and humus fractionation during composting are considered together.

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1. Introduction

The agricultural use of anaerobically digested sewage sludge as an organic amendment to improve soil fertility is becoming increasingly important. The management of the raw sludge involves many problems such as pathogens, plant seeds, odors, and

a pasty structure with high water content. This later feature makes it hard to store and may lead to immobilization and volatilization of plant nutrients (Cambardella et al., 2003). One of the possibilities to convert sewage sludge into a marketable organic amendment or fertilizer is to co-compost it with different bulking agents, such as wood chips, thus obtaining a humus-like material that is easy to store (Gallardo et al., 2007). The addition of such a bulking agent for composting may optimize substrate properties such as air space, moisture content, C/N ratio, particle density, pH and mechanical structure, affecting positively the decomposition rate. In this sense, ligno-cellulosic by-products such as wood chips and sawdust are commonly used as bulking agents (Maboeta and van Rensburg, 2003; Pasda et al., 2005; Neves et al., 2009). In the case of anaerobically digested sewage sludges with high contents of nitrogen, heavy metals, and other toxic or phytotoxic substances, bulking

Abbreviations: C_{HA} , Carbon in humic acids; C_{FA} , Carbon in fulvic acids; C_{Humin} , C in hydrolysable humin; C_{humus} , (sum of C contents in FAs, HAs and hydrolyzable humin); TOC, Total organic carbon; X_{MB} , mobile and mobilisable metal form; X_{LB} , low bioavailability metal form.

* Corresponding author. Centro de Investigaciones sobre Desertificación, CIDE (CSIC-UVEG-GV), Apartado Oficial, 46113 Moncada, Valencia, Spain. Tel.: +34961220540; fax: +34961270967.

E-mail addresses: florencio.ingelmo@uv.es (F. Ingelmo), maria.j.molina@uv.es (M.J. Molina).

agents like sawdust are recommended because of the dilution effect (Banegas et al., 2007).

Composting of organic wastes has been defined as a well-established method, which leads to a stabilized product rich in humic substances that resemble native soil humic substances (De Bertoldi et al., 1996). In addition, the agricultural use of stable, mature sewage sludge-based compost means knowing its content (Cec, 1986; Royal Decree, 1990), and the biogeochemical forms of the heavy metals present (Hsu and Lo, 2001).

Most of the studies on the speciation of heavy metals have been carried out in raw or composted sludges-amended soils (Petruzzelli et al., 1994; Kunito et al., 2001; Maboeta and van Rensburg, 2003; Hanc et al., 2009). Only a few were dedicated specifically to study the bioavailability of the heavy metals during composting of sewage sludges (Amir et al., 2005; Liu et al., 2007; Tandy et al., 2009). Studies relating changes in bioavailability of heavy metals with changes in humus fractions during composting are scarce (Amir et al., 2005; Liu et al., 2007).

The speciation of each metal in the sewage sludge-based compost may depend not only on its initial chemical state in the sewage sludge which also depends on their nature and processing (Fuentes et al., 2004; Walter et al., 2006), but also on the organic matter transformations during composting. These could influence the metal distribution through metal interaction with the newly formed humic substances (Petruzzelli et al., 1994; Amir et al., 2005; Liu et al., 2007).

The objective of this work was to examine the relationships between the changes in the organic matter content and humus fractions, and the bioavailability of heavy metals. This was tested in a 70:30 (on wet basis) mixture of ADSS and wood chips with an initial C/N ratio of 30.4, during its aerobic batch composting at 30 °C of external temperature in an open type lab-scale reactor without lixiviation.

2. Materials and methods

2.1. Composting

The raw material used in the composting process was a mixture of sewage sludge and wood chips as bulking agent, in the ratio 70:30 (on a wet basis). Sewage sludge was an anaerobically digested dewatered cake of sludge (FACSA Sewage Treatment Plant in Castellón, Spain). The characteristics of the raw sludge were 94.3% moisture content, pH 8.51, EC 1.51 dS m⁻¹; 42.2% TOC; 6.37% total N; C/N 6.62; and total Zn, Pb, Cu, Ni, and Cd contents of 1660, 310, 256, 16.0, and 1.95 mg kg⁻¹, respectively (all results expressed in dry basis). The C/N ratio of the wood chips was 64.5, its moisture content was 8.5%, and its total N content was 0.83% (Gallardo et al., 2007). Normally, bulking agents have high C/N ratios, which can compensate for the low values of the sewage sludge because of the dilution effect (Banegas et al., 2007; Neves et al., 2009).

The pilot-scale composting experiments were carried out in five 65 L capacity open type lab-scale reactor without drainage of lixiviates. Aeration was controlled daily, moisture every five days, and mixture turned every 15 days. Composting was monitored for 140 days, when oxygen consumption finished (García et al., 1992). According to temperature measurements (Gallardo et al., 2007) composting developed in a first very active phase with high oxygen consumption until day 20; a second phase in which the activity dropped to a medium level until day 90; and a third phase with low activity, which lasted until day 140.

2.2. Physico-chemical analysis

To obtain representative samples for the physico-chemical analysis of the sludge based compost during the time of

composting, good homogenization was ensured, and five aliquots of about 80 g (on dry basis) were taken and mixed at every sampling date. Three replicates of each composite sample were analysed at 0, 14, 84, and 140 days of composting. The time intervals were determined according to the changes of composting temperature and oxygen consumption (Gallardo et al., 2007). To determine their main physico-chemical properties we followed standard methods (MAFF, 1996): organic carbon by partial oxidation with potassium dichromate, total nitrogen by the Kjeldahl method, and pH and electrical conductivity (EC), respectively, in a 1/2.5 and a 1/5 sample/water ratios. The total concentrations of metals were determined through inductively coupled plasma-ICP (EPA, 1990) using a Perkin Elmer ICP-5500 after the microwave digestion of the samples with HNO₃:HClO₄ (Polkowska-Motrenko et al., 2000).

Compost samples were extracted with 0.1 M NaP₂O₇ (pH 9.8) at room temperature using a sample/extractant ratio of 1/10. Each extraction was repeated 3 times. For each extraction step, the mixture was shaken for 3 h, centrifuged at 15,000 g for 15 min and the supernatant was filtered through a Whatman 31 filter paper. The combined alkaline extracts (soluble humic substances) were then acidified with concentrated H₂SO₄ to pH 1, left standing for 24 h in a refrigerator to allow the complete precipitation of HAs, and then centrifuged at 15,000 g for 30 min to separate the supernatant FAs fraction. Since the alkali-insoluble humin fraction may contain humic-like substances (i.e. proteinaceous compounds linked to decomposed ligno-cellulosic materials), we determined the hydrolyzed humin (Zaccheo et al., 2002). The hydrolyzed humin was obtained after acidification of the sample retained in the filter paper with concentrated 6 N HCl for 10 h, filtration and washing with deionized water. This fraction was considered as forming part of the humus in the compost. The total alkali extractable (soluble humic substances), the FAs, and the hydrolyzed humin were analyzed for C. The C in HAs was obtained by the difference between C in the total alkali extractable and C in FAs (MAFF, 1996). C_{humus} was obtained as the sum of C contents in FAs, HAs and hydrolysable humin.

Heavy metal fractionation for Zn, Cu, Pb, Ni, and Cd was determined according to Sposito's procedure (Amir et al., 2005). In each of the three replicates taken from the composite samples, a series of reagents were sequentially applied with a compost/extractant ratio of about 1/4. The sequence of reagents application to collect the medium -bioavailable fraction MB (mobile and mobilisable) was: H₂O (shaking during 2 h at 20 °C, three times); KNO₃ 0.5 M (shaking during 16 h at 20 °C); NaOH 0.5 M (shaking during 16 h at 20 °C), and EDTA 0.05 M (shaking during 16 h at 20 °C). Finally, to collect the low-bioavailable fraction LB (bound to sulphides; hardly mobilisable) the samples were treated with HNO₃ 4 M (shaking during 16 h at 80 °C). Metal concentration was measured after each step treatment, and referred to dry weight. All filtered supernatants were analyzed by ICP (EPA, 1990).

The levels of bioavailability considered in this work are:

- 1) Medium, MB (mobile and mobilisable fractions):

$$MB = X - H_2O + X - KNO_3 + X - NaOH + X - EDTA$$

- 2) Low, LB (Sulphides. Hardly mobilisable fraction):

$$LB = (X - HNO_3)$$

2.3. Statistical analysis

Statistical analyses were performed with the SPSS v.17.0 statistical software. A one-way ANOVA was used to detect the significant

effect of time of composting on different compost parameters. The Tukey's *t*-test was used for mean comparison and significant differences at 95% level on data obtained at the different composting times. To describe more clearly the metal and humus fraction variations through time, also linear and curvilinear adjustments were performed. In order to ascertain the best-fit model between variations in the metal fractions during composting and changes in the organic fractions, stepwise linear regressions and quadratic curve estimations were performed on the MB and LB fractions of each metal as dependent variables. The independent organic variables were C_{FA} , C_{HA} , C_{Humin} , and C_{FA}/C_{HA} , C_{HA}/C_{Humin} , and C_{humus}/TOC ratios.

3. Results and discussion

3.1. Compost properties

The main physico-chemical properties of the composted sludge at different times of the process, the ANOVA and the Tukey's *t*-test results are presented in Table 1.

Because of the high moisture content of the raw sludge, the ratio of sewage sludge and wood chips on a dry weight basis was 15:85. As shown by Pasda et al. (2005) this product is not easy to decompose because its high lignin/tannins ratio. This fact likely provoked that temperature in the reactors during the composting process was always below 35 °C. No significant changes were detected for pH, EC and total N. The high value of pH in the raw sewage sludge may compensate for the decrease of this parameter during composting (Amir et al., 2005; Liu et al., 2007). The pH during composting was in the optimal range for the development of bacteria and fungi (Zorpas et al., 2003).

Total organic C content (TOC) decreased significantly during composting (Table 1), which is consistent with the decomposition of the organic matter through microbe respiration in the form of CO₂ and even through mineralization. The overall decomposition observed in this work (37%) contrasts with the 60% observed by Jouraiphy et al. (2005) during 135 days of composting of a mixture of sewage sludge and green plant waste, and the 19.6% of Amir et al. (2005) during 180 days with straw as bulking agent. At difference with other authors (Soumaré et al., 2002), the organic matter decomposition during composting did not cause an increase in total N during the process. Although N variations were not significant, the trend was to decrease. In agreement with the results by García et al. (1995), it is interpreted because heavy metal concentration of the sewage sludge may have affected to certain extent the mineralization rate of N in our sewage sludge based compost.

The C/N ratio significantly decreased from 30.4 in the initial mixture to 21.6 at 140 days. This relatively high C/N ratio at the end of composting indicates that organic matter has not achieved an adequate stabilization (De Bertoldi et al., 1996), likely due to the quality of the bulking agent (Pasda et al., 2005).

Since metal loss by leaching did not occur in our experiment, we observed a continuous increase of total heavy metal concentration in the compost due to the weight loss during composting, the release of carbon dioxide and water, and the mineralization process as shown by Lazzari et al. (2000). Although the trend was to increase, no significant differences with time were detected for total Zn. The increase of the total metal concentration during composting was significant for other metals which had high concentration in the sludge (Pb and Cu), but also for metals with low concentrations (Ni, Cd). The total heavy metal concentrations in the obtained compost were below the maximum permitted in Spain for application of sewage sludge in soils (Royal Decree, 1990).

3.2. Heavy metals bioavailability

The sum of the amounts extracted by sequential extraction (MB and LB fractions, Table 2) for the most abundant metals (Zn, Pb, Cu, Ni), and also for Cd, was, respectively, almost four or two times lower than the total amount of metal. This result indicates that most metals are mainly bound to residual forms. Our results agree with those by Amir et al. (2005), who found recoveries of 20–30% using this sequential extraction procedure. The fact that the residual fraction is so abundant indicates that an important proportion of metal is probably occluded in minerals present in the sludge as has been referred by Wong et al. (2001) for some metals such as Pb.

For all metals except Pb, the concentration of the MB forms is higher than that in the LB forms (Table 2). This suggests that composting enhances the availability of most of metals. The MB and LB fractions of Zn vary in a quadratic function with time. The amount of Zn_{MB} increases to a maximum at day 84 and decreases thereafter to concentrations that are similar to those at day 14. Zn_{LB} follows the reverse trend (Table 2). It decreases to a minimum at day 84, and increases at day 140 to concentrations that are similar to those at the beginning of composting. This result suggests that important changes in the Zn speciation occur in the final period.

For Cu, Ni and Cd, the concentrations of the MB fraction follow a linear significant increase with time of composting. Cu_{LB} also linearly decreases with time although with the *b* parameter (absolute value) lower than the corresponding parameter of the Cu_{MB} model. This result indicates that the increase in the MB fraction of Cu occurs at expenses of both the LB fraction and the residual fraction. The changes in the LB fractions of Ni and Cd during composting follow a curvilinear trend, reaching a maximum at day 84 and decreasing thereafter, especially Ni. The decrease of the Ni_{LB} in the last period of composting suggests that some moieties of the Ni_{LB} become more available and increase the Ni_{MB} fraction, whereas some other could join the residual fraction. For Cd, the decrease in the LB fraction is lower than the corresponding increase of the MB fraction, and suggests that some Cd_{MB} forms also at expenses of residual Cd. The MB fraction of Pb remains constant

Table 1

Physico-chemical properties (*n* = 3) of the sludge based compost at different days of composting. All results expressed in dry basis.

Time ^a	Moisture (%)	pH	EC (dSm ⁻¹)	TOC (%)	Total N (%)	C/N	Total Zn mg kg ⁻¹	Total Pb	Total Cu	Total Ni	Total Cd
0	71.8 a	7.07 a	1.06 a	50.0 a	1.64 a	30.4 a	259.8 a	45.3 a	37.7 a	2.24 a	0.29 a
14	71.0 a	7.03 a	1.14 a	45.2 b	1.54 a	29.3 ab	262.1 a	49.5 ab	41.3 a	2.38 a	0.33 ab
84	69.0 b	7.01 a	1.16 a	37.4 c	1.51 a	24.8 ab	267.1 a	53.7 ab	43.1 ab	2.69 b	0.40 ab
140	68.8 b	7.01 a	1.12 a	31.3 d	1.45 a	21.6 b	278.2 a	57.4 b	49.5 b	2.76 b	0.45 b
ANOVA											
F	15.942	1.848	0.243	1960.23	1.896	14.340	2.924	14.141	12.446	19.310	12.365
p	0.001	0.217	0.864	0.000	0.209	0.001	0.100	0.001	0.002	0.001	0.002

^a Days of composting. Mean value followed by different letters is statistically different (Tukey's *t*-test, *p* < 0.05).

Table 2
Evolution of heavy metals in medium bioavailable forms (MB) and in low bioavailable forms (LB), and evolution of humic (FA, HA), humic-like substances (hydrolysable humin), and their ratios during composting. All metal concentrations are expressed in mg kg⁻¹ dry matter ($n = 3$).

Dependent Variable	Means and Tukey's <i>t</i> -test Time of composting (days)				ANOVA curvefit		Best-fit Model parameters			
	0	14	84	140	F	P	a	b	c	R ²
Zn _{MB}	39.4 a	46.2 b	53.6 c	46.1 b	80.533	<0.001	40.41	0.347	−0.002	0.947
Zn _{LB}	25.6 a	19.4 b	13.2 c	23.4 a	94.132	<0.001	24.77	−0.343	0.002	0.954
Cu _{MB}	5.09 a	6.15 b	7.00 c	8.92 d	153.23	<0.001	5.349	0.024		0.939
Cu _{LB}	4.34 a	4.18 a	3.78 b	3.45 c	160.85	<0.001	4.300	−0.006		0.941
Pb _{MB}	5.27 a	5.42 a	5.43 a	5.25 a		0.7540				
Pb _{LB}	6.06 a	6.96 b	7.99 c	8.49 d	87.080	<0.001	6.263	0.031	−0.0001	0.951
Ni _{MB}	0.27 a	0.24 ab	0.29 ab	0.46 b	34.852	<0.001	0.234	0.001		0.777
Ni _{LB}	0.31 a	0.36 ab	0.38 ab	0.14 b	16.597	0.0010	0.309	0.001	−0.00004	0.787
Cd _{MB}	0.06 a	0.07 b	0.09 c	0.13 d	222.03	<0.001	0.065	0.0004		0.957
Cd _{LB}	0.08 a	0.09 b	0.11 c	0.10 d	882.99	<0.001	0.081	0.0001	−0.000004	0.995
C _{FA}	5.80 a	6.72 b	8.80 c	9.07 c	64.860	<0.001	6.217	0.023	−0.0002	0.866
C _{HA}	2.11 a	2.24 a	4.82 b	5.87 c	354.86	<0.001	2.057	0.029		0.973
C _{Humin}	7.30 a	8.34 a	11.4 b	13.0 c	100.51	<0.001	7.616	0.040		0.941
C _{FA} /C _{HA}	2.75 a	3.02 a	1.82 b	1.54 c	84.349	<0.001	2.904	−0.010		0.894
C _{HA} /C _{Humin}	0.29 a	0.27 a	0.42 b	0.45 c	104.43	<0.001	0.277	0.001		0.913
C _{humus} /TOC	0.30 a	0.38 b	0.67 c	0.89 d	1383.5	<0.001	0.315	0.004		0.993

Metal fraction (mg kg⁻¹ dry basis): MB = Mobile + Mobilisable (Bioavailable), LB = low bioavailability. FA = % C Fulvic acids; HA = % C Humic acids; TOC = % Total organic carbon. Means in a row followed by the same letter are not significantly different at $\alpha = 0.05$ according to the Tukey's *t*-test. P = P values of the F test in ANOVA curvefit for linear and quadratic models. Model parameters: a = constant; b = coefficient of x in linear and quadratic models; c = coefficient of x² in the quadratic model. Independent variable = Time of composting (days).

during composting, but the curvilinear trend of increase observed for Pb_{LB} indicates that this form likely increases at expenses of the residual forms during composting and reaches a maximum at the end of the process. Although the quadratic fit may indicate a further decrease, this cannot be tested because of the lack of data beyond 140 days. Overall, the results for Pb indicate that this metal accumulates in the very stable organic fractions and unavailable mineral forms.

Increased availability for Zn and Cu through composting is in agreement with results obtained by several authors (Wong et al., 2001; Amir et al., 2005). The observed increase of Cd availability is in agreement with results by McGrath and Cegarra (1992), who found high extractable Cd levels in sludge-amended soils. For composted sludges Walter et al. (2006) found increased mobility for Zn, Cu and Cd during composting. Richards et al. (1997) found reductions in Pb mobility and an increase in Cd and Cu mobility because of the composting process.

3.3. Humic substances

The changes in C in humic (FAs, HAs) and humic-like substances (hydrolyzed humin) and their ratios during composting are shown at the bottom of Table 2. All of them changed significantly with time of composting indicating that transformations of the organic matter and humification have occurred. C in FAs increased mainly during the initial phases of composting. The best model describing the FAs changes is quadratic and reflects that stop increase beyond day 84 of composting. C in FAs was higher than that in HAs thus indicating that among the soluble humic substances the most abundant are those of low molecular weight. Some of them may have polymerized in the last phases of composting likely forming more condensed structures such as HAs thus explaining some of the increase of C in HAs and also the linear decrease of the C_{FA}/C_{HA} ratio (Table 2).

C in HAs increased linearly during composting. Its rate of increase was similar or even higher than that of C in FAs, as deduced from the b parameters of models. The linear rate of increase observed for C in hydrolyzed humin is higher than that of C in HAs as deduced by comparison of the corresponding b parameters. This suggests that both FAs and HAs of the sludge may polymerize in the form of humin. The abundance of aliphatic compounds in sewage sludges may have

a negative effect on the formation of the condensed structures typical of the true HAs (García et al., 1989). Likely, the dilution of the sludge with the bulking agent in our compost has lowered this negative effect and even facilitated HAs and likely humin formation through polymerization of FAs with some ligno-cellulosic derivatives coming from the partial degradation of the wood chips. The slope of the linear model describing the changes of the HA/Humin ratio (Table 2) is an order magnitude lower (absolute value) than that of FA/HA ratio. This result may indicate that the transformation of FAs into HAs is higher than that of HAs into humin, but also that some compounds in humin may transform into HAs.

Finally, the progressive increase of the C_{humus}/TOC ratio indicates that the proportion of humified organic matter (sum of FAs, HAs, and hydrolyzed humin) increases linearly through composting.

3.4. Metal fraction-humic substances relationships

Table 3 summarizes the best-fit models (highest R²) containing the humus fractions in the compost that are most related to the

Table 3
Best-fit models for major metal fractions varying in the sewage sludge compost and C in humus fractions during composting.

Dependent variable	Model	Best-fit Model parameters			
		Coefficient	SE	p-value	R ²
Zn _{MB}	Constant	8.271	2.897	0.019	0.975
	C _{FA}	14.919	1.412	<0.001	
	C _{Humin}	−7.515	0.848	<0.001	
Cu _{MB}	Constant	3.538	0.304	<0.001	0.965
	C _{humus} /TOC	5.785	0.499	<0.001	
	Constant	3.578	0.533	<0.001	0.982
Pb _{LB}	C _{humus} /TOC	10.330	2.051	0.011	
	(C _{humus} /TOC) ²	−5.418	1.710	<0.001	
Ni _{MB}	Constant	0.588	0.031	<0.001	0.990
	C _{humus} /TOC	0.908	0.041	<0.001	
	C _{FA}	−1.030	0.007	<0.001	
Cd _{MB}	Constant	0.033	0.004	<0.001	0.951
	C _{humus} /TOC	0.101	0.007	<0.001	
	Constant	−0.084	0.104	0.440	0.990
C _{humus} /TOC	C _{Humin}	0.090	0.030	0.018	
	C _{HA}	0.073	0.029	0.036	
	C _{FA}	−0.069	0.032	0.065	

changes in the main metal forms during composting. FAs and humin explained the Zn_{MB} fraction. The stepwise regression procedure selected C_{humus}/TOC ratio as the independent variable predicting metal fractions of the rest of metals. The C_{humus}/TOC ratio was also dependent on the three humus fractions considered in this work. The best model explaining the variations of the C_{humus}/TOC is shown at the bottom of the table. C_{humus}/TOC ratio increases when C in hydrolyzed humin and HAS increase, and when C in FAs decreases.

Overall, results in Table 3 suggest that the bioavailability of metals clearly depend on the organic compounds present and formed during composting, which may increase or restrict it.

Regressions indicate that Zn availability is positively associated with the FA fraction and negatively with humin. This result agrees with those by Moreno et al. (1996) who questioned the capability of Zn to form complexes with organic compounds. Alloway and Jackson (1991) found Zn associated to organic matter of low molecular weight. The negative dependence with humin may be indicating a decrease in Zn bioavailability at the end of the composting process due to a relative decrease of FAs amount in much more stable forms such as humin.

The C_{humus}/TOC ratio explains both Cu_{MB} and Cd_{MB} fractions. Cu_{MB} increases at expenses of the LB fraction (Table 2), and this increase is explained by the increase of C in humin and HAS at expenses of transformations of the sulphide forms. It follows that more than a half of Cu_{MB} must be attached to alkali-insoluble EDTA extractable organic forms such as humin, and the rest bound to alkali soluble HAS. This explanation also follows for Cd, although, as deduced from data (Table 2), Cd_{MB} increases at expenses of the residual form of this metal.

Ni_{MB} increases when C_{humus}/TOC ratio increases and C in FAs decreases because some FAs polymerize into HAS and humin. These results suggest that Ni_{MB} follows the same trend as Cu and Cd, and the reverse trend as Zn_{MB} .

As indicated in Table 1, the order of abundance of Cu, Ni, and Cd in the sludge is $Cu > Ni > Cd$. However, comparing the amount of metal in the MB fraction at the beginning and at the end of composting, their relative availability increase in the order $Cd_{MB} > Ni_{MB} = Cu_{MB}$. This suggests that the main factor explaining their bioavailability during composting was not the initial metal concentration but the stability of complexes with humic-like substances and HAS, which likely increase in the order $Cu > Ni > Cd$. Other authors (Canet et al., 1997) have also attested to the high stability of Cu-organic matter complexes. Soler Rovira et al. (2010) found that the complexing capacity of Cu (II) increased as the humification degree increased. Our results suggest that it may occur also for Ni and Cd.

Finally, the model for Pb differs from the rest of metals. Since the MB fraction did not change through composting (Table 2), the increase of the Pb_{LB} fraction in a quadratic model with the C_{humus}/TOC ratio may indicate the amount of residual Pb changing to LB forms. The quadratic fit would open the question to a further decrease of Pb_{LB} forms (decreasing branch of the curve) depending on the compost maturation.

4. Conclusions

The 140 days composting process of a mixture of sewage sludge and wood chips (C/N ratio of 30.4), resulted in a product with a relatively high C/N ratio of 21.6, a relatively low stabilization of the organic matter if considered the dominance of FAs over HAS, and total heavy metal concentrations below the maximum permitted for land application. With exception of Pb, the relative bioavailability of metals increased with composting. Zn bioavailability was mainly associated to percentage C in FAs. Bioavailability of Cu, Ni and Cd during composting was associated to percentage C in humin

and HAS. Pb concentration increased in unavailable forms, and followed a quadratic function of the C_{humus}/TOC ratio.

Our results suggest that the composting process renders the metal in more available forms. The main forms of metal binding in the sludge and their availability in the final compost may be better described when metal fractionation obtained in sequential extraction and humus fractionation during composting are considered together.

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