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EFFECT OF TEMPERATURE CONDITIONS ON Cu, Ni, Zn AND Fe COMPLEXATION BY HUMIC SUBSTANCES DURING SEWAGE SLUDGE COMPOSTING

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Abstract

The aim of this study was to determine how temperature conditions (max. 72°C, 81°C and 43.5°C) during sewage sludge composting affect the distribution of organically bound heavy metals in humic substances (HS). To accomplish this, the content of Cu, Ni, Zn and Fe was measured in the two main fractions of HS, humic acids (HAs) and the fulvic fraction (FF). In the feedstock, the FF was mainly responsible for binding all metals except Ni. During composting in the two series with thermophilic conditions, the content of Cu and Ni in HAs increased considerably. However, in the third series this increase did not take place. Zn content in HAs nearly doubled in the first two series, but 92-94% of Zn still remained in the FF. Although Cu, Ni, and Zn content in stable-HAs increased in all three series, the increase was much greater in the two series with higher temperatures. Fe distribution was not affected by composting. These results may indicate that thermophilic conditions during composting of sewage sludge should be favored to reduce the risk of heavy metal pollution when it is used as a soil amendment.

Key words: fulvic fraction, heavy metals complexation, labile humic acids, sewage sludge compost, stable humic acids

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1. Introduction

Composting is one of the most desirable methods of disposal for many organic wastes, including sewage sludge. Compost is an organic fertilizer containing humic substances and nutrients (Li et al., 2017; Rivero et al., 2004; Warman and Termeer, 2005). Soil amendment with composts provides a natural source of macronutrients (N, P, K) for soils that are deficient in these components. The value of compost as fertilizer is also due to its high content of micronutrients (Ayuso et al., 1996). Moreover, compost improves soil structure by improving the aeration of heavy soils or increasing the water capacity of light soils (Stuckey and Hudak, 2001). However, it should not be used in agriculture

or reclamation of degraded soils if it has a high total metal concentration. It is known that the bioavailability and mobility of heavy metals and their related eco-toxicity depends more on their specific chemical forms or ways of binding than on total metal content (Gupta and Sinha, 2007). In sewage sludge composts, the chemical forms of metals depend less on their initial chemical state in the sewage sludge used as a feedstock, and more on processes during composting, e.g. stabilization and humification of organic matter, metal solubilization by pH decrease, metal biosorption by microbial biomass or metal complexation with newly formed humic substances (Amir et al., 2005). These processes redistribute metals from their original fractionation pattern to a new one during the process of composting. One of the

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characteristics of the composting process is the humification of organic matter, which increases HS content, including HAs. Both humic and fulvic acids are known to significantly influence the mobility and bioavailability of heavy metals in soils (Stevenson, 1994). This is due to the presence of many functional groups in HAs and fulvic acids (FAs). It is mainly humic carboxylic -COOH and phenolic -OH groups that are involved in the formation of metal-humic complexes (Spark et al., 1997).

Because of the properties of humic substances, they are considered to be important for the distribution and mobility of metals in the environment, especially as metal-HS complexes. So far, research on these complexes has been mainly conducted with various kinds of soils (Borůvka and Drábek, 2004; Donisa et al., 2003; Zacccone et al., 2008) or soils amended with various organic materials (Wang et al., 2010). Some data are also available on metal complexation with humic or fulvic fractions isolated from water (Hiraide, 1992) or solid materials, e.g. coal (Erdogan et al., 2007) and corn tissue (Evangelou and Marsi, 2001). However, there are not many investigations of metal binding to HS during composting, despite the fact that compost is commonly used as a fertilizer or a soil amendment. A detailed study of metal distribution in composts should include not only conventional sequential extraction procedures as has been done in other studies (Iwegbue et al., 2007; Smith, 2009), but should focus on metal distribution among the individual fractions of humic substances, i.e. humic and fulvic acids. In addition to metal distribution in finished composts, it is important to obtain knowledge of how the process of composting and humification changes metal distribution over time.

It is known that during composting the concentration of FAs decreases, while that of HAs increases. Because metals are more stable when bound to HAs, higher HA concentrations could increase the stability of metals in compost or soils amended with compost. A better understanding of this process would provide scientific and practical benefits, such as the ability to determine compost quality and to predict metal leaching and bioavailability. Therefore, this study aimed to determine how the distribution of organically bound heavy metals in HS fractions changes during sewage sludge composting at different temperatures. The distribution of Cu, Ni, Zn and Fe among the FF, and among both labile (L-HAs) and stable (S-HAs) humic acids was determined.

2. Material and methods

2.1. Sewage sludge composting

Composting was performed in a two-stage system: the first stage was an aerated bioreactor, and the second, a windrow that was turned weekly. The bioreactor was equipped with an aerated system that enabled regulation of the quantity of air supplied. Air was pumped into the bioreactor through its floor. Inside the bioreactor, temperature sensors were

installed, enabling measurement of temperature with a precision of 0.1°C. The bioreactor was covered with a 10 cm layer of mature compost to insulate it and minimize water loss. This cover also acted as a biofilter to minimize odor emissions. A detailed description of the bioreactor/windrow has been presented in an earlier paper by Kulikowska and Klimiuk (2011).

2.2. Feedstock materials

For composting, dewatered sewage sludge was obtained from a municipal wastewater treatment plant (MWTP), working as an SBR system. The sewage sludge was characterized by high moisture (83-84%), low TOC concentration (33-35%) and high nitrogen concentration (6-7%). The concentrations of other components in the sewage sludge were as follows: P, 1.51-1.98% d.w.; K, 0.51-0.62% d.w.; Ca, 2.04-2.32% d.w.; Mg, 0.43-0.72% d.w.; Mn, 0.25-0.33% d.w.; Cd, 0.36-0.43 mg kg d.w.⁻¹; Cu, 79.3-112.4 mg kg d.w.⁻¹; Ni, 22.3-41.2 mg kg d.w.⁻¹; Pb, 9.8-13.2 mg kg d.w.⁻¹; Zn, 321-387 mg kg d.w.⁻¹; Hg, 0.02-0.04 mg kg d.w.⁻¹. Due to the duration of composting, sewage sludge for each series was collected at different time intervals, which affected its characteristics. Owing to the high moisture content and low C/N ratio (5-6) of the sludge, it was mixed with lignocellulosic materials. Rape straw, grass and wood chips were used as structural materials. There were three series, differed in feedstock composition (Table 1). In each series, the content of sewage sludge was 60% (w/w), rape straw from 2 to 10% (w/w) and grass between 0 and 15% (w/w). To obtain different maximum temperatures and durations of the thermophilic phase, the composition of the feedstock and aeration intensity were varied because these are the main factors that influence the temperature and duration of the thermophilic phase. Series 1-3 differed in the share of lignocellulosic materials (wood chips, rape straw, grass) (Table 1) and aeration rate (0.5–1.5 l kg⁻¹ min⁻¹). The relatively large share of grass and rape straw in series 3 was chosen because the author's previous study found that this composition (15% grass, 7% rape straw) resulted in the lack of a thermophilic phase (Kulikowska and Klimiuk, 2011). The low aeration intensity in series 2 was chosen because it has been found to cause compost overheating (Kulikowska and Gusiatin, 2015). Addition of lignocellulosic materials decreased the moisture content to 64-74% and raised the TOC/N ratio to 12.2-16.1. The ratio between organic matter and nitrogen (OM/N) ranged from 22.7 to 29.9 (Table 2). The composting process was conducted for 170 d, including decomposition of organic materials and sanitation in the bioreactor (10 d), and compost maturation in the windrow (160 d).

2.3. Analytical methods

Samples were taken throughout 170 days of composting. Each time approximately 1.5 kg of material was collected from different points of the bioreactor/windrow.

Table 1. Percentage of individual components in the feedstocks in series 1-3

Series No.	Sewage sludge (% w/w)	Inoculation (% w/w)	Wood chips (% w/w)	Rape straw (% w/w)	Grass (% w/w)
1	60	15	15	10	0
2	60	3	20	2	15
3	60	3	15	7	15

Table 2. Selected physico-chemical properties of the feedstock in series 1-3

Series No.	Moisture (%)	OM ^a (%)	TOC (%)	Total N (%)	OM/N	TOC/N
1	68.8 (±4.2)	61.6 (±3.2)	33.2 (±1.2)	2.06 (±0.22)	29.9	16.1
2	63.7 (±3.9)	62.3 (±3.9)	30.9 (±1.6)	2.42 (±0.19)	25.7	12.8
3	74.0 (±3.2)	58.0 (±4.4)	32.5 (±2.6)	2.55 (±0.12)	22.7	12.2

Note: a - organic matter

The samples were mixed, dried at 105°C and passed through a 0.5 mm sieve. Total organic matter was determined by ignition of the samples at 550°C (PN-Z-15011-3:2001) and total organic carbon with a TOC analyzer (VCSN, Shimadzu). Total nitrogen was determined by the Kjeldahl method (PN-Z-15011-3:2001).

2.3.1. Extraction of humic substances (HS)

Humic substances (HS) and individual fractions (HAs and FF) were isolated by conventional procedures described by Boratyński and Wilk (1965). Additionally, before extracting the HS, the compost samples were washed with distilled water in order to eliminate soluble non-humic substances (e.g. sugars and proteins), which can interact with HS (Amir et al., 2006). Then, the samples were defatted with a mixture of chloroform and methanol (Jouraiphy et al., 2005) in a microwave oven. After that, first 0.1 M Na₄P₂O₇ at pH=7, and then 0.1 M NaOH at pH=12 were used as extractants. Na₄P₂O₇ can dissolve the free macromolecules ionized at neutral pH, and the macromolecules weakly bound to mineral surfaces through cation bridges (i.e. L-HAs). NaOH dissociates all acidic functional groups and dissolves macromolecular structures (i.e. S-HAs). Sequential extraction using pyrophosphate, followed by sodium hydroxide, enabled separation of L-HAs and S-HAs from total HAs. A detailed procedure for the extraction is described in an earlier study by Kulikowska and Klimiuk (2011).

2.3.2. Analysis of heavy metals

To determine total metal (Cu, Ni, Zn and Fe) concentration in the feedstocks and composts, the dried samples were digested in a microwave oven (MARSXpress, CEM, USA) using a HCl:HNO₃ mixture at a 3:1 ratio (V/V), filtrated through Whatman 42 filter papers and analyzed by a flame atomic absorption spectrometer (FAAS) (Varian AA280FS, Australia), equipped with a sample introduction pump system. The accuracy of metal analysis using the FAAS method was checked by analysis of CRM 142 R reference material. Metal content in L-HAs, S-HAs and FF was directly measured in supernatants. For preparation of calibration curves, stock standard

solutions of a given metal were purchased from Sigma-Aldrich Co. (Poland). All measurements were performed in three repetitions. Data was statistically evaluated using STATISTICA 10.0 (StatSoft, Inc.). The ANOVA test was used, and p<0.05 was considered to be significant.

3. Results and discussion

3.1. Organic matter and temperature evolution during sewage sludge composting

During the first stage of composting, thermophilic conditions occurred after 2 days in both series 1 and 2 (Fig. 1). In series 1, the temperature of the composted materials reached a maximum of 72°C. In series 2, the materials remained at 81°C for 3 consecutive days, which means that in this series the compost was overheated. After moving the composted biomass to the windrow, the temperature remained high in series 1 for a further 12 d, whereas in series 2 for over 20 days. Then, in both series, the temperature progressively decreased until it was similar to that of the ambient air. In series 3, the temperature reached a maximum of 43-43.5°C in the bioreactor, and never exceeded 39°C in the windrow (Fig. 1). This indicates that the feedstock was not hygienized because thermophilic conditions were not obtained.

During composting, the loss of OM was calculated according to the equation given by Paredes et al. (2000). In all series, OM concentration decreased notably; the greatest decrease took place in the first 50 days of composting (Fig. 2). However, due to the absence of thermophilic conditions, OM loss in series 3 (33%) was less than in series 1 (41%) and series 2 (43%). During composting, organic matter is not only mineralized, but also humified, increasing HS concentration. This has been confirmed by numerous investigations (Amir et al., 2006; Domeizel et al., 2004; Jouraiphy et al., 2005; Paredes et al., 2002).

Because HS interact with metal ions, humification and the resulting increase in HS concentration affect metal speciation and mobility in compost. Changes in HS concentrations and their fractions (HAs and FF) are presented in Table 3. In the feedstocks, the highest concentration of HS was observed in series 3 (165.4 mg C/g OM).

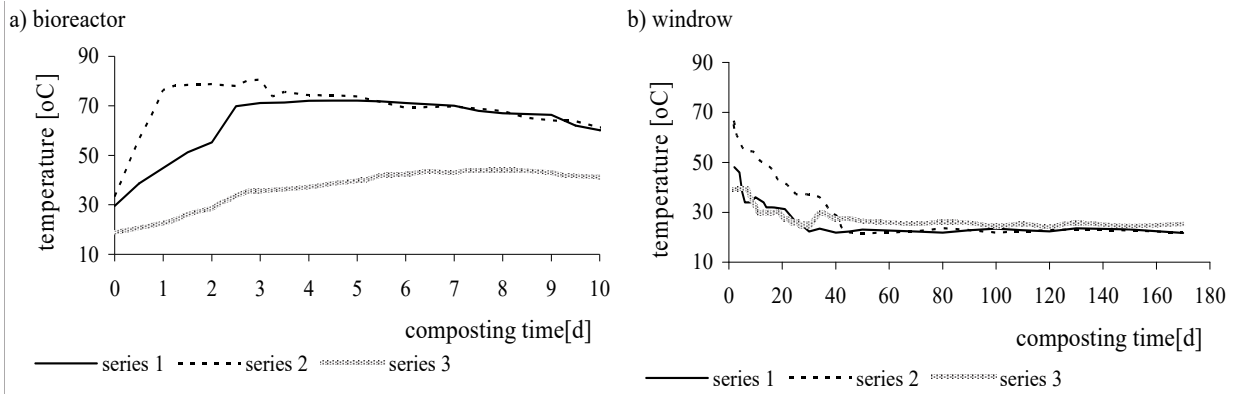


Fig. 1. Temperature profiles during sewage sludge composting

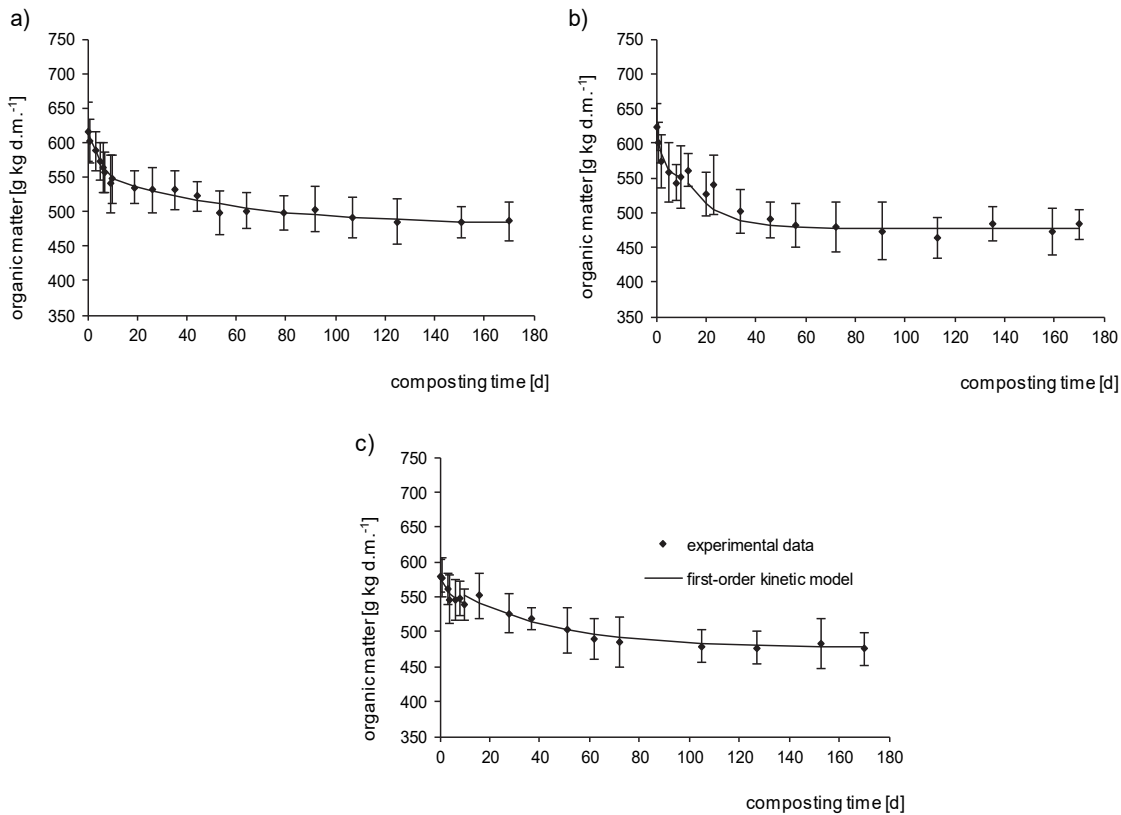


Fig. 2. Changes in organic matter during sewage sludge composting: a) series 1, b) series 2, c) series 3 (error bars represent standard deviation, $n = 3$)

In series 1 and 2 it was 1.6-1.7-times lower than in series 3. During composting, HS concentrations increased considerably in series 1 and 2, by 46 and 43%, respectively. In series 3, however, this increase was less than 20%. The low degree of humification (i.e. the relatively low increase in HS content) in series 3 is likely due to the lack of a thermophilic phase. These temperatures favor the growth of microorganisms that decompose lignocelluloses and form humus precursors.

The most intense lignin degradation occurs in the thermophilic phase because the microorganisms responsible for this process need carbon sources other than these provided by lignin, especially carbohydrates produced by degradation of cellulose

and hemicellulose (Sánchez-Monedero et al., 1999). In the feedstock in series 1 and 3, the FF predominated in HS (high FF/HAs ratio); whereas in series 2, the concentrations of HAs and the FF were similar (FF/HAs ratio of 1.03). In all series, HAs increased and the FF decreased during humification, with a corresponding decrease in FF/HAs ratio. In mature composts, however, only series 2 contained more HAs than FF (FF/HAs = 0.64); the FF/HAs ratio was 1.02 in series 1, and 1.25 in series 3. The high FF/HAs ratio in series 3 could have been caused by the lack of thermophilic conditions, which can reduce the polymerization of the FF to HAs (Kulikowska and Klimiuk, 2011). Composts contain some labile HAs (L-HAs) and/or stable HAs (S-HAs).

Table 3. Changes in humic substances (HS) concentration during sewage sludge composting in series 1-3

Series 1					Series 2					Series 3				
Time (d) ^a	HS ^b	HA in HS (%)	FF/HA	S-HA in HA (%)	Time (d) ^a	HS ^b	HA in HS (%)	FF/HA	S-HA in HA (%)	Time (d) ^a	HS ^b	HA in HS (%)	FF/HA	S-HA in HA (%)
0	97.3 (±3.4)	35.5 (±0.7)	1.81	85.4 (±1.6)	0	104.3 (±5.4)	49.2 (±0.5)	1.03	79.3 (±1.3)	0	165.4 (±4.4)	48.3 (±1.4)	1.54	89.0 (±0.9)
16	105.4 (±5.4)	36.0 (±0.9)	1.77	87.2 (±1.9)	14	108.5 (±3.4)	50.9 (±0.7)	0.96	76.0 (±1.2)	16	165.9 (±4.1)	37.6 (±0.9)	1.66	87.4 (±1.0)
26	105.5 (±5.8)	40.7 (±1.1)	1.45	85.2 (±2.1)	18	109.9 (±4.1)	53.1 (±1.3)	0.88	71.3 (±1.9)	23	175.0 (±6.4)	40.0 (±1.1)	1.50	87.4 (±1.2)
35	114.0 (±4.3)	43.2 (±0.8)	1.32	87.9 (±2.4)	24	121.7 (±4.2)	52.4 (±1.1)	0.91	74.2 (±1.6)	28	171.0 (±4.8)	43.6 (±0.9)	1.29	87.3 (±1.3)
44	116.3 (±3.8)	43.9 (±0.9)	1.28	85.6 (±2.4)	34	123.5 (±3.5)	55.0 (±1.4)	0.82	71.2 (±1.5)	37	176.0 (±5.2)	41.1 (±0.9)	1.43	87.0 (±1.2)
53	120.0 (±6.2)	44.0 (±1.2)	1.28	86.6 (±2.6)	44	125.0 (±5.1)	56.0 (±1.7)	0.79	78.4 (±1.9)	51	187.0 (±4.0)	41.1 (±0.9)	1.43	89.0 (±1.4)
64	118.8 (±4.1)	44.1 (±0.8)	1.27	87.5 (±2.5)	60	128.7 (±3.8)	55.7 (±1.5)	0.80	71.6 (±2.1)	62	181.8 (±6.4)	44.0 (±1.4)	1.27	88.2 (±1.5)
79	124.4 (±5.7)	45.3 (±1.2)	1.21	86.5 (±2.4)	73	147.5 (±1.9)	56.1 (±1.3)	0.78	71.1 (±1.9)	72	179.0 (±6.3)	46.0 (±1.5)	1.17	88.2 (±1.5)
92	125.0 (±6.1)	47.2 (±1.1)	1.12	88.2 (±1.9)	88	150.1 (±3.4)	56.1 (±1.7)	0.78	73.1 (±2.0)	90	182.3 (±5.1)	45.6 (±1.3)	1.19	88.2 (±1.4)
107	145.1 (±7.2)	48.7 (±1.3)	1.05	88.1 (±1.7)	106	149.4 (±3.2)	58.6 (±1.7)	0.71	76.4 (±1.8)	105	179.3 (±4.5)	44.5 (±1.0)	1.25	89.2 (±1.6)
125	143.1 (±6.9)	49.2 (±1.3)	1.03	87.5 (±2.1)	132	146.1 (±3.6)	60.6 (±1.4)	0.65	73.8 (±2.1)	127	194.2 (±6.5)	43.5 (±1.1)	1.30	87.4 (±1.4)
151	141.7 (±4.8)	49.8 (±0.8)	1.01	87.5 (±2.4)	159	147.5 (±3.9)	61.1 (±1.5)	0.64	78.6 (±2.2)	153	198.0 (±6.6)	42.4 (±2.4)	1.36	86.1 (±1.3)
170	142.5 (±5.4)	49.5 (±1.1)	1.02	87.6 (±1.9)	170	149.4 (±3.8)	59.5 (±1.4)	0.64	78.2 (±2.1)	170	182.0 (±5.1)	42.7 (±1.8)	1.25	87.1 (±1.3)

Note: Values are given as means ($n = 3$, \pm standard deviation); a - composting time; b - mg C g OM⁻¹

Although it is often omitted from studies, the content of L-HAs and S-HAs is important, especially when using compost as a soil amendment. L-HAs are weakly bound macromolecules of small or medium size with an aromatic character; S-HAs are larger macromolecules with aliphatic character (Giegużyńska et al., 2009; Schnitzer and Schuppli, 1989). Some investigations have indicated that L-HAs from soils bond more metal (e.g. Cu) than S-HAs under the same reaction conditions, which is probably connected with the acidity of L-HAs caused by the presence of acidic functional groups.

Thus, complexes of metals with L-HAs are more soluble and mobile in soil profile than complexes with S-HAs (Giegużyńska, 2002). In the present study, most humic acids were S-HAs: in series 1 and 3, L-HAs content averaged 12-13%; in series 2, over 25% (Table 3).

3.2. Distribution of heavy metals between HAs and FF

3.2.1. Total metal concentration

The total metal concentrations in the feedstocks and mature composts in series 1-3 are presented in Table 4. In the feedstocks, the most abundant metal was Fe, followed by Zn, Cu and Ni. The main source of heavy metals in the feedstocks was sewage sludge (data not shown). Because the sewage sludge used for feedstock preparation in individual series was collected from the same MWTP, but with long time intervals between collections due to the composting time for each series, metal concentrations

varied. Therefore, when comparing how temperature conditions during composting affect metal distribution between HAs and FF, the results will be presented as % content, not as concentration.

In mature composts, the order of metal abundance was the same as in the feedstocks, although their total concentrations had changed. During composting, metal concentration can increase or decrease; such fluctuations are a typical phenomenon. An increase in metal concentration results from loss of organic matter as heavy metals cannot be degraded or volatilized. A decrease may be connected with the fact that during composting leachate can be generated and may leach metals from the composted mass. Leaching happens mainly in the initial phase of composting; in this phase volatile fatty acids are produced during degradation of organic matter, which lowers the pH, increasing the solubility of metals. In our investigations, the concentration of all the analyzed metals was greater in the mature composts than in the feedstocks: 7.1-20.3% greater for Fe, 9.5-18.8% for Zn, 3.1-17.3% for Cu, and 19.3-47.3% for Ni. The increase in metal concentration can be explained by two mechanisms: i) losses of organic matter during mineralization, or ii) the incorporation in the organic fractions of metals that previously were incorporated in other fractions (Ciavatta et al., 1993).

3.2.2. Metals in HS

Concentrations of heavy metals bound to humic substances in mature composts are shown in Table 5.

Table 4. Total metal concentrations in the feedstock and mature composts in series 1-3

Heavy metal	Concentration (mg kg d.w. ⁻¹)					
	series 1		series 2		series 3	
	0 d	170 d	0 d	170 d	0 d	170 d
Fe	13.3×10 ³ (±0.66) ^a	14.2×10 ³ (±0.89) ^a	9.3×10 ³ (±0.34) ^a	10.9×10 ³ (±0.71) ^b	7.9×10 ³ (±0.74) ^a	9.5×10 ³ (±0.53) ^b
Zn	309.9 (±11.6) ^a	339.3 (±18.8) ^a	248.9 (±9.5) ^a	295.7 (±14.1) ^b	238.1 (±6.5) ^a	270.6 (±10.2) ^b
Cu	68.2 (±5.5) ^a	70.3 (±4.8) ^a	57.9 (±4.2) ^a	67.3 (±5.8) ^a	52.1 (±2.6) ^a	61.1 (±3.2) ^b
Ni	14.8 (±1.3) ^a	21.8 (±3.3) ^b	24.4 (±4.1) ^a	29.1 (±1.6) ^a	28.3 (±0.9) ^a	35.6 (±2.7) ^b

Note: Values are given as means ($n = 3$, \pm standard deviation). Within a column, means followed by the same letter are not significantly different at $P < 0.05$, using the ANOVA test.

Table 5. Metal concentrations bound to HS in series 1-3

Heavy metal	Concentration (mg kg d.w. ⁻¹)		
	series 1	series 2	series 3
Fe	2435 (±183) ^a	2904 (±357) ^a	1553 (±135) ^b
Zn	192.2 (±19.1) ^a	206.7 (±25.1) ^a	236.0 (±19.4) ^a
Cu	53.1 (±2.2) ^a	54.1 (±3.6) ^a	45.7 (±4.0) ^a
Ni	10.6 (±1.4) ^a	41.6 (±4.4) ^b	31.2 (±4.1) ^c

Note: Values are given as means ($n = 3$, \pm standard deviation). Within a column, means followed by the same letter are not significantly different at $P < 0.05$, using the ANOVA test.

In all composts, the abundance of heavy metals in HS followed this order: Fe > Zn > Cu > Ni, which is similar to the total concentrations in the mature compost as a whole (Table 4).

It is worth mentioning that metal concentrations in HS, with the exception of Fe, were higher than those in the oxidizable (organic) fraction, which were determined using the BCR sequential extraction procedure (Pueyo et al., 2008). For the sake of comparison, the concentrations in the oxidizable fraction were: 12.0-21.3 mg Zn/kg, 42.2-49.2 mg Cu/kg and 3.4-4.6 mg Ni/kg (full data not shown). The higher metal concentrations in HS may be due to the extraction procedures that were used. The HS fraction was extracted with a sodium hydroxide/pyrophosphate solution, whereas metals in the oxidizable fraction were extracted with ammonium acetate. The former method not only extracts metals from the HS fraction, but also leaches a portion of the exchangeable metals, thus increasing the apparent metal concentration. Moreover, some metals bound to the Fe-oxide fraction may have also been dissolved by this method (Borůvka and Drábek, 2004; Donisa et al., 2003). A similar phenomenon has been noted by Wang et al. (2010) with chicken manure compost. They found that the average content of Cu in HS as determined by Na₄P₂O₇-NaOH extraction accounted for 46.8% of the total Cu in the compost.

In both the feedstock and mature composts in all series, Fe, Zn and Cu were bound mainly to the FF of the HS, but Ni was predominately bound to HAs. In the feedstock, the mean content of Fe in the FF was 95% (Fig. 3), Zn was 96% (Fig. 4) and Cu was 81% (Fig. 5). In mature composts, metal content in the FF was similar to that in the feedstock for Fe (95%) and Zn (93%), but lower for Cu (67-74%) (Figs. 3-5). The highest metal content in the FF was observed in series 3, which had the lowest degree of humification. Borůvka and Drábek (2004) have reported similar

results with heavily polluted soils of Fluvisol type. In their study, 98, 82, and 96% of organically bound Cd, Pb, and Zn, respectively, were in the fulvic fraction. Donisa et al. (2003) reported that 11 elements, with the exception of Ca, were more abundant in the fulvic than in the humic fraction in natural soils (andosol, podzol, cambisol).

These reports show that fulvic acids are generally the main fraction of HS that reacts with metals, irrespective of the acids origin (natural/polluted soils or compost). The FF has a higher capacity for metal binding because of its higher number of reactive groups. In addition, mainly due to their lower molecular weight and higher content of functional groups, fulvic acids are assumed to form more soluble, mobile and bioavailable metal complexes than HAs. Therefore, fulvic acids can act as metal carriers, whereas HAs tend to immobilize and accumulate metals in the solid phase of composts or soils.

Ni content in HAs ranged from 61 to 77% of the total amount in HS. The ability of different kinds of composted waste to complex Ni can differ, depending on the amounts of the HAs and FF that it contains. Tejada et al. (2008) found that Ni toxicity decreased more in soils amended with crushed cotton gin compost than in poultry manure compost; the cotton gin compost contained over 4 times more humic acids than the poultry manure compost, in which fulvic acids predominated. According to the authors, the reason for this may be the greater chelation of Ni by HAs than by fulvic acids.

The fact that in the present study most metals were bound to the FF, but not to the HAs, might be explained in two ways. First, HAs were extracted from HS at pH~1.0 (Sec. 2.3.1). The metal-humic acid stability constants available in the literature (Stevenson, 1976, Stevenson, 1977 cited in Dionisa et al., 2003) suggest that only a few, if any, humus-metal

complexes are stable at such a low pH. From purely thermodynamic considerations, therefore, most metals bound to HAs in the pyrophosphate/sodium hydroxide solution might be expected to remain in solution after precipitation of HAs. This might mean that in the present study, some of the metal extracted from the FF could have been originally bound to the HAs.

Second, when organo-metallic complexes are sufficiently inert, the metal may not be extracted, but instead be retained in the HA precipitates. Österberg et al. (1999) (cited in Donisa et al., 2003) found that Cu-HA complexes were characterized by a very slow rate of dissolution, presumably because Cu ions diffuse into HA particles and bind to sites from which they cannot be readily released. Similar results have been obtained for calcium; because of the substantial diffusion of calcium ions into HAs, they have been suggested to be able to retain metal ions even at relatively high acidity (Österberg and Wei, 1999 cited in Donisa et al., 2003).

Retention of metal in complexes with HA precipitates might have occurred in our study. Although Zn and Cu predominated in the FF, an increase in the concentration of these metals in HAs was noticed during composting (Figs. 3-4), especially in series 1 and 2. Ni concentration in HAs also increased although its content in this fraction was already high in the feedstock (Fig. 6).

Composting had almost no effect on Fe content in organic fractions, despite the increase of HAs content. This may indicate that Fe remained in the FF (due to low pH) or that its affinity to HAs was lower than to the FF. During humification, Zn, Cu and Ni content in HAs increased considerably, but Fe content did not. In series 1 and 2, the percent content of Zn nearly doubled (Fig. 3a, 3b). However, 92-94% of all Zn in HS was still found in the FF, due to the low content of Zn in the feedstock (2.7-4.3% of HS content). Thus, it appears that the amount of Zn bound by HAs was only slightly affected by the degree of compost maturity and the content of HAs. The increase of Cu and Ni content in HAs, and the corresponding decrease in the FF were especially notable in series 1 and 2, where the concentration of HAs was higher because higher temperatures resulted.

The increase of metal content in HAs during composting may be attributed to the fact that O-containing functional groups are formed that have a high affinity for heavy metals. Xiong et al. (2010) showed that phenolic and benzene carboxylic groups have a high affinity for Cu and Cd and that the composting process improved the capacity of HAs to form complexes with both metals. Taken together, these data indicate that the concentration of HAs in compost and the maturity of compost affect Cu and Ni content in HAs.

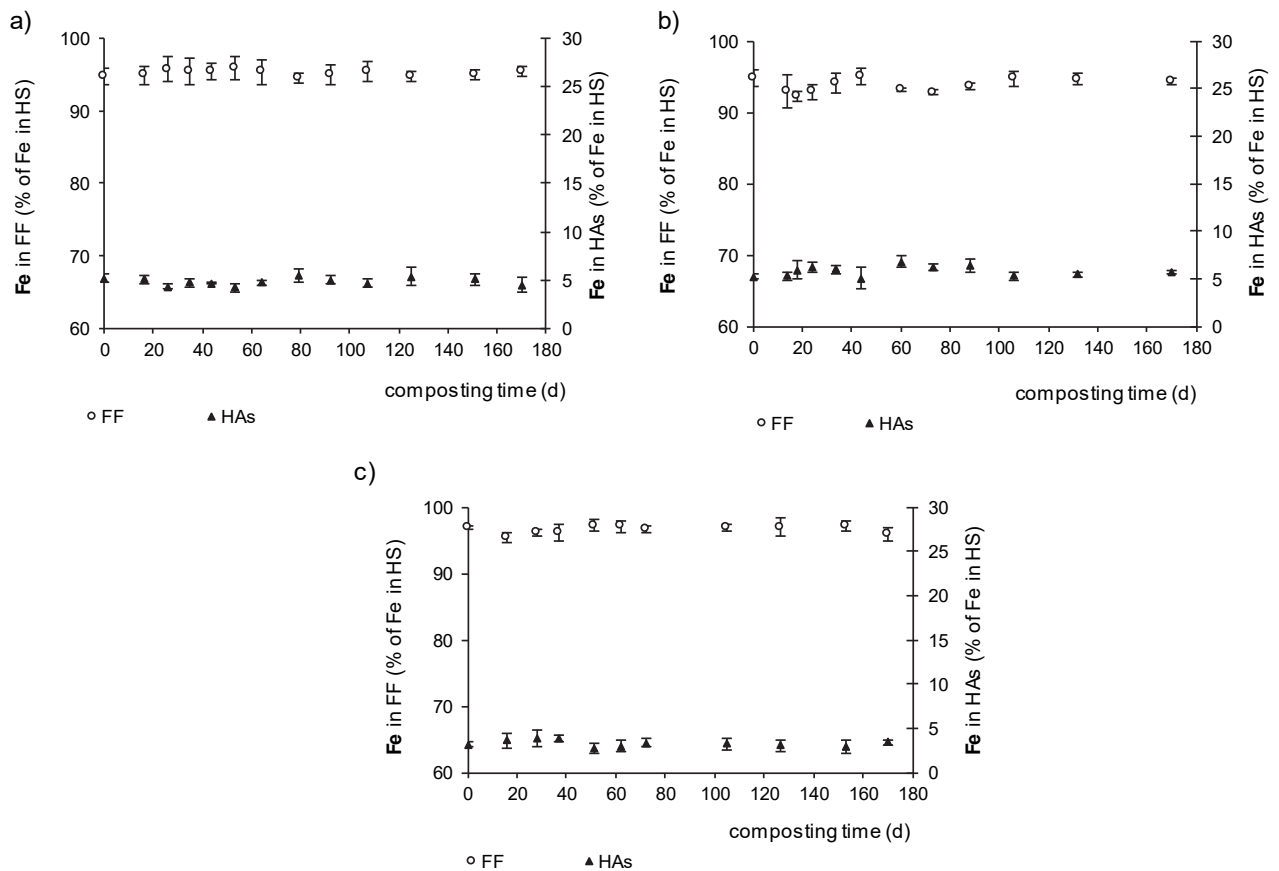


Fig. 3. Changes of Fe content in FF and HAs during sewage sludge composting: a) series 1, b) series 2, c) series 3 (error bars represent standard deviation, $n = 3$)

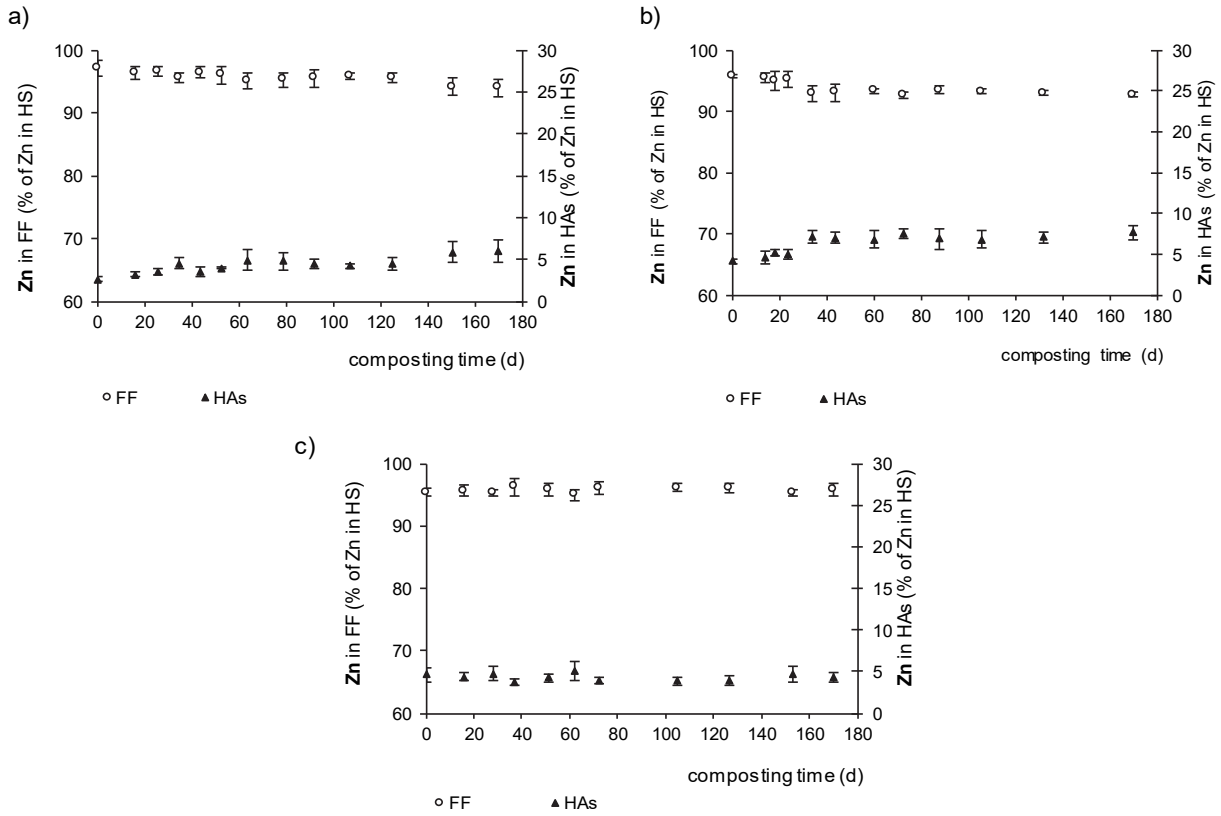


Fig. 4. Changes of Zn content in the FF and HAs during sewage sludge composting: a) series 1, b) series 2, c) series 3 (error bars represent standard deviation, $n = 3$)

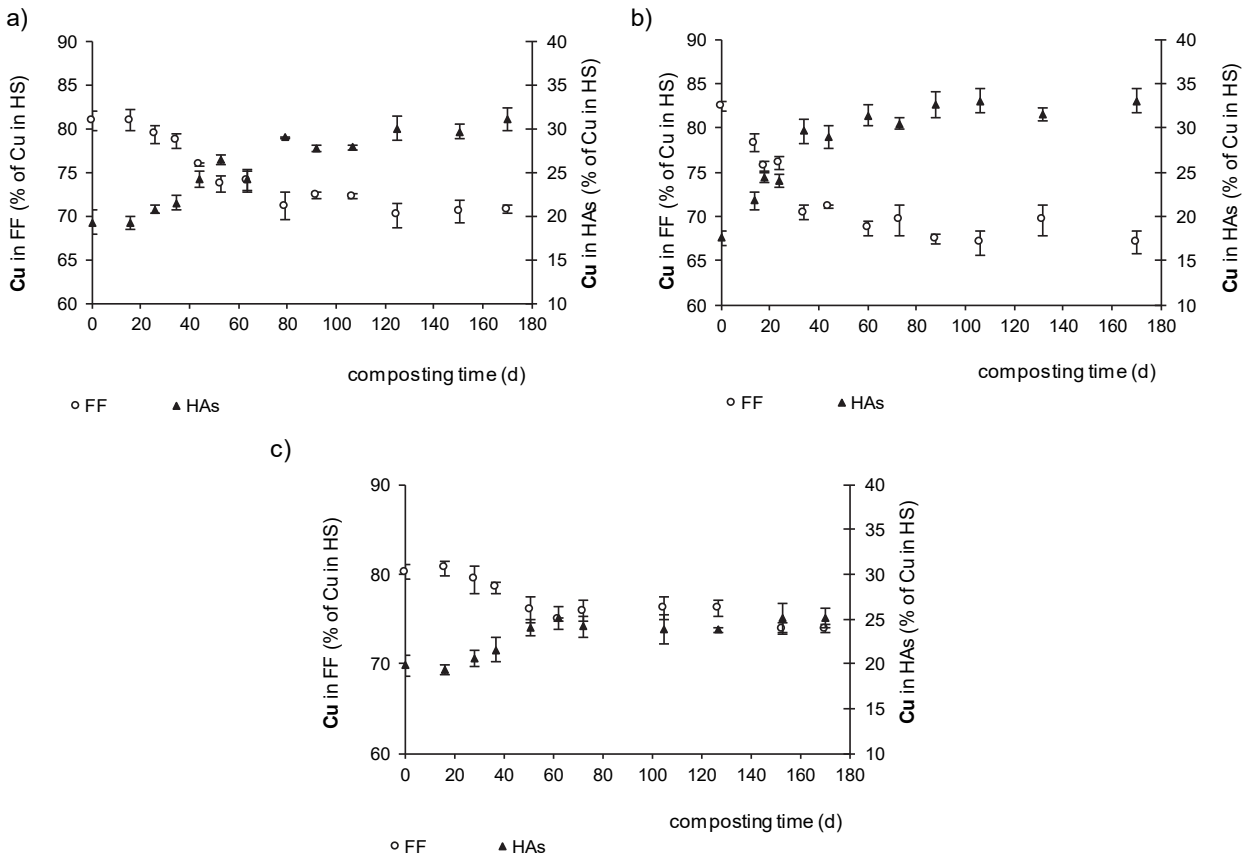


Fig. 5. Changes of Cu content in the FF and HAs during sewage sludge composting: a) series 1, b) series 2, c) series 3 (error bars represent standard deviation, $n = 3$)

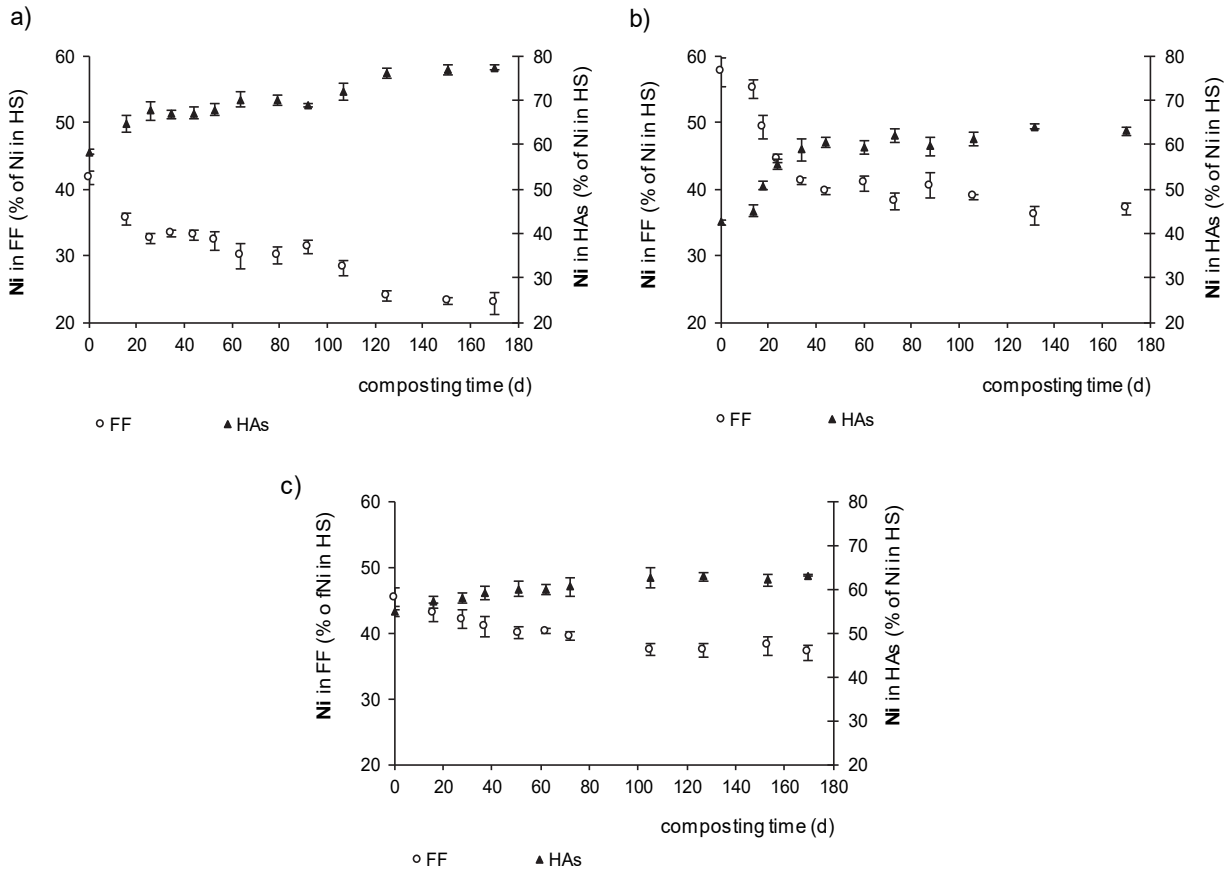


Fig. 6. Changes of Ni content in the FF and HAs during sewage sludge composting: a) series 1, b) series 2, c) series 3 (error bars represent standard deviation, $n = 3$)

Table 6. Metal (Me) content in S-HAs (as % of HAs) in feedstock and mature compost in series 1-3

Heavy metal	Me in S-HA (%)					
	series 1		series 2		series 3	
	feedstock	mature compost	feedstock	mature compost	feedstock	mature compost
Fe	24.9 (± 2.1) ^a	24.2 (± 0.9) ^a	31.0 (± 1.3) ^a	31.9 (± 1.2) ^a	21.2 (± 1.6) ^a	21.7 (± 0.5) ^a
Zn	19.7 (± 1.5) ^a	40.7 (± 2.3) ^b	39.3 (± 0.8) ^a	65.2 (± 1.6) ^b	43.8 (± 2.3) ^a	60.7 (± 1.3) ^b
Cu	19.2 (± 1.7) ^a	31.0 (± 0.7) ^b	24.5 (± 0.4) ^a	54.2 (± 2.4) ^b	21.8 (± 0.6) ^a	28.4 (± 2.1) ^b
Ni	76.2 (± 2.9) ^a	81.0 (± 2.2) ^b	76.1 (± 1.3) ^a	97.8 (± 2.5) ^b	82.9 (± 1.7) ^a	87.0 (± 2.5) ^b

Note: Values are given as means ($n = 3$, \pm standard deviation). Within a column, means followed by the same letter are not significantly different at $P < 0.05$, using the ANOVA test.

This investigation and a previous investigation (Kulikowska and Klimiuk, 2011) also show that the concentration of humic acids in compost is closely related to the technological conditions of composting. Thus, it can be assumed that through appropriate process control, metal content in HAs can be increased. Thus, it can be assumed that providing thermophilic conditions during composting favors the formation of HAs, which can increase metal content in HAs.

3.2.3. Metals in stable and labile HAs

Metal content in S-HAs and L-HAs depended mainly on metal type. Fe content in HAs was 4-6%, of which only 21.7-31.0% was found in S-HAs (Table 6), which is considered the most stable organic fraction. The content of Fe in S-HAs did not change during composting, whereas the content of Zn and Cu in S-

HAs in mature compost was greater than in the feedstock. The amount of Ni in mature composts was also greater than in the feedstock, with the greatest increase occurring in series 2.

Cu and Ni content in HAs increased in series where thermophilic conditions were achieved. This can be explained by the need to polymerize FAs, and then create HAs. Metal-HA complexes have a high molecular weight and are relatively stable with regard to metal-exchange reactions (Koukal et al., 2003) when compared to complexes with fulvic acids. Plaza et al. (2005) found that the stability constants (as $\log K$) for Cu(II) complexes with HAs and FAs isolated from soil were 7.04 and 6.41, respectively. In the same soil, amended with pig slurry at a rate of $150 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$, a similar trend in metal stability was observed, even over the long term. Similarly, Zaccone et al. (2008) have shown that in peat organic matter, HAs were

involved in the formation of more stable organo-mineral complexes with Ni, while fulvic acids increased the mobility of trace elements throughout the soil profile. When compost is used as soil fertilizer, complexes between metals and HAs are more desirable than those with FF due to less metal bioavailability.

To sum up, these findings may preliminarily validate the strategy of adjusting technological parameters of composting to reduce the pollution risk of heavy metals in sewage sludge land-application.

4. Conclusions

In this study, Cu and Ni were more stable after composting in thermophilic conditions or when compost overheated than in mesophilic conditions. With higher temperatures, more HAs and metal-HA complexes formed, including complexes with stable-HAs. Metal complexes with HAs are more stable than complexes with the FF.

Thus, to reduce the risk of heavy metal pollution when using sewage sludge compost as a soil amendment, it appears to be advisable to favor thermophilic conditions during composting.

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References

- Amir S., Hafidi M., Lemee L., Merlina G., Guiesse M., Pinelli E., Revel J.-C., Bailly J.-R., Ambles A., (2006), Structural characterization of humic acids, extracted from sewage sludge during composting, by thermochemolysis - gas chromatography - mass spectrometry, *Process Biochemistry*, **41**, 410-422.
- Amir S., Hafidi M., Merlina G., Revel J.-C., (2005), Structural changes in lipid-free humic acids during composting of sewage sludge, *International Biodeterioration and Biodegradation*, **55**, 239-246.
- Ayuso M., Hernández T., Garcia C., Pascual J.A., (1996), Stimulation of barley growth and nutrient absorption by humic substances originating from various organic materials, *Bioresource Technology*, **57**, 251-257.
- Boratyński K., Wilk K., (1965), Studies on organic matter, Part IV, Fractionation of humic substances using complexing solutions and diluted alkali, (in Polish), *Soil Science Annual XV*, **1**, 5363.
- Bortůvka L., Drábek O., (2004), Heavy metal distribution between fractions of humic substances in heavily polluted soils, *Plant, Soil and Environment*, **50**, 339-345.
- Ciavatta C., Govi M., Simoni A., Sequi P., (1993), Evaluation of heavy metals during stabilization of organic matter in compost produced with municipal solid wastes, *Bioresource Technology*, **43**, 147-153.
- Domeizel M., Khalil A., Prudent P., (2004), UV spectroscopy: a tool for monitoring humification and for proposing an index of the maturity of compost, *Bioresource Technology*, **94**, 177-184.
- Donisa C., Mocanub R., Steinnes E., (2003), Distribution of some major and minor elements between fulvic and humic acid fraction in natural soils, *Geoderma*, **111**, 75-84.
- Erdogan S., Baysal A., Akba O., Hamamci C., (2007), Interaction of metals with humic acid isolated from oxidized coal, *Polish Journal of Environmental Studies*, **16**, 671-675.
- Evangelou V.P., Marsi M., (2001), Composition and metal ion complexation behavior of humic fractions derived from corn tissue, *Plant and Soil*, **229**, 13-24.
- Giegużyńska E., (2002), *Studies on the differentiation of fractional composition, structure and physico-chemical properties of humic acids in deluvial soils of Dłusko catena in Inskie Lakeland* (in Polish), PhD Thesis, Agricultural Academy in Szczecin, Faculty of Environmental Management and Agriculture.
- Gieguzynska E., Amine-Khodja A., Trubetskoy O.A., Trubetskaya O.E., Guyot G., ter Halle A., Golebiowska D., Richard C., (2009), Compositional differences between soil humic acids extracted by various methods as evidenced by photosensitizing and electrophoretic properties, *Chemosphere*, **75**, 1082-1088.
- Gupta A.K., Sinha S., (2007), Phytoextraction capacity of the plants growing on tannery sludge dumping sites, *Bioresource Technology*, **98**, 1788-1794.
- Hiraide M., (1992), Heavy metals complexed with humic substances in fresh water, *Analytical Sciences*, **8**, 453-459.
- Iwegbue C.M.A., Emuh F.N., Isirimah N.O., Egun A.C., (2007), Fractionation, characterization and speciation of heavy metals in composts and compost-amended soils - review, *African Journal of Biotechnology*, **6**, 67-78.
- Jouraiphy A., Amir S., Gharous M., Revel J.-C., Hafidi M., (2005), Chemical and spectroscopic analysis of organic matter transformation during composting of sewage sludge and green plant waste, *International Biodeterioration and Biodegradation*, **56**, 101-108.
- Koukal B., Guéguen C., Pardos M., Dominik J., (2003), Influence of humic substances on the toxic effects of cadmium and zinc to the green alga *Pseudokirchneriella subcapitata*, *Chemosphere*, **53**, 953-961.
- Kulikowska D., Gusiatin Z.M., (2015), Sewage sludge composting in a two-stage system: carbon and nitrogen transformations and potential ecological risk assessment, *Waste Management*, **38**, 312-320.
- Kulikowska D., Klimiuk E., (2011), Organic matter transformations and kinetics during sewage sludge composting in a two-stage system, *Bioresource Technology*, **102**, 10951-10958.
- Li H., Li Y.K., Li C.C., (2017), Evolution of humic substances during anaerobic sludge digestion, *Environmental Engineering and Management Journal*, **16**, 1577-1582.
- Paredes C., Bernal M.P., Cegarra J., Roig A., (2002), Biodegradation of olive mill wastewater sludge by its co-composting with agricultural wastes, *Bioresource Technology*, **85**, 1-8.
- Paredes C., Roig A., Bernal M.P., Sánchez-Monedero M. A., Cegarra J., (2000), Evolution of organic matter and nitrogen during co-composting of olive mill wastewater with solid organic wastes, *Biology and Fertility of Soils*, **32**, 222-227.
- Plaza C., Senesi N., García-Gil J.C., Polo A., (2005), Copper(II) complexation by humic and fulvic acids from pig slurry and amended and non-amended soils, *Chemosphere*, **61**, 711-716.

- Pueyo M., Mateu J., Rigol A., Vidal M., López-Sánchez J.F., Rauret G., (2008), Use of the modified BCR three-step sequential extraction procedure for the study of trace element dynamics in contaminated soils, *Environmental Pollution*, **152**, 330-341.
- Rivero C., Chirenje T., Ma L.Q., Martinez G., (2004), Influence of compost on soil organic matter quality under tropical conditions, *Geoderma*, **123**, 355-361.
- Sánchez-Monedero M.A., Roig A., Cegarra J., Bernal M.P., (1999), Relationships between water-soluble carbohydrate and phenol fractions and the humification indices of different organic wastes during composting, *Bioresource Technology*, **70**, 193-201.
- Schnitzer M., Schuppli P., (1989), The extraction of organic matter from soils with 0.5 M NaOH and 0.1 M Na₂P₂O₇ solutions, *Canadian Journal of Soil Science*, **69**, 253-262.
- Smith S.R., (2009), A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge, *Environment International*, **35**, 142-156.
- Spark K.M., Wells J.D., Johnson B.B., (1997), The interaction of a humic acid with heavy metals, *Australian Journal of Soil Research*, **35**, 89-101.
- Stevenson F.J., (1994), *Humus Chemistry, Genesis, Composition, Reactions*, 2nd Edition, John Wiley Sons Inc., Canada.
- Stuckey H.T., Hudak P.F., (2001), Effects of compost on Loblolly pine tree growth in Northeast Texas, *Compost Science and Utilization*, **9**, 121-128.
- Tejada M., Moreno J.L., Hernández M.T., García C., (2008), Soil amendments with organic wastes reduce the toxicity of nickel to soil enzyme activities, *European Journal of Soil Biology*, **44**, 129-140.
- Wang X.-D., Chen X., Ali A.S., Liu S., Lu L., (2010), Dynamics of humic substance-complexed copper and copper leaching during composting of chicken manure, *Pedosphere*, **20**, 245-251.
- Warman P.R., Termeer W.C., (2005), Evaluation of sewage sludge, septic waste and sludge compost applications to corn and forage: Yields and N, P and K content of crops and soils, *Bioresource Technology*, **96**, 955-961.
- Xiong X., Yan-Xia L., Ming Y., Feng-Song Z., Wei L., (2010), Increase in complexation ability of humic acids with the addition of ligneous bulking agents during sewage sludge composting, *Bioresource Technology*, **101**, 9650-9653.
- Zaccone C., Coccozza C., Cheburkin A.K., Shotyk W., Miano T.M., (2008), Distribution of As, Cr, Ni, Rb, Ti and Zr between peat and its humic fraction along an undisturbed ombrotrophic bog profile (NW Switzerland), *Applied Geochemistry*, **23**, 25-33.