

## Research Article

# Bioavailability and Solubility of Heavy Metals and Trace Elements during Composting of Cow Manure and Tree Litter

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**Objectives.** To characterize the total content of heavy metals (Cd, Cr, Cu, Hg, Mo, Ni, Pb, and Zn) and trace elements (As and Se) of interest, their bioavailability and solubility during the composting of cow manure and tree litter in piles inoculated with beneficial microorganism (IBM), or not inoculated (NBM), on the university campus of the Universidad Nacional Agraria La Molina (UNALM). **Methods.** The investigation evaluated composting in six piles, three IBM piles and three NBM piles, for 120 days. Every 30 days, a composite sample was taken from each pile. The raw materials used were cattle manure and tree litter. The variables analyzed were the total concentrations (content) of metals and trace elements of interest, extracted with diethylenetriaminepentaacetic acid/pentatetic acid (DTPA) (for the bioavailability study) and extracted with deionized water (for the solubility study). **Results.** The average values ( $n = 3$ ) of the total content ( $\text{mg}\cdot\text{kg}^{-1}$ ) on day 120 in IBM were in the following order: Zn (404.33) > Cu (86.33) > Pb (71.2) > Cr (34.33) > As (28.0) > Ni (13.83) > Mo (2.86) > Se (1.38) > Cd (1.32) > Hg (0.39) and in NBM: Zn (466.0) > Cu (112.23) > Pb (73.23) > Cr (35.33) > As (29.67) > Ni (14.37) > Mo (3.23) > Se (1.55) > Cd (1.38) > Hg (0.38). The values complied with the Austrian Compost Standard for Landscaping and Land Rehabilitation and the Peruvian Standard for Nonorganic Agriculture. Significant differences were observed in some elements ( $p < 0.05$ ) between 0 and 120 days in both types of pile (IBM and NBM). **Conclusions.** For nonorganic agriculture in Peru, urban compost is recommended, whereby raw organic materials are inoculated with beneficial microorganisms during the composting process to reduce the bioavailability of Cu and Zn, as these elements showed less bioavailability than noninoculated piles. The composting process for both piles (IBM and NBM) reduced the solubility in Cu, Pb, and Zn.

## 1. Introduction

The heavy metals present in plants and their bioavailability are a concern because of the multiple adverse effects on human health that these chemical elements have [1–3] as well as carcinogenic and neurological effects [4, 5]. The heavy metals present in plants in urban areas come mainly from air pollution generated by burning fuel and emissions from the manufacturing industry [6–9]. Other forms of contamination include irrigation water [10], inorganic fertilizer [11], and organic fertilization with compost [12, 13].

The world's population has migrated mainly from rural areas to urban areas [14], thus increasing the demand for food in the latter. Due to this need, the practice of urban agriculture is recommended [15] to produce plant food. However, research has shown that there is contamination in crops in these areas [8, 16].

Compost is an amendment that is used in urban agriculture [15], especially in poorer areas, because it is a low-cost technique for recycling organic matter and is easy to handle [17]. Compost has the virtue of being a soil improver and also provides nutrients to plants [18, 19]. However, due

to the raw materials used, it can contain significant contents of heavy metals [18, 20–24]. It is known that green spaces with arboreal vegetation in an urban environment retain heavy metals in the foliage due to the precipitation of atmospheric dust produced mainly by the use of fossil fuels by vehicular traffic [7, 25–31]. It is also known that cattle manure contains significant amounts of some heavy metals [32–36] which is due to the fact that cattle feed has nutrients fortified with these elements and that, in some cases, the quantities supplied are greater than the absorption in the digestive process, whereby these are excreted [34, 35].

Humic and fulvic acids are generated during the composting process, which can influence the mobility of metals and trace elements, thus reducing their bioavailability for plants [24, 37–39]. The total content of heavy metals and trace elements analyzed during the composting process is not an adequate indicator of their bioavailability [18, 21]. Therefore, it is important to use an extractant such as pentatetic acid (DTPA) to determine their bioavailability [21, 40] or deionized water to determine their solubility [24, 37, 41, 42].

Inoculation with beneficial microorganisms (BMs) is a bioremediation technique used in areas with soil contaminated by heavy metals whereby their bioavailability and solubility are reduced [43, 44]. Due to the presence of these polluting elements in the raw materials used in composting in urban areas, it could be a useful technique to reduce their availability in the compost produced [45]. It is known that BM can improve the composting process, reducing the required time and increasing the contents of macroelements such as N, P, and K [46, 47]. One of the metabolic properties of these organisms is that they can promote changes in the speciation of metals towards less polluting forms, and they can adsorb them on their own structures [48]. BMs are considered to be microbial consortia made up of yeasts and bacteria that increase the metabolism of the degradation of the raw organic components of the composting piles, especially the lignocellulose elements, towards more assimilable intermediates in the food chain of the microbiological ecosystem [49].

The objective of this investigation was the characterization of the total content of heavy metals and trace elements of interest, as well as their bioavailability and solubility, during the composting of cow manure and tree litter in piles inoculated, or not inoculated, with beneficial microorganisms on the university campus of the Universidad Nacional Agraria La Molina (UNALM).

## 2. Materials and Methods

**2.1. Location.** The university campus of UNALM is located in the district of La Molina, in the province of Lima, department of Lima, in Peru. It is at an altitude of 246 meters above sea level, and the average temperature is between 15 and 26°C (Figure 1).

### 2.2. Materials

**2.2.1. Raw Materials for the Investigation Stages.** The research had two distinct stages. The characterization stage

analyzed the total contents of heavy metals, Cd, Cr, Cu, Hg, Mo, Ni, Pb, and Zn, and of trace elements, As and Se, of interest (HM&TE) in the products from co-composting cow manure (COW-M) and grass clipping (GRASS-C) and from composting of tree litter (TREE-L), two types of compost that are produced on the UNALM campus. The evaluation stage investigated the total contents, bioavailability, and solubility of the HM&TE during the composting of COW-M and TREE-L in piles inoculated with beneficial microorganisms (IBMs) and in piles which were not inoculated with microorganisms (NBM). The piles were of pyramidal form, 0.8 m high with a 3 m base for the co-composting (manure and vegetal material) and 1.5 m high with a 5 m base in the case of the composting (only tree litter). Table 1 shows the raw materials used, their weights, proportion, and composting days.

**2.2.2. Characterization of the Raw Materials of the Evaluation Stage.** For the evaluation stage, physicochemical variables and the total contents of heavy metals and trace elements of interest ( $HM_{tot}$ ) were recorded in the raw materials prior to the beginning of the composting process, as shown in Table 2. Most of the physicochemical values for COW-M were higher than for TREE-L, except for humidity (H%) and ammoniacal nitrogen ( $NH_4^+-N$ ). The organic matter (OM) content was 26.6% higher in COW-M than in TREE-L. The electrical conductivity (EC) in the COW-M was higher, due to the type of cattle feed. Similar results were found in Cáceres and Marfa [50]. TREE-L concentrations for most  $HM_{tot}$  exceeded COW-M, except for Cu, Se, and Zn. The total Pb content in the TREE-L was more than five times the value in COW-M. Ahmed et al. [51] also found an important difference between Pb in poultry manure ( $1.3\text{ mg}\cdot\text{kg}^{-1}$ ) and in sawdust ( $16.0\text{ mg}\cdot\text{kg}^{-1}$ ), more than twelve times the value. Soobhany et al. [52] obtained average total concentrations of Cu and Zn lower values in leaves and dry branches ( $84.3\text{ mg}\cdot\text{kg}^{-1}$  and  $65.3\text{ mg}\cdot\text{kg}^{-1}$  respectively) than in cattle manure ( $137.1\text{ mg}\cdot\text{kg}^{-1}$  and  $596.0\text{ mg}\cdot\text{kg}^{-1}$  respectively) which is corroborated in the present results where the TREE-L had lower values than the COW-M in Cu and Zn (see Table 3), which, as mentioned above, may be due to the feed received by cattle and especially those used for milk production.

**2.2.3. Generation, Inoculation, and Characterization of Beneficial Microorganisms in the Evaluation Stage.** The generation of inoculum with BM was performed in two stages: the first was the generation of the stock solution (SS) and the second was the activation and enrichment of the SS for inoculation in the field. The SS or “efficient EM1 microorganisms” as Dr. Teruo Higa calls it [53] was prepared from cabbage leaf extract (*Brassica oleracea* L. var. *capitata*). In the second stage, nutrients were added and a bundle of plants. These plants were wrapped in silk textile to produce a filtrate that was decomposing and possibly contributing in the generation of BM in the SS. The plants were “rue” (*Ruta graveolens* L.), “horsetail” (*Equisetum arvense* L.), and “rosemary” (*Rosmarinus officinalis* L.). The plants selected

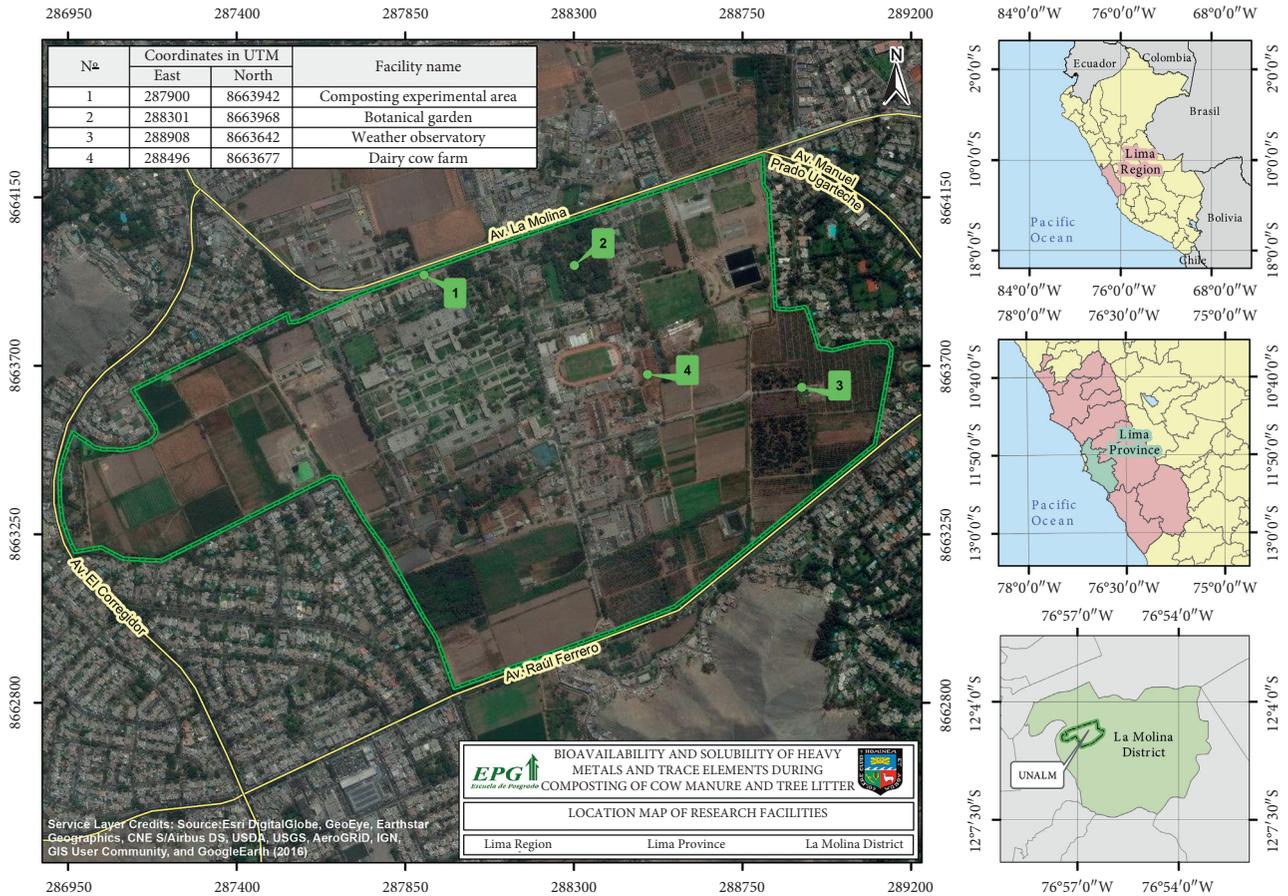


FIGURE 1: Satellite image of the university campus with the facilities which contributed to the investigation.

TABLE 1: Raw materials for composting used in the investigation stages.

Stage	Type of compost	Raw material	Quantity in compost pile (kg)	Proportion (%)	Composting time (days)
Characterization	Co-compost of cow manure and grass clipping	Cow manure	200	40	111
		Grass clipping	300	60	
	Compost of tree litter	Tree litter	3000	100	
Evaluation	Co-compost of cow manure and tree litter IBM	Cow manure	240	40	120
		Tree litter	360	60	
	Co-compost of cow manure and tree litter NBM	Cow manure	240	40	
		Tree litter	360	60	120

TABLE 2: Physicochemical variables of raw materials used in the evaluation stage composting (n = 6).

Physicochemical variable	Tree litter (TREE-L)	Cattle manure (COW-M)
	P 1-6 (mean ± st. dev.)	P 1-6 (mean ± st. dev.)
pH <1/5>	6.62 ± 0.12	7.77 ± 0.14
H (%)	37.21 ± 2.04	16.46 ± 1.19
EC <1/5> (dS m <sup>-1</sup> )	6.40 ± 0.89	14.17 ± 0.80
TOC (%)	29.01 ± 4.36	36.41 ± 2.02
TN (%)	1.45 ± 0.10	2.16 ± 0.11
C/N	20.14 ± 3.16	16.87 ± 1.34
HA (%)	1.61 ± 0.40	2.33 ± 0.31
FA (%)	2.04 ± 0.66	3.56 ± 0.28
NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	4.49 ± 0.22	2.30 ± 0.23
OM (%)	59.06 ± 3.82	74.25 ± 0.92

TABLE 3: Heavy metals and trace elements of interest of the raw materials used in the evaluation stage composting ( $n=6$ ).

Total contents of heavy metals and of trace elements of interest (HMtot)	Tree litter (TREE-L) P 1-6 (Mean $\pm$ St. Dev.)	Cattle manure (COW-M) P 1-6 (Mean $\pm$ St. Dev.)
As ( $\text{mg}\cdot\text{kg}^{-1}$ )	28.17 $\pm$ 3.13	8.83 $\pm$ 2.93
Cd ( $\text{mg}\cdot\text{kg}^{-1}$ )	1.29 $\pm$ 0.05	0.34 $\pm$ 0.03
Cr ( $\text{mg}\cdot\text{kg}^{-1}$ )	26.50 $\pm$ 3.02	6.33 $\pm$ 1.03
Cu ( $\text{mg}\cdot\text{kg}^{-1}$ )	49.00 $\pm$ 4.00	94.67 $\pm$ 27.85
Hg ( $\text{mg}\cdot\text{kg}^{-1}$ )	0.30 $\pm$ 0.03	0.06 $\pm$ 0.02
Mo ( $\text{mg}\cdot\text{kg}^{-1}$ )	4.46 $\pm$ 0.82	4.27 $\pm$ 0.17
Ni ( $\text{mg}\cdot\text{kg}^{-1}$ )	7.18 $\pm$ 0.57	3.13 $\pm$ 0.32
Pb ( $\text{mg}\cdot\text{kg}^{-1}$ )	59.00 $\pm$ 5.34	9.43 $\pm$ 1.60
Se ( $\text{mg}\cdot\text{kg}^{-1}$ )	0.56 $\pm$ 0.04	1.41 $\pm$ 0.45
Zn ( $\text{mg}\cdot\text{kg}^{-1}$ )	268.00 $\pm$ 17.57	313.00 $\pm$ 70.24

for enrichment have medicinal properties and generate BM [54–58]. The activation procedure is as suggested by Dr. Teruo Higa [53] prior to definitive field application. The first inoculation was carried out with a 20% dilution. In other words, 4 L of the activated solution was taken and diluted in 20 L of water for the irrigation of piles P1, P2, and P3. Next, in each turning over, which was performed weekly, during the first month inoculation it was with a 1% dilution (200 mL in 20 L). That is to say, four inoculations were made at 1% (Figure 2). This inoculation process followed the guidelines mentioned by APROLAB [59] and OISCA-BID [53]. On days 0 (T0) and 30 (T30), samples were taken in triplicate from the activated stock solution (ASS) for microbiological analysis. On both occasions, the pH was measured, and an average of 3.21 and 3.19, respectively, was obtained (Figure 2). Figure “a” of supporting information shows an image of the triplicate samples of the activated stock solution.

The average results are shown in Table 4 where it is observed that the presence of BM is in the order of millions, whereas the presence (Table 5) of pathogenic microorganisms (PM) is almost nil or minimal. On day 0 (T0) and day 30 (T30), the molds and yeasts were, respectively, independently counted. Alvarez [56] found on average for the yeasts in rue  $5.5 \times 10^4$  UFC·mL<sup>-1</sup> and in rosemary  $5.6 \times 10^5$  UFC·mL<sup>-1</sup>, for *Bacillus* spp.  $1.46 \times 10^7$  UFC·mL<sup>-1</sup> in rue and  $6.67 \times 10^5$  UFC mL<sup>-1</sup> in rosemary, for *Lactobacillus* spp.  $6.43 \times 10^{12}$  UFC·mL<sup>-1</sup> in rue and  $3.47 \times 10^{12}$  UFC·mL<sup>-1</sup> in rosemary, and for actinomycetes  $8.67 \times 10^3$  UFC·mL<sup>-1</sup> in rue and  $1.6 \times 10^5$  UFC·mL<sup>-1</sup> in rosemary. The results for the ASS of cabbage enriched with rue, rosemary, and horsetail are slightly higher in yeasts, slightly lower in *Bacillus* spp. and actinomycetes and significantly lower in *Lactobacillus* spp. than analyzed by Alvarez [56] for rue and rosemary but even so representing important values.

**2.3. Methods.** The variables analyzed and the methods of analysis are given below, followed by the chronology of the samples taken in each type of compost (Table 6). The results were obtained for homogenized samples composed of five subsamples (four vertices and crown) from the COW-M co-composting piles (Figure “b” of supporting information shows an image of the 120 day sampling), and for TREE-L alone, samples were composed of three subsamples taken from the base of the pile. The 500 g samples were collected in Ziplock™

polypropylene bags which were sent to a laboratory for analysis in triplicate. In the case of microbiological variables, they were also analyzed in triplicate. The viability time was 24 hours, and all were delivered within 12 hours of collection.

**2.3.1. Analyzed Secondary Variables.** Twenty-three secondary variables were analyzed, eleven physicochemical and twelve microbiological ones.

**(1) Physicochemical Variables.** Made up of eleven (11) variables: moisture (**H**%) using TMECC method 03.09-A-total solids and moisture at  $70 \pm 5^\circ\text{C}$  [60]; hydrogen potential (pH) using the TMECC method 04.11-A electrometric pH determination for compost [60]; electrical conductivity (**EC**) using the TMECC method 04.10 electrical conductivity for compost 1:5 [60]; total organic carbon (TOC) using the Walkley and Black method [61]; organic matter (**OM**) by TMECC 05.07-A loss on ignition method [60]; total nitrogen (**TN**) using the Kjeldahl method [62]; ammonium nitrogen (**NH<sub>4</sub><sup>+</sup>-N**) using method 8.2.1 Rev. 2005 [63]; and humic acid fraction (**HA**) and fulvic acid fraction (**FA**) using the standardized KononovaBelchikova method [64]. Apparent density (**Da**) was determined by the known volume cylinder method [65]. The temperature (**T<sup>o</sup>**) was recorded *in situ* using a digital thermometer, ST-9265 multithermometer (13 cm long sensor and 1°C precision), taking measurements in five zones of each pile (four vertices and crown) from which a mean was obtained.

**(2) Microbiological Variables.** Consists of twelve variables: mesophilic aerobes; thermophilic aerobes; *Lactobacillus* spp.; molds and yeasts; total coliforms; fecal coliforms; *Escherichia coli*; and *Salmonella* spp. using ICMSF test method [66]; *Pseudomonas* spp., *Bacillus* spp., and actinomycetes by the APHA analysis methods [67] and helminths using the standards methods for the recovery and enumeration of helminth ova in wastewater, sludge, compost, and urine-diversion waste in South Africa [68].

**2.3.2. Analyzed Primary Variables.** The thirty primary variables are made up of three groups that group the ten elements under analysis (As, Cd, Cu, Cr, Hg, Ni, Mo, Pb, Se, and Zn).

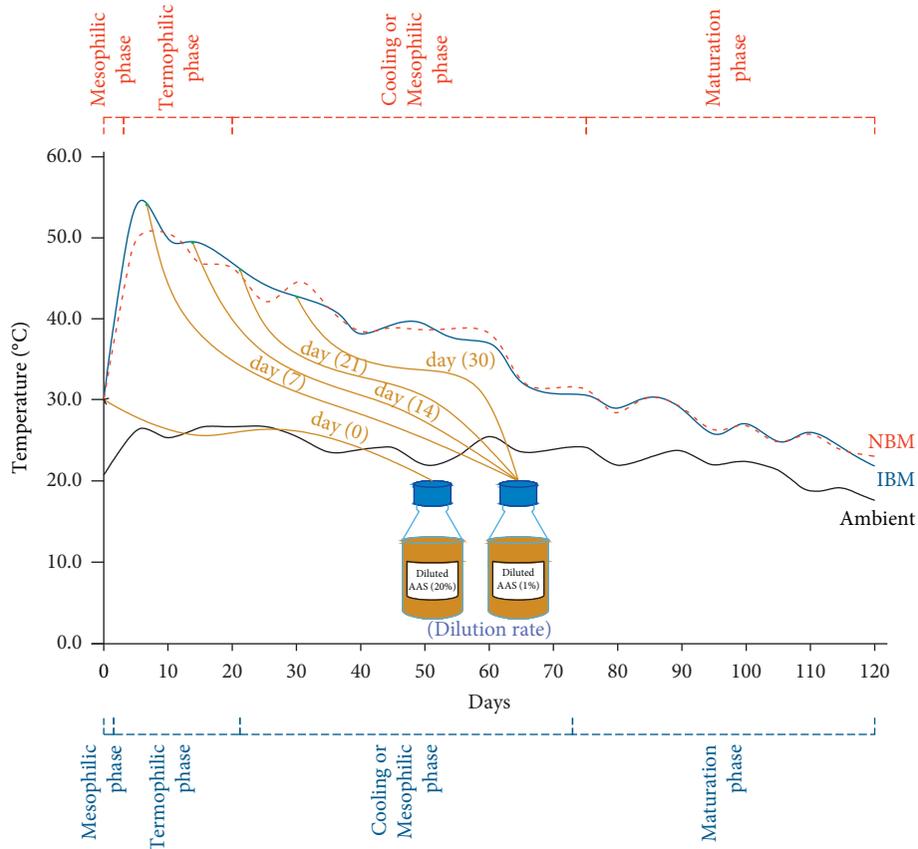


FIGURE 2: Days of inoculation of the compost piles with diluted activated stock solution (ASS), phases of the composting process and temperatures of the inoculated (IBM), and noninoculated (NBM) piles and the ambient temperature.

TABLE 4: Microbiological analysis of beneficial micro-organisms—BM in the activated stock solution—ASS ( $n = 3$ ).

Parameters	T0	T30
	Mean $\pm$ st. dev.	Mean $\pm$ st. dev.
Mesophilic aerobes (UFC·mL <sup>-1</sup> )	$1.25 \times 10^7 \pm 4.01 \times 10^6$	$4.33 \times 10^6 \pm 1.10 \times 10^6$
Thermophilic aerobes (UFC·mL <sup>-1</sup> )	$3.00 \times 10^4 \pm 4.35 \times 10^4$	$5.00 \times 10^3 \pm 4.23 \times 10^3$
<i>Lactobacillus</i> spp. (UFC·mL <sup>-1</sup> )	$1.27 \times 10^7 \pm 2.08 \times 10^6$	$1.00 \times 10^7 \pm 5.33 \times 10^6$
Molds and yeasts (UFC·mL <sup>-1</sup> )	$<1.00 \times 10^2 \pm 0.00$	$1.27 \times 10^6 \pm 8.50 \times 10^5$
<i>Bacillus</i> spp. (UFC·mL <sup>-1</sup> )	$1.00 \times 10^2 \pm 0.00$	$1.77 \times 10^6 \pm 3.06 \times 10^6$
Actinomycetes (UFC·mL <sup>-1</sup> )	$1.01 \times 10^5 \pm 1.11 \times 10^5$	$1.60 \times 10^3 \pm 5.66 \times 10^2$

TABLE 5: Microbiological analysis of pathological microorganisms PM in the activated stock solution ASS ( $n = 3$ ).

Parameters	T0	T30
	Mean $\pm$ st. dev.	Mean $\pm$ st. dev.
Total coliforms (NMP·mL <sup>-1</sup> )	$<3.00 \pm 0.00$	$<3.00 \pm 0.00$
Fecal coliforms (NMP·mL <sup>-1</sup> )	$<3.00 \pm 0.00$	$<3.00 \pm 0.00$
<i>Pseudomonas</i> spp. (NMP·mL <sup>-1</sup> )	$<3.00 \pm 0.00$	$<3.00 \pm 0.00$
<i>Escherichia coli</i> (NMP·mL <sup>-1</sup> )	$<3.00 \pm 0.00$	$<3.00 \pm 0.00$
Detection of <i>Salmonella</i> spp. (25 mL)	Absent $\pm$ --	Absent $\pm$ --
Larvae and eggs of helminths, cysts, and oocysts of pathogenic protozoa (N/4 mL)	$0.67 \pm 1.15$	$1.87 \times 10^1 \pm 6.11$

(1) Total Concentration of Heavy Metals and Trace Elements of Interest ( $HM_{tot}$ ). Analysed by the method 3050B [69] acid digestion of sediments, sludge, and soils and method 6020A [70] inductively coupled plasma mass spectrometer.

(2) Concentration of Heavy Metals and Trace Elements of Interest Extracted with Pentatetic Acid ( $HM_{DTPA}$ ). Solid/extractant ratio 1:5 ( $p/v$ ) for two hours with 0.005 M DTPA (diethylenetriamine pentaacetic acid acquired from MERCK KGaA™) plus 0.1 M TEA (triethanolamine acquired from

TABLE 6: Recorded parameters and measurement dates for each stage of the investigation.

Stage	Primary variables	Secondary variables	Measurement date
Characterization	Total content ( $HM_{tot}$ )	Four physicochemical variables (pH, EC, TOC, and TN)	February 13, 2014 (T111/T240)
Evaluation	Total content ( $HM_{tot}$ ), Bioavailability ( $HM_{DTPA}$ ) and solubility ( $HM_{H2O}$ )	Eleven physicochemical variables ( $T^{\circ}$ , H%, pH, EC, TOC, TN, $NH_4^+-N$ , HA, FA, OM, and Da)	February 2, 2016 (T0);
		Twelve microbiological variables (mesophilic aerobes, thermophilic aerobes, <i>Lactobacillus</i> spp., molds and yeasts, total coliforms, fecal coliforms, <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Pseudomonas</i> spp., <i>Bacillus</i> spp., <i>actinomycetes</i> , and <i>helminths</i> )	March 3, 2016 (T30);
			April 2, 2016 (T60); May 2, 2016 (T90); June 1, 2016 (T120)

MERCK KGaA™) and 0.01 M  $CaCl_2$  at pH 7.3 with diluted HCl. The compost extract was stored in plastic vials at 4°C for further analysis [21].

(3) *Concentration of Heavy Metals and Trace Elements of Interest Extracted with Deionized Water ( $HM_{H2O}$ )*. 1 g of compost sample was taken. The sample was extracted with 15 mL of deionized water (by reverse osmosis and ion exchange with  $EC < 1 \mu S \cdot m^{-1}$ ) by mechanical agitation at 120 rpm for two hours and then centrifuged at 4000 rpm for 5 minutes. It was filtered with a 0.45  $\mu m$  nylon membrane. The compost extract was stored in plastic vials at 4°C for further analysis [21].

Analyses of the digestates of the primary variables were performed on a Perkin Elmer spectrometer, model Elan DRC-e. Table 6 shows the stages of the investigation, primary variables, secondary variables, and measurement dates.

2.4. *Statistical Analysis (Evaluation Stage)*. The experimental design of the investigation in its evaluation stage was completely randomized (completely randomized design). The investigation considered the IBM and NBM factors, five treatments (composting days: 0, 30, 60, 90, and 120), and three repetitions (composting piles). Fifty-three variables have been evaluated, divided into thirty main variables and twenty-three (23) secondary variables. Table “a” of supporting information shows factors, treatments, and their respective coding.

2.4.1. *Secondary Variables*. Twelve physicochemical variables (H%, pH, EC, TOC, TN, C/N ratio, HA, FA, HA/FA ratio,  $NH_4^+-N$ , OM, and Da) were registered.  $T^{\circ}$  was discarded due to the nonexistence in the literature of information concerning its influence on the bioavailability of the heavy metals and trace elements of interest, and the C/N and HA/FA rates used to determine the maturation of compost were incorporated [17]. Eleven microbiological variables include mesophilic aerobes, thermophilic aerobes, *Lactobacillus* spp., molds and yeasts, *Pseudomonas* spp., *Bacillus* spp., *actinomycetes*, total coliforms, fecal coliforms, *Escherichia coli*, and *helminths*. The variable *Salmonella* spp. was withdrawn from the statistical analysis as its results are qualitative.

2.4.2. *Primary Variables*. Primary variables are thirty principal variables considering the three analysis groups ( $HM_{tot}$ ,  $HM_{DTPA}$  and  $HM_{H2O}$ ) and the ten elements under analysis (As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, and Zn).

2.4.3. *Analysis of the Variables*. The analysis of the (primary and secondary) variables was carried out using the decomposition technique of the variability of the data called analysis of variance (ANOVA). This test allowed the comparison of the mean concentration or units of each variable at different composting days (treatments) on the basis of the completely randomized design, or factor design, used in this investigation. For correct application, the ANOVA of a factor requires compliance with the assumption of normal distribution and homogeneity of variances [71]. The AndersonDarling test was used or the verification of normality and the Bartlett test for homogeneity. For the compliance with both assumptions, the  $p$  value associated with each of these tests should be greater than the level of significance, 0.05. The ANOVA analysis was performed when it was verified that the two assumptions, variance homogeneity and normal distribution, were met. When these assumptions were not met, it was decided to use the KruskalWallis Test or nonparametric method or free distribution [71]. These analyses were carried out using the statistical software R™ Version 3.3.2.

In order to analyze how primary and secondary physicochemical variables vary with respect to the application factors of beneficial microorganisms and treatments, general linear and mixed models were made to consider the heterogeneity of variance during the analyzed period. Then, the most appropriate model was selected using the values of Akaike (AIC), Schwarz (BIC), and the maximum likelihood test (LRT) [72]. For all measured variables, normality was analyzed using the ShapiroFrance test [73]. In the case of microbiological secondary variables, generalized linear binomial negative models were used [72]. In order to study the degree of association between primary and secondary variables, the Pearson linear correlation coefficient was used in those variables that did not show influence by the inoculation factor. This comparative value is reinforced with the  $p$  value (if it is  $< 0.05$ , it is significant and if it is  $> 0.05$ , it is not significant). This correlation was obtained by processing the information with the InfoStat™ software, version 2017.1.2. Correlations were evaluated between the 53 variables, all against all. For the present article, the primary variables were

selected that exceeded the correlation of 0.5, positive or negative, in relation to the fraction of humic acids (AHs).

Using the InfoStat™ software, version 2017.1.2, an unrestricted sorting analysis was made of the principal components. Fifty-three variables were used for this (primary and secondary, excepting *Sallmonella* spp. as it has qualitative values) initially through a first run, where abundant values greater than 1 were obtained. From this, a reduction of variables was made, through the ordering of the variables and their weights in the first components, working with the absolute values and observing which variables have greater weight in the first two components. From this new arrangement, two autovalues with values greater than 1 were obtained (the variables that had less weight in the first two axes were eliminated). Finally, the variables obtained for components 1 and 2 explain 78% of the variance.

### 3. Results

#### 3.1. Characterization Stage

**3.1.1. Secondary Variables (Physicochemical).** The results of the recorded physicochemical variables (pH, EC, TOC, TN, and C/N ratio) of the compost products (T111/T240) of the characterization stage are shown in Figure 3. It is observed that, in all mean values, manure compost exceeds tree litter. Compared with the Chilean standard [74], we have the pH complying for both types of compost and both classes (A and B); for the EC, only tree litter complies for class B compost; TN is complied with for both types of compost and for both classes, and the C/N ratio is met in both types of compost and for both classes. It is important to emphasize the value of CE, which can be associated with the type of cattle feed [75]. This presence of EC in this type of manure can generate the increase of this value in the soils where it is applied, in the medium or long term, which can generate processes of salinization and infertility [76, 77].

**3.1.2. Primary Variables (Total Content of Heavy Metal and Trace Elements of Interest).** The  $HM_{tot}$  of the TREE-L composting product (T120) showed the following order in its mean values ( $mg \cdot kg^{-1}$ ): Zn (533.79) > Pb (173.32) > Cu (83.32) > As (73.78) > Cr (31.739) > Ni (8.49) > Se (3.02) > Cd (2.10) > Mo (2.04) > Hg (0.76). In the COW-M and GRASS-C T120 compost: Zn (404.56) > Pb (67.26) > Cu (59.91) > As (38.09) > Cr (9.54) > Ni (6.64) > Mo (2.14) > Se (1.65) > Cd (1.15) > Hg (0.35). The  $HM_{tot}$  values in the TREE-L compost are higher than in the COW-M and GRASS-C compost. In the cases of Cr and Pb for TREE-L compost, it exceeds by more than three and two times the mean value of the COW-M and GRASS-C compost, respectively (Figure 4). The higher  $HM_{tot}$  value in the compost generated from the TREE-L of the Botanical Garden of the UNALM shows what was pointed out by several authors [7, 25–30] on the retention of these elements in the foliage due to the precipitation of atmospheric dust. The botanical garden, due to its

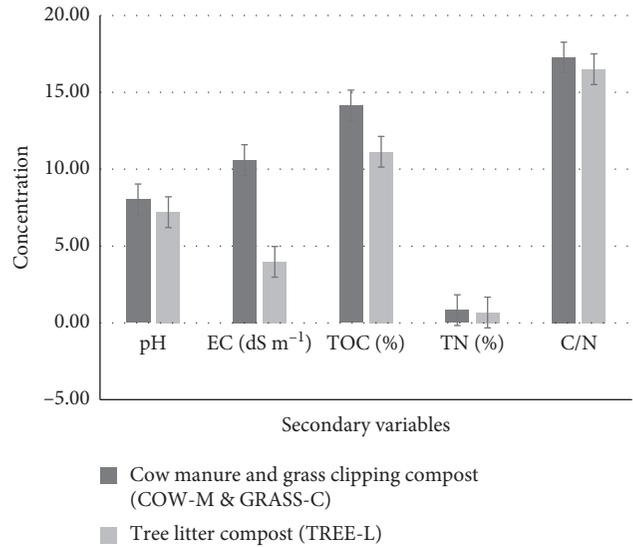


FIGURE 3: Mean values of physicochemical variables of cow manure and grass clipping compost, and tree litter compost ( $n = 3$ ).

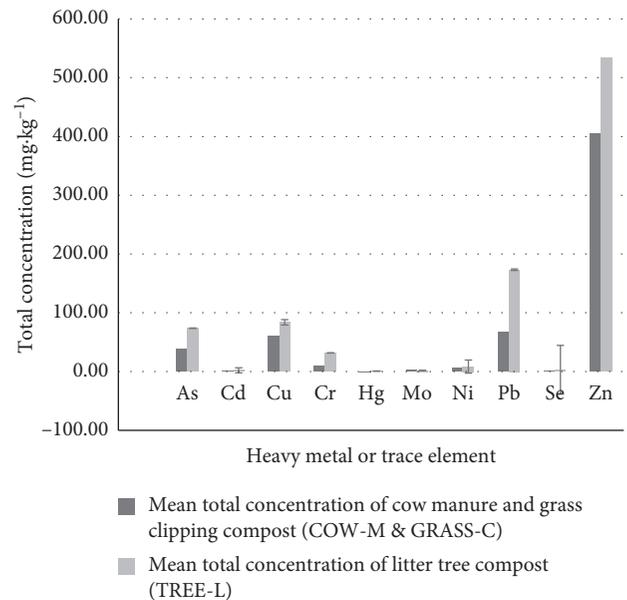


FIGURE 4: Mean concentration of heavy metals and trace elements of interest in cow manure compost and grass clipping vs tree litter compost ( $n = 3$ ).

proximity to La Universidad Avenue, acts as a “biofilter” for the university due to the retention of these pollutants. On the other hand, the compost generated from COW-M and GRASS-C, not being exposed at the same intensity and frequency to air pollution, shows lower levels of heavy metals, but Cu and Zn do show a significant presence due to the feeding of dairy cattle [32–36].

This stage was carried out 24 months before the evaluation stage, giving knowledge of the concentration of heavy

metals and trace elements of interest in significant amounts in the TREE-L compost, so it was decided to use this raw material for the next (evaluation) stage mixed with COW-M.

### 3.2. Evaluation Stage

**3.2.1. Secondary Variables (Microbiological).** Changes were observed in the distribution of the microbial population during the composting process influenced by the physico-chemical variables, in principle, the temperature, the presence of nutrients (C/N rate variation), and the pH, which have been properly documented and referenced by Moreno and Moral [17] and Ch'ng et al. [78] in the normal development of aerobic composting. It can be inferred that the levels of heavy metals and trace elements of interest, due to the recorded concentrations, should not have significantly negatively influenced the metabolic activity of microbial populations, and, it is possible, it rather influenced in the opposite direction. For the latter, Reddy et al. [79] mention that metal tolerances of bacteria were for As 255–355 mg·kg<sup>-1</sup>, for Cd 10–35 mg·kg<sup>-1</sup>, for Zn 215–320 mg·kg<sup>-1</sup>, for Hg 278–315 mg·kg<sup>-1</sup>, and for Pb 3450–4010 mg·kg<sup>-1</sup>. Only in the case of Zn are there recorded values that exceeded these tolerance levels.

Two figures of microbiological variables with the physicochemical variables HA, pH, ratio C/N, and T° have been added in order to be able to see the behavior during the composting process of the average values in the IBM and NBM piles. Figure 5 groups the microbiological variables that were counted as colony-forming units per gram (CFU·g<sup>-1</sup>) and Figure 6 as most probable number per gram (MPN·g<sup>-1</sup>). These grouped figures do not consider the variable *Salmonella* spp. as it has qualitative values nor the helminths as they have a differentiated accounting to the groupings. The first group figure, considered also as BM, is made up of mesophilic aerobes and thermophilic aerobes, *Bacillus* spp., *Lactobacillus* spp., molds and yeasts, and actinomycetes, considering the order of descending population magnitude, and the second figure, considered also as PM, of *Pseudomonas* spp., total coliforms, fecal coliforms, and *Escherichia coli*, considering the order of descending population magnitude. As can be seen at the beginning of the process (Day 0), the population of microorganisms is higher than that observed at the end of the composting process (Day 120). In the cases of the second grouping, it is important to observe the reduction of pathogens such as fecal coliforms, which are the only microbiological variables that showed a statistically significant ( $p < 0.05$ ) reduction in its population in the IBM and NBM piles. These population changes with significant values at the beginning and decreasing during the composting process have also been observed by Ahmed et al. [80] and Haroun et al. [81]. The sterilization of pathogens in composting piles is related to the maximum temperatures reached and the retention time [17, 41, 51]. In both types of piles, the temperature reached 56°C (Figure 2), but in IBM, the thermophilic phase lasted 20 days, while in NBM, it lasted 18 days and in no case was the temperature in the range of 60 to 70°C, which is recommended for the sterilization of the

majority of pathogens [17, 51]. Despite this, the maximum values recommended by the Peruvian regulations [82, 83] were met in the IBM piles, except for helminths, and the NBM piles also did not reach the value for eliminating total coliforms in the composting product (Day 120).

**3.2.2. Primary Variables.** The results of the 10 metals and trace elements of interest, the main variables of this investigation, are presented graphically and described in grouped and differentiated form in HM<sub>tot</sub>, HM<sub>DTPA</sub>, and HM<sub>H2O</sub> for the IBM and NBM piles and the physicochemical variables (pH, T°, C/N ratio, and HA).

The mean values of the total concentrations of heavy metals and trace elements of interest in the products of the composting (Day 120) of the COW-M and the TREE-L IBM have the following order (mg·kg<sup>-1</sup>): Zn (404.33) > Cu (86.33) > Pb (71.2) > Cr (34.33) > As (28.0) > Ni (13.83) > Mo (2.86) > Se (1.38) > Cd (1.32) > Hg (0.39). In the products of composting of COW-M and TREE-L NBM, there was the following order (mg·kg<sup>-1</sup>): Zn (466.0) > Cu (112.23) > Pb (73.23) > Cr (35.33) > As (29.67) > Ni (14.37) > Mo (3.23) > Se (1.55) > Cd (1.38) > Hg (0.38) (Figure 7). Cd, Pb, and Zn exceeded the values of the Austrian Standard [84] for compost with organic agriculture in IBM and NBM, just as it is found with Cu in NBM. In addition, As also exceeded the Brazilian Standard [85] for compost with organic agriculture in IBM and NBM. Nevertheless, the compost complied with it for landscaping and land rehabilitation of the Austrian Standard, and also with Peru's regulations [82, 83] for compost for nonorganic agriculture.

The hierarchical distribution of the elements differs from the one observed in the products of composting in the characterization stage, as although for the four composts Zn is the highest, Pb is the second, displacing Cu to third place, followed by As in fourth place (Section 3.1.2). Ciavatta et al. [40] found distributions of the average concentration (mg·kg<sup>-1</sup>) of heavy metals, in compost from municipal household waste in Italy during 55 days of composting in an aerated static pile, in summer: Zn (1 037) > Pb (981) > Cu > (737) > Cr (162) > Cd (6, 7) and in winter: Zn (981) > Cu (399) > Pb (287) > Cr (69) > Ni (47) > Cd (3.9). Hanc et al. [24] recorded at the end of 84 days of composting household waste with garden waste in a reactor with medium aeration that the average concentration values (mg·kg<sup>-1</sup>) were Zn (167.1) > Cu (29.1) > Pb > (16.9) > Cd (0.27). In both investigations, Zn leads the total concentration values, and the other three are Cu, Pb, and Cr, similar to those recorded in this investigation. The sums of the means of the ten elements of interest on day 0 for IBM and NBM (mg·kg<sup>-1</sup>) were 501.03 ± 65.77 and 520.53 ± 35.22, and on day 120, they presented values of 643.98 ± 20.72 and 737 ± 60.95, respectively. This meant an average increase of 28.53% and 41.66% for IBM and NBM, respectively.

The percentage increase of the concentration from day 0 to 120 in IBM piles was in the following order: Se (112.3%) > Ni (78.0%) > Pb (49.5%) > Cr (43.0%) > Zn (41.5%) > Cd (40.4%) > Hg (34.5%) > As (9.1%). The concentration

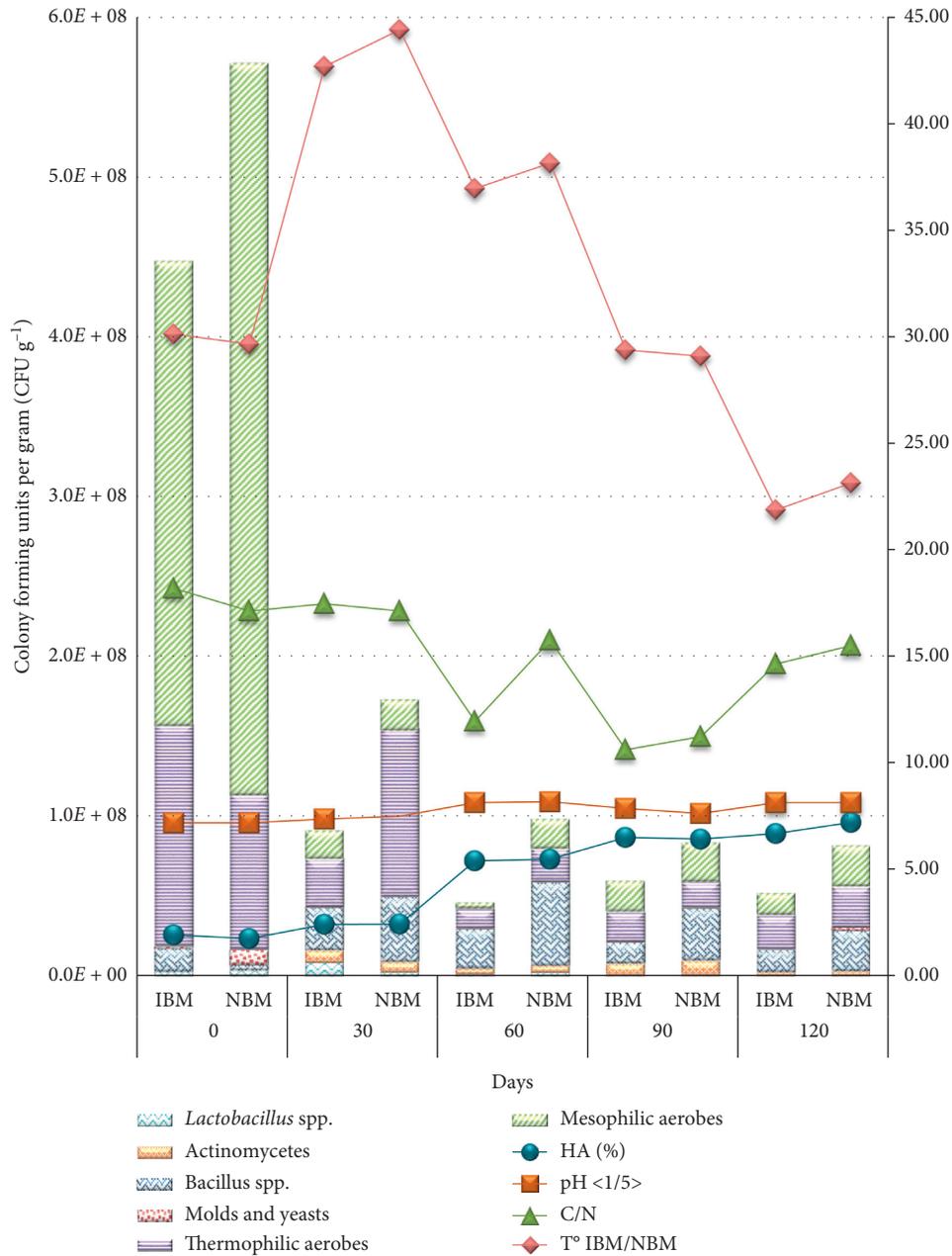


FIGURE 5: Microbiological variables counted as colony-forming units per Gram (CFU·g<sup>-1</sup>) during composting of cow manure and tree litter in inoculated (IBM) and noninoculated (NBM) piles and physicochemical variables (HA, pH, C/N ratio and T°). \* For the purposes of the composition of the figure, the data for molds and yeasts on day 0 in the noninoculated piles (NBM) were divided by 1000.

reduction for these piles is Mo (43.7%) > Cu (16.5%). The percentage increase of the concentration from day 0 to 120 in NBM piles was in the following order: Ni (105.3%) > Se (96.2%) > Cu (48.3%) > Zn (46.2%) > Cd (36.6%) > Hg (35.7%) > Cr (35.9%) > Pb (27.7%) > As (2.3%). The only concentration reduction for these piles was of Mo at 32.6%. Statistical significance ( $p < 0.05$ ) was observed in the increase of Hg, Se, and Zn and the reduction in Mo for both types of pile. There is no trend in the variations indicating any influence of inoculation with BM, but there was found differences among 120 days samples of Cu. The increase in concentration could be due to the loss of mass resulting from mineralization and volatilization of carbon

and nitrogen [17, 21, 52, 86, 87]. The reduction in Mo may be associated with the effect of the pH on its mobility and possible leaching [88]. Ciavatta et al. [40] showed an increase in the summer in the sequence: Ni (752%) > Pb (554%) > Cd (379%) > Cu (303%) > Zn (66%); in winter: Cd (550%) > Ni (104%) > Zn (77%) > Cr (47%) > Cu (19%) > Pb (6%). Hanc et al. [24] observed that the order of increases was Zn (84%) > Cu (49%) > Pb (22%); in the case of Cd, there was a reduction of 4%. Similar to this investigation, most of the average total concentrations of heavy metals increased at the end of the composting process, although with differences in the incremental values, by type of compost and season.

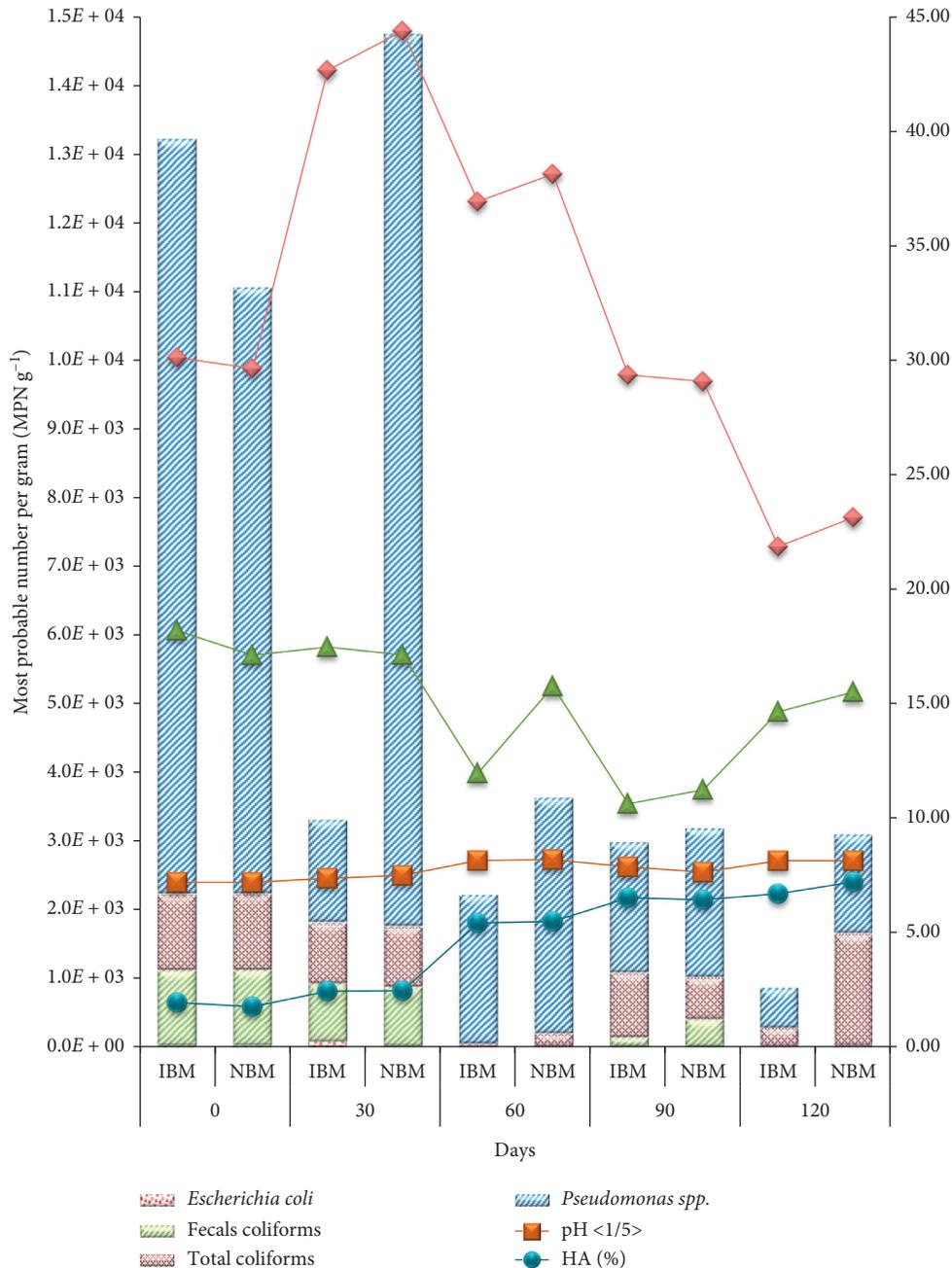


FIGURE 6: Microbiological variables counted as most likely number per gram (NMP·g<sup>-1</sup>) during composting of cow manure and tree litter in piles inoculated with beneficial microorganisms (IBM) and in noninoculated piles (NBM) and physicochemical variables (HA, pH, temperature, and C/N ratio).

The mean values of concentration of heavy metals and trace elements of interest extracted with DTPA from the COW-M and the TREE-L IBM composts (Day 120) had the following order (mg·kg<sup>-1</sup>): Zn (91.59) > Pb (24.50) > Cu (11.2) > As (2.55) > Cd (0.77) > Mo (0.37) > Cr (0.35) > Ni (0.33) > Se (0.07) > Hg (0.001). In the COW-M and TREE-L NBM composts, they had the following order (mg·kg<sup>-1</sup>): Zn (118.9) > Pb (27.21) > Cu (16.15) > As (2.57) > Cd (0.83) > Mo (0.41) > Ni (0.37) > Cr (0.32) > Se (0.07) > Hg (0.001). Figure 8 shows the evolution of the average values of the heavy metals and trace elements of interest extracted with

DTPA during the composting process in IBM and NBM piles and physicochemical variables (pH, T°, C/N ratio, and HA). The sums of the means of the ten elements of interest on day 0 for IBM and NBM (mg·kg<sup>-1</sup>) were 119.69 ± 7.50 and 123.13 ± 8.14, and on day 120, they presented values of 131.76 ± 7.00 and 166.90 ± 12.95, respectively. There was an average increase of 10.09% and 35.55% for IBM and NBM, respectively.

During composting, comparing the concentrations at day 0 and day 120 in both IBM and NBM piles, five of the elements had an increasing trend and the other five had a

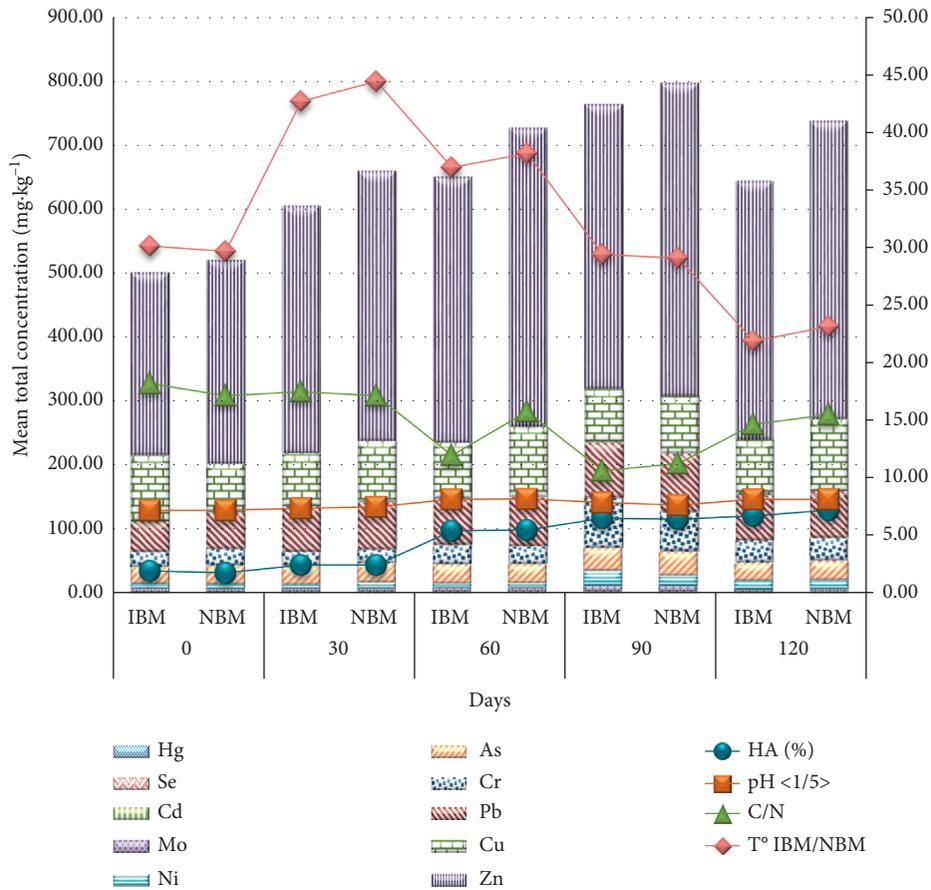


FIGURE 7: Total concentration of heavy metals and trace elements of interest and physicochemical variables (HA, pH, C/N ratio, and T° ratios) during the composting of cattle manure and tree litter in piles inoculated with beneficial microorganisms (IBM) and in non-inoculated (NBM) piles.

decreasing trend. The increases in the IBM piles had the following order: Mo (353.7%) > Cu (62.7%) > Cd (13.2%) > Zn (9.9%) > Pb (0.8%). The reductions in the IBM piles had the following order: Hg (83.3%) > Ni (68.0%) > Se (45.7%) > Cr (32.7%) > As (5.9%). The increases in the NBM piles had the following order: Mo (182.1%) > Cu (162.2%) > Zn (39.7%) > Cd (13.5%) > Pb (1.0%). The reductions in the NBM piles had the following order: Hg (85.7%) > Cr (43.9%) > Se (42.6%) > Ni (38.3%) > As (4.8%). Statistical significance ( $p < 0.05$ ) was observed in the increase in Cu and Mo and in the reduction of Cr, Ni, and Se for both pile types. In the variations, there is no trend indicating any influence of the inoculation with BM. Ciavatta et al. [40] observed in 60-day compost increases in the concentration of Cu, Pb, Ni, and Cd, but for Zn and Cr, it remained almost constant. The opposite was found by Huang et al. [21] as they showed a 63-day composting reduction in Cu and Zn extracted with DTPA. The results are dissimilar to what was mentioned by the authors and the investigation. However, they do agree in the case of Cu and that mentioned by Ciavatta et al. [40].

The mean values of concentration of heavy metals and trace elements of interest extracted with deionized water from the COW-M and TREE-L IBM composts (Day 120)

had the following order ( $\text{mg}\cdot\text{kg}^{-1}$ ): As (3.08) > Zn (1.69) > Mo (0.71) > Cu (0.62) > Pb (0.44) > Ni (0.26) > Cr (0.21) > Se (0.09) > Cd (0.007) > Hg (0.002). In the COW-M and the TREE-L NBM composts, they had the following order ( $\text{mg}\cdot\text{kg}^{-1}$ ): As (3.14) > Zn (1.85) > Mo (0.74) > Cu (0.7) > Pb (0.36) > Ni (0.29) > Cr (0.2) > Se (0.09) > Cd (0.007) > Hg (0.002). Figure 9 shows the evolution of the average values of the heavy metals and trace elements of interest extracted with deionized water during the composting process in IBM and NBM piles and physicochemical variables (pH, T°, C/N ratio, and HA). The sums of the means of the ten elements of interest on day 0 for IBM and NBM ( $\text{mg}\cdot\text{kg}^{-1}$ ) were  $13.09 \pm 2.53$  and  $11.64 \pm 1.94$ , and on day 120, they presented values of  $7.10 \pm 0.62$  and  $7.38 \pm 0.44$ , respectively. This meant an average reduction of 45.74% and 36.57% for IBM and NBM, respectively.

During the composting of COW-M and TREE-L, there was a decreasing trend in most of the concentrations of the analyzed elements extracted with deionized water, except Mo which showed an increasing trend comparing the values of day 0 and 120. The reductions in the IBM piles had the following order: Cu (76.3%) > Hg (75.0%) > Cd (68.2%) > Ni (66.4%) > Zn (64.5%) > Pb (63.8%) > Cr (41.7%) > Se (28%) > As (5.2%). Mo increased by 46.6%. The reductions in the

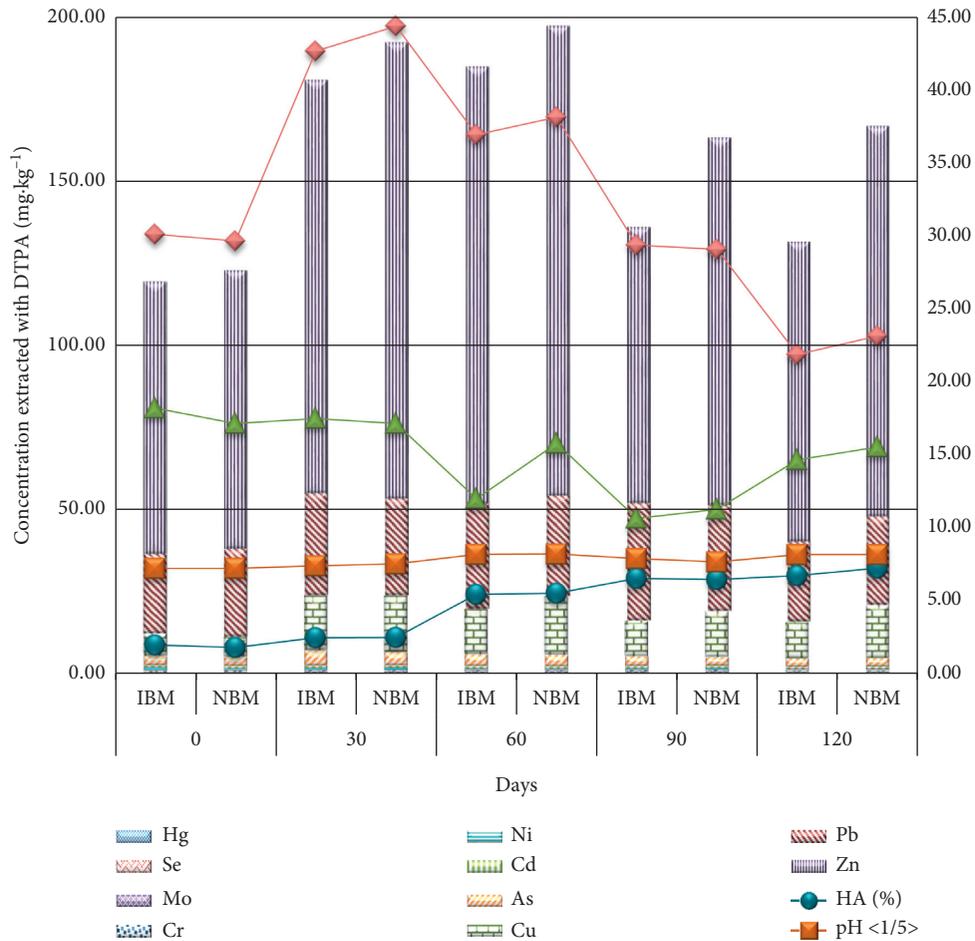


FIGURE 8: Concentration of heavy metals and trace elements of interest extracted with DTPA and physicochemical variables (HA, pH, temperature, and C/N ratio) during the composting of cow manure and tree litter in piles inoculated with beneficial microorganisms (IBMs) and in noninoculated (NBM) piles.

NBM piles had the following order: Cu (68.9%) > Hg 60.0%) > Ni (55.0%) > Cd (53.3%) > Zn (52.9%) > Pb (47.9%) > Se (36.6%) > Cr (25.9%) > As (5.7%). Mo increased by 42.0%. These results indicate that the composting process reduced the solubility and leaching of the metals other than Mo. Statistical significance ( $p < 0.05$ ) was observed in the reduction in Cu, Ni, Pb, and Zn for both types of piles, from which it can also be stated, without statistical significance, that in absolute terms, there are higher solubility reduction values for these elements in IBM piles than in NBM piles. Ciavatta et al. [40], Huang et al. [21], Castaldi et al. [37], Hargreave et al. [41], and Ramdani et al. [89] showed a reduction in the concentration of Cd, Cr, Cu, Ni, Pb, and Zn extracted with deionized water in composting between 60 and 100 days, which resembles the results found in the present investigation.

### 3.2.3. Statistical Analysis of the Primary and Secondary Variables of the Evaluation Stage

(1) *Analysis of the Primary Variables.* Zn, Cu, and Pb are the three elements with the highest total concentration, and they also exceeded the maximum values for organic agriculture [84, 85]. The Cu showed a significant difference, in both types of piles (IBM and NMB), between day 0 and 120 in the concentration extracted with DTPA and deionized water. For Pb, the difference was shown in the extraction with deionized water. For Zn, there were differences in total concentration and in extraction with deionized water (Figure 10).

3.3. *Correlation of Variables.* On the basis of those variables that were significant ( $p$  value > 0.05) in the Pearson correlation with a coefficient value greater than 0.5 and did not show any difference between the factors (inoculation and noninoculation), it was observed for the elements Cu, Ni, Zn, Cd, Pb, Se, and Hg extracted with water that they have a significant negative correlation with the humic acid fraction (Table 7). The same happens with Se, Ni, and Hg extracted

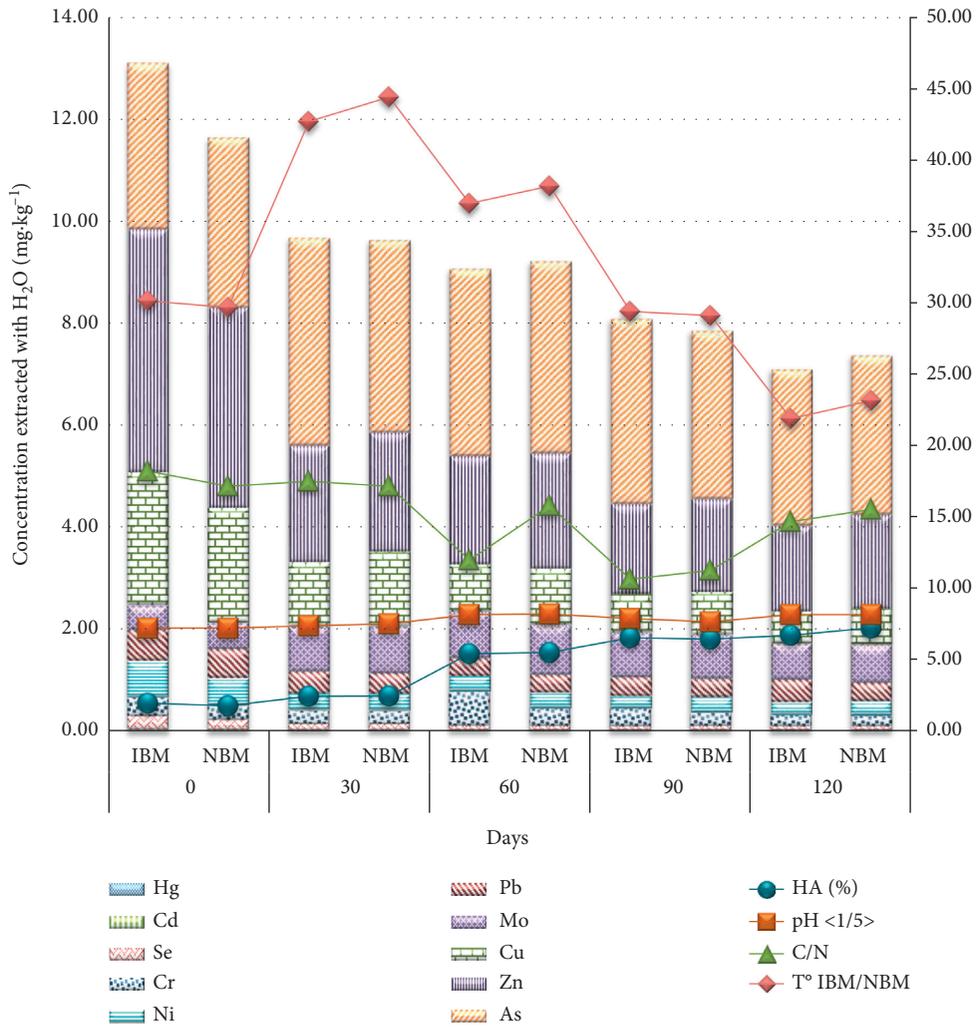


FIGURE 9: Concentration of heavy metals and trace elements of interest extracted with deionized water and physicochemical variables (HA, pH, C/N ratio, and T°) during the composting of cow manure and tree litter in piles inoculated with beneficial microorganisms (IBM) and noninoculated (NBM) piles.

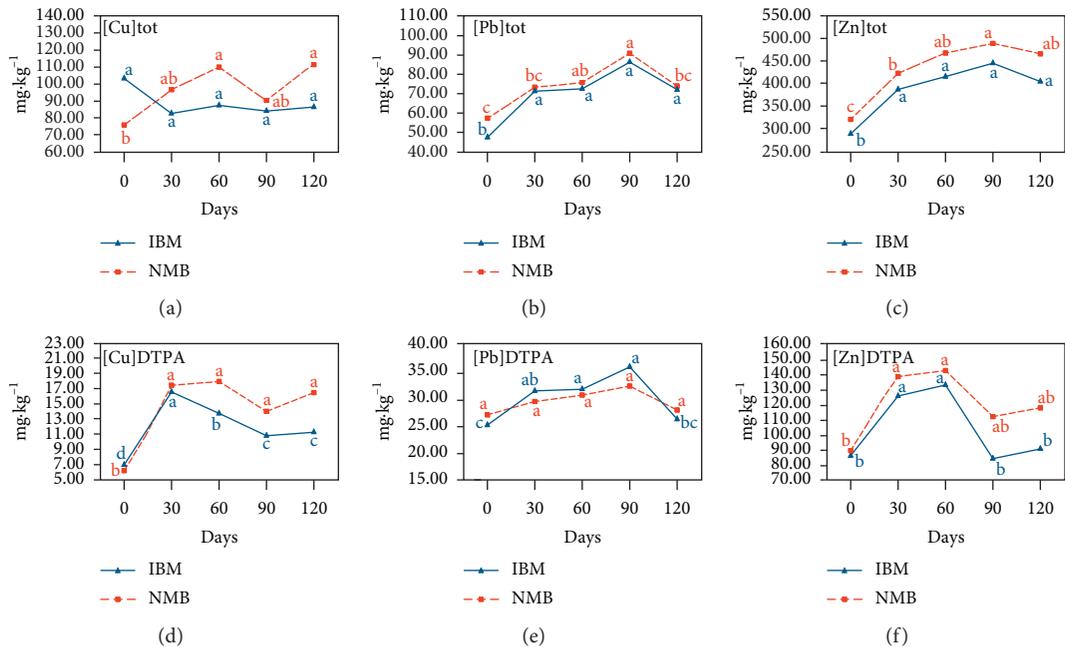


FIGURE 10: Continued.

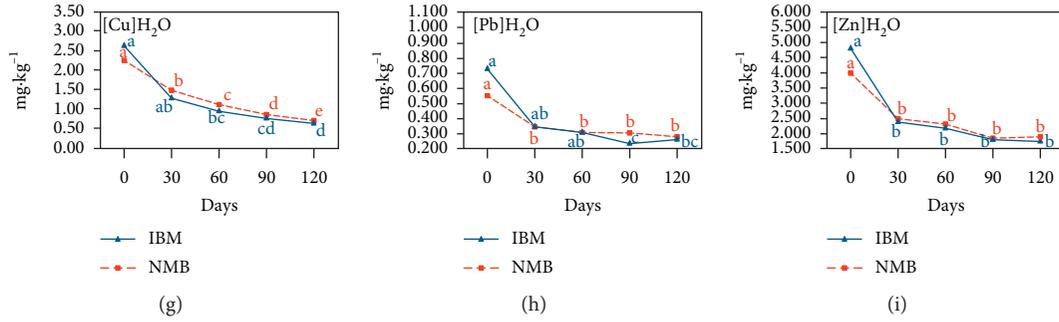


FIGURE 10: Total concentrations, extracted with DTPA and extracted with deionized water, of Cu, Pb, and Zn during the composting of cattle manure and tree litter in IBM and NMB piles. Between points on the lines with different letters the differences are statistically significant ( $p < 0.05$ ).

TABLE 7: Negative values of Pearson's correlation between primary variables and the AH, with significance without influence of the inoculation factor.

Primary variable ( $\text{mg} \cdot \text{kg}^{-1}$ )	Secondary physicochemical variable (%)	$n$	Pearson	$p$ value
$[\text{Cu}]_{\text{H}_2\text{O}}$	HA	30	-0.8410	$5.94 \times 10^{-9}$
$[\text{Se}]_{\text{DTPA}}$	HA	30	-0.8382	$7.45 \times 10^{-9}$
$[\text{Ni}]_{\text{DTPA}}$	HA	30	-0.7680	$7.26 \times 10^{-7}$
$[\text{Ni}]_{\text{H}_2\text{O}}$	HA	30	-0.7550	$1.43 \times 10^{-6}$
$[\text{Zn}]_{\text{H}_2\text{O}}$	HA	30	-0.7377	$3.29 \times 10^{-6}$
$[\text{Cd}]_{\text{H}_2\text{O}}$	HA	30	-0.6705	$5.04 \times 10^{-5}$
$[\text{Hg}]_{\text{DTPA}}$	HA	30	-0.6615	$6.88 \times 10^{-5}$
$[\text{Pb}]_{\text{H}_2\text{O}}$	HA	30	-0.6269	$2.10 \times 10^{-4}$
$[\text{Se}]_{\text{H}_2\text{O}}$	HA	30	-0.6266	$2.12 \times 10^{-4}$
$[\text{Hg}]_{\text{H}_2\text{O}}$	HA	30	-0.5201	$3.22 \times 10^{-3}$

[·] total concentration; [ $\cdot$ ]<sub>DTPA</sub>: concentration extracted with pentatetic acid; [ $\cdot$ ]<sub>H<sub>2</sub>O</sub>: concentration extracted with deionized water; HA: fraction of humic acids.

TABLE 8: Positive values of Pearson's correlation between primary variables and the AH with significance without influence of the inoculation factor.

Primary variable ( $\text{mg} \cdot \text{kg}^{-1}$ )	Secondary physicochemical variable (%)	$N$	Pearson	$p$ value
$[\text{Cd}]_{\text{tot}}$	HA	30	0.5176	$3.40 \times 10^{-3}$
$[\text{Cr}]_{\text{tot}}$	HA	30	0.5788	$8.06 \times 10^{-4}$
$[\text{Ni}]_{\text{tot}}$	HA	30	0.5864	$6.61 \times 10^{-4}$
$[\text{Mo}]_{\text{DTPA}}$	HA	30	0.6223	$2.41 \times 10^{-4}$
$[\text{Pb}]_{\text{tot}}$	HA	30	0.6254	$2.19 \times 10^{-4}$
$[\text{Hg}]_{\text{tot}}$	HA	30	0.6633	$6.47 \times 10^{-5}$
$[\text{Zn}]_{\text{tot}}$	HA	30	0.7385	$3.18 \times 10^{-6}$
$[\text{Se}]_{\text{tot}}$	HA	30	0.8024	$9.65 \times 10^{-8}$

[·] total concentration; [ $\cdot$ ]<sub>DTPA</sub>: concentration extracted with pentatetic acid; [ $\cdot$ ]<sub>H<sub>2</sub>O</sub>: concentration extracted with deionized water; HA: fraction of humic acids.

with pentatetic acid. The opposite occurs with Mo extracted with pentatetic acid which shows a positive correlation with AH and in the total concentration of Cd, Cr, Ni, Pb, Hg, Zn, and Se (Table 8). Castaldi et al. [37] found a correlation of -0.95; -0.95; -0.92, and -0.90 ( $p < 0.01$ ), respectively between the Pb, Cd, Zn, and Cu extracted with deionized water and the AH after 100 days of composting of solid municipal waste in Italy, values higher than those found in this investigation, but which

confirm the affinity between these two variables for these elements.

3.3.1. *Unrestricted Sorting Analysis with Examination of Principal Components.* Figure 11 shows that the variables C/N and OM (%) were highly correlated, as well as Da ( $\text{g} \cdot \text{cm}^{-3}$ ) with  $[\text{Cr}]_{\text{tot}}$  ( $\text{mg} \cdot \text{kg}^{-1}$ ) and that those variables with a negative correlation were  $[\text{Zn}]_{\text{DTPA}}$  ( $\text{mg} \cdot \text{kg}$ ) and FA (%). In the majority

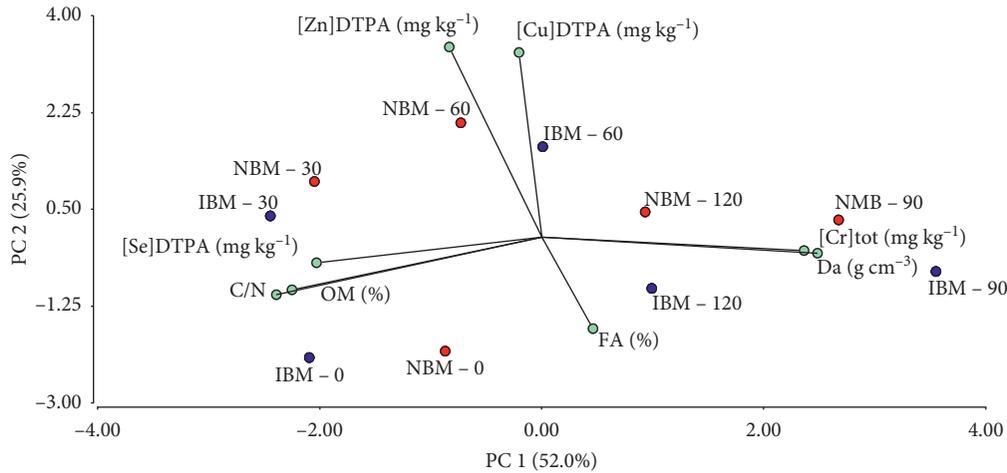


FIGURE 11: Unrestricted sorting analysis (with examination of principal components).

of cases, the effect of adding microorganisms was not observed to affect the results with respect to days (NBM and IBM). In most cases, the points are close. With respect to the treatment days, the difference between the grouping of the days 0 and 30, of day 60, and of the grouping 120 and 90 is remarkable. The variables C/N, OM (%), and  $[\text{Se}]_{\text{DTPA}}$  ( $\text{mg}\cdot\text{kg}^{-1}$ ) are more related to days 0 and 30 (especially IBM-30). The Da variables ( $\text{g}\cdot\text{cm}^{-3}$ ) with  $[\text{Cr}]_{\text{tot}}$  ( $\text{mg}\cdot\text{kg}^{-1}$ ) are related to days 90 and 120; the  $[\text{Zn}]_{\text{DTPA}}$  variables ( $\text{mg}\cdot\text{kg}^{-1}$ ) and  $[\text{Cu}]_{\text{DTPA}}$  ( $\text{mg}\cdot\text{kg}^{-1}$ ) are more related to day 60.

## 4. Discussions

**4.1. On the Variation of the Total Content, Bioavailability, and Solubility of the Heavy Metals and Trace Elements of Interest during the Composting of Cow Manure and Tree Litter.** There is variation in total content between day 0 and 120 with a statistical significance ( $p < 0.05$ ) in Hg, Mo, Se, and Zn for both pile types (IBM and NBM). In **Hg**, with 34.5% increase (from 0.29 to  $0.39\text{ mg}\cdot\text{kg}^{-1}$ ) for IBM and 35.7% increase (from 0.28 to  $0.38\text{ mg}\cdot\text{kg}^{-1}$ ) for NBM. In **Se**, with 112.3% increase (from 0.65 to  $1.38\text{ mg}\cdot\text{kg}^{-1}$ ) in IBM and 96.2% increase (from 0.79 to  $1.55\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM, and, in **Zn**, with 41.5% increase (from 285.67 to  $404.33\text{ mg}\cdot\text{kg}^{-1}$ ) in IBM and 46.2% increase (from 318.67 to  $466.0\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM. In **Mo**, 43.7% reduction (de 5.08 a  $2.86\text{ mg}\cdot\text{kg}^{-1}$ ) was observed in IBM and 32.6% reduction (from 4.79 to  $3.23\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM.

There is variation in bioavailability between day 0 and 120 with a statistical significance ( $p < 0.05$ ) in Cu, Cr, Mo, Ni, and Se for both pile types (IBM and NBM) in **Cu**, with 62.7 % increase (from 6.89 to  $11.21\text{ mg}\cdot\text{kg}^{-1}$ ) in IBM and 162.2 % increase (from 6.16 to  $16.15\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM.; in **Mo**, with 353.7% increase (from 0.082 to  $0.372\text{ mg}\cdot\text{kg}^{-1}$ ) in IBM and 182.1% increase (from 0.145 to  $0.409\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM; in **Cr**, with 32.7% reduction (from 0.52 to  $0.35\text{ mg}\cdot\text{kg}^{-1}$ ) for IBM and 43.9 % reduction (from 0.57 to  $0.32\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM; in **Ni**, with 68.0% reduction (from 1.03 to  $0.33\text{ mg}\cdot\text{kg}^{-1}$ ) in IBM and 38.3% reduction (from 0.60 to  $0.37\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM; in **Se**, with 45.7% reduction (from

0.13 to  $0.07\text{ mg}\cdot\text{kg}^{-1}$ ) for IBM and 42.6% reduction (from 0.12 to  $0.07\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM.

There is variation in solubility between day 0 and 120 with a statistical significance ( $p < 0.05$ ) in Cu, Ni, Pb, and Zn for both pile types (IBM and NBM) in **Cu**, with 76.3% reduction (from 2.62 to  $0.62\text{ mg}\cdot\text{kg}^{-1}$ ) in IBM and 68.9% reduction (from 2.25 to  $0.70\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM; in **Ni**, with 66.4% reduction (from 0.268 to  $0.09\text{ mg}\cdot\text{kg}^{-1}$ ) in IBM and 55.0 % reduction (from 0.2  $\text{mg}\cdot\text{kg}^{-1}$  to  $0.09\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM; in **Pb**, with 63.8% reduction (from 0.719 to  $0.26\text{ mg}\cdot\text{kg}^{-1}$ ) in IBM and 47.9% reduction (from 0.547 to  $0.285\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM; in **Zn**, with 64.5% reduction (from 4.764 to  $1.689\text{ mg}\cdot\text{kg}^{-1}$ ) in IBM and 52.9 % reduction (from 3.933 to  $1.852\text{ mg}\cdot\text{kg}^{-1}$ ) in NBM.

The increase in the total content of Hg, Se, and Zn is due to the loss of mass resulting from mineralization and volatilization of carbon and nitrogen [24]. The reduction in the total Mo content may be due to mobility resulting from the increase in pH [1]. The increased bioavailability of Cu and Mo is linked to mineralization and chelation processes that in some cases increase availability [1] and also to higher pH (>5.5) which influences availability in Mo [3]. The composting process reduced the solubility and leaching of Cu, Ni, Pb, and Zn, and there were higher solubility reduction values in the inoculated piles than in the noninoculated piles. It is understood that the oxidation process and the formation of organometallic complexes influenced the reduction of the soluble content of the mentioned elements [21, 37, 42, 90, 91].

**4.2. On the Influence of Humic and Fulvic Acids on Total Concentration, Bioavailability, and Solubility of the Heavy Metals and Trace Elements of Interest during the Composting of Cow Manure and Tree Litter.** There is a positive correlation between the humic acid fraction and the total concentration of Se, Zn, Hg, Pb, Ni, Cr, and Cd in the following order: Se (+0.80) > Zn (+0.73) > Hg (0.66) > Pb (+0.63) > Ni (+0.59) > Cr (+0.58) > Cd (+0.52). From the records, it can be concluded that humic acids could support the retention in

the compost matrix of the mentioned elements, which would reduce their mobility and increase their concentration (bioaugmentation) while the organic matter mineralizes [3, 24, 92–94]. This affinity is confirmed by comparing the results of variation between days 0 and 120 for IBM and NBM. It should be noted that the As, which had the smallest increase in total concentration (<10%) for both piles, confirming the nonaffinity with the AH [1, 3].

There is a negative correlation between the concentration of the humic acid fraction and the concentration extracted with pentatetic acid for Se, Ni, and Hg in the following order: Se (−0.84) > Ni (−0.77) > Hg (−0.66). A chelating or sequestering effect in the megastructure of the humic acid fraction can be understood, which generated a phenomenon of “immobility” in the compost matrix [90]. This is confirmed by the analysis of the variation between days 0 and 120 for inoculated and noninoculated piles because these three elements present the greatest reductions in their extracted concentrations. It is highlighted that the As, which had the lowest reduction extracted with pentatetic acid (<6%) for both piles, confirming the nonaffinity with the AH [1, 3].

There is a positive correlation (+0.62) between the concentration of the humic acid fraction and the concentration of Mo extracted with pentatetic acid. This can infer a possible affinity in the disposition of this element for plants in the presence of humic acids [3, 88]. This is confirmed by the analysis of the variation between days 0 and 120 for inoculated and noninoculated piles because this element presents the greatest increase of its extracted concentration.

There is a negative correlation between the concentration of the humic acid fraction and the concentration from the extraction with deionized water of Cu, Ni, Zn, Cd, Pb, Se, and Hg in the following order: Cu (−0.84) > Ni (−0.76) > Zn (−0.74) > Cd (−0.67) > Pb (−0.63) > Se (−0.63) > Hg (−0.52). It is possible that the increased concentration of the humic acid fraction during the composting process influenced the reduction of the solubility of these elements, which infers a redistribution of the water-soluble fractions to more stable forms strongly bound to the humic acid fractions [12, 37, 90]. This is confirmed by the analysis of the variation between days 0 and 120 for IBM and NBM, due to the fact that these elements present the greatest reductions of their extracted concentrations. It should be noted that the As, which had the smallest reduction in the concentration extracted with deionized water (<6%) for both piles, confirms the nonaffinity with the HA [1, 3].

The concentration of the fulvic acid fraction showed no significant correlation with any of the analyzed primary variables. Humic and fulvic acids are organic substances that can have a high capacity for generating bonds with heavy metals, due to the large number of functional groups. In this investigation, this was only indicated with humic acid for some heavy metals, which may be due to the raw materials and the types of extractants which were used [21, 95].

#### 4.3. On the Influence of Inoculation with Beneficial Microorganisms on Total Concentration, Bioavailability, and Solubility of the Heavy Metals and Trace Elements of Interest.

Comparing day 0 (T0) and day 120 (T120), the total Cu content was reduced by 16.45% (from 103.33 to 86.33 mg·kg<sup>−1</sup>) in IBM and increased by 48.32% (from 75.67 to 112.23 mg on the influence of inoculation with beneficial microorganisms on total concentration, bioavailability, and solubility of the heavy metals and trace elements of interest kg<sup>−1</sup>) in NBM. Comparing the concentration in the final product (T120) of IBM and NBM, it was observed that the former registered 23.08% less concentration of total Cu. Based on what has been recorded, it can be concluded that the inoculation of beneficial microorganisms reduced the total Cu concentration.

Comparing day 0 (T0) and day 120 (T120), the bioavailability of Cu increased 62.7% (from 6.89 to 11.21 mg·kg<sup>−1</sup>) in IBM and 162.2% (from 6.16 to 16.15 mg·kg<sup>−1</sup>) in NBM. Comparing the bioavailability of the Cu extracted in the final product (T120), we have 30.6% less concentration in IBM. From the above, it is concluded that the inoculation of the beneficial microorganisms reduced the bioavailability of Cu.

Comparing day 0 (T0) and day 120 (T120), the bioavailability of Zn increased by 9.94% (from 83.32 to 91.60 mg·kg<sup>−1</sup>) in IBM and by 39.71% (from 85.14 to 118.95 mg·kg<sup>−1</sup>) in NBM. Comparing the bioavailable concentration of Zn in the final product (T120), we have 23% less in IBM. From the above, it is concluded that the inoculation of the beneficial microorganisms reduced the bioavailability of Zn.

The inoculation of beneficial microorganisms did not influence, with statistical significance ( $p < 0.05$ ), the extraction with deionized water of the heavy metals and trace elements of interest or the solubility of these elements.

The lower bioavailability of Cu and Zn in inoculated piles than in noninoculated piles may be due to the fact that inoculation increased the bioaugmentation of these elements in the cells of microorganisms, or the chelation with organic functional groups, or the sequestration in dead cells [45]. While the reduction in the concentration of the total Cu content remains unknown because of its low probability of volatilization, one possibility is that refractory compounds are generated that prevent its quantification [3].

## 5. Conclusions

The composting product made with tree litter and cattle manure from an urban area cannot be used in organic agriculture because As, Cd, Cu, Pb, and Zn do not comply with the maximum allowable values of Brazil and Austria. However, it is possible to use it in land rehabilitation and landscaping considering the Austrian standard and also in nonorganic agriculture in Peru. The presence of Cu and Zn in the composting product is due to the type of cattle feed and to atmospheric contamination, while for As, Cd, and Pb, it is mainly due to the atmospheric contamination. Inoculation of beneficial microorganisms reduces the bioavailability of Cu and Zn in comparison with the compost from piles which were not inoculated. The composting process reduced in both pile types (IBM and NBM) and the solubility of Cu, Pb and Zn. Fulvic acids did not show a significant

correlation with any of the elements; however, humic acids did show affinity with Cd, Cu, Pb, and Zn but not with As. This affinity confirms that the soils of green spaces can be sinks for heavy metals. Likewise, we can infer that the compost of the cattle manure can be recommended as an amendment to soils to reduce the leaching of Cu and Zn.

The responses of the ten elements under analysis are different, as we have observed. It is therefore unlikely that their bioavailability and solubility can be reduced with a single treatment technique. Specifically, in the case of As, its behavior associated with an increase in pH must be taken into account, for which other techniques such as the aggregation of biochar, zeolites, or vermicomposting could be considered. Regarding the presence of helminth oocysts, consideration should be given to increasing the frequency of turning and to inoculation with a greater activated stock solution of beneficial microorganisms in order to reach temperatures between 60 and 70°C and so eliminate them.

### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

### Conflicts of Interest

The authors declare that there are no conflicts of interest.

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### Supplementary Materials

Figure a: triplicate samples of the activated stock solution (ASS) of beneficial microorganisms at day 0. Figure b: collection of samples from the compost piles on day 120 (T120) of the evaluation stage. Table a: treatments investigated at the evaluation stage. (*Supplementary Materials*)

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