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Extractability, Distribution Among Different Particle Size Fractions, and Phytotoxicity of Cu and Zn in Composts Made With the Separated Solid Fraction of Pig Slurry

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The presence of elevated concentrations of heavy metals (Cu and Zn) in pig slurry and, particularly, in the solid fraction obtained after solid-liquid separation is a limiting factor for their use in agricultural soils. These metals are further concentrated if compost is produced from solid pig slurry. This paper studies the influence of the composting of the separated solid phase of pig slurry on the concentrations and solubility of Cu and Zn, and their distribution in the different particle size fractions, as well as evaluating their potential toxic effects on seed germination and seedling growth. Two composts were prepared with the solid fraction of pig slurry in a piglets and sows farm, using two different bulking agents (cereal straw and cotton gin waste). The concentrations of Cu and Zn in the mature compost were higher due to organic matter degradation; however, their solubility decreased from 0.72 and 1.76% in the solid fraction of pig slurry to 0.18 and 0.30% of total Cu and Zn, respectively, in the compost prepared with cotton gin waste. Zinc was concentrated in the smallest particle size fraction, while the Cu concentration was highest in the largest particles, and associated to the organic matter/humic fraction. The elimination of the smallest particle size fraction would not reduce significantly the total heavy metal concentration of the composts. Nevertheless, the low solubility of both metals in the composts avoided any significant toxic effect on seed germination and also in the growth test when compost was present at low rates.

Keywords: composting, germination index, heavy metals, pig slurry, solubility

INTRODUCTION

The intensive and large-scale development of pig farming has led to the concentration of pig slurry production in small areas that offer limited land for its disposal and use in agriculture (FAO, 2009; Sáez et al., 2017). In addition, the application of pig slurry to agricultural soil is limited to 170 kg N ha^{-1} in areas vulnerable to nitrate pollution (CEE, 1991). These facts have led both farmers and researchers to seek alternative uses and management options for pig slurry, in order to avoid the need to transport, or the excessive accumulation of this material in the farm, with the consequent environmental concern that this may pose (Burton and Turner, 2003). Frequently, pig slurry is separated into its solid and liquid fractions through different physico-chemical processes in farms (Popovic et al., 2012), which allows the management of both fractions separately (Sáez et al., 2017). While the liquid fraction can be used as nutrient-rich irrigation water on agricultural land in the vicinity of the farm, the solid fraction can be either transported further for landspreading or subjected to microbial stabilization processes (i.e., composting; Santos et al., 2016; Sáez et al., 2017).

However, the concentrations of potentially toxic heavy metals (mainly Cu and Zn) in the slurry may prevent its use in agricultural soils devoted to food production (Moral et al., 2008). Such metals, despite being essential micronutrients for plant and crop growth, can become toxic above their corresponding threshold concentrations. The high concentrations of these metals in pig slurry derive from their use as ZnO and $CuSO_4$ in feed additives in pig farms (especially during pregnancy and in the post-weaning phase; Carlson et al., 1999; Woodworth et al., 1999). After solid-liquid separation, the metals from the slurry are recovered mostly in the solid fraction (Popovic et al., 2012; Sáez et al., 2017), which can be a major factor limiting the use of this waste material for direct agricultural use or for preparation of high quality compost (Ko et al., 2008; Bernal et al., 2017; Sáez et al., 2017). Therefore, repeated application of the solid fraction of pig slurry or its derived composts to soil, as fertilizer in crop production, can build up metals in the soil with the corresponding environmental risk, as has been found already for sewage sludge and municipal solid waste (MSW) and their derived composts (Hargreaves et al., 2008; Smith, 2009). The use of compost rich in heavy metals would affect not only soil, but also surface and groundwater and the surrounding environment, and may end in the introduction of the contaminants into the food chain (Smith, 2009).

A potential alternative use for pig slurry is as a soil improver in the restoration of trace element contaminated soils (Clemente et al., 2012; Martínez-Fernández et al., 2014), where the addition of low amounts of metals with the slurry would not significantly increase their contents in the soil and would, therefore, suppose no environmental risk (Pardo et al., 2014). The use of fresh, raw pig slurry, or its separated solid fraction in contaminated soils has been shown to be an effective alternative for the recycling of this material, as it helps to stabilize the contaminants in the soil (Clemente et al., 2019) and promotes the establishment of a self-sustainable and long-lasting vegetation cover (Pardo et al., 2014) in otherwise completely bare soils. Compost from MSW, green waste, or olive-mill wastes has been found to be efficient in reducing the availability and solubility of heavy metals during the remediation of contaminated soils (Farrell and Jones, 2010; Pardo et al., 2016). The use of compost made with the solid fraction of pig slurry as a component of the substrate used for the cultivation of bioenergy crops (Sáez et al., 2016) has also been shown to be a valid management alternative for pig slurry solids.

The bioavailable fraction of the contaminants (the one that can be easily taken up by the plants and other living organisms present in the soil) has been recently proposed as the fraction that has to be regulated and monitored in remediation procedures and for which the corresponding legislation and maximum allowed limits have to be defined (Alvarenga et al., 2018). Notwithstanding, the fact that the bioavailability of the metals in the soils may change over time must be also contemplated, especially after the application of biodegradable organic materials, and total concentrations equally limited and regulated. Therefore, the availability (solubility and/or extractability) of the contaminants present in the organic materials used in remediation procedures may have to be taken into account—in addition to the total contents in these materials—when precise restoration plans are designed. The solubility or extractability of heavy metals in compost and other organic materials can be assessed by the use of single extraction reagents, such as neutral salt solutions, and sequential extraction procedures (Walter et al., 2006; Smith, 2009; Alvarenga et al., 2015). In any case, these extraction procedures are operationally defined, and bioassays are strongly recommended to complement chemical information in toxicity/risk assessment (van Gestel et al., 2001).

The heavy metal concentrations, both total and bioavailable, in compost from organic wastes have lately received increasing attention. This is a relevant issue in the European Union, where environmental policies aim to increase the recycling of biodegradable wastes and composts on land and to prevent inputs of contaminants entering the soil (Smith, 2009). Most of the studies carried out in this regard have focused on sewage sludge, MSW, and the composts derived from them. For instance, heavy metals are mainly associated with finer particle size classes in mechanically-segregated MSW (Sharma et al., 1997). Consequently, the mechanical screening of these composts to remove the fine fraction has been considered as a useful technique to reduce heavy metal concentrations in the marketed composts and therefore to increase their agricultural value (Smith, 2009). Regarding bioavailability, most studies have found that the extractability and availability of the metals decrease throughout the composting and maturation periods (Eneji et al., 2003; Amir et al., 2005).

The concentrations of metals in the feedstock used for composting, not the type of feedstock, affect the final concentrations in the compost (Smith, 2009). However, the availability of metals can depend on both the content in the feedstock and the maturation state of the compost. In fact, Soumaré et al. (2003) reported higher concentrations of Zn in the water-soluble and exchangeable fractions of a peat-based substrate, compared to green waste compost, despite a lower total Zn concentration in the former, which highlights the importance of the physico-chemical characteristics of the organic materials regarding metal bioavailability.

Therefore, the objective of this work was to study the influence of the bio-oxidation/maturation processes on the concentrations and solubility of heavy metals (Zn and Cu) during the composting of the separated solid phase of pig slurry, and their distribution in the different particle size fractions, evaluating their potential toxic effects on seed germination and seedling growth. We hypothesized that the bioavailability and toxicity of Cu and Zn in pig slurry can be reduced through the stabilization of the organic matter during composting, and that Cu and Zn may be associated mainly with a specific particle size (fine) fraction of the compost that could be easily removed by sieving for reducing their concentration in the full compost.

MATERIALS AND METHODS

Compost Samples

The composts studied in the experiment were prepared with a mixture of the solid fraction (SF) of pig slurry obtained from a piglets and sows farm located in southeast Spain and two different bulking materials: cereal straw and cotton gin waste. The SF was isolated using a screw-press system for solid-liquid separation (without flocculants). Two composting piles were prepared within the farm facilities: Pile 1, mixing the separated SF (SF1, solid fraction stored for one month) with cereal straw, as bulking agent, in a trapezoidal pile (3:2, v:v; 4.8 m^3); and Pile 2, mixing SF (SF2, freshly separated) with cotton gin waste (2:1, v:v; 19.3 m^3). The windrow system with aeration by mechanical turning was used for both piles. The bio-oxidative phase of Pile 1 lasted for 75 days (with 3 mechanical turning events in total) and that of Pile 2 for 120 days (with 5 mechanical turning events in total); the total composting times (including the maturation period) were 170 and 187 days, respectively. Compost samples were obtained by mixing seven sub-samples taken from seven representative sites of the corresponding pile, covering the whole profile (from the top to the bottom of the pile). Further details of the composting process can be found in Sáez et al. (2017).

The original solid fractions (SF1 and SF2) and the final mature composts (1 and 2) were characterized physico-chemically (**Tables 1**, **2**). The moisture content was determined after drying at 105°C for 24 h and the organic matter (OM) concentration by loss on ignition at 550°C for 24 h. The electrical conductivity (EC) and pH were measured in 1:10 water extracts (w/v). The water-soluble organic carbon (C_w) was determined in 1:20 (w/v) water extracts, using an automatic analyzer for liquid samples (TOC-V CSN Analyzer, Shimadzu, Japan). The total organic carbon (TOC) and total nitrogen (TN) were determined in an automatic elemental microanalyzer (EuroVector Elemental Analyser, Milano, Italy). The humic-like fractions were extracted with 0.1 M NaOH (C_{EX}) from the composts and from the different particle size fractions, and from these the fulvic acid-like C (C_{FA}) was separated through acid precipitation of the humic acid-like C (C_{HA}); C_{EX} and C_{FA} were analyzed in an automatic carbon analyzer for liquid samples, and C_{HA} was calculated as the difference.

The solid fractions, final compost samples, and initial mixtures for composting were analyzed for their pseudo total heavy metal concentrations through microwave (ETHOS1, Milestone) assisted acid digestion (HNO_3/H_2O_2; 4:1, v/v) followed by measurement in an atomic absorption spectrophotometer (Thermo iCE 3000 series).

Particle Size Fractionation

The mature composts were mechanically sieved at different particle sizes (mm): < 0.05; 0.05–0.5; 0.5–1.0; 1–2; and >2, using a vibratory sieve shaker (RETAC-3D, RETSCH). The different particle size fractions of the mature composts were analyzed for total Cu and Zn as indicated previously, and for soluble and exchangeable Cu and Zn through 0.1 M $CaCl_2$ extraction (1:10, w/v; 16 h) and measurement by atomic absorption spectrophotometry.

TABLE 1 | Characteristics of the composts and the solid fractions of pig slurry used for composting (SF1 and SF2).

	SF1	SF2	Compost 1	Compost 2
pH	8.2	6.8	6.5	7.3
Moisture (%)	83.6	85.8	50.2	31.8
EC (dS m^{-1})	2.7	3.0	4.52	4.79
OM (%)	81.1	80.5	54.4	59.7
TOC (g kg^{-1})	432	390	250	288
C_w (g kg^{-1})	30.3	50.4	0.48	0.75
C_{HA} (g kg^{-1})	–	–	13.4	18.1
C_{EXT} (g kg^{-1})	–	–	24.5	29.3
TN (g kg^{-1})	22.6	28.4	26.5	28.1
NH_4^+-N (g kg^{-1})	13.2	19.2	0.059	1.96
P (g kg^{-1})	24.4	15.4	29.1	27.8
K (g kg^{-1})	11.5	13.8	9.6	20.8
Fe (mg kg^{-1})	2090	1117	4762	4247
Mn (mg kg^{-1})	578	395	737	739
Ni (mg kg^{-1})	4	4	11	12
Pb (mg kg^{-1})	3	6	8	15

The concentrations of Cu and Zn are shown in ***Table 2****.*
EC, electrical conductivity; OM, organic matter; TOC, total organic carbon; C_w, water-soluble carbon; TN, total nitrogen.

TABLE 2 | Total and 0.1 M $CaCl_2$-extractable metal concentrations in the solid fractions of pig slurry (SF1 and SF2), the initial composting mixtures (Pile 1 and Pile 2), and the mature composts (Compost 1 and Compost 2).

	Total Zn	Zn $CaCl_2$		Total Cu	Cu $CaCl_2$	
	(mg kg^{-1})	(mg kg^{-1})	(% Total)	(mg kg^{-1})	(mg kg^{-1})	(% Total)
SF1	3098 ± 32	56 ± 1.4	1.81	249 ± 3.8	1.9 ± 0.08	0.76
Pile 1	2931 ± 99	10 ± 0.7	0.34	205 ± 21	0.4 ± 0.02	0.20
Compost 1	5552 ± 32	47 ± 1.6	0.85	351 ± 17	1.5 ± 0.08	0.43
SF2	3270 ± 91	56 ± 1.4	1.71	269 ± 4.2	1.8 ± 0.06	0.67
Pile 2	4720 ± 12	41 ± 0.6	0.87	173 ± 5.5	1.2 ± 0.06	0.69
Compost 2	5651 ± 40	17 ± 2.6	0.30	335 ± 17	0.6 ± 0.02	0.18

Phytotoxicity Test

The potential phytotoxicity of the composts was evaluated through a direct acute toxicity plant growth test (ISO 15 799.1999), using different proportions of compost with an artificial soil (100/0, 50/50, 25/75, 12.5/87.5, 6/94, 3/97, and 0/100, w/w%). The artificial soil was prepared according to OECD 207.1984. Around 80 g of the corresponding mixtures (at 70% of their WHC) were placed in plastic seedbeds (10 replicates for each mixture). One seed of *Zea mays* L. was sown in each hole and the plants were left to grow for 2 weeks after 50% of the plants had emerged in the controls (artificial soil without compost), in a growth chamber with controlled temperature and relative humidity (11/13 h day/night cycles at 25/18°C and 58/70% relative humidity). For each treatment the number of emerged seedlings was recorded and the fresh weight of the plants (aboveground and roots) determined. The growth index for the fresh weight (g per aboveground of the plants) was expressed as a percentage with respect to the control (artificial soil without compost). The EC50 and LC50 (the compost concentration, in v/v%, at which plant fresh weight and seedling emergence, respectively, were reduced by 50%) were calculated by applying a linear regression analysis to the relationship between the logarithm of the percentage compost concentration and the percentage toxic effect on plant growth (fresh weight) and seedling emergence.

An indirect seed germination index (GI) test was performed using compost water extracts (1:10, w/v; 24 h) and *Lepidium sativum* L. (cress) seeds (Zucconi et al., 1981; de Bertoldi et al., 1983). Ten seeds were placed on filter paper in plastic Petri dishes, 2 ml of the water extracts were carefully added, the seeds were covered with a new layer of filter paper, and the dishes were closed. The Petri dishes (10 per extract and dilution, including deionized water controls) were wrapped in aluminum foil and incubated for 3 days at 21°C. The germination index (GI) was calculated from the percentage of seed germination (G) and root elongation (R), determined in comparison with the results for the controls, as: $GI = [G \times R]/100$.

Statistical Analysis

The IBM SPSS Statistics 22.0 software was used for the statistical analysis. The standard deviation of the means was calculated for the chemical composition of the compost. A one-way ANOVA was performed to determine the effects of the different particle size fractions on the chemical parameters for each compost; and data were also subject to two-way ANOVA (General Linear Model) using particle size fraction and compost as independent factors. Differences between means were determined using Tukey's test. Before the statistical analysis, the data were tested for normality using the Kolmogorov–Smirnov test. Pearson's correlation coefficients between the chemical parameters and the TE concentrations of the different fractions and composts were also determined.

RESULTS AND DISCUSSION

Evolution of Metals Availability During Composting

The solid fractions of slurry obtained by screw press separation on the farm showed elevated concentrations of Zn and Cu (**Table 1**). The concentrations of Cu in SF1 and SF2 were similar to those reported in other pig slurry separation studies (Popovic et al., 2012), but the concentrations of Zn were higher in the present study (>3,000 mg kg^{-1} dw). This was probably because the pig slurry studied here was mainly produced by piglets, and this slurry has a higher concentration of Zn than that from fattening pigs (Moral et al., 2008), since Zn is provided as a feed additive (ZnO) to piglets in order to avoid digestion problems and to improve nutrients assimilation (Carlson et al., 1999; Woodworth et al., 1999). Also, Zn and Cu are excreted at relatively higher rates than other feed constituents (Shepard and Sapinelli, 2012), and over 90% of Cu and Zn has been reported to be retained in the solid fraction of pig slurry after solid-liquid separation using different technologies (Riaño and García-González, 2014).

The total concentrations of Cu and Zn increased in both piles during composting (**Table 2**) due to the degradation of organic matter (OM) and the consequent loss of weight in the piles (Ko et al., 2008; Smith, 2009). According to Sáez et al. (2017), the increase is most evident in the earlier stages of composting, when the rate of OM degradation is highest, in agreement with previous observations (Hsu and Lo, 2000; Ko et al., 2008). The Zn concentrations were greater than those found in compost prepared with pig slurry from fattening pigs (Santos et al., 2016), confirming its origin from the piglet diet. Therefore, the total concentrations of Cu and, especially, Zn were very high in both composts; the values for Zn were well above the limit established for compost in Spain and for fertilizer products in Europe (Ministerio de la Presidencia, 2017; EU, 2019).

In the present experiment, the concentrations of the metals (Cu and Zn) in soluble and exchangeable forms (0.1 M $CaCl_2$-extractable) were very low in comparison with the total values, showing low percentages of extractability, and both tended to decrease from the solid fraction to the mature compost, especially in compost 2 (**Table 2**). The values found for Cu were lower than those reported by Alvarenga et al. (2015) for compost from MSWs with lower total-Cu concentrations (180 mg kg^{-1}), giving an extractable/total Cu concentration ratio similar to that in the initial untransformed wastes (solid fraction and initial mixtures) in the present experiment. However, Alvarenga et al. (2015) found values of 0.1 M $CaCl_2$-extractable Zn in compost from MSW and from agricultural wastes similar to those found here, although their composts had lower total-Zn concentrations. This indicates that the Cu and Zn in the pig slurry composts have very low solubility, compared to other common composted materials. Decreases in heavy metal availability in different organic residual materials, especially MSW, have been found during the composting process, including the maturation period (Smith, 2009). Amir et al. (2005), using a sequential extraction, found that <2% of the total heavy metals were easily available (extracted in dilute KNO_3) in mature sewage sludge compost, and that the Cu and Zn were mainly associated with organic and carbonate fractions. The OM stabilization and humification processes, which occur during composting, could facilitate heavy metal (especially Cu) interaction with ligands of greater complexing strength, rendering them less bioavailable (Smith, 2009). Humic substances have been suggested as the main sites of metal sorption in compost (Song and Greenway, 2004), which is greater for Cu than for Zn. Then, the lower concentration of humic-like substances (NaOH-extractable C and C_{HA}; **Table 1**) found in compost 1 (prepared with cereal straw), with respect to compost 2 (prepared with cotton gin waste), could be responsible for the slightly greater proportion of available Cu and Zn in compost 1 (**Table 2**). Therefore, the bulking agent used had a significant effect on the solubility of metals, which may have affected both the degradation of the OM during composting and the OM humification process (Sáez et al., 2017). Also, the lower value of pH found in compost 1, with respect to compost 2, could have contributed to the higher solubility of Cu and Zn in the former. So, in addition to the

TABLE 3 | Characteristics of the different particle size fractions in Compost 1.

Particle size (mm)	<0.05	0.05–0.2	0.2–0.5	0.5–1	1–2	>2	ANOVA
Size distribution (%)	0.14	4.11	13.20	18.32	18.28	45.94	n.d.
OM (%)	45.2c	45.3c	52.9b	57.2a	57.4a	55.8ab	***
TOC (g kg^{-1})	200b	206b	256a	284a	273a	270a	**
TN (g kg^{-1})	31.2a	21.9b	24.2b	26.1b	25.4b	25.8b	**
C_{HA} (g kg^{-1})	n.d.	5.33b	5.15b	5.14b	6.83a	7.27a	***
C_{FA} (g kg^{-1})	n.d.	5.28b	6.09a	6.50a	6.41a	6.13a	**
Zn (mg kg^{-1})	10239a	6141b	5351bc	4325d	4510cd	5246c	***
Cu (mg kg^{-1})	246d	291c	333b	330b	350ab	368a	***
$CaCl_2$-Zn (mg kg^{-1})	n.d.	53.7a	39.6c	46.2b	38.3c	42.7bc	**
$CaCl_2$-Cu (mg kg^{-1})	n.d.	1.22b	1.27ab	1.41ab	1.34ab	1.42a	*

*n.d., not determined. Values followed by the same letter in each row are not significantly different according to Tukey's test at p < 0.05. *, **, and *** indicate significant at p < 0.05, 0.01, and 0.001, respectively.*

TABLE 4 | Characteristics of the different particle size fractions in Compost 2.

Particle size (mm)	<0.05	0.05–0.2	0.2–0.5	0.5–1	1–2	>2	ANOVA
Size distribution (%)	0.5	5.0	14.8	20.3	23.6	35.6	n.d.
OM (%)	44.4d	42.9d	51.0c	59.9ab	62.2a	58.8b	***
TOC (g kg^{-1})	211b	208b	220b	268a	268a	277a	**
TN (g kg^{-1})	32.1a	27.5c	23.7c	26.5c	30.2ab	27.6bc	***
C_{HA} (g kg^{-1})	n.d.	11.40c	10.43c	13.70b	13.53b	16.85a	***
C_{AH} (g kg^{-1})	n.d.	6.70	6.63	7.77	6.74	7.75	n.s.
Zn (mg kg^{-1})	7202a	5316bc	4957bc	4573c	5465b	4964bc	***
Cu (mg kg^{-1})	270d	286cd	293bc	291cd	350a	314b	***
$CaCl_2$-Zn (mg kg^{-1})	10.3b	17.6a	18.3a	20.6a	17.5a	19.4a	**
$CaCl_2$-Cu (mg kg^{-1})	1.0	0.9	1.0	1.1	0.9	1.0	n.s.

*n.d., not determined. Values followed by the same letter in each row are not significantly different according to Tukey's test at p < 0.05. n.s., ** and *** indicate not significant and significant at p < 0.01 and 0.001, respectively.*

TABLE 5 | Significance of the ANOVA factors for the characteristics of the composts (General Linear Model).

Factors	OM	TOC	TN	C_{HA}	C_{FA}	Total-Zn	Total-Cu	$CaCl_2$-Zn	$CaCl_2$-Cu
Particle size	***	***	***	***	**	***	***	***	n.s.
Compost	*	n.s.	***	***	***	***	***	***	***
P×C	***	n.s.	**	***	*	***	***	**	n.s.

*n.s., *, **, and *** indicate not significant and significant at p < 0.05, 0.01, and 0.001, respectively.*

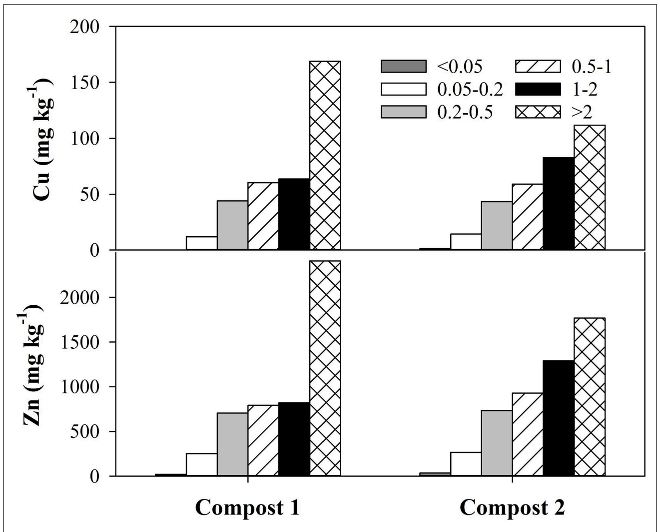

FIGURE 1 | Contribution (mg kg^{-1}) of each particle size fraction to the total Cu and Zn concentrations in the composts (concentration in the fraction × proportion of the fraction in the compost).

feedstock for composting, the physico-chemical characteristics of the final compost affected the solubility and availability of Zn and Cu.

The particle size distribution of both mature composts followed a similar pattern: the coarsest fraction (>2 mm) was the most abundant and the finest fraction (<0.05 mm) the least abundant, with a tendency for the abundance to increase with the particle size (**Tables 3**, **4**). The OM and TOC concentrations (as well as C_{HA} and C_{FA}) decreased with the particle size, which indicates that the inorganic components dominated in the smallest particles with the highest TN concentrations (**Table 5**).

In both composts, the highest Zn concentrations were found in the smallest particle size fraction (<0.05 mm), with the lowest TOC and C_{HA} concentrations, while, contrastingly, the concentrations of Cu were highest in the largest particle size fraction (>2 mm) that had the highest TOC, C_{HA}, and C_{FA} concentrations (**Tables 3–5**). In fact, Zn is added to the piglet diet as ZnO (Carlson et al., 1999), which is mainly excreted and recovered in the slurry (Moral et al., 2008), unaltered by the animal. This together with the low Zn(II) complexing capacity of the humic fraction (Hernández et al., 2006) and its ability to precipitate as Zn oxides at high pH values, suggest that the Zn in mature compost was mainly linked to hardly soluble inorganic forms. Total Zn concentration was correlated positively total-N ($r = 0.555$, $p < 0.01$ data of both compost), and negatively with OM and TOC (all at $p < 0.001$). Regarding extractable-Zn concentrations, negative correlations were found with C_{HA}, C_{FA} and C_{EX} (all at $p < 0.001$), all supporting the idea of Zn being scarcely retained in the organic fraction, and mainly linked with inorganic compounds. The highly significant correlations found between the Cu concentration and both TOC ($r = 0.850$ and 0.656 for composts 1 and 2, significant at $p < 0.001$ and 0.05, respectively; $r = 0.756$ at $p < 0.001$ for the data of both composts) and OM ($r = 0.855$ and 0.757 for composts 1 and 2, significant at $p < 0.001$ and 0.01, respectively; $r = 0.692$ at $p < 0.001$ for the data of both composts) reveal the suitability of this metal for retention by the OM. Also, the correlation between the concentration of extractable-Cu and that of C_{HA} and extractable-C was significant in both cases at $p < 0.01$ ($r = -0.590$ and -0.558, respectively), but the negative relationship indicates the retention of Cu by the humified OM produced during composting. Hernández et al. (2006) concluded that the stability constant of Cu complexes with humic acids are much

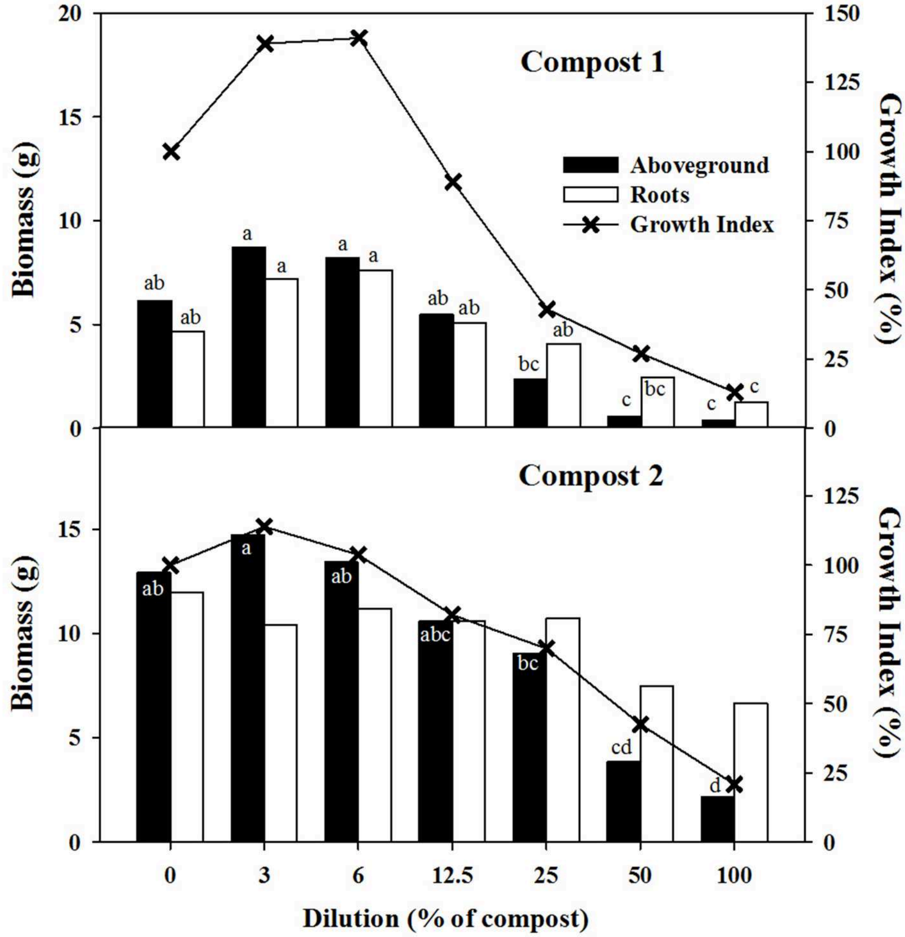

FIGURE 2 | Fresh weight of the aboveground and roots of *Zea mays* (g per treatment) and the Growth Index, at different proportions of compost, in the toxicity test. Bars (aboveground or roots) with the same letter are not significantly different according to Tukey's test at $p < 0.05$.

larger than the corresponding values for Zn, even in pig slurry organic matter.

Due to the largest concentrations of Zn in the smallest particle size fraction, the removal of this fraction by mechanical screening of the composts could be seen as an option to reduce the concentration of this metal in marketable compost, as usually done for MSW compost. However, its elimination would remove only a small amount of the total metal content of the composts (0.28 and 0.64% of total Zn in Compost 1 and Compost 2, and 0.10 and 0.52% for Cu, respectively; **Figure 1**), maintaining virtually unaltered metal concentrations, since this fraction represented only a minor part of the compost (**Tables 3**, **4**).

Phytotoxicity Test and Germination Index

The GI increased for both piles throughout the composting process, from scarce seed germination in the initial mixtures (GI<1% in both composts) to 87.8 and 80.2% in final composts 1 and 2, respectively, which indicates the absence of phytotoxicity. Therefore, in spite of the relatively high total concentrations of Zn and Cu in the composts, the low solubility of the metals meant that they did not have any relevant toxic effects on *L. sativum* seed germination, underlining the relevance of metal availability to phytotoxicity.

In the growth test performed with *Z. mays* (**Figure 2**), there were significant decreases in aerial biomass when compost was present at ≥12.5% in the substrate, with root growth affected at 50% compost. Both composts stimulated the growth of maize seedlings when present at up to 3–6%, with respect to the control soil without compost; Compost 1 also stimulated root growth (**Figure 2**). Sáez et al. (2016) found that the presence of low rates of pig slurry compost (<20%) in a growing medium was beneficial for plant growth, as the compost provided essential plant nutrients (mainly N, P, and K). But, when high proportions of the composts were applied in the present experiment, Compost 1 was not able to maintain correct root growth, which resulted in a greater decrease in the growth index than for Compost 2.

The calculated values of EC_{50} were 29% for Compost 1 and 40% for Compost 2, which indicates greater toxicity of Compost 1. In addition, the values of LC_{50} (related to seedling emergence) indicated that Compost 1 applied at 62% can provoke a lethal effect on 50% of the seeds, while for Compost 2 no harmful effects were found.

Sáez et al. (2016) found that maize seed germination was delayed in growing media prepared with pig slurry compost mixed with coir or biochar at proportions >20% (v:v), with reduced plant growth; however, the percentage seed germination was reduced only at proportions >60%. In that study, the reduction of salinity and of the available Cu and Zn concentrations (so that they did not provoke plant metal stress), as well as the removal of the volatile organic compounds from the compost, were identified as the main benefits of the use of biochar in substrates prepared with pig slurry compost. In agreement with the present results, the authors concluded that the use of pig slurry compost as a component of growing media was only feasible at low ratios (<20%), to avoid phytotoxic effects.

All these results indicate that the presence of Zn and Cu at high concentrations in these composts did not negatively affect seed germination and plant growth when the composts were used at low rates, compatible with agricultural doses. But, the application of high proportions of both composts in the soil under real field conditions can cause the long-term accumulation of Cu and, mainly, Zn in the soil and changes in their solubility and availability leading to negative effects on plants.

CONCLUSIONS

The high total concentrations of Cu and, especially, Zn in the solid phase of the pig slurry led to metal-rich composts. The highest concentration of Zn occurred in the smallest particles whereas Cu remained preferentially in the largest particles with the highest contents of OM and the humified fraction. The elimination of the smallest particle size fraction by mechanical screening would not reduce significantly the total Zn concentration of the composts. Despite the elevated total concentrations, the low solubility of both metals in the mature composts avoided any significant toxic effect on seed germination and also on plant growth when used up to 3–6% in the growing media, according to the short-term phytotoxicity tests. Long-term (repeated) compost application could build up the levels of Cu and Zn in the soil, and changes in availability or solubility may occur during organic matter degradation process.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

RC performed the statistical analysis and wrote the manuscript. JS-T carried out the experimental work under the supervision of MB who revised and corrected the manuscript.

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REFERENCES

Alvarenga, P., Clemente, R., Garbisu, C., and Becerril, J. M. (2018). "Indicators for monitoring mine site rehabilitation," in *Bio-Geotechnologies for Mine Site Rehabilitation, 1st Edn*., eds M. N. V. Prasad, P. J. de Campos Favas, and S. K. Maiti (Amsterdam: Elsevier), 49–66. doi: 10.1016/B978-0-12-812986-9.00003-8

Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., et al. (2015). Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: benefits *versus* limiting factors. *Waste Manag.* 40, 44–52. doi: 10.1016/j.wasman.2015.01.027

Amir, S., Hafidi, M., Merlina, G., and Revel, J. C. (2005). Sequential extraction of heavy metals during composting of sewage sludge. *Chemosphere* 59, 801–810. doi: 10.1016/j.chemosphere.2004.11.016

Bernal, M. P., Sommer, S. G., Chadwick, D., Qing, C., Guoxue, L., and Michel, F. C. Jr. (2017). Current approaches and future trends in compost quality criteria for agronomic, environmental, and human health benefits. *Adv. Agron.* 144, 143–233. doi: 10.1016/bs.agron.2017.03.002

Burton, H., and Turner, C. (2003). *Manure Management.* Treatment strategies for sustainable agriculture, second ed. Silsoe Research Institute, Lister and Durling Printers, Flitwick, Bedford, UK.

Carlson, M. S., Hill, G. M., and Link, J. E. (1999). Early- and traditionally weaned nursery pigs benefit from phase-feeding pharmacological concentrations of zinc oxide: effect on metallothionein and mineral concentrations. *J. Animal Sci.* 77, 1199–1207. doi: 10.2527/1999.7751199x

CEE (1991). Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. *Off. J. Eur. Commun. L* 375, 1–8.

Clemente, R., Arco-Lázaro, E., Pardo, T., Martín, I., Sánchez-Guerrero, A., Sevilla, F., et al. (2019). Combination of soil organic and inorganic amendments helps plants overcome trace element induced oxidative stress and allows phytostabilisation. *Chemosphere* 223, 223–231. doi: 10.1016/j.chemosphere.2019.02.056

Clemente, R., Walker, D. J., Pardo, T., Martínez-Fernández, D., and Bernal, M. P. (2012). The use of a halophytic plant species and organic amendments for the remediation of a trace elements-contaminated soil under semi-arid conditions. *J. Hazard. Mat.* 223–224, 63–71. doi: 10.1016/j.jhazmat.2012.04.048

de Bertoldi, M., Vallini, G., Pera, A. (1983). The biology of composting: a review. *Waste Manag. Res.* 1, 157–176. doi: 10.1177/0734242X8300100118

Eneji, A. E., Honna, T., Yamamoto, S., Masuda, T., Endo, T., and Irshad, M. (2003). The relationship between total and available heavy metals in composted manure. *J. Sustain. Agric.* 23, 125–34. doi: 10.1300/J064v23n01_09

EU (2019). Regulation (EU) 2019/1009 of The European Parliament and of The Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003. *Off. J. Eur. Union L* 170, 1–114.

FAO (2009). *The State of Food and Agriculture. Livestock in the Balance*. Available online at: http://www.fao.org/docrep/012/i0680e/i0680e.pdf (accessed October 11, 2019).

Farrell, M., and Jones, D. L. (2010). Use of composts in the remediation of heavy metal contaminated soil. *J. Hazard. Mat.* 175, 575–582. doi: 10.1016/j.jhazmat.2009.10.044

Hargreaves, J. C., Adl, M. S., and Warman, P. R. (2008). A review of the use of composted municipal solid waste in agriculture. *Agric. Ecosyst. Environ.* 123, 1–14. doi: 10.1016/j.agee.2007.07.004

Hernández, D., Plaza, C., Senesi, N., and Polo, A. (2006). Detection of copper(II) and zinc(II) binding to humic acids from pig slurry and amended soils by fluorescence spectroscopy. *Environ. Pollut*. 143, 212–220. doi: 10.1016/j.envpol.2005.11.038

Hsu, J. H., and Lo, S. L. (2000). Effect of composting on characterization and leaching of copper, manganese, and zinc from swine manure. *Environ. Pollut*. 114, 119–127. doi: 10.1016/S0269-7491(00)00198-6

Ko, H. J., Kim, K. Y., Kim, H. T., Kim, C., Kim, C. N., and Umeda, M. (2008). Evaluation of maturity parameters and heavy metal contents in composts made from animal manure. *Waste Manag*. 28, 813–820. doi: 10.1016/j.wasman.2007.05.010

Martínez-Fernández, D., Arco-Lázaro, E., Bernal, M. P., and Clemente, R. (2014). Comparison of compost and humic fertiliser effects on growth and trace elements accumulation of native plant species in a mine soil phytorestoration experiment. *Ecol. Eng.* 73, 588–597. doi: 10.1016/j.ecoleng.2014.09.105

Ministerio de la Presidencia (2017). Real Decreto 999/2017 de 24 de noviembre, por el que se modifica el Real Decreto 506/2013, de 28 de junio, sobre productos fertilizantes. *BOE* 296, 119396–119450.

Moral, R., Pérez-Murcia, M. D., Pérez-Espinosa, A., Moreno-Caselles, J., Paredes, C., and Rufete, B. (2008). Salinity, organic content, micronutrients and heavy metals in pig slurries from South-eastern Spain. *Waste Manag.* 28, 367–371. doi: 10.1016/j.wasman.2007.01.009

Pardo, T., Bes, C., Bernal, M. P., and Clemente, R. (2016). Alleviation of environmental risks associated with severely contaminated mine tailings using amendments: modelling of trace elements speciation, solubility and plant accumulation. *Environ. Toxicol. Chem*. 35, 2874–2884. doi: 10.1002/etc.3434

Pardo, T., Clemente, R., Epelde, L., Garbisu, C., and Bernal, M. P. (2014). Evaluation of the phytostabilisation efficiency in a trace elements contaminated soil using soil health indicators. *J. Hazard. Mater*. 268, 68–76. doi: 10.1016/j.jhazmat.2014.01.003

Popovic, O., Hjorth, M., and Jensen, L. (2012). Phosphorus, copper and zinc in solid and liquid fractions from full-scale and laboratory-separated pig slurry. *Environ. Technol.* 33, 2119–2131. doi: 10.1080/09593330.2012.660649

Riaño, B., and García-González, M. C. (2014). On-farm treatment of swine manure base on solid-liquid separation and biological nitrification-denitrification of liquid fraction. *Environ. Manag.* 132, 87–93. doi: 10.1016/j.jenvman.2013.10.014

Sáez, J. A., Belda, R. M., Bernal, M. P., and Fornes, F. (2016). Biochar improves agro-environmental aspects of pig slurry compost as a substrate for crops with energy and remediation uses. *Ind. Crops Prod*. 94, 97–106. doi: 10.1016/j.indcrop.2016.08.035

Sáez, J. A., Clemente, R., Bustamante, M. A., Yañez, D., and Bernal, M. P. (2017). Evaluation of the slurry management strategy and the integration of the composting technology in a pig farm – Agronomical and environmental implications. *J. Environ. Monitor*. 192, 57–67. doi: 10.1016/j.jenvman.2017.01.040

Santos, A., Bustamante, M. A., Tortosa, G., Moral, R., and Bernal, M. P. (2016). Gaseous emissions and process development during composting of pig slurry: the influence of the proportion of cotton gin waste. *J. Clean. Prod*. 112, 81–90. doi: 10.1016/j.jclepro.2015.08.084

Sharma, V. K., Canditelli, M., Fortuna, F., and Cornacchia, G. (1997). Processing of urban and agro-industrial residues by aerobic composting: review. *Energy Convers. Manag*. 38, 453–78. doi: 10.1016/S0196-8904(96)00068-4

Shepard, S. C., and Sapinelli, B. (2012). Trace elements in feed manure and manured soils. *J. Environ. Qual*. 41, 1846–1856. doi: 10.2134/jeq2012.0133

Smith, S. R. (2009). A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared with sewage sludge. *Environ. Int.* 35, 142–156. doi: 10.1016/j.envint.2008.06.009

Song, Q. J., and Greenway, G. M. (2004). A study of the elemental leachability and retention capability of compost. *J. Environ. Monit.* 6, 31–37. doi: 10.1039/b310840f

Soumaré, M., Tack, F. M. G., and Verloo, M. G. (2003). Characterisation of Malian and Belgian solid waste composts with respect to fertility and suitability for land application. *Waste Manag.* 23, 517–22. doi: 10.1016/S0956-053X(03)00067-9

van Gestel, C. A. M., van der Waarde, J. J., Derksen, J. G. M., van der Hoek, E. E., Veul, M. F. X. W., Bouwens, S., et al. (2001). The use of acute and chronic bioassays to determine the ecological risk and bioremediation efficiency of oil-polluted soils. *Environ. Toxicol. Chem*. 20, 1438–1449. doi: 10.1002/etc.5620200705

Walter, I., Martínez, F., and Cala, V. (2006). Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses. *Environ. Pollut*. 139, 507–14. doi: 10.1016/j.envpol.2005.05.020

Woodworth, J. C., Tokach, M. D., Nelssen, J. L., Goodband, R. D., O'Quinn, P. R., and Fakler, T. M. (1999). The effects of added zinc from an organic zinc complex or inorganic sources on weanling pig growth performance. *J. Animal Sci*. 77(Suppl 1):61.

Zucconi, F., Pera, A., Forte, M. V., and de Bertoldi, M. (1981). Evaluating toxicity of immature compost. *BioCycle* 22, 54–57.

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