Water Air Soil Pollut (2017) 228: 152
DOI 10.1007/s11270-017-3345-1

Copper/Zinc Bioaccumulation and the Effect of Phytotoxicity on the Growth of Lettuce (*Lactuca sativa* L.) in Non-contaminated, Metal-Contaminated and Swine Manure-Enriched Soils

Mariane Wolf · Dilmar Baretta · Valter A. Becegato · Vitor de C. Almeida · Alexandre T. Paulino

Received: 30 December 2016 / Accepted: 16 March 2017 / Published online: 23 March 2017

Abstract Copper/zinc bioaccumulation and the effect of phytotoxicity on the growth of lettuce (*Lactuca sativa* L.) were studied in plastic vessels containing (i) non-contaminated soil, (ii) copper-contaminated soils at concentrations of 75.0 and 125.0 mg kg^{-1}, (iii) zinc-contaminated soils at concentrations of 1200 and 2400 mg kg^{-1}, and (iv) soil enriched with swine manure. Copper and zinc concentrations in lettuce leaves were determined by flame atomic absorption spectrometry during 42 days of growth. Copper concentrations from 0.92 to 13.06 mg kg^{-1} were found in lettuce leaves grown in copper-contaminated soils and zinc concentrations from 58.13 to 177.85 mg kg^{-1} were found in lettuce leaves grown in zinc-contaminated soils. Copper and zinc concentrations in lettuce leaves grown in swine manure-enriched soils ranged from 0.82 to 8.33 and 0.68 to 13.27 mg kg^{-1}, respectively. Copper and zinc bioaccumulation caused a decrease in lettuce growth in metal-contaminated soils and an increase in phytotoxicity effects when compared to growth in non-contaminated and manure-enriched soils. These findings were confirmed by measuring leaf areas and biomasses. Copper was less toxic to lettuce than zinc due to the different concentrations in the soil. Lettuce growth and development was better in the swine manure-enriched soil than non-contaminated soil, which indicates that swine manure is a safe agricultural biofertilizer when used in appropriate amounts to avoid metal bioaccumulation in soil and plants.

Keywords Copper · Zinc · Bioaccumulation · Soil · Phytotoxicity

M. Wolf · A. T. Paulino
Department of Food and Chemical Engineering, Santa Catarina State University, BR 282, Km 574, Pinhalzinho, SC 89870-000, Brazil

D. Baretta
Department of Zootechny, Santa Catarina State University, Rua Beloni Trombeta Zanin, 680E, Chapeco, SC 89815-630, Brazil

V. A. Becegato · A. T. Paulino (✉)
Graduate Program in Environmental Sciences, Santa Catarina State University, Av. Luis de Camões, 2090, Conta Dinheiro, Lages, SC 88520-000, Brazil
e-mail: alexandre.paulino@udesc.br

V. d. C. Almeida
Department of Chemistry, State University of Maringa, Av. Colombo, 5790, Jardim Universitário, Maringa, PR 87020-900, Brazil

1 Introduction

The bioaccumulation of copper and zinc in soil and plants poses risks for animals, humans, and natural resources, compromising their functionality and sustainability (Carvalho and Orsine 2011; Lago-Vila et al. 2016). These metals can be ingested by animals and humans through the consumption of contaminated plants and water. Therefore, rigorous control is necessary in contaminated areas, with the avoidance of certain soils and sources of water for the purposes of growing vegetables (Carvalho and Orsine 2011). The bioaccumulation of metals in human organs due to the ingestion of contaminated plants can have harmful effects on health and increase the risk of cancer (Leite et al. 2015). To diminish toxicity to humans and animals, international regulations establish maximum limits for metals, such as copper and zinc, in foods (Wuana and Okieimen 2011).

Lettuce (*Lactuca sativa* L.) is widely consumed around the world and is also an excellent bioindicator for investigating the contamination of soil and water. This vegetable is an annual plant of the *Asteracea* family that is normally cultivated in tropical climates (Henz and Suinaga 2009). Lettuce has been employed to study the effects of copper and zinc toxicities on plants after biofertilization of the soil using swine manure. In order to increase the swine production, micronutrients such as copper and zinc are commonly added to animal feed (Muniz et al. 2010). High dosages of copper and zinc prevent diarrhea and enhance animal growth (Perdomo et al. 2001). As a consequence, these metals are found in the manure, with the excretion/bioaccumulation rates ranging from 70 to 95%, depending on the type of diet employed (Kornegay and Harper 1997; Paulino et al. 2005; Schmidt et al. 2007; Santos et al. 2011). Swine manure also contains other essential micronutrients, such as manganese and iron, which are important to the growth and development in both animals and plants (Schmidt et al. 2007). However, high concentrations of copper, zinc, manganese, and iron can be toxic to plants, animals, and humans. It is therefore important to control the micronutrient concentration in the feed composition to avoid environmental pollution when using swine manure for the purposes of soil enrichment (Segat et al. 2015).

Environmental pollution in agricultural soils is generally associated with the frequent application of swine manure as a biofertilizer, metal bioaccumulation, and pesticide concentrations in plants (Luo et al. 2014). The inappropriate use of swine manure has negative impacts on the water quality of rivers, lakes, and groundwater as well as the growth and development of various plants (Mattias et al. 2010; Smanhotto et al. 2010). Even small amounts of copper and zinc are bioavailable in soil and water (Costa et al. 2007). Bioaccumulation in plants can lead to significant changes in metabolic processes. This is known as phytotoxicity and varies depending on the type of metal. As a result, changes occur in leaves and roots, which are evidenced by changes in the characteristics of these parts of the plant as well as reductions in leaf area and biomass (Kabata-Pendias 2011; Nascimento et al. 2014a).

The aim of the present study was to investigate copper and zinc bioaccumulation and the effect of phytotoxicity on the growth of lettuce (*L. sativa* L.) in non-contaminated, metal-contaminated, and swine manure-enriched soils to establish parameters for using manure as biofertilizer. Copper and zinc were measured in lettuce leaves using flame atomic absorption spectrometry. The phytotoxicity study involved the use of digital photographs as well as measurements of leaf area and biomass.

2 Materials and Methods

2.1 Experimental Design

Twenty-one vessels were separated into groups of seven to perform experiments in triplicate, as indicated in Fig. 1. The vessels received approximately 500.0 g of non-contaminated soil (vessel a), metal-contaminated soil (vessels from b to f), or swine manure-enriched soil (vessel g). Vessels b and c contained copper-contaminated soils at concentrations of 75 and 125 mg kg^{-1}, respectively. Vessels d and e contained zinc-contaminated soils at concentrations of 1200 and 2400 mg kg^{-1}, respectively. Vessel f contained soil with a mixture of copper and zinc at concentrations of 125 and 1200 mg kg^{-1}, respectively. These concentrations corresponded to copper and zinc concentrations added during the production of swine feed. Each soil sample was prepared considering 75 m^3 of either metal solution or swine manure per hectare of land.

Lettuce (*L. sativa* L.) supplied by a local market was planted in the vessels, which were protected from strong sunlight and rain by a plastic screen. The vessels containing lettuce were irrigated three times a day with potable water. Copper and zinc bioaccumulation and the effect of phytotoxicity on lettuce growth were monitored for 42 days. Phytotoxicity results were monitored using digital photographs and the determination of leaf area and biomass. Leaf area was measured using a CI 203 handheld laser leaf area meter. Next, all leaves were dried in an oven at 65 °C for 24 h and weighed under anhydrous conditions for the determination of biomass. The climate conditions (precipitation, maximum, minimum and average temperatures, and relative humidity) throughout the study were obtained from the State of Santa Catarina Center for Environmental Resources and Hydrometeorology, Brazil.

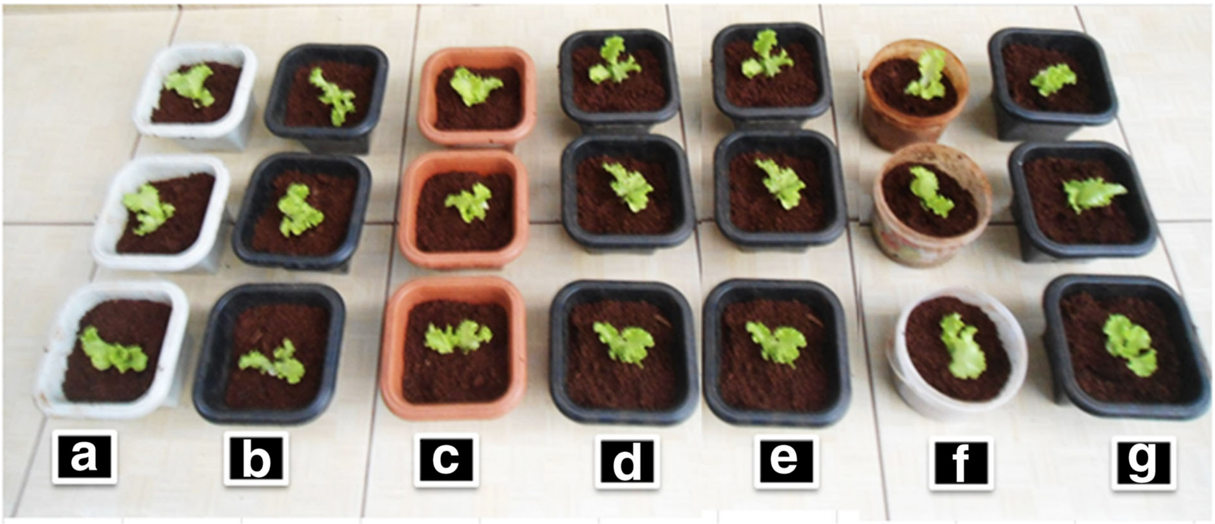

Fig. 1 Preparation of plastic vessels for lettuce growth

2.2 Climate Variables

Temperature during lettuce growth ranged from 14.06 ± 2.58 to 24.58 ± 2.80 °C (mean 18.75 ± 2.28 °C). Mean precipitation and relative humidity were 5.68 ± 14.14 mm and 72.47 ± 10.67%, respectively. These climate conditions are favorable to the growth and development of different vegetables, including lettuce (Henz and Suinaga 2009).

2.3 Characterization of Swine Manure

Manure was collected once after the end of the first phase of swine growth and dried at room temperature for 5 days to reduce the water content prior to use. The first phase of swine growth lasts 21 days. pH was measured using a Tecnopon mPA 210 pH meter with automatic temperature compensation, ±0.01 resolution, and a pH combination glass electrode. Moisture was determined based on Tedesco et al. (1995) with some modifications. Carbon and nitrogen contents were determined using a CHNS elemental analyzer (Vario EL cube). Copper, zinc, iron, and manganese were determined using flame atomic absorption spectrometry (FAAS AA-6300, Shimadzu, air-acetylene flame) with a detection limit of 0.0200 mg L^{-1} and wavelengths of 324.7, 213.9, 248.3, and 279.5 nm, respectively. Bioavailable potassium and phosphorus were determined using flame photometry and UV-Vis spectrophotometry, respectively, after extraction with Mehlich-1 solution.

2.4 Swine Manure Properties

The physicochemical properties and macro-micronutrient concentrations in the swine manure used for lettuce growth were pH 7.50, 49.5% moisture, 43.5% total carbon content, 2.58% total nitrogen content, and 16.9/1 carbon/nitrogen rate. Phosphorus, potassium, manganese, copper, zinc, and iron concentrations were 3.39, 3.12, 0.021, 0.072, 0.038, and 0.437 mg kg^{-1}, respectively. The copper concentration was higher than the zinc concentration. Moreover, significant concentrations of total nitrogen, total carbon, phosphorus, potassium, manganese, and iron were determined. These results are associated with different diets employed during the growth and development of animals to produce manure with similar properties. The physicochemical composition of swine manure depends on feed digestibility and the bioavailability of phosphorus, potassium, manganese, copper, zinc, and iron (Muniz et al. 2010; Pomar et al. 2009). The properties of swine manure need to be defined before application as a biofertilizer to avoid metal bioaccumulation and phytotoxicity in plants (Souza et al. 2009).

2.5 Sample Preparation Method

Triplicate lettuce samples were collected from soils containing copper, zinc, swine manure, or a mixture of copper and zinc every 7 days for 42 days. The leaves, roots, water, and soils (non-contaminated, metal-contaminated, and manure-enriched) were appropriately separated, and each sample was prepared by acid digestion. For the digestion of leaves, roots, and soils, 1.0-g samples were placed into 87.0-mL open glass flasks containing 10 mL of 1:1 (*v*/*v*) diluted nitric acid. The flasks were placed into a digestion block and heated to 95 ± 5 °C for 10 min. Subsequently, 5.0 mL of concentrated nitric acid was added with the temperature kept at 95 ± 5 °C for 2 h. After cooling to room temperature, 2.0 mL of Mili-Q® water and 7.0 mL of 30% (*v*/*v*) hydrogen peroxide were added, followed by heating to 95 ± 5 °C for 30 min to complete the decomposition of organic matter. Finally, 10.0 mL of concentrated hydrochloric acid was added, with the temperature kept at 95 ± 5 °C for 15 min for complete metal solubilization. The final solution was cooled to room temperature and placed into a 50.0-mL volumetric flask, which was filled to the maximum volume with Milli-Q® water. For the digestion of water, 500.0 mL of sample was placed in a 1000-mL beaker and concentrated nitric acid was added up to pH 2.0. The beaker was kept on a hot plate at 60.0 °C for several hours for the complete decomposition of organic matter or up to final volume of approximately 25.0 mL. The remaining solution was cooled to room temperature and placed into a 50.0-mL volumetric flask, which was then filled to the maximum volume with Milli-Q® water. The aqueous sample solutions were stored in amber glass flasks prior to analysis by flame atomic absorption spectrometry, flame photometry, and UV-Vis spectrophotometry.

2.6 Copper and Zinc Concentrations in Non-contaminated Soil and Water

The respective copper and zinc concentrations were 1.95 ± 0.20 and 1.75 ± 0.15 mg kg^{-1} in non-contaminated soil and 0.21 ± 0.05 and 0.17 ± 0.06 mg kg^{-1} in water. Respective maximum copper and zinc concentrations are 200 and 450 mg kg^{-1} for agricultural soils and 2.0 and 1.1 mg kg^{-1} for groundwater (Cetesb 2005; Conama 2013). Thus, the copper and zinc concentrations in the soils and water used during the growth and development of lettuce are in agreement with values stipulated by Brazilian regulations. High copper and zinc concentrations in soils are predominant factors with regard to metal bioaccumulation in vegetables and an increase in phytotoxicity.

2.7 Statistical Analysis

Analysis of variance (ANOVA) of the results (expressed as mean ± standard deviation) was performed with the aid of the ASSISTAT® 7.7 program and the use of the *t* test with a 95% significance level ($p < 0.05$).

3 Results and Discussion

3.1 Phytotoxicity Assays

Figure 2 displays the phytotoxicity results for lettuce growth in non-contaminated soil (Fig. 2a), soils contaminated with copper at concentrations of 75 mg kg^{-1} (Fig. 2b) and 125 mg kg^{-1} (Fig. 2c) and soils contaminated with zinc at concentrations of 1200 mg kg^{-1} (Fig. 2d) and 2400 mg kg^{-1} (Fig. 2e) for 42 days. Phytotoxicity was greater in the zinc-contaminated soils than copper-contaminated soils. Moreover, the toxic effects were much more significant with 2400 mg of zinc per kilogram of soil.

Table 1 displays the mean leaf area (square inch) and leaf mean biomass (g) of lettuce grown in

Fig. 2 Phytotoxicity results for lettuce grown in non-contaminated soil (**a**), soils contaminated with copper at concentrations of 75 mg kg^{-1} (**b**) and 125 mg kg^{-1} (**c**) and soils contaminated with zinc at concentrations of 1200 mg kg^{-1} (**d**) and 2400 mg kg^{-1} (**e**) for 42 days

Table 1 Leaf mean area (square inch) and leaf mean biomass (g) for lettuces collected from non-contaminated, metal-contaminated, and swine manure-enriched soils

Days	Non-contaminated		Copper-contaminated (125 mg kg^{-1})		Zinc-contaminated (2400 mg kg^{-1})		Swine manure-enriched	
	Area	Biomass	Area	Biomass	Area	Biomass	Area	Biomass
7	2.54a ± 0.20	1.95a ± 0.05	1.95a ± 0.05	0.29a ± 0.01	0.89a ± 0.02	0.09a ± 0.01	2.55a ± 0.13	0.67a ± 0.03
14	5.43b ± 0.33	3.34b ± 0.07	3.34b ± 0.07	0.33b ± 0.03	0.94a ± 0.03	0.13a ± 0.03	6.67b ± 0.32	0.94b ± 0.03
21	10.3c ± 0.43	8.43c ± 0.04	8.43c ± 0.04	0.56c ± 0.02	0.99c ± 0.01	0.15c ± 0.01	12.89c ± 0.21	1,32c ± 0.02
28	14.6d ± 1.21	10.9d ± 0.07	10.9d ± 0.07	1.01d ± 0.03	1.03d ± 0.01	0.18c ± 0.04	17.8d ± 0.19	1.55d ± 0.05
35	19.4e ± 1.18	13.3e ± 0.04	13.3e ± 0.04	1.21e ± 0.04	1.05d ± 0.00	0.20e ± 0.03	21.4e ± 0.55	2.08e ± 0.02
42	20.2f ± 1.90	15.4f ± 0.03	15.4f ± 0.03	1.43f ± 0.03	1.04d ± 0.02	0.19e ± 0.02	23.6f ± 0.43	2.25f ± 0.03

Means with different letters in the vertical columns are significantly different by *t* test at a 95% confidence level

non-contaminated, metal-contaminated, and swine manure-enriched soils. Larger leaf areas and biomasses were found when the lettuce was grown in the manure-enriched and non-contaminated soils. A reduction in leaf area and biomass was observed in lettuce grown in soil contaminated with copper at a concentration of 125 mg kg^{-1}. Concentrations of nutrients, such as calcium, potassium, magnesium, and manganese decrease with the increase in the copper bioaccumulation, resulting in a smaller leaf area and biomass (Sheldon and Menzies 2005). Lettuce growth was significantly affected in soils contaminated with zinc at a concentration of 2400 mg kg^{-1}. Zinc and aluminum are efficient inhibitors of nutrients, leading to reductions in plant growth, leaf area, and biomass (Sheldon and Menzies 2005). Similar results were found for the soils containing 1200 mg kg^{-1} of zinc and 75 mg kg^{-1} of copper (data not shown). The metal bioaccumulation mechanism modifies structural, biochemical, and physiological properties in vegetables. These modifications depend on the metal chemical species, concentration, and exposure time. Exposure time and type of contamination can increase the toxic effects on plants due to the efficient bioaccumulation of metals (Macêdo and Morril 2008).

Figure 3 displays the phytotoxicity results for lettuce grown in non-contaminated soil (Fig. 3a), soil containing a mixture of 125.0 mg kg^{-1} of copper and 1200 mg kg^{-1} of zinc (Fig. 3f), and swine manure-enriched soil (Fig. 3g) for 42 days. Leaf area and biomass were lower for lettuce grown in soil containing the mixture of copper and zinc in comparison to the non-contaminated soil (data not shown). Lettuce leaves from metal-contaminated soil exhibited discoloration and poor development, indicating more significant phytotoxicity. Similar results were previously observed for lettuce grown in soils containing copper and zinc separately.

Lettuce growth and development was lower in the non-contaminated soil than manure-enriched soil. This finding can be attributed to the high amount of organic matter in swine manure, which favors the growth and development of vegetables (Nookabkaew et al. 2016; Gallo et al. 2015). Leaf area and biomass were also larger for lettuce grown in the manure-enriched soil. Copper and zinc concentrations were lower in swine manure than metal-contaminated soils, which translated to lower toxicity.

The high concentrations of potassium, phosphorus, and nitrogen in swine manure are essential to the growth and development of plants (Girotto et al. 2010;

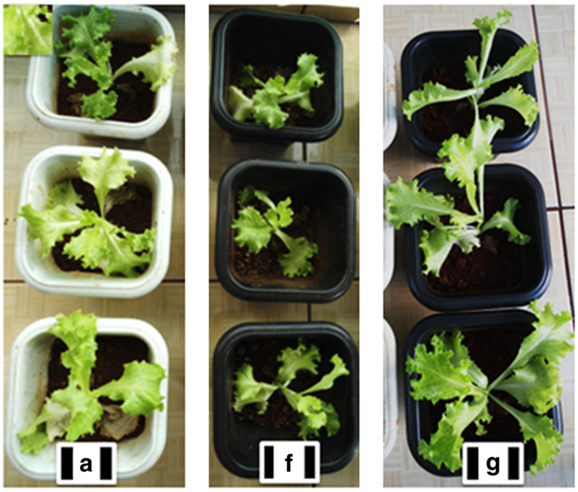

Fig. 3 Phytotoxicity results for lettuce grown in non-contaminated soil (**a**), soil containing a mixture of 125.0 mg kg^{-1} of copper and 1200 mg kg^{-1} of zinc (**f**) and swine manure-enriched soil (**g**) for 42 days

Nookabkaew et al. 2016). Copper and zinc affect the growth, development, and phytotoxicity of vegetables by inhibiting the root functions, thereby increasing chlorosis (Andrade et al. 2010; Broadley et al. 2007). The effects of metal toxicity in vegetables are evident in the abnormal growth of leaves and roots, as observed experimentally. Moreover, darker leaves due to poor stretching and a lower amount of biomass may be observed (Sheldon and Menzies 2005). Lastly, a high concentration of copper in roots and leaves hinders electron transport during photosynthesis, thereby decreasing the amount of chlorophyll and modifying the chloroplast structure (Yruela 2005; Cambrollé et al. 2013).

3.2 Copper and Zinc Bioaccumulation in Lettuce Leaves

Table 2 displays the copper and zinc concentrations in the lettuce leaves collected from metal-contaminated soils at 7-day intervals for 42 days. Bioaccumulated metal concentrations increased with the increase in the growth time, and zinc concentrations were always higher than copper concentrations. These results are associated with higher zinc concentrations in the soil and different affinities for active sites in lettuce leaves and roots (Nascimento et al. 2014a; Junio et al. 2011).

Copper and zinc are adsorbed to organic and inorganic compounds in soils, forming either soluble complexes or insoluble precipitates. Physicochemical interactions during the formation of complexes and precipitates generally alter the bioavailability of metals (Kabata-Pendias and Pendias 2001), thereby decreasing bioaccumulation in plants. Other parameters that affect metal bioaccumulation include (i) the concentration and speciation of the metal, (ii) metal movement in the soil and on the root surface, (iii) mass transference to the roots, and (iv) translocation from the roots to the leaves (Alloway 1995).

The significant zinc bioaccumulation in lettuce leaves may be associated with the bioavailability of this metal in the soil, since organic acids produced from the decomposition of organic matter solubilize high amounts of zinc (Melo et al. 2008; Nascimento et al. 2014b). Consequently, the bioavailable zinc concentration increases because there is no complexation of this metal with hydroxyl ions at low pH values (Santos et al. 2002). As basaltic soils have pH values around 5.0 (Melo et al. 2012), the high bioaccumulation in lettuce leaves is explained by the significant concentrations of bioavailable zinc. In contrast, copper interacts strongly with minerals and organic acids (Girotto et al. 2010). Thus, the lower mobility of copper and its strong interaction with organic and inorganic colloids in soil lead to lower bioaccumulation in lettuce leaves. The fact that both copper and zinc bioaccumulation increased with the increase in the metal concentrations in soil indicates that the predominant factor for the bioaccumulation process is the affinity of these metals for active sites in lettuce leaves (Malavolta 1980; Macêdo and Morril 2008). Maximum copper and zinc limits in foods are 30.0 and 50.0 mg kg^{-1}, respectively (Brasil 1965). As bioaccumulated zinc concentrations were higher than the maximum limit permitted, the lettuce produced in this experiment was inappropriate for consumption.

3.3 Simultaneous Copper and Zinc Bioaccumulation in Lettuce Leaves

Table 3 displays the copper and zinc concentrations in lettuce leaves collected from the soil containing a

Table 2 Copper and zinc bioaccumulation in lettuce leaves collected from soils containing 75.0 and 125.0 mg kg^{-1} of copper and 1200 and 2400 mg kg^{-1} of zinc for 42 days

Days	75.0 mg kg^{-1} Copper concentration (mg kg^{-1})	125.0 mg kg^{-1}	1200 mg kg^{-1} Zinc concentration (mg kg^{-1})	2400 mg kg^{-1}
7	0.97a ± 0.15	0.92a ± 0.17	58.13a ± 1.87	69.95a ± 1.36
14	5.77b ± 0.88	5.95b ± 0.54	62.62a ± 0.85	95.12b ± 2.25
21	8.95c ± 0.38	8.90c ± 0.38	71.48b ± 1.30	118.32c ± 2.19
28	9.31c ± 0.07	9.32c ± 0.27	88.10c ± 3.25	152.23d ± 0.27
35	10.68d ± 0.28	10.63d ± 0.20	111.08 d ± 2.81	166.17e ± 2.43
42	13.06e ± 0.45	13.02e ± 0.40	119.60e ± 3.93	177.85f ± 1.87

Means with different letters in the vertical columns are significantly different by *t* test at a 95% confidence level

Table 3 Simultaneous copper and zinc bioaccumulation in lettuce leaves collected from soil containing a mixture of 1200 mg kg^{-1} of zinc and 125.0 mg kg^{-1} of copper for 45 days

Days	Zinc Concentration (mg kg^{-1})	Copper
7	32.44a ± 1.98	Not detected
14	47.30b ± 1.84	Not detected
21	68.90c ± 1.23	1.1a ± 0.06
28	92.72d ± 3.10	3.8b ± 0.36
35	120.12e ± 4.22	5.1c ± 0.12
42	155.87f ± 2.70	6.5d ± 0.18

Means with different letters in the vertical columns are significantly different by *t* test at a 95% confidence level

mixture of 125.0 mg kg^{-1} of copper and 1200 mg kg^{-1} of zinc collected at 7-day intervals for 42 days. The bioaccumulated concentrations of these metals increased with the increase in lettuce growth time, as observed for other plants (Basahi and Hassan 2013; Jordão et al. 2013). In addition, the bioaccumulated concentrations were higher for zinc than copper due to different initial concentrations in the soil, confirming that zinc has greater affinity for active sites in the vegetable, as observed elsewhere (Malavolta 1980; Macêdo and Morril 2008).

In general, vegetables have different bioaccumulation capacities due to physicochemical interactions between metals and organic matter (Barceló and Poschenrieder 2003). These interactions may be independent, antagonist, additive, or synergistic and depend on the both chemical speciation of the metal as well as the properties of the metal-contaminated soil (Menegale et al. 2015). Copper bioaccumulation rates in lettuce leaves collected from the soil containing a mixture of copper and zinc were lower than those observed for soil containing copper alone, whereas the zinc bioaccumulation rates were higher than those found for soils containing zinc alone, demonstrating that copper bioaccumulation was inhibited by the presence of zinc, which suggests a relationship of antagonism (Alloway 1995; Silva et al. 2007). Moreover, lettuce may act as an exclusory plant, with higher metal concentrations bioaccumulated in roots. As a result, this vegetable becomes tolerant to toxic metals, with a decrease in the translocation of metals from the roots to the leaves (Kabata-Pendias 2011).

3.4 Copper and Zinc Bioaccumulation in Lettuce Leaves Grown in Manure-Enriched Soil

Table 4 displays the copper and zinc concentrations in lettuce leaves collected from soil enriched with swine manure at 7-day intervals for 42 days. Copper and zinc were only detected beginning in the second and third week, respectively, and the bioaccumulation of these metals was influenced by lettuce growth time.

The growth and development of vegetables in swine manure-enriched soils depend on the amount of organic matter and minerals present, which alter metal bioavailability for plants (Macêdo and Morril 2008; Havlin et al. 2013). Copper and zinc bioaccumulation was lower in lettuce leaves grown in the manure-enriched soils than metal-contaminated soils, which is associated with the bioavailability and different concentrations of metal in soil. Moreover, zinc bioaccumulation was higher than copper bioaccumulation, even with a higher copper concentration in the swine manure. This may be related to zinc-plant complexes, which are more stable than copper-plant complexes (Seidel et al. 2009; Andrade et al. 2010; Veiga et al. 2012; Nookabkaew et al. 2016).

The frequent use of swine manure in soils may significantly increase copper and zinc concentrations in soluble and exchangeable forms, thereby increasing toxicity to plants as well as resulting in the transference of these metals to rivers, lakes, and groundwater (Girotto et al. 2010). Most natural minerals in soils are not bioavailable, because they remain complexed to inorganic and organic matters by means of high-energy physicochemical bonds. However, copper and zinc from anthropogenic sources may remain as low-energy complexes or high-solubility precipitates,

Table 4 Copper and zinc bioaccumulation in lettuce leaves collected from swine manure-enriched soil for 42 days

Days	Zinc Concentration (mg kg^{-1})	Copper
7	Not detected	Not detected
14	Not detected	0.82a ± 0.24
21	0.68a ± 0.08	3.98b ± 0.33
28	9.03b ± 0.25	5.17c ± 0.58
35	10.13c ± 0.21	6.65d ± 0.13
42	13.27d ± 0.08	8.33e ± 0.15

Means with different letters in the vertical columns are significantly different by *t* test at a 95% confidence level thereby increasing bioavailability, toxicity, and bioaccumulation in plants (Kabata-Pendias and Pendias 2001; Conte et al. 2003).

4 Conclusion

Bioavailable metal fractions in soils can cause phytotoxicity to plants and contaminate rivers, lakes, and groundwater. A high zinc concentration decreases the production and quality of vegetables. While the use of swine manure as biofertilizer improves production indices, it may also lead to higher bioaccumulation levels of metals in plants, thereby increasing phytotoxicity. Alternative methods for growing vegetables should be studied to prevent the environmental pollution of soils, rivers, lakes, and groundwater. Lastly, it is necessary to apply adequate amounts of swine manure to soils with the aim of improving vegetable production while avoiding metal bioaccumulation.

Acknowledgments The authors gratefully acknowledge the Brazilian fostering agency CAPES for research support. ATP acknowledges grants awarded by CNPq (Nos. 479402/2013-2 and 312356/2015-3).

References

Alloway, B. J. (1995). *Heavy metals in soil*. London: Blackie Academic.

Andrade, A. F. M., Sobrinho, N. M. B. A., & Mazur, N. (2010). Contents of zinc, cadmium and lead in rice plants in soils incubated with slag. *Revista Brasileira de Engenharia Agrícola e Ambiental, 14*, 1087–1093.

Barceló, J., & Poschenrieder, C. (2003). Phytoremediation: principles and perspectives. *Contributions to Science, 2*, 333–344.

Basahi, J. M., & Hassan, I. A. (2013). Assessing roadside conditions and vehicular emissions using roadside lettuce plants. *Polish Journal of Environmental Studies, 22*, 387–393.

Brasil. (1965). Decreto n°. 55.871, de 26 de março de 1965, Diário Oficial da União, Brasília.

Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I., & Lux, A. (2007). Zinc in plants. *The New Phytologist, 173*, 677–702.

Cambrollé, J., García, J. L., Ocete, R., Figueroa, M. E., & Cantos, M. (2013). Growth and photosynthetic responses to copper in wild grapevine. *Chemosphere, 93*, 294–301.

Carvalho, J. C. B., & Orsine, J. V. C. (2011). Contamination of environment by different sources and harm the health of the population. *Enciclopédia Biosfera, 7*, 1107–1118.

Cetesb. (2005). Companhia ambiental do Estado de São Paulo. http://www.cetesb.sp.gov.br. Accessed 19 April 2005.

Conama. (2013). *Conselho Nacional do Meio Ambiente. Resolução no 460 de 30 de dezembro de 2013*. Brasília: Diário Oficial da República Federativa do Brasil.

Conte, E., Anghinoni, I., & Rheinhemer, D. S. (2003). Phosphorus accumulation fractions in a clayey oxisol in relation to phosphate doses under notillage. *Revista Brasileira de Ciência do Solo, 27*, 893–900.

Costa, C. N., Meurer, E. J., Bissani, C. A., & Tedesco, M. J. (2007). Sequential fractionation of cadmium and lead in soils. *Ciência Rural, 37*, 1323–1328.

Gallo, A. S., Guimarães, N. F., & Souza, M. D. B. (2015). Productivity of culture of bean after green manure, with the addition of slurry liquid of swine. *Revista de la Faculdad de Agronomía, 114*, 45–51.

Girotto, E., Ceretta, C. A., Brunetto, G., Santos, D. R., Silva, L. S., Lourenzi, C. R., Lorensini, F., Vieira, R. C. B., & Schmatz, R. (2010). Copper and zinc forms and accumulation in soil after successive pig slurry applications. *Revista Brasileira de Ciência do Solo, 34*, 955–965.

Havlin, J. L., Tisdale, S. L., Nelson, W. L., & Beaton, J. D. (2013). *Soil fertility and fertilizers: An introduction to nutrient management*. London: Pearson.

Henz, G. P., & Suinaga, F. A. (2009). Tipos de alfaces cultivados no Brasil. *Embrapa, 75*, 1–7.

Jordão, C. P., Andrade, R. P., Cotta, A. J. B., Cecon, P. R., Neves, J. C. L., Fontes, M. P. F., & Fernandes, R. B. A. (2013). Copper, nickel and zinc accumulations in lettuce grown in soil amended with contaminated cattle manure vermicompost after sequential cultivations. *Environmental Technology, 34*, 765–777.

Junio, G. R. Z., Sampaio, R. A., Santos, G. B., Nascimento, A. L., Prates, F. B. S., & Fernandes, L. A. (2011). Heavy metals concentrations in corn fertilized with phosphate rock and sewage sludge compost. *Revista Brasileira de Engenharia Agrícola e Ambiental, 15*, 1082–1088.

Kabata-Pendias, A. (2011). *Trace elements in soils and plants*. New York: CRC Press.

Kabata-Pendias, A., & Pendias, H. (2001). *Trace elements in soils and plants*. New York: CRC Press.

Kornegay, E. T., & Harper, A. F. (1997). Environmental nutrition: nutrient management strategies to reduce nutrient excretion of swine. *The Professional Animal Scientist, 13*, 99–111.

Lago-Vila, M., Rodríguez-Seijo, A., Arenas-Lago, D., Andrade, L., & Veja, M. F. L. (2016). Heavy metal content and toxicity of mine and quarry soils. *Journal of Soils and Sediments*. doi:10.1007/s11368-016-1354-0.

Leite, A., Silva, R., & Cunha, E. (2015). Occupational disease: medico legal importante: a case report. *Arquivos de Medicina, 29*, 93–97.

Luo, Y., Stichnothe, H., Schuchardt, F., Guoxue, L., Huaitalla, R. M., & Xu, H. (2014). Life cycle assessment of manure management and nutrient recycling from a Chinese pig farm. *Waste Management and Research, 32*, 4–12.

Macêdo, L. S., & Morril, W. B. B. (2008). Origem e comportamento dos metais fitotóxicos: Revisão da Literatura. *Tecnologia e Ciência Agropecuária, 2*, 29–38.

Malavolta, E. (1980). *Elementos de nutrição mineral de plantas*. São Paulo: Ceres.

Mattias, J. L., Ceretta, C. A., Nesi, C. N., Girotto, E., Trentin, E. E., Lourenzi, C. R., & Vieira, R. C. B. (2010). Copper, zinc and manganese in soils of two watersheds in Santa Catarina with intensive use of pig slurry. *Revista Brasileira de Ciência do Solo, 34*, 445–1454.

Melo, E. E. C., Nascimento, C. W. A., Accioly, A. M. A., & Santos, A. C. Q. (2008). Phytoextraction and fractionation of heavy metals in soil after multiple applications of natural chelants. *Scientia Agricola, 65*, 61–68.

Melo, V. F., Uchôa, S. C. P., Dias, F. O., & Barbosa, G. F. (2012). Levels of finely ground basalt rock in the chemical properties of a yellow latosol of the savannah of Roraima. *Acta Amazonica, 42*, 471–476.

Menegale, M. L. C., Castro, G. P. A., & Mancuso, M. A. C. (2015). Silicon: interaction with soil-plant system. *Journal of Agronomic Sciences, 4*, 435–454.

Muniz, M. H. B., Berto, D. A., Augusto, R. M. N., Neto, M. A. T., Wechsler, F. S., Tierzo, V. L., & Hauptli, L. (2010). Organic and inorganic mineral sources for weanling piglets. *Ciência Rural, 40*, 2163–2168.

Nascimento, A. L., Sampaio, R. A., Junio, G. R. Z., Carneiro, J. P., Fernandes, L. A., & Rodrigues, M. N. (2014a). Heavy metal contents in soil and in sunflower fertilized with sewage sludge. *Revista Brasileira de Engenharia Agrícola e Ambiental, 18*, 294–300.

Nascimento, R. S. M. P., Skorupa, A. L. A., Passos, L. P., & Marques, J. J. (2014b). Extraction and plant availability of zinc and lead in a latosol amended with steel industry residues. *Revista Brasileira de Ciências Agrárias, 9*, 322–329.

Nookabkaew, S., Rangkadilok, N., Prachoom, N., & Satayavivad, J. (2016). Concentrations of trace elements in organic fertilizers and animal manures and feeds and cadmium contamination in herbal tea (Gynostemma pentaphyllum Makino). *Journal of Agricultural and Food Chemistry, 64*, 3119–3126.

Paulino, A. T., Tessari, J. A., Nogami, E. M., Lenzi, E., & Nozaki, J. (2005). Lipid increase induced by lead accumulation in tilapia *Oreochromis niloticus*. *Bulletin of Environmental Contamination and Toxicology, 75*, 42–49.

Perdomo, C.C., Lima, G.J.M.M., Nones, K. (2001). Produção de suínos e meio ambiente. In: Celant TMB ed. Seminário nacional de desenvolvimento da suinocultura. Gramado. Brazil: Abps.

Pomar, C., Hauschild, L., Zhang, G.-H., Pomar, J., & Lovatto, P. A. (2009). Applying precision feeding techniques in growing-finishing pig operations. *Revista Brasileira de Zootecnia, 38*, 226–237.

Santos, G. C. G., Abreu, C. A., Camargo, O. A., & Abreu, M. F. (2002). Flue dust as zinc source to corn and its effect in heavy metals availability. *Bragantia, 61*, 257–266.

Santos, L. B., Paulino, A. T., Tambourgi, E. B., & Almedia, V. C. (2011). Toxic effects of dietary of Al^{3+} ions in tilapias (*Oreochromis niloticus*) and protective effect of Zn^{2+} ion. *Toxicological and Environmental Chemistry, 93*, 92–101.

Schmidt, V., Gottardi, C. P. T., & Nadvorny, A. (2007). Segurança sanitária durante a produção, o manejo e produção, o manejo e a disposição final de dejetos suínos. In M. A. Seganfredo (Ed.), *Gestão Ambiental na Suinocultura* (pp. 251–258). Brazil: Embrapa.

Segat, J. C., Alves, P. R. L., Baretta, D., & Cardoso, E. J. B. N. (2015). Ecotoxicological evaluation of swine manure disposal on tropical soils in Brazil. *Ecotoxicological and Environmental Safety, 122*, 91–97.

Seidel, E. P., Costa, A. C. S., & Lana, M. C. (2009). Copper phytoavailability and corn dry biomass production after swine manure application. *Revista Brasileira de Ciência do Solo, 33*, 1871–1878.

Sheldon, A. R., & Menzies, N. W. (2005). The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Knuth) in resin buffered solution culture. *Plant and Soil, 278*, 341–349.

Silva, M. L. S., Vitti, G. C., & Trevisam, A. R. (2007). Concentration of heavy metals in grain of plants cultivated in soil with different contamination levels. *Pesquisa Agropecuária Brasileira, 42*, 527–535.

Smanhotto, A., Sousa, A. P., Sampaio, S. C., Nóbrega, L. H. P., & Prior, M. (2010). Copper and zinc in the lixiviated material and in the soil application of swine wastewater on soil cultivated with soybean. *Engenharia Agrícola, 30*, 346–357.

Souza, C. F., Carvalho, C. C. S., Campos, J. A., Matos, A. T., & Ferreira, W. P. N. (2009). Characterization of waste from swine finishing phase. *Ceres, 56*, 128–133.

Tedesco, M. J., Gianello, C., Bissani, C. A., Bohnen, H., & Volkmeiss, S. J. (1995). *Análises de solo, plantas e outros materiais*. Porto Alegre: UFRGS.

Veiga, M., Pandolfo, C. M., & Junior, A. A. B. (2012). Zinc and copper in the soil and in the crop biomass after nine years of the use of nutrients sources associated to soil management systems in a Hapludox. *Revista Agropecuária Catarinense, 25*, 79–84.

Wuana, R.A., Okieimen, F.E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology, Article ID 402647, 20 pages.

Yruela, I. (2005). Copper in plants. *Brazilian Journal of Plant Physiology, 17*, 145–156.