DOI: 10.5433/1679-0359.2015v36n4p2483

Lead and nutrient allocation in vegetables grown in soil from a battery site

Alocação de chumbo e nutrientes em hortaliças cultivadas em solo contaminado por baterias

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Abstract

The steady growth of the Brazilian automotive industry and the resulting development of the battery market, which represent a large proportion of the lead (Pb) used in the country, have made battery recycling one of the main sources of Pb soil contamination in Brazil. Plants cultivated in Pb-contaminated soil can take up this metal, which can affect the plant's nutritional metabolism. The Pb can also be transferred into the edible parts of plants, thereby imposing threats to human health. This study was conducted to evaluate the concentration of Pb in edible parts of vegetables grown on soil contaminated by battery recycling activities. This study also investigated the effects of Pb on nutrient concentrations in plants. Plant species biomass, Pb concentration, and concentrations of macronutrients (P, K, Ca, Mg) and micronutrients (Fe, Mn, Zn, Cu) in plant parts were measured. The results showed that Pb concentrations in the edible parts of vegetables grown in contaminated soil were above the threshold acceptable for human consumption. Among the vegetables evaluated, only lettuce dry matter production was reduced because of the high concentration of Pb in soil. The presence of Pb altered the concentration of micronutrients in the edible parts of kale, carrots, and okra, stimulating higher Mn and Cu concentrations in these plants when cultivated in contaminated soil.
Key words: Edible parts, heavy metals, nutrients uptake

Resumo

O pleno crescimento brasileiro da indústria automobilística e a concomitante ampliação do mercado de baterias, que utiliza grande quantidade do Pb consumido no país, tornaram a sua reciclagem uma das principais formas de contaminação dos solos por chumbo. Plantas cultivadas em solo contaminado por Pb podem absorver esse metal que, além de afetar o metabolismo nutricional, pode ser transferido às partes comestíveis dos vegetais afetando a saúde humana. O presente trabalho objetivou avaliar o efeito do Pb na concentração de nutrientes em olerícolas, bem como o potencial de transferência desse elemento de um solo contaminado pela reciclagem de baterias automotivas às partes comestíveis das hortaliças. Os resultados demonstraram que, com relação as concentrações de Pb observadas nas partes comestíveis dos vegetais crescidos em solos contaminados, todos os vegetais apresentaram concentrações de Pb acima do limite aceitável para consumo humano. Entre os vegetais avaliados, somente a alface reduziu a produção de matéria seca devido a alta concentração de Pb no solo. Chumbo alterou a concentração de micronutrientes nas partes comestíveis, estimulando o aumento do teor de Mn e Cu em couve, cenoura e quiabo cultivados em solo contaminado.
Palavras-chave: Partes comestíveis, metais pesados, absorção de nutrientes

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Recebido para publicação 09/04/14 Aprovado em 14/01/15

Introduction

Heavy metals naturally occur in low concentrations in soils, as a result of weathering and other pedogenetic processes. However, these concentrations can be amplified by biogeochemical processes and especially by anthropogenic activities (KEDE et al., 2008; FREITAS et al., 2009). Among the main environmental contaminants, lead (Pb) is the second most hazardous element on the EPA priority list (ATSDR 2010) and poses a significant threat because of its high toxicity (YADAV et al., 2002; WANG et al., 2006; KEDE et al., 2008).

Plants grown in contaminated soils can absorb and accumulate heavy metals (LUI et al., 2006), resulting in photosynthesis inhibition, alterations in the plant structure and permeability of the membrane, and disturbances in mineral nutrition (SARMA et al., 2006). Pb can decrease cation (K, Ca, Mg, Mn, Zn, Cu, Fe) and anion (NO_3^-) absorption by the root system (SHARMA; DUBEY, 2005).

Increased Pb concentrations in vegetable crops can be harmful to humans (RATTAN et al., 2005). Impaired cognitive development and intellectual performance in children have been associated with high levels of Pb consumption. Adults can suffer from reproductive and renal problems, as well as increased blood pressure (WHO, 1993). Babies can ingest Pb through breast-feeding, and in pregnant women, Pb is easily transferred to unborn babies through the placenta (RABINOWITZ et al., 1985).

The existing use of Pb-contaminated soil to grow vegetable crops provides an ideal setting to study the Pb uptake potential of different vegetable species. The greater the Pb transfer into edible parts, the greater the risk is to consumers. This study evaluated the concentration of Pb in the edible parts of vegetables grown on soil contaminated by battery recycling activities and also investigated the effects of Pb on the concentration of nutrients in plants.

Materials and Methods

The experiment was conducted in a greenhouse between May and July 2008 using soil classified as a distrofic Spodosol, collected near an automobile battery recycling facility. The site is located in Rio Tinto, Paraiba state, Northeast Brazil (latitude 6º48’21.48’’N and longitude 35º04’32.14’’W). Samples of the same soil type were also collected from a non-contaminated area for use as a control. For the chemical and physical analyses, the soil samples were air-dried and sieved through a 2 mm mesh sieve (EMBRAPA, 1997, 1999) (Table 1). The total Pb concentration was obtained by *aqua regia* (HCl:HNO_3, molar ratio 3:1) The *aqua regia* extractant was added to the soil sample and the mixture was left at room temperature in a beaker covered with a watch glass for 16 h. The samples were then heated to 80°C for 2 h, cooled, brought to 50 mL in a volumetric flask, and filtered (PEREIRA et al., 2007). After filtering, the extracts were transferred to PET flasks, labeled, and kept at 4°C in a refrigerator until they were placed in an atomic absorption spectrophometer. Soil acidity was very high (pH 3.9) and was therefore adjusted to a pH of approximately 6.5 using calcium carbonate and magnesium oxide (3:1 molar ratio).

The four vegetables chosen for this study are among the most abundant ones in the typical Brazilian diet, and represent different edible parts (tubers, leaves, and fruits). Seedlings of carrot (*Daucos carota L.*), kale (*Brassica oleracea* L. var. *acephala*), okra (*Abelmoschus esculentus* L.), and lettuce (*Lactuca sativa L.*) were grown in polystyrene trays in a greenhouse, using vermicompost as the substrate. To prepare the experiment, 5.5 kg of soil was transferred into plastic pots and fertilized prior to seedling planting. The soil samples were fertilized as follows: 250, 240, 150, and 100 mg kg^{-1} of N, P, K, and S, respectively, added as NH_4SO_2, $NH_4H_2PO_4$, and KNO_3; the micronutrients Fe ($FeSO_4.7\ H_2O$), Mn ($MnCl_2.4H_2O$), Zn ($ZnSO_4.7H_2O$), Cu ($CuSO_4$), B (H_3BO_3), and Mo ($Na_2MoO_4.2H_2O$) were applied at the concentrations of 2, 4, 4, 1.5, 1, and 0.2 mg kg^{-1}, respectively (NASCIMENTO et al., 2006). Twenty days after sowing, the seedlings were transferred into the pots, and 15 days later, they were thinned out, leaving four plants per pot. During the experiment, pots were irrigated daily with distilled water to maintain the soils at approximately 80% of water retention capacity.

Table 1. Chemical and physical characteristics of the Pb-contaminated and non-contaminated soil samples used for growing the vegetables.

Characteristic	Soil	
	Contaminated	Non-contaminated
pH (water – 1:2,5)	3.89	5.1
P (mg dm^{-3})	2.30	8.30
Na^+ ($cmol_c$ dm^{-3})	0.28	0.31
K^+ ($cmol_c$ dm^{-3})	0.05	0.10
Ca^{+2} ($cmol_c$ dm^{-3})	4,6	4,4
Mg^{+2} ($cmol_c$ dm^{-3})	0.90	2.90
Al^{+3} ($cmol_c$ dm^{-3})	0.50	0.15
H+Al ($cmol_c$ dm^{-3})	3.30	2.80
Pb (mg kg^{-1})	413.0	9.20
M.O (g kg^{-1})	14.73	26.9
Sand (g kg^{-1})	943.0	940.0
Silt (g kg^{-1})	27.0	27.0
Clay (g kg^{-1})	30.0	33.0

The experiment was conducted in a factorial randomized blocks design of 2 × 4 × 4. The treatments consisted of two soil conditions (contaminated and non-contaminated), four vegetable species (lettuce, kale, carrot, and okra) and four plant parts (root, stem, leaf, and edible parts), with four replications. The plants were separated into roots, stems, leaves, and edible parts, washed thoroughly in tap water, and then in distilled water, to remove traces of nutrients and Pb ions from the surface (LIU et al., 2009). The collected parts were weighed, put in paper bags, and dried in an oven at 65-70°C until they reached a constant weight. The dried samples were weighed once more, ground in a Wiley cutting mill, and were then submitted to a nitro-perchloric digestion (EMBRAPA, 1999) to determine concentrations of Ca, Mg, Fe, Mn, Zn, Cu, and Pb by atomic absorption spectrophotometry, P by colorimetry, and K by flame photometry. Soil samples from each pot were taken to measure available Pb by EDTA and DTA (LINDSAY; NORVELL, 1978; LANTMANN; MEURER, 1982). The data obtained for dry matter, Pb content, and concentrations of macro and micronutrients in the various plant parts were submitted to analysis of variance (ANOVA) and the measurements were compared using the Scott-Knot test at 5% probability. Using the same soil used in our experiment, Freitas et al. (2014) reported that lime amendment decreased the soluble amounts of Pb in soil from up to 361 mg L^{-1} to 168 mg L^{-1}.

Results and Discussion

The presence of Pb significantly altered the production of leaf and edible part dry matter for plants grown in contaminated soil, but had no apparent effect on the production of root dry matter for any of the vegetable species studied (Table 2). For lettuce, a 14.55% reduction was observed in the dry matter production of leaves. Okra plants showed greater production of fruit dry matter when grown in Pb-contaminated soil. Considering the strong adsorptive capacity of Pb by iron oxides, clay minerals, and organic matter (BOSSO; ENZWEILER, 2008), the reduced phytotoxic effects of Pb in the soil indicate that the overall high soil concentrations do not necessarily imply high toxicity of plants. Additionally, adjusting the pH to approximately 6.5 probably contributed to decreased Pb availability in the soil.

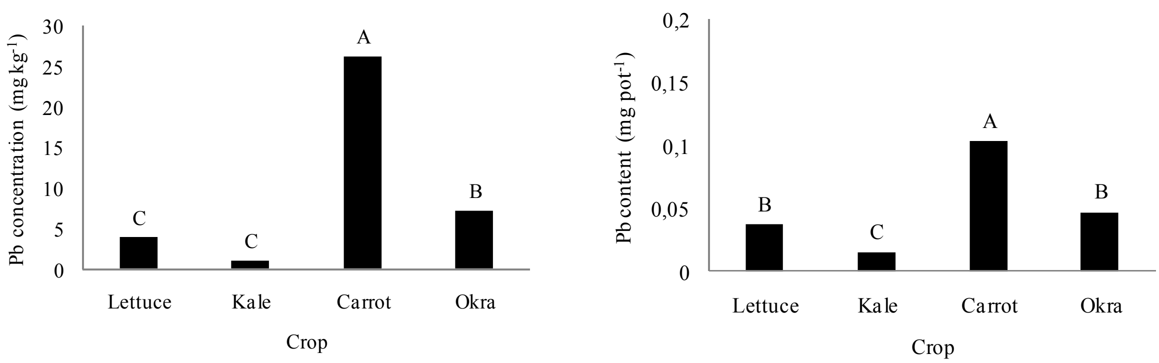
Table 2. Dry matter production (g^{-1} per pot) for vegetables grown in Pb-contaminated and non-contaminated soils.

Part / Soil	Lettuce		Kale		Carrot		Okra	
	C	NC	C	NC	C	NC	C	NC
Root	0.43Ac	1.01Ac	2.71Ac	2.02Ac	0.75Ac	0.54Ac	1.45Ac	1.55Ac
Stem	2.18Ab	3.70Ab	4.56Ab	4.54Ab	NA	NA	2.98Ab	4.11Ab
Leaf	9.45Ba	11.06Aa	14.28Aa	13.28Ba	14.82Aa	13.08Ba	6.67Aa	7.54Aa
Edible part	*	*	*	*	3.93Ab	2.51Ab	6.54Aa	3.86Bb
Total	12.07B	15.77A	22.25A	19.84B	19.51A	16.14B	17.64A	17.06A
CV%	17.05							

*=Leaf itself is the edible part.
C= contaminated soil; NC = non-contaminated soil; NA = non-applicable.
Means followed by the same letters are not significantly different by the Scott & Knott test at 5% probability. Capital letters compares plant parts, between a contaminated and a non-contaminated soil; small letters compares each plant part for each soil condition.

The Pb concentrations in the edible parts of vegetables grown in contaminated soil varied between 1.03 and 26.19 mg kg^{-1} of dry matter, in the following order: carrot > okra> lettuce > kale (Figure 1). Carrot, despite having only 20.15% of matter in the edible parts (Table 2), had the greatest potential to transfer Pb from the soil into the food chain because of the high concentrations of this metal in the edible parts. The edible parts of kale cultivated in contaminated soil had the highest dry weight compared to the weights of the other plant parts (Table 2). However, kale had the lowest accumulation of Pb among the crops, and it also transferred the lowest quantity of Pb from the soil to the plant (Figure 1). Kale concentrated only 1.03 mg kg^{-1} of Pb in its edible parts, and because of the high quantity of dry matter produced (Table 2), it did not extract significant amounts of Pb from the soil.

Figure 1. Pb concentration (mg kg^{-1}) and content (mg pot^{-1}) in edible plant parts grown in contaminated soil. Means followed by the same letters are not significantly different by the Scott & Knott test at 5% probability.



The high concentrations of Pb in the edible parts of the vegetable species (lettuce: 2.01 mg kg^{-1} FW; kale: 0.56 mg kg^{-1} FW; carrot: 14.17 mg kg^{-1} FW; and okra: 3.97 mg kg^{-1} FW) are above the allowable legal limits for healthy human consumption, according to Codex Alimentarius, 2014. Even though the Pb concentrations were higher than those permitted for agricultural soils according to Brazilian regulations (CONAMA, 2014), the vegetables contained acceptable and different amounts of Pb in their edible parts (Figure 1). This indicates that Pb transfer to these parts is highly dependent on species.

The presence of Pb in the soil reduced the concentration of Ca in lettuce (Table 3). The presence of Pb generates antagonism in terms of Ca absorption, inhibiting this function because of the blocking of cationic channels through competitive transport (SHARMA; DUBEY 2005; LIU et al., 2009). However, the presence of Pb in the soil prompted increased Ca concentrations in kale and carrot roots. No significant effect of Pb on the concentration of this element was found for the other vegetable parts. Pb can affect the natural processes of absorption and translocation of nutrients in plants grown in contaminated soil (SINHA et al., 2006; GOPAL; RIZVI, 2008), possibly by altering the selectivity and permeability of the plasma membrane. This can apply to increased uptake of some elements, especially for those absorbed by passive routes (SHARMA; DUBEY, 2005; LIU et al., 2009; SARMA et al., 2006), thereby contributing, in some cases, to increased concentrations of certain nutrients in plants.

The presence of Pb in the soil increased the concentration of Mg in lettuce stems, kale leaves, and carrot roots (Table 3). However, Pb did not affect Mg concentrations in lettuce, carrot, and okra leaves. Díaz-Aguilar et al. (2001) reported that Pb did not affect Mg concentrations in wheat leaves grown in soil with a high Pb concentration.

Pb caused significant increases in P concentrations in kale and carrot roots, although the concentration of this element was lower in kale and okra leaves (Table 3). Some authors (GOPAL; RIZVI, 2008) have observed that the presence of Pb provokes synergism in P absorption in radish plants, because of the direct interference of this metal with the metabolism of P in the plant. It is also likely that P added via fertilization can precipitate Pb by forming lead phosphates in the roots (MUÑOZ-BERTOMEU et al., 2009).

Except for the edible parts of carrots, the presence of Pb altered K concentrations in all plant parts for plants grown in contaminated and non-contaminated soils (Table 3). Pb promoted reduction in K concentrations in lettuce roots, although the opposite condition was observed in other vegetable parts. This increase in K concentrations in plant parts may be related to the loss of selectivity in cellular membranes (SARMA et al., 2006) provoked by Pb, thereby favoring the passive uptake of K. This may be the reason for the increased concentration of K in plant aerial parts, i.e., the increase in the root K uptake was probably related to K redistribution in plants.

K concentrations in plant parts grown in contaminated soil varied from 13.58 to 55.51 mg kg^{-1} (lettuce), 22.60 to 50.91 mg kg^{-1} (kale), 15.95 to 36.72 mg kg^{-1} (carrot), and 33.23 to 44.29 (okra) (Table 3). K was concentrated mainly in the leaves of the plants, except in the case of okra. The okra plants grown in contaminated soil contained the greatest K concentrations in their stems, at 1.41 times the amount found in the fruit, reflecting low transfer of this element to edible plant parts.

Table 3. Ca, Mg, P and K concentration in lettuce, kale, carrot and okra, grown in Pb-contaminated and non-contaminated soil.

Crop / Soil	Lettuce		Kale		Carrot		Okra	
	C	NC	C	NC	C	NC	C	NC
				Ca (g kg^{-1})				
Root	9.09Bb	12,66Ab	13,55Ab	3,97Bb	15,41Aa	12,29Bb	13,27Ab	13,28Ab
Stem	5.80Bc	14,06Ab	3,74Ac	3,51Ab	NA	NA	14,29Ab	14,39Ab
Leaf	14.72Ba	18.77Aa	17.88Aa	17.31Aa	16.19Aa	16.40Aa	40.39Aa	42.02Aa
Edible plant	*	*	*	*	3.92Ab	3.75Ac	4.42Ac	4.46Ac
*CV (%)				10.23				
				Mg (g kg^{-1})				
Root	33.52 Ab	35.92Ab	16.94Ab	15.96Ab	52.39Aa	39.70Ba	34.62Ac	34.93Ac
Stem	38.69Aa	17.89Bc	15.87Ab	15.80Ab	NA	NA	39.01Ab	38.50Ab
Leaf	41.27Aa	42.08Aa	39.10Aa	35.27Ba	34.35Ab	31.95Ab	53.48Aa	54.56Aa
Edible plant	*	*	*	*	18.48Bc	26.78Ac	29.10Ac	30.74Ad
*CV (%)				19.93				
				P (g kg^{-1})				
Root	15.25Aa	13.83Aa	13.22Aa	8.36Bb	12.08Aa	9.54Ba	8.80Aa	9.21Aa
Stem	8.93Ab	8.33Ab	6.13Ac	6.40Ac	NA	NA	5.94Ab	6.06Ab
Leaf	7.63Ab	8.23Ab	11.60Bb	15.40Aa	7.52Ab	7.48Ab	8.25Ba	9.99Aa
Edible plant	*	*	*	*	6.73Ab	6.97Ab	6.28Ab	6.33Ab
*CV (%)				18.78				
				K (g kg^{-1})				
Root	13.58Bc	22.10Ac	22.60Ab	17.80Bb	15.95Ac	6.90Bb	35.71Ab	28.91Ba
Stem	35.01Ab	30.72Bb	50.91Aa	31.13Ba	NA	NA	44.29Aa	29.05Ba
Leaf	55.51Aa	46.58Ba	50.19Aa	30.76Ba	36.72Aa	30.86Ba	33.23Ac	27.32Ba
Edible plant	*	*	*	*	28.00Ab	27.71Aa	31.37Ac	27.32Ba
*CV (%)				10.72				

*=Leaf itself is the edible part.
C= contaminated soil; NC = non-contaminated soil; NA = non-applicable.
*CV = Coefficient of Variation.
Means followed by the same letters are not significantly different by the Scott & Knott test at 5% probability. Capital letters in the line compare the same crop and the same plant part; small letters in the column compares parts of same crop for each soil condition.

Pb increased Fe concentrations in the roots of lettuce, kale, carrot, and okra. However, concentrations of Fe were 18% lower in the leaves of lettuce cultivated in contaminated soil (Table 4). Cabbage plants grown in Pb-contaminated soil showed reduced concentrations of Fe in the leaves (SINHA et al., 2006). The Fe reduction in leaves results in a loss of vitality and capacity to resist stressful conditions (GEEBELEN et al., 2002). An increase in Fe concentrations in roots was also noted by Gopal and Rizvi (2008) who evaluated alterations to radish plants submitted to excess Pb. The increase in Fe concentrations in the roots may be caused by higher Fe flow resulting from Pb-induced loss of membrane selectivity (SHARMA; DUBEY, 2005).

A high concentration of Mn was recorded in the roots and stems of plants grown in contaminated soil (Table 4). In contrast with Pb-induced low concentration of Mn in lettuce leaves, kale and okra leaves grown in contaminated soil displayed greater concentrations of this metal. Mn was mainly concentrated in the leaves of all the plants. The presence of Pb in the soil also increased Mn concentrations in wheat plant parts (DÍAZ-AGUILAR et al., 2001).

Although Pb prompted an increase in Zn concentrations in lettuce roots grown in contaminated soil, a 28.50% reduction was noted in the concentration of Zn in lettuce leaves (Table 4). The low concentration of Zn in leaves is related to the reduction in the stability of the plasma membrane, thus making plants less tolerant to biotic stresses such as metal toxicity (GEEBELEN et al., 2002).

Table 4. Concentration of Fe, Mn, Zn and Cu in lettuce, kale, carrot and okra grown in Pb-contaminated and non-contaminated soil.

Crop / Soil	Lettuce		Kale		Carrot		Okra	
	C	NC	C	NC	C	NC	C	NC
				Fe (mg kg^{-1})				
Root	233.15Aa	189.60Ba	244.57Aa	149.93Ba	268.92Aa	149.31Ba	235.57Aa	133.70Ba
Stem	43.11Ac	52.57Ac	21.01Ac	27.05Ac	NA	NA	26.20Ac	25.52Ad
Leaf	136.41Bb	167.06Ab	83.89Ab	85.02Ab	71.51Ab	76.90Bb	74.88Ab	85.92Ab
Edible part	*	*	*	*	48.51Ac	51.89Ac	36.34Ac	47.16Ac
*CV (%)				14.22				
				Mn (mg kg^{-1})				
Root	77.23Ab	35.89Bb	144.45Aa	16.28Bb	525.19Aa	128.98Ba	103.57Ab	40.46Bb
Stem	30.73Ac	14.61Bc	13.95Ab	4.64Bc	NA	NA	39.90Ac	18.52Bc
Leaf	126.49Ba	270.04Aa	142.12Aa	78.28Ba	182.51Ab	133.31Aa	260.83Aa	159.24Ba
Edible part	*	*	*	*	42.38Ac	18.10Bb	40.88Ac	24.09Bc
*CV (%)				11.46				
				Zn (mg kg^{-1})				
Root	392.82Aa	188.92Bb	612.54Aa	227.17Bb	909.97Aa	253.03Ba	395.91Ab	255.01Ba
Stem	236.81Ab	293.26Aa	263.36Ab	154.54Bc	NA	NA	226.44Ac	101.72Bc
Leaf	227.70Bb	318.36Aa	617.88Aa	325.97Ba	178.73Ab	179.17Ab	486.81Aa	178.26Bb
Edible part	*	*	*	*	171.16Ab	143.17Ab	164.08Ad	180.26Ab
*CV (%)				8.28				
				Cu (mg kg^{-1})				
Root	48.27Aa	22.54Ba	34.36Ba	38.30Aa	35.33Aa	18.59Ba	19.36Aa	7.71Ba
Stem	9.96Ac	10.80Ac	14.49Ac	14.49Ab	NA	NA	5.89Ac	2.39Bb
Leaf	13.48Ab	12.98Ab	19.27Ab	14.49Bb	8.88Ab	4.14Bb	8.63Ab	3.64Bb
Edible part	*	*	*	*	8.13Ab	4.39Bb	6.14Ac	1.89Bb
*CV (%)				8.05				

*=Leaf itself is the edible part. C= contaminated soil; NC = non contaminated soil; NA = non-applicable. *CV = Coefficient of Variation.
Means followed by the same letters are not significantly different by the Scott & Knott test at 5% probability.
Capital letters compare each plant part between a contaminated and a non-contaminated soil; and small letters compare each plant part for each soil condition.

Similar to lettuce, greater concentrations of Zn were observed in kale, carrot, and okra roots grown in contaminated soil. The lettuce, kale, and carrot plants grown in contaminated soil had highest Zn concentrations in the roots, whereas in okra plants the highest concentrations were in the leaves.

Pb caused high Cu concentrations in lettuce, carrot, and okra roots, but was related to reductions in Cu concentration in kale roots (Table 4). In general, an imbalance in Cu allocation promoted by Pb was observed in plant parts, and was also reported by Sharma and Dubey (2005). It was also found that, in both contaminated and non-contaminated soils, the highest and lowest Cu concentrations in plant parts were observed in roots and stems, respectively. Pb also prompted a significant increase in the concentration of Cu in carrot roots, leaves, and edible parts (Table 4). The increase in Cu concentrations in the plant parts may be caused by higher Cu flow resulting from the Pb-induced loss of membrane selectivity (SHARMA; DUBEY, 2005).

Conclusions

1. The edible parts of vegetables grown in contaminated soils all had Pb concentrations above the acceptable threshold for human consumption.

2. Among the vegetables evaluated, only the lettuce dry matter production was reduced because of high concentrations of soil Pb.

3. Pb altered the concentration of micronutrients in the edible parts of kale, carrots, and okra, thus stimulating higher Mn and Cu concentrations in these plants when cultivated in contaminated soil.

4. In general, Pb toxicity changed the macronutrient concentrations and distributions in the plants. For example, although P root uptake increased, the amounts of P transferred to shoots decreased. This effect was probably caused by damages to the plasma membrane and precipitation of Pb phosphates in the roots. In contrast, K had increased uptake because of higher membrane permeability, as reflected by higher concentrations in other plant parts.

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