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# Lead and cadmium accumulation potential and toxicity threshold determined for land cress and spinach

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### **Abstract**

Soil contamination with potentially toxic elements (PTEs) in agricultural lands, in part, is responsible for limiting the crop productivity and the food chain contamination. The objective of this study were to asses the limiting of crop productivity by cadmium (Cd) and lead (Pb), the potential transfer and bioaccumulation of these PTEs in plants, and ultimately the food chain contamination to ensure that the pre-established soil threshold concentrations for Cd and Pb are enough to control food chain exposure to them. Therefore, land cress and spinach were grown in some pots containing a sandy loam soil contaminated with increasing concentrations of Pb and Cd. The concentrations of Pb and Cd in land cress and spinach at any level of soil contamination were compared with the threshold concentrations of Pb and Cd in leafy vegetables as established by the Codex Alimentarius Commission (CAC). A bioaccumulation factor was calculated to estimate the potential transfer of Pb and Cd to the food chain. According to the results, Pb was of more phytotoxicity than Cd. The lower limit of the maximum acceptable concentration of Pb in soil was safe enough to ensure the prevention of the food chain contamination to Pb. Results also showed that growing land cress on contaminated soils was of great potential risk of Pb transfer to the human food chain when compared to spinach. The pre-established maximum acceptable concentration of Cd in soil of 1-20 mg kg<sup>-1</sup> was not safe to prevent the contamination of food chain. Cd was of a greater potential of entering the human food chain than Ph

Keywords: Bioconcentration; Cd; Contamination; Human food chain; Pb; Phytotoxicity.

# Introduction

Vegetables are vital to the human diet, particularly, to provide the essential trace elements. However, their contamination with potentially toxic elements (PTEs) including lead (Pb) and cadmium (Cd), even at quite low concentrations, can be harmful not only to plants itself but also to the humans who use these vegetables (Bowen, 1979; Mohamed et al., 2003). The main causes of vegetables contamination with PTEs are irrigation with

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water from streams contaminated by industries, the cultivation on former industrial land contaminated by spilled oil and industrial wastes, the pollution of crops by heavy traffic, the application of contaminated compost (de Zeeuw, 2004). The PTEs may accumulate in the edible parts of vegetables that are directly consumed by people. Generally, the highest amounts of PTEs accumulate in the leaves, whereas the lowest contents are located in seeds, making the leafy vegetables are of more concern regarding the human food chain contamination.

PTEs cannot be chemically degraded or destroyed. Therefore, PTEs tend to bioaccumulate once entered the organisms (Zhuang et al., 2009). Bioaccumulation refers to an increase in the concentration of a given chemical in a biological organism over time, compared to the chemical's concentration in the environment. There are many publications to deal with PTEs in vegetables (e.g. Sherif et al., 1979; Tahvonen and Kumpulainen, 1991; Stalikas et al., 1997; Pinochet et al., 1999; Mohamed et al., 2003). Cadmium and lead, as common PTEs in urban agricultural soils, are biopersistent and, once absorbed by an organism, remains resident for many years (over decades for humans) although it is eventually excreted.

Few countries have established guidelines for threshold concentrations of PTEs to regulate metal levels in soils. Warnings and critical limits are often based on total soil PTE content. However, it is widely accepted that the total soil PTE concentrations are poor indicators of their potential effects and their toxicity to terrestrial organisms (Abollino et al., 2005; Finžgar et al., 2007). In fact, to determine the potential for PTEs to confer health problems to consumers of crops grown on contaminated soils, bioavailability and food chain transfer need to be considered. Metal mobility and bioavailability in soil varies significantly with soil properties for similar total soil metal concentrations. Most of the international guidelines enacted to regulate metal levels in agricultural soils relate to the land application of sewage sludge ('biosolids'). An in-depth review of this area has been published earlier (McLaughlin et al., 2000). There is a great disparity between countries as to the maximum acceptable concentrations of metals established by them for agricultural soils receiving anthropogenic inputs of metals (McLaughlin et al., 2000). The maximum acceptable concentrations of Pb and Cd, among different countries, are in the ranges of 50-300 and 1-20 mg/kg, respectively (Council Directive 86/278/EEC, 1986; McLaughlin et al., 2000).

Few countries have also established guidelines for allowable concentrations of metals in such foods as leafy vegetables. According to these guidelines, allowable concentrations of Pb and Cd in leafy vegetables are 0.3 and 0.2 mg metal/kg fresh weight, respectively (Hamon and McLaughlin, 2003).

Some PTEs pose little hazard of food chain contamination due to their strong phytotoxic effects (i.e., increasing metal concentrations cause plant mortality before transfer to the next trophic level, e.g., people or grazing animals, has an opportunity to occur). This is known as the soil-plant barrier (Hamilton et al., 2007). Cd has been identified as the major heavy metal of health concern in contaminated lands as it is, relative to most other metals, more available to plants and is found in concentrations in harvestable parts of the crops that could be harmful to humans but are not toxic to the plant (Stevens and McLaughlin, 2006). On the other hand, Pb is not readily soluble in soil and generally has a low rate of transfer to the human food. Therefore, a risk analysis might be required in cases such as limiting the

crop productivity by PTEs, the transfer potential and bioaccumulation of PTEs in plants, and ultimately the food chain contamination to ensure that the pre-established soil threshold concentrations for PTEs are enough to control food chain exposure to PTEs.

The aims of this study were (i) to assess the effect of soil contamination with Pb and Cd on the yield reduction of land cress and spinach, (ii) to determine the bioconcentration of Pb and Cd by land cress and spinach and the risk of food chain contamination, and (iii) to test the utility of using the pre-established soil threshold concentrations for Pb and Cd as a substitute for plant metal thresholds to control food chain exposure to such PTEs as Pb and Cd.

## **Materials and Methods**

Land cress and spinach were grown in some pots containing a sandy loam soil contaminated with different levels of Pb (0, 150, 300, 600, 800, 1000, and 1500 mg kg<sup>-1</sup>) or Cd (0, 10, 20, 40, 60, 80, and 100 mg kg<sup>-1</sup>) under controlled greenhouse condition. The edible parts of the plants were harvested 42 days after seeds germination which is the best harvest time for using them by human as a food. The plant samples were washed, ovendried and grinded. The total amounts of Pb and Cd in plants were extracted and analyzed using a graphite furnace atomic absorption spectrometer to ensure that the PTEs are not transferred to the food chain. Details on the experiments could be found in Khodaverdiloo (2007) and Khodaverdiloo and Homaee (2008).

In this study, the concentrations of Pb and Cd in land cress and spinach at any level of soil contamination with these metals were compared with the threshold concentrations of Pb and Cd in leafy vegetables as established by the Codex Alimentarius Commission (CAC) (Hamon and McLaughlin, 2003). From this comparison the maximum allowable concentrations of these metals in the soil (i.e. the total Pb or Cd in soil which lead to their threshold concentrations in plants) were defined. This way, the utility of using the pre-established soil threshold concentrations for Pb and Cd (Hamon and McLaughlin, 2003) as a substitute for plant metal thresholds to control food chain exposure to metals was tested. To test the phytotoxicity of the metals, the plants yield reduction was also determined at the applied soil Pb or Cd concentrations. Furthermore, the plant yield corresponds for the threshold concentrations in the plants when used as food, was defined.

To estimate the potential transfer of Pb and Cd to the food chain the bioconcentration of soil metal by plant is also calculated as follow:

$$BCF = \frac{total\ metal\ in\ plant\ fresh\ matter(mg\ kg^{-1})}{total\ metal\ in\ soil\ (mg\ kg^{-1})}$$

where BCF (-) is biconcentration factor.

The allowable concentrations of metals in foods, almost in all countries, are given in fresh weight basis (Hamon and McLaughlin, 2003). Consequently, the Pb and Cd concentrations in shoot dry matter were multiplied by the appropriate DW/FW ratio to give the concentrations in fresh matter. The mean DW/FW ratio was  $0.081\pm0.003$  and  $0.121\pm0.007$  for land cress and spinach, respectively.

#### **Results and Discussion**

Soil properties

The experimental soil was a sandy loam with an alkaline pH of soil saturated paste of 8.2. The bulk density of soil was 1.4 gr cm<sup>-3</sup>. The electrical conductivity of soil saturated paste extract (EC<sub>e</sub>) was 0.9 dSm<sup>-1</sup>, thus, no salinity stress was affecting plant for the whole growth period.

# Uptake of Pb and Cd

Figure (1) show the Pb (Figures 1a and 1b) and Cd (Figures 2a and 2b) concentrations in dry weight of the plant material at different levels of soil contamination with these metals, respectively, for land cress and spinach. The maximum accumulation of Pb was 87.3 mg kg<sup>-1</sup> dry weight of the land cress material at soil total Pb of 1500 mg kg<sup>-1</sup> and 89.7 mg kg<sup>-1</sup> dry weight of the spinach material at soil total Pb of 1000 mg kg<sup>-1</sup>. In the case of Cd, however, this was 745.6 and 128.5 mg kg<sup>-1</sup> dry weight for land cress and spinach, respectively. These results show a relatively linear trend between Pb in land cress and soil Pb concentration (Figure 1a), while, for spinach (Figure 1b) there is a threshold soil Pb concentration (600 mg kg<sup>-1</sup>), beyond which the plant is more efficient to take up Pb. Figures 2a and 2b indicate that land cress is tending to take up more Cd than spinach. For land cress, the shoot Cd content increased up to soil Cd-contamination of 40 mg kg<sup>-1</sup> and remained relatively constant up to 60 mg kg<sup>-1</sup>. However, there was a sharp decrease from the first contamination level for spinach. This indicates that land cress can accumulate and tolerate soil-Cd up to 60 mg kg<sup>-1</sup>. While the threshold soil Cd concentration for spinach remained unknown, the first applied level of Cd (10 mg kg<sup>-1</sup>) resulted in shoot Cd content decrease

As presented in table (1), at all soil Pb concentrations the BCF values were below 0.005 and 0.011 for land cress and spinach, respectively. In the case of soil contamination with Cd, the BCF ranged from 2.731 (with 10 mg Cd kg $^{-1}$  soil) to 0.060 (with 100 mg Cd kg $^{-1}$  soil) for land cress and from 1.542 (with 10 mg Cd kg $^{-1}$  soil) to 0.013 (with 60 mg Cd kg $^{-1}$  soil) for spinach.

Table 1. Bioconcentration factor (BCF) of soil Pb and Cd by land cress and spinach at different levels of soil contamination.

Soil Total Pb (mg kg <sup>-1</sup> )						
	150	300	600	800	1000	1500
land cress	0.004 <sup>a</sup>	0.003	0.003	0.004	0.005	0.005
spinach	0.000	0.001	0.001	0.007	0.011	_b
		Soil	Total Cd (mg k	(g <sup>-1</sup> )		
	10	20	40	60	80	100
land cress	2.731	1.845	1.491	0.892	0.108	0.060
spinach	1.542	0.547	0.075	0.013	-	-

<sup>&</sup>lt;sup>a</sup> The BCF values were calculated as the ratio of (metal in fresh weight of the plant material (mg kg<sup>-1</sup>)):(soil metal concentration(mg kg<sup>-1</sup>)).

<sup>&</sup>lt;sup>b</sup> plants not grown as a result of metal phytotoxicity.

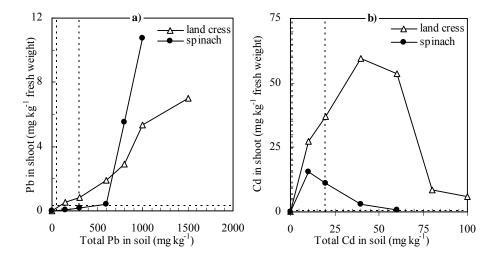


Figure 1. Pb (a) and Cd (b) concentration in shoot fresh matter of land cress and spinach vs. different levels of soil contamination. The horizontal dashed-lines are the allowable concentrations of Pb and Cd in fresh weight of leafy vegetables and the vertical dashed-lines are the range for the maximum acceptable concentrations of Pb (50 to 300 mg kg<sup>-1</sup>) and Cd (1 to 3 mg kg<sup>-1</sup>) established by various countries for agricultural soils receiving anthropogenic inputs of metals (from McLaughlin et al., 2000).

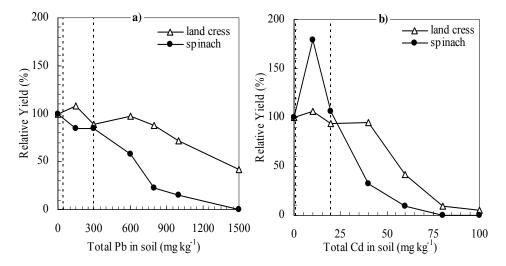


Figure 2. Relative experimental yield  $Yc/Y_0$  of land cress and spinach vs. soil Pb (a) and Cd (b) concentrations. The vertical dashed-lines are the range for the maximum acceptable concentrations of Pb (50 to 300 mg kg<sup>-1</sup>) and Cd (1 to 3 mg kg<sup>-1</sup>) established by various countries for agricultural soils receiving anthropogenic inputs of metals (from McLaughlin et al., 2000).

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#### Relative Yield

The effect of soil Cd and Pb on relative yield of land cress and spinach is presented in figure (2). Decline in relative yield ranged from 11% (with 300 mg Pb kg<sup>-1</sup> soil) to 57% (with 1500 mg Pb kg<sup>-1</sup> soil) for land cress and from 15% (with 150 mg Pb kg<sup>-1</sup> soil) to 100% (with 1500 mg Pb kg<sup>-1</sup> soil) for spinach. In the case of soil contamination with Cd, this decline ranged from 6% (with 20 mg Cd kg<sup>-1</sup> soil) to 94% (with 100 mg Cd kg<sup>-1</sup> soil) for land cress and from 67% (with 40 mg Cd kg<sup>-1</sup> soil) to 100% (with 100 mg Cd kg<sup>-1</sup> soil) for spinach. These results show that while, at the same levels of soil contamination, the Pb-tolerance of land cress was higher as compared to spinach, the latter is more tolerant to the lower levels of soil Cd contamination (i.e. when soil total Cd is less than 40 mg kg<sup>-1</sup>). However, at higher levels of soil total Cd (> 40 mg Cd kg<sup>-1</sup> soil), land cress grew better than spinach.

As shown in figure (2b), a statistically significant yield increase of spinach (in comparison with the control) was found where the soil contained 10 mg Cd kg<sup>-1</sup>. This favorable effect of soil Cd and Pb at low concentrations on plant yield also was found for land cress (Figures 2a and 2b), but it was not statistically significant. Such a stimulating effect of Cd on plant yield was previously found by many researchers (e.g., Bosiacki, 2008; John et al., 2009), but, so far it has not been explained.

To test the metal phytotoxicity the reductions in plant yield were assessed at the upper and lower limits of pre-established maximum acceptable concentration of the metals in soil (50-300 mg kg<sup>-1</sup> for Pb and 1-20 mg kg<sup>-1</sup> for Cd). The yield reductions, as an indication of metal phytotoxicity, were between  $\approx 0$  to 11% and  $\approx 0$  to 15% for land cress and spinach, respectively, when soil total Pb was in the range of pre-established maximum acceptable concentration of Pb in soil (i.e. 50-300 mg kg<sup>-1</sup>) (Figure 2a). This reduction was < 6% when land cress grown on a soil with 20 mg Cd kg<sup>-1</sup> (i.e. the upper limit of the maximum acceptable concentration of Cd in soil) (Figure 2b). Soil Cd concentrations below 20 and 10 mg kg<sup>-1</sup>, respectively for spinach and land cress, not only had no phytotoxicity, but also a stimulating effect of Cd on the plant growth was observed (Figure 2b).

### Risk of Food Chain Contamination

To assess the risk of human food chain contamination, the metal concentration of plant's fresh matter, when grown on a soil which the Pb or Cd concentrations were in the range of upper and lower limits of pre-established maximum acceptable concentration of the metals in soil (i.e. 50-300 mg kg<sup>-1</sup> for Pb and 1-20 mg kg<sup>-1</sup> for Cd), was compared with the allowable concentration of these metals in leafy vegetables (0.3 and 0.2 mg kg<sup>-1</sup> for Pb and Cd, respectively). Land cress shoots exceeded the allowable concentration for Pb in leafy vegetables of 0.3 mg kg<sup>-1</sup> when grown on a soil contained 300 mg Pb kg<sup>-1</sup> (Figure 1a). However, Pb concentration in land cress was below the allowable concentration when grown on a soil with 50 mg Pb kg<sup>-1</sup> (Figure 2a). In the case of spinach, Pb concentration in plant was below the allowable concentration either when grown on a soil with 50 or even 300 mg Pb kg<sup>-1</sup> (Figure 2a). These results showed that, for the soil in this study, the lower limit of the maximum acceptable concentration of Pb in soil is safe enough to ensure the prevention of the food chain contamination to Pb. These results also showed that growing

land cress on contaminated soils is of great potential risk of Pb transfer to the human food chain when compared to spinach.

For Cd, the land cress and spinach concentrations of Cd were fairly greater than the allowable concentration of Cd in leafy vegetables of 0.2 mg kg<sup>-1</sup>, even when they were grown on a soil with 1 mg Cd kg<sup>-1</sup> (Figure 1b). These results showed that, for the soil in this study, the pre-established maximum acceptable concentration of Cd in soil of 1-20 mg kg<sup>-1</sup> is not safe to prevent the contamination of food chain. These results also showed that the Cd is of a greater potential of entering the human food chain than Pb.

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