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Lead Bioaccumulation and Translocation in Herbaceous Plants Grown in Urban and Peri-Urban Soil and the Potential Human Health Risk

Ada Baldi [1], Stefano Cecchi [2], Chiara Grassi [1,*], Camillo A. Zanchi [1], Simone Orlandini [1] and Marco Napoli [1]

1 Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Piazzale delle Cascine, 18-50144 Firenze, Italy; ada.baldi@unifi.it (A.B.); camillo.zanchi@unifi.it (C.A.Z.); simone.orlandini@unifi.it (S.O.); marco.napoli@unifi.it (M.N.)
2 Institute for Bioeconomy (IBE) of National Research Council (CNR), Via Madonna del Piano, 10-50019 Florence, Italy; stefano.cecchi@ibe.cnr.it
* Correspondence: chiara.grassi@unifi.it

Abstract: Lead (Pb) contamination risks to crops grown in urban and peri-urban soils is a great concern that should be better evaluated to define the Pb maximum levels in soils for safe cultivation and to identify suitable strategies to remediate Pb polluted urban soils. The objective of this work was to evaluate the potential risk for human health from the ingestion of the edible portions of barley, castor bean, common bean, Indian mustard, sorghum, spinach, and tomato grown in an unpolluted soil (initial Pb content 32.6 mg kg^{-1}) spiked with 0, 300, 650, 1000 mg Pb kg^{-1}, respectively. The potential possibility of using these plants to phyto-remediate the soil of Pb was also assessed. Pot trials were conducted for two years (2008 and 2009). Results highlighted that all the investigated species were able to attain growth to maturity in high Pb spiked soil, although Pb influenced dry matter accumulation. Even in soils with low Pb concentrations, Pb accumulated the edible parts. Noteworthy, even in untreated control soils, all tested species revealed a Pb concentration in the edible parts that was higher than the safe limit set by FAO/WHO. None of the investigated species were considered Pb hyperaccumulators, but all were shown to be potentially suitable for phyto-stabilization.

Keywords: lead toxicity; urban agriculture; soil pollution; lead bioconcentration; foodstuff

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1. Introduction

Urban and peri-urban agriculture are expanding worldwide, and are emerging as an integral component of urban planning policies in both developed and developing countries, attributable to a multifunctional role [1–3]. One of the greatest concerns relating to urban and peri-urban agriculture is the contamination of soil with heavy metals (HM) [4,5].

Lead (Pb) is a toxic HM for human health [6,7]. Inhalation and ingestion of Pb, even at low concentrations, can be very harmful to human health [8,9]. Given that Pb has been extensively used since ancient times, nowadays Pb contaminated soils are widespread on a global scale [10,11]. In urban and peri-urban soils, the presence of this metal was mainly shown to be dependent on the past use of Pb as an antiknock agent in gasoline and as a base for exterior paints [12]. For uncontaminated agricultural soil a Pb concentration ranging from 2 to 300 mg kg^{-1} was reported by Alloway (1995) [13], which designed a level of 100 mg kg^{-1} as a dangerous threshold for both humans and the environment. A study conducted to evaluate the HM content in agricultural soils of the European Union [11] reported that the highest percentage of analyzed samples displayed a concentration of Pb below 60 mg kg^{-1}. In contrast, Ajmone-Marsan and Biasioli (2010) [4], after reviewing studies conducted in a total of 86 cities around the world, highlighted an extreme variability of Pb contamination in the soil.

By passing from the soil to the edible plant, HMs then enter the food chain [14]. The methodologies commonly adopted to assess the risk associated with the intake of HM contaminated food are based on provisional tolerable weekly intake (PTWI) and/or oral reference dose (RfD) [15–17]. In the year 2011, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) stated that there was no evidence for establishing a threshold for critical Pb-induced effects, so it concluded that the previously established PTWI (25 μg/kg body weight) could no longer be considered health-protective and withdrew it. Moreover, the United States Environmental Protection Agency (US EPA) has no consensus RfD for Pb. Therefore, to date, as reference values for Pb concentration in food commodities, those fixed by the FAO/WHO Codex Alimentarius Commission can be considered [18]. The Codex sets the maximum limit of Pb in vegetables at 0.10 mg kg^{-1} (fresh weight), with the exception of brassica and leafy vegetables for which 0.30 mg kg^{-1} is permissible. However, no limit for kale and spinach has yet been provided. Generally, vegetables grown in soil where Pb levels do not exceed 300 mg kg^{-1} can be safely eaten [19]. The Italian law does not establish limits for Pb concentration in agricultural soil. Limits are, however, provided for public green and residential area soils (100 mg kg^{-1}), as well as industrial and commercial use soils (1000 mg kg^{-1}) [20]. Further, a maximum concentration of 100 mg kg^{-1} was fixed as a topsoil threshold concentration for the distribution of compost or sewage sludge [20]. Instead, the European Directive 86/278/CEE [21] sets a threshold of 300 mg kg^{-1} Pb in the soil [22].

The ability of plants to absorb pollutants, such as Pb, can be exploited for soil phytoremediation [23,24]. This technology involves the use of plants capable of absorbing HM through their roots and then of translocating and accumulating contaminants in their harvestable tissues [25–27]. To date, only a few plants may be considered Pb hyperaccumulators and among these the majority belong to the Brassicaceae Family [28–30]. However, several studies have been conducted to identify plants with phytoremediation potential, which compensate for a lower concentration of Pb in their tissues with fast growth, high biomass production, and ease of both cultivation and harvest [28,30,31]. Nevertheless, the topic is still warranting further investigation, primarily to evaluate the relationship between different soil Pb levels and Pb concentrations found in the roots and aerial parts of plants.

Therefore, the present study was conducted to investigate plant growth, as well as the bioaccumulation and translocation capacity of Pb by various herbaceous crops normally grown in urban and peri-urban areas around the world. The growth response of barley, castor bean, common bean, Indian mustard, sorghum, spinach, and tomato to different soil Pb levels were evaluated and then both the accumulation of Pb in roots, stems and leaves, and fruits were assessed. The results were envisioned to evaluate the suitability of the investigated species to remediate Pb spiked soil, as well as the potential risk for human health due to the ingestion of the edible portions of the respective plants.

2. Materials and Methods

2.1. Reagents

Lead (II) sulphate (>98.0% *w*/*w*) was from Sigma-Aldrich, Saint Louis, MO, USA. Super-pure nitric acid (HNO_3) for trace metal analysis and HCl for analytic analysis were from Carlo Erba, Rodano, Milan, Italy. A 10% (*v*/*v*) HNO_3 and a 5% (*v*/*v*) HCl solutions were prepared by HNO_3 and HCl in distilled water, respectively.

2.2. Experimental Set-Up

A pot experiment was carried out for two years (January 2008–December 2009) under outdoor conditions in Montepaldi (San Casciano Val di Pesa, Italy). The climate was sub-Mediterranean with dry summers and wet autumns and springs, respectively [32]. During the study period, yearly average temperature values were 15.3 and 15.7 °C for 2008 and 2009, respectively. The yearly rainfall was 703.8 and 671 mm for 2008 and 2009, respectively. More detailed information on the climatic conditions recorded during the study period can be found in [33].

The soil (Table 1) was collected in a vineyard (0–15 cm depth) and then prepared as reported in [34].

Table 1. The physical and chemical characteristics of the soil used in the experiment.

Properties	Measure Unit	Value
Clay	%	40.8
Silt	%	40.1
Sand	%	19.1
Organic carbon	%	1.21
Total nitrogen	%	0.08
Available phosphorous	mg kg^{-1}	7.5
Total calcium carbonate	%	19.1
pH		7.9
Cation Exchange Capacity	cmol kg^{-1}	15
Total copper	mg kg^{-1}	55.5
Total lead	mg kg^{-1}	32.6
Total nickel	mg kg^{-1}	62.2
Total zink	mg kg^{-1}	87.2

The experiment included seven crop species tested on four soil Pb levels (treatments). Each treatment was replicated 20 times. The four Pb levels were realized by spiking and homogeneously mixing the soil with a solution containing 0, 9.879, 21.405, 32.931 g of lead (II) sulphate corresponding to 0 (control), 300, 650, and 1000 mg kg^{-1} Pb added to soil, respectively. The 300 and 1000 mg kg^{-1} of Pb levels were selected from a consideration of the maximum Pb levels in the soil [21] and the limits for Pb in the soils intended for commercial or industrial areas set by the Italian law [20], respectively.

In both years, a total of 560 plastic pots (30 cm diameter; 30 cm depth; 18 L volume) were filled with 22.5 kg of soil. For each pot, the spiking was performed as follows: the soil was grinded using a soil crusher and pass over a 2 mm sieve. The Pb containing solution was added to the soil and the soil mixed carefully in a bucket for 2 min with a trowel. After a day of air drying, the soil was passed again thought the soil crusher (5 min) to be grinded and to mix the HM. Finally, the soil was transferred in a pot. Soil samples (10 g) from each pot were analysed to determine the actual DTPA-exchangeable Pb. The average DTPA-exchangeable Pb in soils was determined to be 2.12, 52.4, 124.1, and 178.7 mg kg^{-1} Pb for the doses from 0 to 1000 mg kg^{-1} Pb added to the soil. The seven crops were: barley (*Hordeum vulgare* L.), castor bean (*Ricinus communis* L.), common bean (*Phaseolus vulgaris* L.), Indian mustard (*Brassica juncea* L. Czern.), sorghum (*Sorghum bicolor* (L.) Moench), spinach (*Spinacia oleracea* L.), and tomato (*Solanum lycopersicum* L.). For each crop, the sowing and transplanting dates are reported in Table 2.

Table 2. The sowing or transplanting date for each crop and experimental year.

Crop	Sowing or Transplanting Date	
	2008	2009
Barley	27 February 2008	2 March 2009
Castor bean	27 February 2008	2 March 2009
Common bean	27 February 2008	2 March 2009
Indian mustrad	27 February 2008	2 March 2009
Sorghum	27 May 2008	20 May 2009
Spinach	20 March 2008	24 March 2009
Tomato	27 May 2008	20 May 2009

Over the entire experimental period, the pots were automatically drip-irrigated (as determined by the soil moisture) to avoid stressing the crops as reported in [34].

2.3. Plant Biomass and Lead Spectrophotometric Determination

The plants were harvested at the end of their respective biological cycles. For each pot, three different parts of plant (roots, stems and leaves, and fruits) were collected separately, weighed (DW, kg) after oven-drying at 105 °C, and ground. To trace the metal content, a sample (1 g of DW) for each of the different plant parts of each crop was ashed in a muffle furnace (550 °C; ~12 h) until the ash colour was light grey. After cooling, the ash content was acid digested with 5 mL HNO_3, and the sample-acid mixture was heated until the residues were dissolved. After cooling, the mixture was filtered (Whatman 42) in a 10 mL flask, made up to volume with distilled water. The HNO_3 solution was used as a blank sample. The HCl solution (5% v/v) was used to clean the poly-propylene containers and test tubes before being used for analytic analysis [35]. The Pb concentration (mg kg^{-1} DW) was determined using an ICP-OES (Optima 7300 DV, PerkinElmer, Inc. Shelton, CT, USA). Details of the operating conditions were as follows: forward power 1450 W, argon (purity grade > 99.99%), argon plasma flow rate 15 L min^{-1}, auxiliary argon flow rate 0.2 L min^{-1}, argon nebulizer flow rate 0.6 L min^{-1} and wavelengths 220.353 nm with axial view. The minimum detectable level was 1.06 µg L^{-1}. The Pb determinations were performed in triplicate for each sample.

2.4. Data Evaluation

For each crop, dry matter (DM) production and DM Pb concentration were analysed for the different parts of the plant. The capacity of each respective crop in taking up Pb from the polluted soil and then translocating this HM from roots to aerial tissues was evaluated utilizing both the bioconcentration factor (BCF; Equation (1)) [36] and the translocation factor (TF; Equation (2)) [37], respectively.

$$BCF = \frac{CPbplant}{CPbsoil} \tag{1}$$

where CPb_{plant} was the Pb concentration in the plant and CPb_{soil} was the Pb concentration in the soil.

$$TF = \frac{CPb_{aereal\ tissues}}{CPb_{roots}} \tag{2}$$

where $CPb_{aerial\ tissue}$ was the Pb concentration in aerial tissue, while CPb_{roots} was the Pb concentration in roots.

Plants with both BCF and TF values higher than 1 were considered as accumulators, suitable for phytoextraction, while the others were evaluated for phyto-stabilization [33,37,38]. The root bioconcentration factor (BCFr) was used to evaluate the efficacy of the plant in concentrating Pb in the roots (Equation (3)).

$$BCFr = \frac{CPb_{roots}}{CPbsoil} \tag{3}$$

The experiment was performed using a two-factor split-plot design, with the year being the main factor (fixed factor) and the Pb level in the soil being the secondary factor (random factor). For each crop, analysis of variance (ANOVA) was performed to evaluate difference between treatment for DW and Pb concentration in different plant parts. The multiple mean comparisons were performed using the Tukey honest significant difference (Tukey's HSD test) at the $p < 0.05$ probability level. Box and whiskers plots were used to represent the Pb concentration and DW values for the different crops and plant tissues, respectively [33].

3. Results and Discussion

3.1. Effect of the Pb Soil Level on DW and Pb Accumulation in Different Plant Parts

All the investigated crop species reached the harvest stage, thereby showing both survival and growth capacity even at the highest Pb levels.

3.1.1. Barley

The Pb in the soil appears to positively influence the increase in leaves and stems DW of barley (Figure 1). Indeed, in both years, the DW of leaves and stems of plants grown in Pb contaminated soil was statistically higher than that of control plants. The maximum roots and fruits DW was in 300 mg Pb kg^{-1} spiked soil, while the DW recorded in 650 and 1000 mg Pb kg^{-1} was statistically equal or lower than that of the control plants (Figure 1). The highest annual DW values for leaves and stems were 24.2 g and 20.5 g, measured in the first year in plants in 1000 mg Pb kg^{-1} spiked soil and the second year in 650 mg Pb kg^{-1} spiked soil, respectively. The highest DW values for both fruits and roots were measured in the 300 mg Pb kg^{-1} spiked soil. Aery and Jagetiya, 1997 [39] reported a significant ($p < 0.01$) reduction of 70.3% in DW of roots and of 41.6% in DW of shoots of 45-day old plants of barley grown in sandy loam soil with Pb concentrations ranging from 10 to 6250 mg Pb kg^{-1} soil. Instead, Ryzhenko et al., 2016 [40] showed Pb stimulating barley growth in chernozem soils with 450 mg Pb kg^{-1}, but depressing barley growth in a sod-podzolic soil spiked with 300 mg Pb kg^{-1} soil.

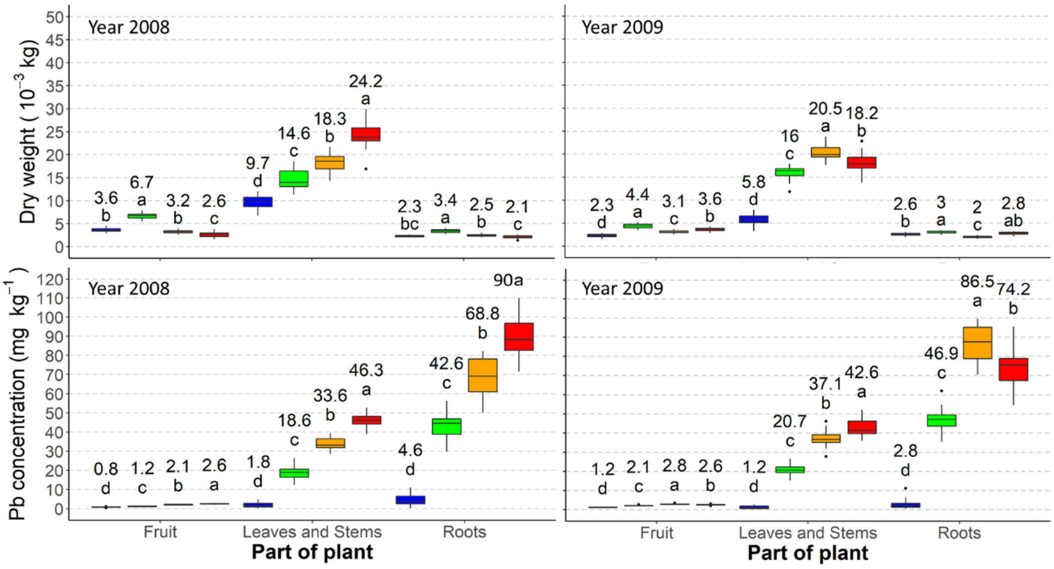

Figure 1. Boxplots of the dry weight (**top**) and Pb concentration (**bottom**) determined in different parts of barley plants in 2008 (**left** panel) and 2009 (**right** panel) for 0 (blue box), 300 (green box), 650 (orange box), and 1000 mg kg^{-1} (red box) of Pb added to soil, respectively. Lowercase letters indicate different means between treatments ($p < 0.05$) according to the post hoc Tukey test.

In 2008, the Pb concentration in the three different plant parts was shown to increase coinciding with increases in the soil Pb concentration (Figure 1). The same trend was observed for leaves and stems in 2009, while in fruits and roots the maximum Pb concentration was attained in soils spiked with 650 mg Pb kg^{-1} (Figure 1) These results were consistent with those reported previously under open field conditions in a sub-alkaline silty loam soil in south Italy [41] and in an acid clay loam soil in the South Island of New Zealand [42]. Our results indicated that barley concentrated between 2 to 35.6 times less Pb in the fruit ($p < 0.05$) than in other tissues. Similarly, the Pb concentration in the fruits (1.8 mg Pb kg^{-1} DW) was previously reported to be seven times lower than in the roots (13.1 mg Pb kg^{-1} DW) of barley plants grown in soil containing 22 mg Pb kg^{-1} soil [43].

3.1.2. Castor Bean

Castor bean has gained considerable importance as a phytoremediation plant in the last two decades, as has been demonstrated by the large number of scientific papers published on this topic since the more recent review by [31]. In both years, fruit DW was significantly higher ($p < 0.05$) in treated plants than in control plants (Figure 2), attaining the maximum value in the 1000 mg kg^{-1} spiked soil. No differences were observed between the second and the third Pb level. In 2008, the DW values in the leaves and stems were significantly lower in treated plants compared to control plants, while in 2009 the DW values of the second Pb level were significantly higher than those in the remaining treatments. The DW of the roots was higher in the control plants than that in 2008 plants grown in 650 mg Pb kg^{-1} and 1000 mg Pb kg^{-1} and that in 2009 plants grown in 300 mg Pb kg^{-1} and 650 mg Pb kg^{-1}. Regarding DW measurements in the literature, conflicting results are evident, since the experimental conditions differ. Four levels of Pb in nutrient solutions (0, 100, 200, and 400 µmol Pb L^{-1}) were shown to result in a decrease in castor bean DW coinciding with the increase in Pb [44]. In contrast, other studies did not show a reduction in castor bean shoot and root DW grown in nutrient solutions spiked with 0, 6, 12, 24, 48 and 96 mg Pb L^{-1} [45] and 25, 50, 100, 150, or 200 µmol L^{-1} [46], respectively. Under the present experimental conditions, castor bean DW varied widely depending on the different parts of the plants. Differences were observed also between years, suggesting the need for further studies. The DW of fruits was higher in the treated plants than in the controls.

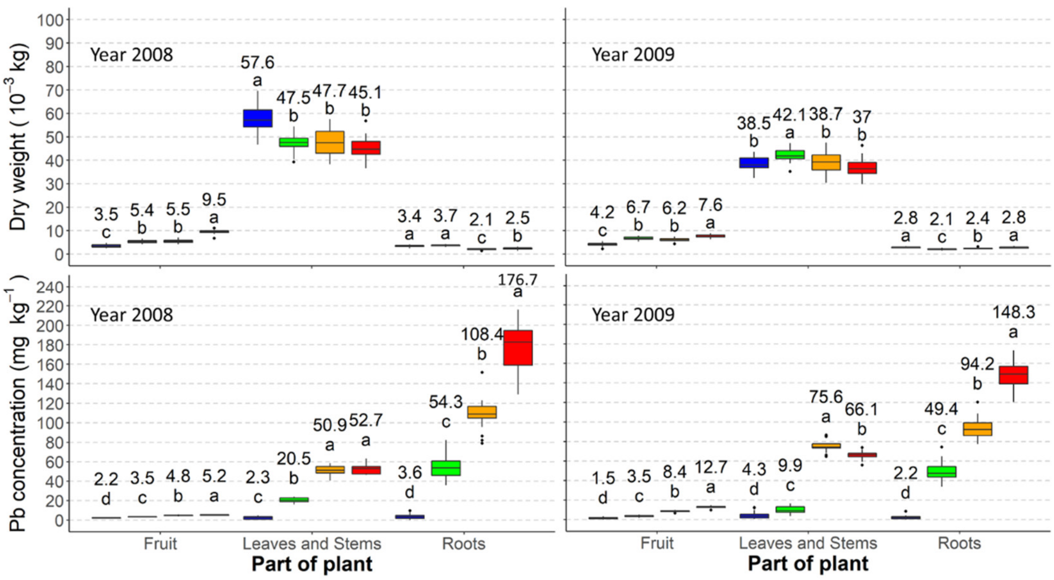

Figure 2. Boxplots of the dry weight (**top**) and Pb concentration (**bottom**) determined in different parts of castor bean plants in 2008 (**left** panel) and 2009 (**right** panel) for 0 (blue box), 300 (green box), 650 (orange box), and 1000 mg kg^{-1} (red box) of Pb added to soil, respectively. Lowercase letters indicate different means between treatments ($p < 0.05$) according to the post hoc Tukey test.

In both years, the Pb concentration in leaves and stems increased along with the Pb concentration in the soil up to the 650 mg Pb kg^{-1} treatment, after which no further increments were found at the 1000 mg Pb kg^{-1} treatment. The root Pb concentration increased from the control up until the third Pb soil level. The increased uptake of Pb by castor bean, coinciding with the increase in Pb concentration in the cultivation media, was observed previously [31,45,47]. The root Pb concentration was 11.7 to 34.0 times higher than that observed in fruits and 2.2 to 3.4 times more than that observed in the leaves and stems. Results showed that castor bean has a great capacity to accumulate Pb in the roots, with reduced efficacy in translocating this element to the aerial tissues. These results are in accordance with those of Alves et al. (2016) [48] and Kiran and Prasad (2017) [31] who showed that roots were able to accumulate 85% and 45% of the Pb absorbed by the whole plant, respectively. Opposite results were obtained by Boda et al. (2017) [49], who stated that castor bean accumulates higher Pb in leaves and stems than in roots.

3.1.3. Common Bean

Inter-annual differences in DW production were found in fruits and leaves and stems (Figure 3). In 2008, the DW of both fruits and leaves and stems significantly increased ($p < 0.05$) in the 300 mg Pb kg^{-1} treatment and decreased ($p < 0.05$) in the 1000 mg Pb kg^{-1} treatment with respect to the control. In 2009, the fruit DW of plants grown in Pb spiked soils was higher ($p < 0.05$) than that of the control plants, with the highest average DW value determined in the 650 mg Pb kg^{-1} treatment. Moreover, the DW of leaves and stems measured in the 300 mg Pb kg^{-1} and 650 mg Pb kg^{-1} treatments were higher than that measured in the control. In both years, root growth significantly decreased ($p < 0.05$) with the increase in soil Pb concentration. In general, results indicated that total plant DW increased with increasing soil Pb concentration compared to the control except for the highest level of soil Pb in 2008. Our results were in contrast with those of Sánchez et al. (1999) [50], who reported that, in a soilless pot experiment, common bean DW significantly ($p < 0.05$) decreased along with increased Pb concentration in the solution. Further investigations must be conducted to better clarify the growth response of common bean in Pb spiked soil.

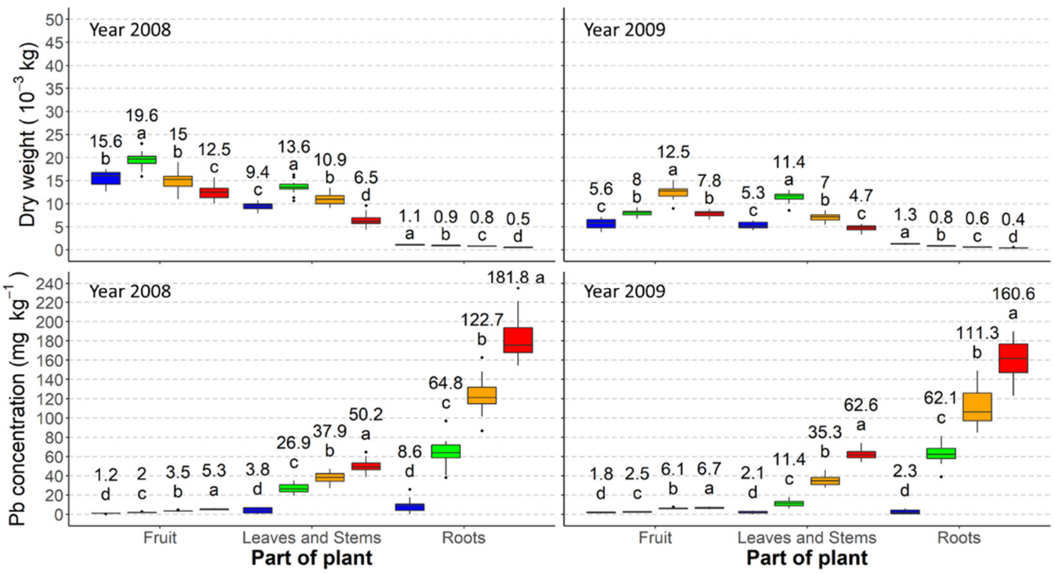

Figure 3. Boxplots of the dry weight (**top**) and Pb concentration (**bottom**) determined in different parts of common bean plants in 2008 (**left** panel) and 2009 (**right** panel) for 0 (blue box), 300 (green box), 650 (orange box), and 1000 mg kg^{-1} (red box) of Pb added to soil, respectively. Lowercase letters indicate different means between treatments ($p < 0.05$) according to the post hoc Tukey test.

In both 2008 and 2009, the Pb concentration in common bean tissues increased ($p < 0.05$) along with the soil Pb concentration. Results indicated that the highest concentration of Pb was in the roots, especially in the 650 and 100 mg kg^{-1} Pb spiked soils, followed by the leaves and stems, and finally the fruits, respectively. These results are in contrast with previous published research conducted in residential area of Chicago (USA) [51]. These authors reported common bean as one of the species with the lowest capacity to accumulate Pb. On the other hand, Finster et al. (2004) [51] and other authors [52–55] evidenced that the majority of the Pb absorbed by common bean was accumulated in roots and that the Pb concentration in plant tissues was significantly correlated to Pb concentration in soil.

3.1.4. Indian Mustard

An increment in the Pb inhibitory effect on DW accumulation of leaves, stems and roots was observed as the Pb concentration in soil increased (Figure 4). In 2008, no differences were observed between the second and the third Pb level. In 2009, the leaves and stems DW was more affected by the addition of 300 and 1000 mg Pb kg^{-1} while the root DW was affected by all Pb levels compared to control. The present results were consistent with those reported previously, showing that shoot and root DW declined linearly when soil Pb concentration increased from 0 to 540 mg Pb kg^{-1} [56]. Other authors showed that in a pot experiment with soil spiked with Pb (0, 100, 250, and 500 μg mL^{-1} of Pb) root growth was reduced at 250 and 500 μg mL^{-1} Pb, with no effect on shoot growth at any of the Pb concentrations applied [57]. In contrast, Bassegio et al. (2020) [56] observed an increase in shoot DW of plants grown in a pots filled with Rhodic Acrudox soil artificially contaminated with incremental Pb levels, from 24 mg Pb kg^{-1} (control) to 1150 mg Pb kg^{-1}.

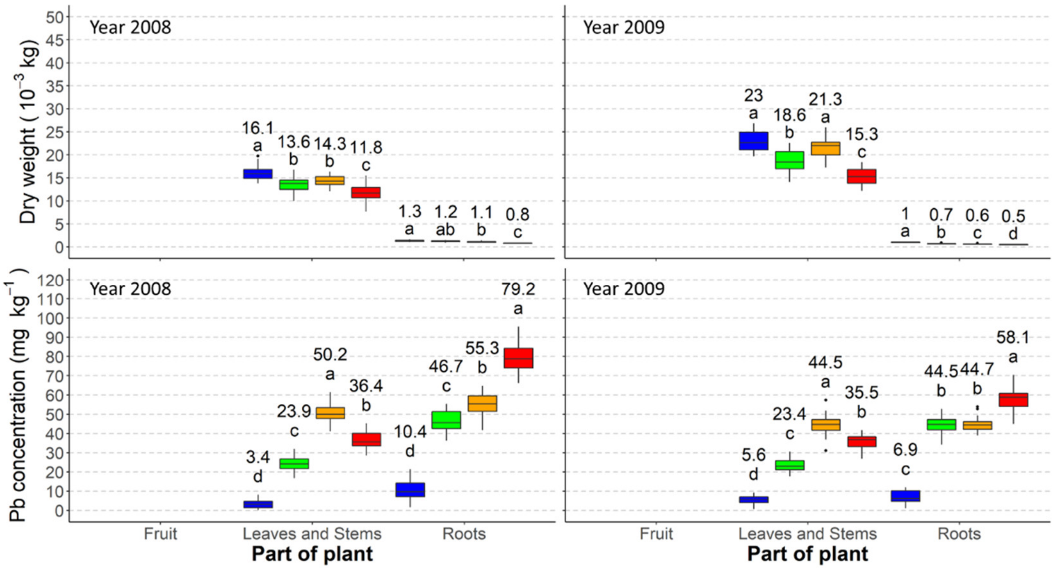

Figure 4. Boxplots of the dry weight (**top**) and Pb concentration (**bottom**) determined in different parts of Indian mustard plants in 2008 (**left** panel) and 2009 (**right** panel) for 0 (blue box), 300 (green box), 650 (orange box), and 1000 mg kg^{-1} (red box) of Pb added to soil, respectively. Lowercase letters indicate different means between treatments (($p < 0.05$) according to the post hoc Tukey test.

Pb-treated plants showed a significantly ($p > 0.05$) higher Pb concentration compared to the control plants. In both years, the leaves and stems of treated plants showed the same trend, with the minimum and maximum Pb concentrations at 300 and 650 mg Pb kg $^{-1}$ soil, respectively. The highest Pb concentration in the roots was shown with 1000 mg kg^{-1} in both years. Inter-annual differences were detected between the 300 and 650 mg kg^{-1} Pb levels. In 2008, the Pb concentration in the roots was higher in 650 mg Pb kg^{-1} than in the 300 mg Pb kg^{-1} spiked soil. However, in 2009, no difference was observed between the two treatments. Moreover, our data indicated that Indian mustard predominantly accumulated Pb in the roots, but that the plant was effective in transporting Pb from the roots to the shoots. These results are consistent with those of some authors who reported an increase of Pb concentration in the shoots and roots of Indian mustard as the Pb concentration in soil increased [57,58], and with authors reporting that these species accumulate Pb more in roots than in shoots [28,57,59–61]. More specifically, in the study conducted in the city of San Petersburg (Russia) by Drozdova et al. (2019) [28], the observed Pb concentration pattern of *B. juncea* was roots > leaves > inflorescence > stems and roots retain about 10 times more Pb than stems and inflorescence and two times more Pb than leaves.

3.1.5. Sorghum

Inter-annual differences in DW production of sorghum were observed for all considered tissues (Figure 5). In 2008, the biomass of the fruits, leaves and stems, and roots were significantly ($p < 0.05$) higher in treated plants than in the control plants. In the fruits, no differences were observed between the 650 and 1000 mg kg^{-1} treatments. In the leaves and stems, DW significantly ($p < 0.05$) increased as the Pb concentration in the soil increased, whereas in the roots, the highest DW was measured in the 650 mg Pb kg^{-1} spiked soil followed by the by 300 mg kg^{-1} and 1000 mg kg^{-1} in decreasing order. In 2009, no common trend was observed in the different parts of the plant in response to the addition of Pb. In the fruits, no differences were observed between the control and treated plants. In leaves and stems, a significant increase in biomass production was detected with 1000 mg Pb kg^{-1} in comparison to the control. In the roots, a decrease in DW ($p < 0.05$) was observed with the increase in soil Pb. Similarly, in a growth chamber experiment, between from 5 mg L^{-1} and 100 mg L^{-1} Pb in solution, the reduction in the shoot and root DW became increasingly more severe even if the plants continued to grow [62]. To the contrary, the present findings suggest that Pb in the soil may have stimulated the growth of sorghum, even at 1000 mg kg^{-1}. However, since no trend in DW was observed between the two years, more research is necessary to better clarify the growth response of sorghum in Pb contaminated soil. In 2008, all our treatments showed an increase in fruit DW compared to the control. In 2009, no differences were observed between the control and treated plants. The DW of the leaves and stems increased with the increase of Pb in the soil in 2008. However, in 2009, no differences were observed between the control, 300 mg kg^{-1} and 650 mg kg^{-1} treatments, respectively. For the roots, the DW of the control was the lowest in 2008 and the highest in 2009.

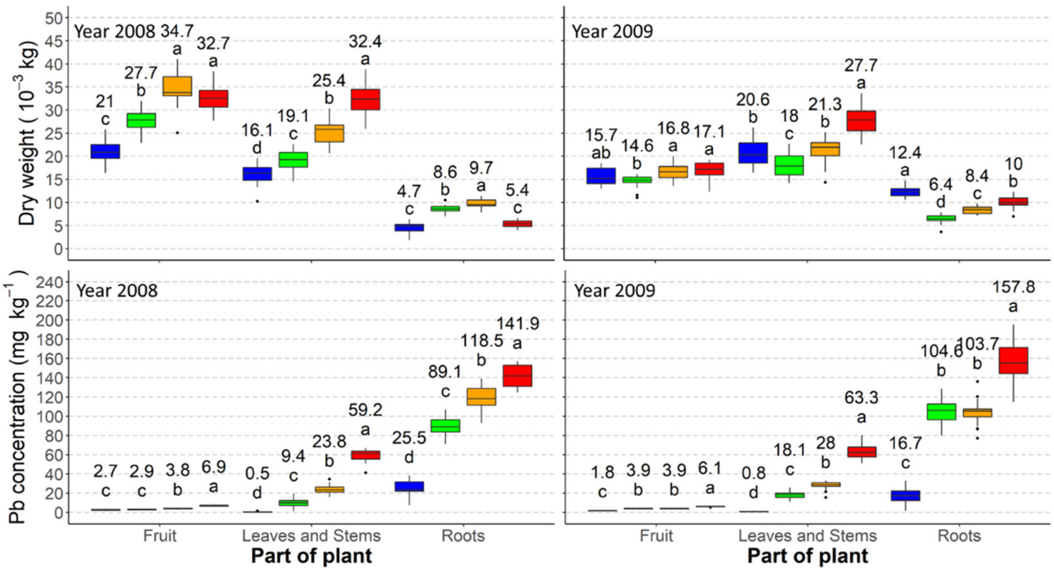

Figure 5. Boxplots of the dry weight (**top**) and Pb concentration (**bottom**) determined in different parts of sorghum plants in 2008 (**left** panel) and 2009 (**right** panel) for 0 (blue box), 300 (green box), 650 (orange box), and 1000 mg kg^{-1} (red box) of Pb added to soil, respectively. Lowercase letters indicate different means between treatments ($p < 0.05$) according to the post hoc Tukey test.

The highest Pb concentration in fruits was recorded in the 1000 mg Pb kg^{-1} spiked soil in 2009. In 2008, no differences were observed between the control and the 300 mg kg^{-1} treatment, whereas in 2009 an increase in Pb in the fruits was evident for all treatments compared to the control. In both years, Pb concentration in the leaves and stems increased significantly with the increase in soil Pb concentration. The same result was observed for the roots in 2008, while for the two intermediate concentrations, no differences were observed in 2009. The highest Pb concentrations in tissues were retained in the roots, ranging from 2.4 to 51.6 times more Pb than that measured in the leaves and stems, and from 9.3 to 31.2 times more Pb than that measured in the fruits, respectively. Consistent with the present results, various authors found that the roots were the principle depot for the accumulation of Pb in sorghum, followed by the leaves and stems, and finally the fruits [62–66]. Blanco et al. (2016) [64], in a study conducted in the peri-urban area of Córdoba city (Argentina), despite observing a negative correlation between Pb concentration in soil and Pb concentration in roots and fruits, stated that sorghum accumulated Pb mainly in roots and only a small amount in fruits. Moreover, Memoli et al. (2017) [66], in the urban agglomeration of Ponticelli-Naples (Italy), detected twenty times more Pb in roots than in leaves.

3.1.6. Spinach

Since roots of spinach were very thin and difficult to separate from the soil, the effect of Pb levels in the soil on biomass production and accumulation in tissue were evaluated only in leaves and stems. The DW was negatively affected ($p < 0.05$) by the soil Pb concentration (Figure 6). In both years, the highest ($p < 0.05$) DW was measured in the control plants, and the DW of the treated plants decreased significantly ($p < 0.05$) as the soil Pb concentration increased. Similarly, Alia et al. (2015) [67] observed a significant reduction in the DW of spinach grown in the Pb spiked soil. In particular, those authors found that in a soil treated with 300 and 500 mg kg^{-1} Pb, shoot DW decreased by 2 and 8%, respectively, compared to the control. The Pb concentration detected in the leaves and stems showed an opposite trend in DW, being lower in the control plants and increasing significantly ($p < 0.05$) along with increased Pb concentration in the soil. In 2009, the Pb concentration detected in the treatment containing 1000 mg kg^{-1} was more than two times higher than that detected in the treatment containing 650 mg kg^{-1}. It has previously been established that spinach has a high capacity for the uptake and translocation of Pb from the soil to aerial tissues, and that the metal content in plants increases with the increasing level of Pb in soil [68–74]. Compared to other leafy vegetables, spinach was shown to possess the greatest ability to accumulate Pb in its tissues. More specifically, from the comparison between ten green leafy vegetables and herbs collected from five different soils in the Markazi province (Iran), spinach results the species with higher Pb tissue concentration [69]. Analogously, in a study conducted in the suburban area of Teheran (Iran) by Souri et al. (2018) [75], spinach showed the higher Pb concentration when compared to garden cress, coriander and lettuce. Spinach exhibited higher levels of Pb (5.97 µg g^{-1}) in leaves (4.37 µg g^{-1}), followed by the stems and roots (4.87 µg g^{-1}) when grown in soil contaminated with 17.5 µg g^{-1} Pb [73].

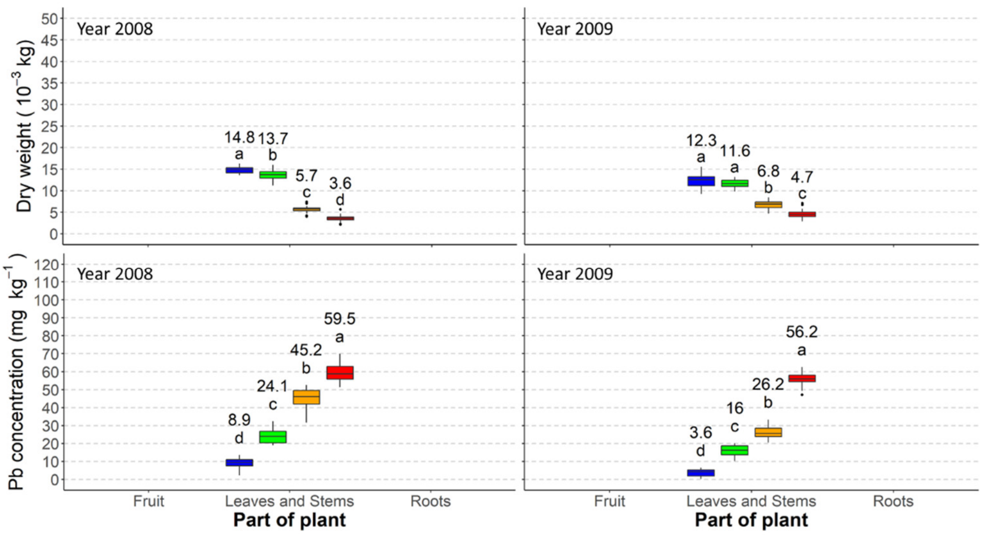

Figure 6. Boxplots of the dry weight (**top**) and Pb concentration (**bottom**) determined in different parts of spinach plants in 2008 (**left** panel) and 2009 (**right** panel) for 0 (blue box), 300 (green box), 650 (orange box), and 1000 mg kg^{-1} (red box) of Pb added to soil, respectively. Lowercase letters indicate different means between treatments ($p < 0.05$) according to the post hoc Tukey test.

3.1.7. Tomato

Inter-annual variability in biomass production for the different parts of the tomato plant was observed (Figure 7). In 2008, the DW of fruits was affected by treatment, with lowest production recorded at 1000 mg Pb kg^{-1} compared to the control. The DW of the leaves and stems were significantly higher only at 1000 mg Pb kg^{-1}. The DW of the roots was increased at 300 mg Pb kg^{-1} and decreased at 1000 mg kg^{-1}, respectively. In 2009, the DW of the fruits was increased at 650 mg Pb kg^{-1} compared to control. No differences were observed in the DW of leaves and stems, whereas the DW of roots was affected by all treatments, with minimum values recorded at 300 mg Pb kg^{-1} and 650 mg Pb kg^{-1}. Khan and Nazar Khan (1983) [76] reported that the application of 75 ppm Pb significantly promoted the growth of tomato plants in pots, while the application of 600 ppm resulted in decreased biomass. Likewise, Akinci et al. (2010) [77] found that tomato seedling shoot growth after treatment with 0, 75, 150, and 300 mg Pb L^{-1} was negatively affected by increasing Pb concentrations. Decreased tomato biomass as a result of increasing soil Pb was also observed previously, showing a significant ($p < 0.05$) reduction in the fruits, leaves, and roots from 450 mg Pb kg^{-1} upwards compared to the control treatment [78].

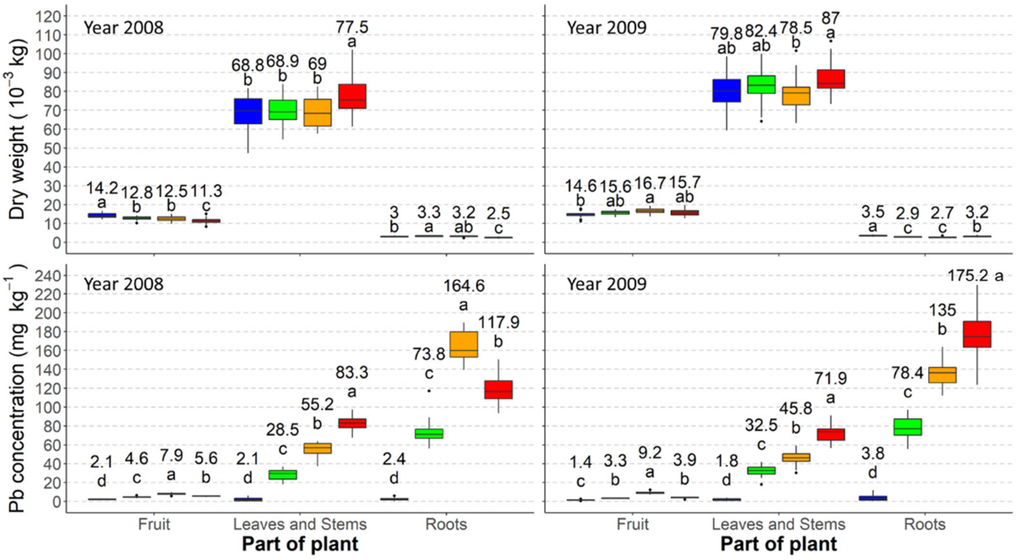

Figure 7. Boxplots of the dry weight (**top**) and Pb concentration (**bottom**) determined in different parts of tomato plants in 2008 (**left** panel) and 2009 (**right** panel) for 0 (dark-grey box), 300 (grey box), 650 (light-grey box), and 1000 mg kg^{-1} (white box) of Pb added to soil, respectively. Lowercase letters indicate different means between treatments ($p < 0.05$) according to the post hoc Tukey test.

In both years, the minimum and maximum Pb fruit concentrations were recorded in the control plants and plants treated with 650 mg Pb kg^{-1}, respectively. The Pb concentration in leaves and stems increased significantly along with the Pb concentration in the soil. In 2009, the same trend was observed in the roots, while in 2008, the maximum Pb concentration was recorded with the 650 mg kg^{-1} treatment, followed by the 1000 mg kg^{-1} and 300 mg kg^{-1} treatments, respectively. Moreover, tomato plants mainly contained Pb in the roots. The levels were approximately two times more than those in the leaves and stems and 20 times more than those contained in the fruit. Similarly, Finster et al. (2004) [51] found that roots of tomato grown in an urban community garden of Kansas City (MO, USA) contained 33 and 72 times more Pb than shoots and fruits, respectively. Maximum Pb concentration in the root tissues, as opposed to the aboveground tissues, was reported for tomatoes by many other authors [52,77–79].

3.2. Crop Bioconcentration Factor and Translocation Factor

Considering the entire period, all the tested crops showed BCF values lower than 1 in Pb spiked soils, but with values higher than 1 in control soils with the exception of tomato in the second year (Table 3). In treated plants, TF values higher than 1 were found for Indian mustard in 2009 at 650 mg kg^{-1} Pb (Table 3). In control plants, TF values lower than 1 were found for barley and Indian mustard in 2008 and for sorghum in both years. As none of the investigated species showed both BCF and TF values higher than 1, they cannot be considered suitable for phytoextraction of soil spiked with at least 300 mg Pb kg^{-1}. However, BCFr results seem to suggest the suitability of some plants for phyto-stabilization. The BCFr was higher than 1 in the control for all plants (Table 3). The BCFr values for barley and Indian mustard plants grown in contaminated soil was far less than 1, except for those grown in soils with 300 mg kg^{-1} Pb (mean values of 0.85 and 0.87, respectively). Castor bean and common bean plants showed BCFr values around 1 in all treatments where Pb was added to soils. The BCFr values for sorghum were just below 1 in soils contaminated with 650 and 1000 mg kg^{-1} Pb (mean values of 0.89 and 0.83, respectively), but higher than 1 (mean value of 1.85) in soil contaminated with 300 mg kg^{-1} Pb. The BCFr values for tomato were higher than 1 for all spiked soils except for soil spiked with 1000 mg kg^{-1} Pb in 2008.

Brunetti et al. (2012) [41] found both BCF and TF lower than 1 in barley. Physiological and biochemical mechanisms that limit the bioaccumulation and translocation from roots to shoots (BCF 0.35; TF ranging from 0.02 and 0.12) were shown to be activated in barley at 300 mg kg^{-1} Pb in soil [80]. Castor bean and common bean were previously shown to be low Pb accumulators and translocators [31,45,81]. BCF and TF values lower than 1 were found for Indian mustard [28] and sorghum [82], respectively. Pelfrêne et al. (2019) [83] reported that spinach and tomato had poor capacity in translocating metals from the roots to aerial tissues. TFs lower than 1 for spinach were found previously [69,71,84] while a TF < 1 was shown to occur in tomatoes [84]. For tomato, BCF values lower than 1 were reported [85]. In contrast, Meck et al. (2020) [86] found BCFs higher than 2 and 5 in tomato plants grown in granitic and greenstone substrates, respectively.

3.3. Human Health Risk Assessment via Consumption of Pb Contaminated Foodstuff

For all the studied species, the Pb concentration in the edible parts was above the maximum allowable safe limit (ML) imposed by the Codex Alimentarius [18], except for Indian mustard (Table 4).

Indian mustard revealed a Pb concentration below the ML at the control level in 2008. In the other treatments, Indian mustard revealed a Pb concentration in the order of 1.3–11.7 times above the ML, similar to that of common bean (1.2–11.9 times) and castor bean (2.1–12.38 times), respectively. The two species of cereal, barley, and sorghum accumulated a Pb concentration 3.4–30.3 and 8–27.2 times higher than the ML, respectively. Tomato was the species that mostly exceeded the safe limit (1.6–63.4 times). Although, a safe limit has not been set for spinach, our data revealed a Pb concentration 1.1–17.5 times higher than that set for leafy vegetables. These results highlighted that even in soils containing a Pb content below the dangerous threshold, plants can accumulate a Pb concentration in edible parts to a level well above the safe limit. Therefore, great attention must be paid, not only to the amount of Pb in the soil, but also to soil properties. Properties, including pH, nutrient and organic matter content and soil texture, increase the availability of this element in the soil and uptake by plants [26,29].

Table 3. Values of the bioconcentration factor (BCF), root bioconcentration factor (BCFr), and translocation factor (TF) calculated for different plants using different amounts of Pb in the soil. Lowercase letters indicate different means between treatments ($p < 0.05$) according to the post hoc Tukey test.

Plant	Lead Added to the Soil (mg kg^{-1})	BCF		BCFr		TF	
		2008	2009	2008	2009	2008	2009
Barley	0	1.11 ± 0.55 a	1.00 ± 0.42 a	2.17 ± 1.41 a	1.32 ± 1.2 a	0.5 ± 0.4 a	1.05 ± 1.39 a
	300	0.38 ± 0.06 b	0.45 ± 0.05 b	0.81 ± 0.13 b	0.9 ± 0.12 b	0.32 ± 0.1 b	0.36 ± 0.07 b
	650	0.3 ± 0.02 b	0.32 ± 0.03 c	0.55 ± 0.08 bc	0.7 ± 0.07 b	0.43 ± 0.07 a	0.38 ± 0.06 b
	1000	0.28 ± 0.02 b	0.25 ± 0.02 c	0.5 ± 0.06 c	0.42 ± 0.05 c	0.47 ± 0.08 a	0.49 ± 0.07 b
Castor bean	0	1.17 ± 0.68 a	1.97 ± 1.52 a	1.7 ± 1.2 a	1.04 ± 0.91 a	4.63 ± 12.21 a	11.23 ± 35 a
	300	0.43 ± 0.05 b	0.21 ± 0.07 c	1.04 ± 0.22 b	0.94 ± 0.19 b	0.36 ± 0.09 b	0.19 ± 0.06 b
	650	0.41 ± 0.04 b	0.57 ± 0.04 b	0.87 ± 0.13 b	0.76 ± 0.09 b	0.43 ± 0.07 b	0.71 ± 0.1 b
	1000	0.29 ± 0.02 b	0.37 ± 0.02 bc	0.99 ± 0.14 b	0.83 ± 0.08 b	0.26 ± 0.05 b	0.39 ± 0.04 b
Common bean	0	1.21 ± 0.49 a	1.05 ± 0.3 a	4.06 ± 2.91 a	1.08 ± 0.91 ab	1.31 ± 3.42 a	2.88 ± 3.65 a
	300	0.27 ± 0.03 b	0.2 ± 0.03 b	1.24 ± 0.25 b	1.19 ± 0.18 a	0.2 ± 0.06 b	0.13 ± 0.03 b
	650	0.18 ± 0.02 bc	0.16 ± 0.02 b	0.99 ± 0.13 b	0.9 ± 0.15 b	0.15 ± 0.02 b	0.15 ± 0.03 b
	1000	0.14 ± 0.02 c	0.18 ± 0.02 b	1.02 ± 0.12 b	0.9 ± 0.11 b	0.12 ± 0.02 b	0.17 ± 0.03 b
Indian mustard	0	1.99 ± 1.16 a	2.78 ± 1.08 a	4.91 ± 2.4 a	3.25 ± 1.56 a	0.46 ± 0.53 a	1.05 ± 0.91 a
	300	0.54 ± 0.08 b	0.48 ± 0.07 b	0.89 ± 0.11 b	0.85 ± 0.1 b	0.52 ± 0.11 b	0.53 ± 0.08 b
	650	0.44 ± 0.04 bc	0.37 ± 0.05 bc	0.45 ± 0.05 b	0.36 ± 0.03 c	0.92 ± 0.17 a	1.01 ± 0.17 a
	1000	0.23 ± 0.03 c	0.21 ± 0.02 c	0.44 ± 0.04 b	0.33 ± 0.03 c	0.46 ± 0.06 b	0.61 ± 0.07 b
Sorghum	0	2.32 ± 0.52 a	3.33 ± 1.47 a	12.01 ± 3.83 a	7.88 ± 3.88 a	0.08 ± 0.05 c	0.14 ± 0.19 bc
	300	0.42 ± 0.07 b	0.62 ± 0.1 b	1.7 ± 0.19 b	2 ± 0.25 b	0.06 ± 0.02 c	0.11 ± 0.02 c
	650	0.25 ± 0.04 c	0.33 ± 0.04 b	0.95 ± 0.1 bc	0.84 ± 0.1 c	0.1 ± 0.01 b	0.17 ± 0.04 b
	1000	0.25 ± 0.03 c	0.43 ± 0.05 b	0.79 ± 0.06 c	0.88 ± 0.11 c	0.23 ± 0.04 a	0.27 ± 0.05 a
Spinach	0	4.2 ± 1.47 a	1.7 ± 0.9 a	0	0	0	0
	300	0.46 ± 0.08 b	0.31 ± 0.06 b	0	0	0	0
	650	0.36 ± 0.04 b	0.21 ± 0.03 b	0	0	0	0
	1000	0.33 ± 0.03 b	0.31 ± 0.02 b	0	0	0	0
Tomato	0	1.03 ± 0.74 a	0.88 ± 0.43 a	1.13 ± 0.67 a	1.79 ± 1.59 a	5.01 ± 16.78 a	1.2 ± 1.53 a
	300	0.53 ± 0.09 b	0.58 ± 0.1 b	1.41 ± 0.25 a	1.5 ± 0.22 a	0.35 ± 0.09 b	0.36 ± 0.09 b
	650	0.44 ± 0.05 b	0.35 ± 0.05 c	1.33 ± 0.13 a	1.09 ± 0.11 b	0.29 ± 0.05 b	0.29 ± 0.06 b
	1000	0.43 ± 0.04 c	0.37 ± 0.04 c	0.66 ± 0.08 b	1.08 ± 0.13 b	0.63 ± 0.1 b	0.36 ± 0.06 b

Table 4. Pb concentration expressed on a fresh weight (f.w.) basis in the edible parts of the seven field crops. The maximum allowable safe limit (ML) is reported (FAO/WHO, 2018).

Crop	Edible Part	Water Content	ML	2008				2009			
		%	mg kg^{-1} f.w.	0	300	650	1000	0	300	650	1000
Barley	fruits	15.0	0.20	0.68	1.02	2.65	2.21	1.02	2.65	2.38	2.21
Castor bean	fruits	92.2	0.08	0.17	0.27	0.37	0.41	0.12	0.27	0.66	0.99
Common bean	fruits	89.7	0.10	0.12	0.21	0.36	0.55	1.19	0.26	0.63	0.69
Indian mustard	leaves	93.0	0.30	0.24	1.67	3.51	2.55	0.39	1.64	3.12	2.49
Sorghum	fruits	11.0	0.20	2.40	2.58	3.38	6.14	1.60	3.47	3.47	5.43
Spinach	leaves	91.2	-	0.78	2.12	3.98	5.24	0.32	1.41	2.31	4.95
Tomato	fruits	94.4	0.05	0.12	0.26	0.44	3.17	0.08	0.18	0.52	0.22

The present results were corroborated by Brunetti et al. (2012) [41], who found Pb concentration ranging from about 1 to 9 mg kg^{-1} f.w.) in barley samples collected in the Apulia Region (South Italy). Additional research reported that 10 out of 40 barley grain samples collected in Scotland in 2000 exceeded the ML, reaching a value of 0.48 mg kg^{-1}, whereas previously in 1998 > 99% of samples collected from throughout Britain were below the ML [87]. Pb levels above the limit were detected in all tested vegetables harvested near a Pb mining area in Zimbabwe, including spinach, tomato, and common bean [86]. In particular, these authors found a concentration of Pb in the order of 1400 times and 360 times above the ML in tomato fruits cultivated in greenstone (69.96 mg kg^{-1}) and granitic (18.09 mg kg^{-1}) soils, respectively. Furthermore, Gan et al. (2017) [88] reported that leafy, fruiting, and root vegetables grown in peri-urban Pb contaminated soil contained much more Pb than those cultivated in rural areas and exceeded the permissible limit set by the Chinese government and also by FAO/WHO. Additionally, Inoiti et al. (2012) [89], in urban soils with a Pb concentration ranging from 57.7 to 693.3 mg kg^{-1}, found that Pb in spinach and tomato varied from 13.3 to 29.5 mg kg^{-1} and 3.5 to 20.5 mg kg^{-1}, respectively. Similar results were found also for tomato and spinach grown in peri-urban soil with a Pb concentration ranging from 2.11 to 30.86 mg kg^{-1} [90]. In a 28.8 mg Pb kg^{-1} spiked soil, 1.89 mg kg^{-1} Pb was detected in tomato, whereas in a 36.0 mg Pb kg^{-1} spiked soil, 1.72 mg kg^{-1} Pb was reported for common bean [91]. On the contrary with our results, sorghum was reported to be safe for direct consumption since it accumulates low levels of Pb in aerial parts [64]. To the best of our knowledge, the present study is the first to evaluate Pb concentration in edible parts of castor bean and Indian mustard in relation to FAO/WHO limits for the corresponding foodstuffs.

4. Conclusions

This study was conducted to investigate the plant growth and the removal and storage capacity of Pb in different parts of seven herbaceous crops normally grown in urban and peri-urban areas around the world. Results showed that all the investigated species are able to grow in high Pb spiked soils and that their phyto-stabilization capability depends both on the crop species and soil Pb concentration. This study also showed that Pb uptake and translocation capacity varies within crops and within plant tissues. Between the investigated species, sorghum was found to accumulate more Pb than the other species at lower Pb levels, whereas both tomato and common bean were the highest accumulators at 650 and 1000 mg Pb kg^{-1}, respectively. The knowledge of how individual crops respond in Pb polluted soils is also essential in order to evaluate the potential health hazard due to their consumption. Our results showed that, despite roots being the predominant Pb plant storage parts, the edible tissue showed a Pb concentration higher than the safe limit set by FAO/WHO even when grown in soils with low Pb concentrations. This result is of great importance from a public health point of view, as human health is directly affected by the consumption of foodstuff. To conclude, we suggest a site-specific risk assessment, as well as the adoption of good agricultural practices. The latter include the addition of organic matter to bind Pb in the soil, and a reduced use of both pesticides and wastewater, in order to permit a safer cultivation.

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