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# Accumulation Ability of Three Heavy Metals in Two Legumes (Bean and Faba Bean) in Vegetative Stage at Different Concentrations

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**Abstract:** Some vegetable crops irrigated with wastewater are highly contaminated with heavy metals and are the main source of human exposure to the contaminants.

In this study accumulation of three heavy metals (Pb, Cu and Cd) in two locale varieties of Vicia faba in vegetative stage, irrigated with two different concentrations of each metal are studied using Atomic Absorption Spectrophotometer. For these heavy metals, the highest concentration was used according to WHO/FAO. For lead, lead nitrate was used at the highest and lowest concentration (6 mg/l and 3 mg/l, respectively), while for copper, we used copper nitrate, the two concentrations were 0.3 and 0.15 mg/l for the highest and the lowest concentration respectively. For cadmium, cadmium acetate was used with 0.03 and 0.01 mg/l for the highest and the lowest concentration respectively. Plants irrigated with tape water are the control.

The most heavily contaminated parts for these two varieties were roots; the accumulation of Cu was 15.56 mg/kg of dray matter (DM) and 15.35 mg/ kg of DM in shoots respectively in bean and faba bean for lower concentration. Heavy metals concentrations were lower in shoot parts than in roots and lower than permissible limits of WHO/FAO either in bean and faba bean.

The concentration of Cu in vegetable parts was the highest, where the accumulation was respectively of 14.48 mg/ kg DM and 16.54 mg/kg DM in bean and faba bean for lower and higher concentration respectively. The present study provides the evidence that the local variety of faba bean could accumulate and tolerate higher concentrations of lead and cadmium in vegetative stage better than the locale variety of bean which accumulate and tolerate higher concentration of copper which is less danger than Pb and Cd which can affect human health and environment.

**Key words:** heavy metals, vegetative stage, different concentrations, leguminous, phytoremediation, tolerance, Tunisia

## 1. Introduction

Vegetables comprise an important component of human diet since they contain carbohydrates, proteins, as well as vitamins, minerals and trace elements [1]. It represents one of the first colonizers of poor or degraded soils, with distinct varieties adapted to very different environments and they have been successfully employed for the re-vegetation and restoration of arid and degraded ecosystems [2]. It was known that legumes have an important capacity to up take atmospheric nitrogen thanks to the aptitude of symbiotic relation with soil bacteria. In the other hand, vegetables take up heavy metals and accumulate them in their edible parts [3] and inedible parts in quantities high enough to cause several clinical and physiological problems both to animals and human beings consuming these metal rich plants [1], especially leafy vegetable irrigated with waste water are the most contaminated with heavy metal. They are of great interest for the phytoremediation of soils contaminated with heavy metals and they are promising candidates

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for the successful re-vegetation and stabilization ecosystems degraded by mining activities [4]. It was reported that bioaccumulation of heavy metals in vegetables are influenced by many factors, climate, atmospheric depositions, the concentrations of heavy metals in soil, the nature of soil on which the vegetables are grown and the degree of maturity of the plants at the time of harvest [5], also anthropogenic activities, such as pesticide and herbicide application, mining, or irrigation with wastewater have significantly enhanced heavy metal levels in soils in many areas in the world, which have imposed adverse environmental problems [6]. The concentrations of heavy metals in the plant vary with the type of plant to classify the species [6]. Their ability to grow in marginal soils is often attributed to the symbiotic associations they established with nitrogen–fixing rhizobia and indeed, some legumes tolerate extreme environmental conditions of salinity, drought or high temperature. It was reported that leguminous are not considered as plants accumulate heavy metals, even if they have an aptitude to reduce the negative effects of many heavy metals in different types of soil, Rashed and Awadallah [7] reported that Fe, Sr, Mn, Ni, Co, Pb and Cr accumulate in faba bean leaves, Fe in pods, while Zn and Cu accumulate in the seed. In this context, leguminous plants can be used as pioneer species.

The aim of this study is to evaluate the accumulation ability of shoots and roots of two leguminous in vegetative stage and know morphological changes since legumes are considered as mediators plants.

## 2. Material and Method

### *2.1 Growing Conditions*

The experiment was carried in a greenhouse at the experimental station of High Agronomic Institute of Chott Mariem (Sousse, Tunisia). Bean (Vicia faba L. cv. Mamdouh) and faba bean (Vicia faba L. cv. Badii) seeds were rinsed in water for 24 hour then were sown into pots ( one seed per pot) containing fresh earth and commercial peat to increase organic matter. During germination and seedlings development, tape water was used for irrigation according needs plants, in pots, soil was added to facilitate a good drainage and prevent water logging.

### *2.2 Treatments Applied and Experimental Design*

Peat used for the experiment was manually mixed and homogenized. Three weeks after, the sowing started treatment, for each metal, the highest concentration according to WHO/FAO, were 6, 0.3 and 0.03 mg/l for lead nitrate, copper nitrate and cadmium acetate respectively, while the lowest concentration were 3, 0.1 and 0.01 mg/l for lead nitrate, copper nitrate and cadmium acetate and treatment with tape water served as control. During the experiment, plants were irrigated with nutrient solution twice a week. Split-split-plot experimental design with three blocks was applied and pots were randomly moved daily to minimize position effects.

### *2.3 Samples Collection*

At the end of the experiment, roots and shoots were collected for vegetative fresh weight and dry weight analysis using ICP-MS analysis. To avoid extra contamination from metals, careful procedures of separation and drying were carried out; roots were cleaned according to the procedure of Wang et al. [6]. Shoots and roots were dried at 60° and were ground using a mortar and stored at ambient temperature in flask for analysis.

### *2.4 Analysis of Heavy Metals Using ICP- MS*

The analysis of samples was carried out by using inductively coupled plasma Mass.

Spectrophotometer (ICP-MS) instrument to analyze levels of various heavy metals (Pb, Cu and Cd) and six point calibration curves were used as a blank. The sample introduced into the ICP-MS in a liquid form, was pumped into the sample introduction system, which was made up of a spray chamber and a nebulizer. The plasma contains different heating zones where the sample was successively dried, vaporized, atomized and ionized, during this time, the sample was transformed from a liquid aerosol of solid particles, then, into a gas contains the highest population of excited atoms and monotonic positively charged ions, representing the elemental composition of the sample. The highly efficient ion extraction through the mass spectrometer and detection was what gives to ICP-MS its ultra-trace elemental detection feature.

### *2.5 Statistical Analysis*

The experiment was arranged as a randomized split-split plot design with variety as the main plot factor and heavy metals as the second factor (sub-plot). Different concentrations represent the smallest experimental unit (sub-sub plot). Data analysis was performed using SAS v. 6.0; one way analysis of variance (ANOVA) was used to separate the means which were compared by Duncan's multiple range test (DMRT) at P = 0.01.

## 3. Results

### *3.1 Simple Effect of Species, Heavy Metals and Concentrations on Biometric Parameters*

Simple effect of species and heavy metals showed a significant difference ($p < 0.01$) on biometric parameters. The local variety of bean, showed an increase on growth parameters compared to the local variety of faba bean (*Badii*) (Table 1). For heavy metals, an increase on plant height was observed with lead and cadmium with a value of the order of (40.31 and 41.68 Cm respectively compared to lead. Concerning the vegetative fresh weight, an increase was observed with cadmium followed by copper and lead. For the two local variety of *Vicia faba*, root fresh weight showed an increase with cadmium with a value of the order of 4.30 g followed by lead and copper with a value of the order of 3.03 and 2.77 g respectively. Vegetative dry matter, showed a gradual increase going from cadmium to lead. Root dry weight showed an increase with cadmium compared to copper and lead (Table 1).

**Table 1 Means effect of species and heavy metals on plant high (PH), vegetative fresh weight (VFW), root fresh weight (RFW), vegetative dry weight (VDW) and root dry weight (RDW).**

| | PH | VFW | RFW | VDW | RDW |
|---|---|---|---|---|---|
| Bean | 45.56a | 15.69a | 5.04a | 2.07a | 0.53a |
| Faba bean | 33.78b | 7.59b | 1.69b | 1.44b | 0.19b |
| Pb | 40.31a | 9.79b | 3.03b | 1.18c | 0.21b |
| Cu | 37.02b | 10.79b | 2.77b | 1.73b | 0.41a |
| Cd | 41.68a | 14.34a | 4.30a | 2.35a | 0.45a |

Values presented are means of three replicates with standard deviation. Values are significant different at $P < 0.01$

Statistical analysis of simple effect of concentrations on plant height, vegetative fresh weight and vegetative dry weight did not show a significant difference, but showed a significant difference at ($p < 0.01$) for root fresh weight and root dry weight. The highest value of root fresh weight was accorded to lower concentration with a value of the order of 3.73 g, whereas, for the highest value of root dry weight was to the highest concentration (Table 2).

**Table 2 Means effect of concentrations on plant high (PH), vegetative fresh weight (VFW), root fresh weight (RFW), vegetative dry weight (VDW) and root dry weight (RDW).**

| | PH | VFW | RFW | VDW | RDW |
|---|---|---|---|---|---|
| Control | n. s | n. s | 3.05b | n. s | 0.30b |
| High concentration | n. s | n. s | 3.32ab | n. s | 0.41a |
| Low concentration | n. s | n. s | 3.73a | n. s | 0.37ab |

Values presented are means of three replicates with standard deviation. Values are significant different at $P < 0.01$, n.s: non-significant.

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### *3.2 Effects of Interaction (Heavy Metals* Concentrations *Species) on Biometric Parameters*

The local variety of bean and the local variety of Faba bean showed high tolerance to Pb, Cu and Cd stress for different concentrations without suffering from obvious phytotoxicity symptoms during the experimental period. However, there was difference in appearance among treatments for the two concentrations of these three heavy metals and the control. Data regarding plant height is represented in Fig. 1. For Mamdouh light increase in plant height was observed with the highest concentration of copper (0.3 mg/l (Cu(NO3)2) compared to control plants and plants treated with the lowest concentration of copper, whereas light decrease in plant height was observed with the highest concentration of lead (6 mg/l (Pb(NO3)2) and the highest concentration of cadmium (0.03 mg/l (CH3CO2)2Cd) compared to control plants. For Faba bean, Badii, showed light increase of 0.89%, 2% and 11% in plant height with all the highest concentration of lead, copper and cadmium respectively compared to control plants.

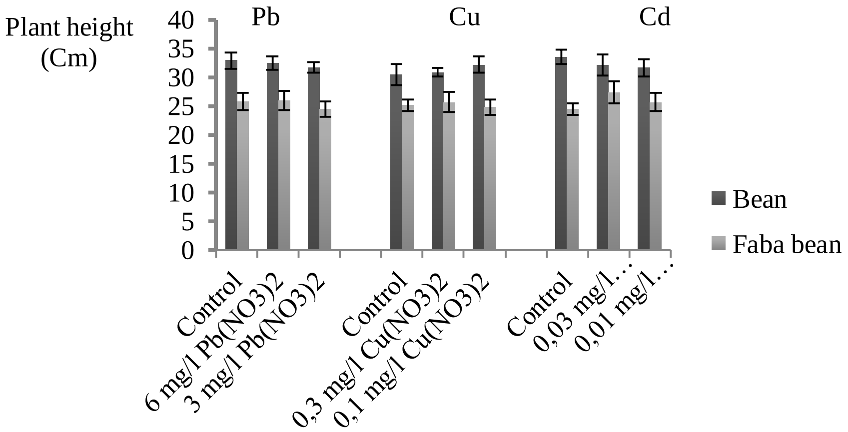


**Fig. 1 Effect of various Pb (6 and 3 Mg/ of Pb(NO3)2), Cu (0.3 and 0.1 Mg/L Of Cu(No3)2) and Cd (0.03 and 0.01 Mg/L of (CH3CO2)2Cd) treatments on plant height of bean and faba bean. Values are expressed as means of three replicates with standard deviations. Values are significant different at P < 0.01.**

For bean, vegetative fresh weight increased with metal level of 0.3 mg /l (Cu(NO3)2), and with metal level of 0.1 mg/l of (Cu(NO3)2) compared to control plants. For lead and cadmium, vegetative fresh weight decreased with the lowest concentration of Pb(NO3)2 and (CH3CO2)2Cd respectively compared to control plants (Fig. 2A). For Faba bean, vegetative fresh weight of Badii, increased at higher metal level of lead and cadmium and showed light decrease at higher metal level of copper compared to control plants (Fig. 2A). Root fresh weight significantly increased with the lowest concentration of lead and cadmium (3mg/l Pb (NO3)2 and (0.01 mg/l (CH3CO2)2Cd) respectively as compared to control plants for Bean. For copper, Mamdouh (Bean), showed a gradual decrease from control plants to plants treated with low concentration (0.1 mg/l Cu(NO3)2). The local variety of faba bean (Badii), showed a significant increase of the root fresh weight with metal levels of 6 mg/l of (Pb(NO3)2) and 0.03 mg/l of (CH3CO2)2Cd compared to control plants, however with metal level of copper (0.3 mg/l Cu(NO3)2), Badii showed a decrease of root fresh weight as compared to control plants (Fig. 2B). By comparing the two concentrations, the local variety of bean (Mamdouh) showed a significant increase of root fresh weight with low concentrations of lead and cadmium compared to high concentrations, whereas, with copper, Mamdouh showed a significant increase of root fresh weight with high concentration of Cu(NO3)2 compared to low concentration of Cu(NO3)2. For local variety of faba bean, Badii showed a significant increase of root fresh weight with high concentration of lead only, but with copper, Badii showed a decrease with metal level of 0.3 mg/l of Cu(NO3)2 compared to 0.1 mg/l of Cu(NO3)2. For cadmium, the local variety of faba bean showed no difference between high and low concentrations (Fig. 2B) for root fresh weight. By comparing the two varieties of bean and faba bean, Badii was more influenced than Mamdouh on root fresh weight (Fig. 2B). At lower and higher levels of lead (6 mg/l of Pb(NO3)2 and 3 mg/l of Pb(NO3)2), the local variety of bean showed a decrease of vegetative dry weight compared to control plants (Fig. 2C). At lower metal stress of lead (3 mg/l of Pb(NO3)2), vegetative dry weight of Mamdouh decreased compared to higher metal stress of lead (6 mg/l of Pb(NO3)2). For copper, Mamdouh, showed an increase of vegetative dry weight for the highest concentration of copper (Cu(NO3)2) compared to control plants (Fig. 2C), however at lower concentration of copper (0.1 mg/l of Cu(NO3)2), there was a significant decrease of vegetative dry weight compared to high concentration of copper. By comparing the two concentrations of copper, vegetative dry weight was more influenced at lower concentration (0.1 mg/l of Cu (NO3)2) (Fig. 2C). The evaluation of vegetative dry weight of local variety of bean at different concentrations of cadmium also indicated that at lower concentration, vegetative dry weight showed a significant increase compared to control plants and to plants treated with high concentration of cadmium (0.03 mg/l of (CH3CO2)2Cd) (Fig. 2C). Concerning the local variety

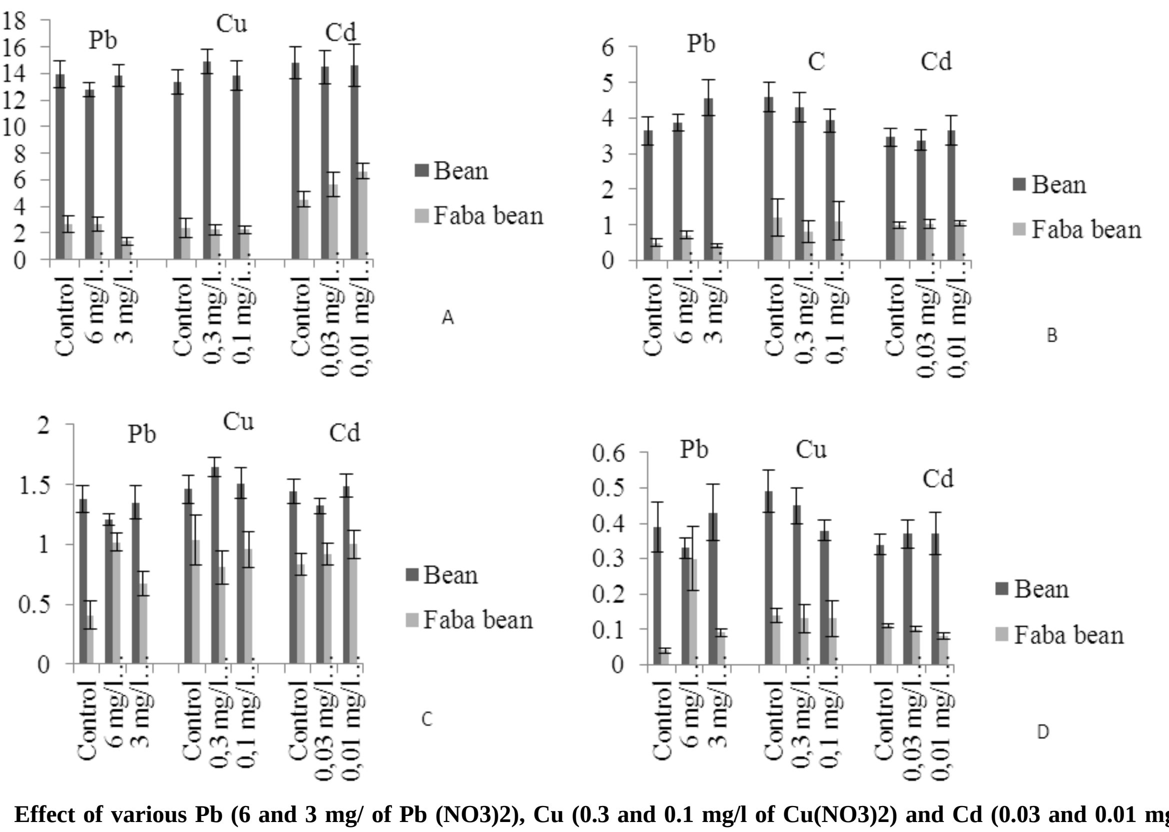


**Fig. 2 Effect of various Pb (6 and 3 mg/ of Pb (NO3)2), Cu (0.3 and 0.1 mg/l of Cu(NO3)2) and Cd (0.03 and 0.01 mg/l of (CH3CO2)2Cd) treatments on vegetative fresh weight (A), root fresh weight (B), vegetative fresh weight (C) and root dry weight (D) of bean and faba bean. Values are expressed as means of three replicates with standard deviations. Values are significant different at P < 0.01.**

of faba bean, Badii showed an increase of vegetative dry weight at higher concentration of lead (6 mg/l of Pb(NO3)2) compared to plants treated with lower concentration (3 mg/l of Pb(NO3)2 and control plants, however for copper, faba bean showed an increase of vegetative dry weight at lower concentration (0.1 mg/l of Cu(NO3)2) compared to plants treated with high concentration of copper (0.3 mg/l of Cu(NO3)2) and control plants (Fig. 2C). Gradual increase in vegetative dry weight was observed with increasing both concentration of cadmium. Overall, vegetative dry weight was higher in bean than in faba bean in metal stress and in untreated plants (Fig. 2C). The evaluation of root dry weight indicated that the local variety of bean had the highest value at lower concentration of lead (3 mg/l of Pb(NO3)2), while for faba bean the highest value was accorded to the highest concentration of lead (6 mg/l of Pb(NO3)2) compared to control plants (Fig. 2D). Gradual increase in root dry weight was observed with decreasing metal stress of copper Mamdouh (local variety of bean), while for faba bean, Badii showed light increase of control plants compared to plants treated with the highest and the lowest concentration of copper (0.3 mg/l of Cu(NO3)2 and 0.1 mg/l of Cu(NO3)2 respectively). For cadmium, Mamdouh showed a significant increase of root fresh weight for plants treated with high and low concentration of cadmium (0.03 mg/l of (CH3CO2)2Cd and 0.01 mg/l of (CH3CO2)2Cd respectively) compared to control plants, while for faba bean, Badii, showed gradual increase of root dry weight with increasing metal stress (Fig. 2D).

### *3.3 Metal Uptake and Accumulation*

#### 3.3.1 Simple Effect of Heavy Metals and Species on Metal Uptake

Statistical analysis of means effect of species and heavy metals showed a significant difference in root and shoot concentrations (Table 3). For the local variety of bean, root concentrations were higher than shoot concentrations similar to the local variety of faba bean. Comparing the two leguminous, the local variety of bean had the highest concentration in roots, while the local variety of faba bean had the highest concentration in shoots. Concerning heavy metals, statistical analysis showed that copper was the first element accumulated in both roots and shoots followed by lead and cadmium.

Means effect of concentrations on root concentrations and shoot concentrations are shown in Table 4. Statistical analysis showed that roots of bean and faba bean accumulated the highest concentration followed by the lowest one, while shoots accumulated the lowest concentration followed by the highest one.

**Table 3 Means effect of species and heavy metals on root concentrations (RC) and shoot concentrations (Sc).**

| | RC | SC |
|---|---|---|
| Bean | 6.26a | 3.79b |
| Faba bean | 6.06b | 4.86a |
| Pb | 5.79b | 1.94b |
| Cu | 11.82a | 10.76a |
| Cd | 0.89c | 0.54c |

Values presented are means of three replicates with standard deviation. Values are significant different at P < 0.01.

**Table 4 Means Effect of concentrations on root concentrations (RC) and shoot concentrations (SC).**

| | RC | SC |
|---|---|---|
| Control | 3.55c | 1.44c |
| High concentration | 8.04a | 5.38b |

| | | |
|---|---|---|
| Low concentration | 6.90b | 6.43a |

Values presented are means of three replicates with standard deviation. Values are significant different at P < 0.01.

Lead, copper and cadmium concentrations in different plant parts of bean are shown in Table 5. For Pb and Cd, both concentrations were higher in roots followed by stem and leaves, while for Cu, and for the highest concentration (0.3 mg/l of Cu (NO3)2, concentrations were higher in stem and leaves followed by roots. Plants treated with the highest concentration of lead (6 mg/l of Pb(NO3)2) increased Pb uptake by 2.7 and 1.08 times in roots and 0.82 times in shoots as compared to plants treated with the lowest concentration of lead (3 mg/l of Pb(NO3)2) and control plant respectively. Plants treated with the highest concentration of copper (0.3 mg/l of Cu(NO3)2) increased Cu uptake by 1.35 and 0.90 times in roots and 3.51 and 1.02 times in shoots as compared to plants treated with the lowest concentration of copper (0.1 mg/l of Cu(NO3)2) and control plants. Plants treated with the highest concentration of cadmium (0.03 mg/l of (CH3CO2)2Cd), increased Cd uptake by 1.44 and 1.20 times in roots and 1.61 times in shoots as compared to plants treated with the lowest concentration of cadmium (0.01 mg/l of (CH3CO2)2Cd) and control plants.

**Table 5 Effect of different concentration of Pb (6 and 3 Mg/ of Pb(NO3)2), Cu (0.3 and 0.1 Mg/L of Cu(No3)2) and Cd (0.03 and 0.01 Mg/L of (CH3CO2)2Cd) on Pb, Cu and Cd concentrations and accumulations/uptake by local variety of bean (mamdouh) in vegetative stage.**

| Treatment | Concentration | Root tissue concentration | Leaf and stem tissue concentration |
|---|---|---|---|
| Lead | Control | 1.70 ± 0.28 | 0.00 ± 0.00 |
| | High concentration | 4.59 ± 0.18 | 1.30 ± 0.30 |
| | Low concentration | 4.24 ± 0.38 | 1.58 ± 0.58 |
| Copper | Control | 10.33 ±1.95 | 4.12 ± 1.63 |
| | High concentration | 14.02 ± 0.16 | 14.48 ± 0.14 |
| | Low concentration | 15.56 ±0.21 | 14.12 ± 0.18 |
| Cadmium | Control | 0.65 ± 0.21 | 0.00 ± 0.00 |
| | High concentration | 0.94 ± 0.05 | 0.92 ± 0.09 |
| | Low concentration | 0.78 ± 0.08 | 0.57 ± 0.09 |

Values presented are means of three replicates with standard deviation. Values are significant different at P < 0.01.

**Table 6 Effect of different concentration of Pb (6 and 3 Mg/L of Pb(No3)2), Cu (0.3 and 0.1 Mg/L of Cu(No3)2) and Cd(0.03 and 0.01 Mg/L of (CH3CO2)2Cd) on Pb, Cu and Cd concentrations and accumulations/uptake by local variety of faba bean (badii) in vegetative stage.**

| Treatment | Concentration | Root tissue concentration | Leaf and stem tissue concentration |
|---|---|---|---|
| Lead | Control | 2.43 ± 0.00 | 0.00 ± 0.00 |
| | High concentration | 3.66 ± 0.47 | 0.98 ± 0.10 |
| | Low concentration | 2.45 ± 0.52 | 1.08 ± 0.63 |
| Copper | Control | 6.05 ± 0.06 | 3.99 ± 0.19 |
| | High concentration | 14.82 ± 0.32 | 13.05 ± 0.19 |
| | Low concentration | 15.35 ± 1.42 | 16.54 ± 0.16 |
| Cadmium | Control | 1.18 ± 0.13 | 0.00 ± 0.00 |
| | High concentration | 1.29 ± 0.13 | 0.91 ± 0.06 |
| | Low concentration | 1.01 ± 0.02 | 0.71 ± 0.11 |

Values presented are means of three replicates with standard deviation. Values are significant different at P < 0.01.

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The highest concentration of cadmium (0.03 mg/l of ( $CH_3CO_2)_2Cd$), increase Cd uptake by 1.09 and 1.27 times in roots and 1.28 times in shoots as compared to plants treated with the lowest concentration (0.01 mg/l of ($CH_3CO_2)_2Cd$) and control plants Overall, the local variety of bean (Mamdouh), accumulated more Pb and Cd in roots than the local variety of faba bean (Badii), whereas, Badii, accumulated more Cu in roots than Mamdouh. In the other hand, Mamdouh accumulated more Cu and Cd in shoots and stem than Badii, while Mamdouh accumulated more Pb in shoots and stem than Badii.

Translocation factor were represented in Table 7. Lead was a metal which was uniform distributed in shoots and stem because the value of TFPb is less than 1 (0.23). The highest value of translocation factor for lead was accorded to the lowest concentration (3 mg/l of $Pb(NO_3)_2$) for the local variety of bean. The translocation factor increased with increasing metal levels of copper and cadmium for the local variety of bean. For Badii, the translocation factor decreased with increasing metal levels of copper and lead, while for cadmium, translocation factor presented fixed value (0.70). Translocation factor was slightly higher in plants treated with the highest concentration of copper (0.3 mg/l of $Cu(NO_3)_2$) for the local variety of bean and was slightly higher in plants treated with the lowest concentration of copper (0.01 mg/l of $Cu(NO_3)_2$) for the local variety of faba bean, and this higher translocation factor indicated that this two local varieties of bean and faba bean are hyper-accumulator of copper for both concentrations in vegetative stage. Higher translocation factor of bean for the highest concentration of cadmium (0.03 mg/l of ($CH_3CO_2)_2Cd$) and for the lowest concentration of copper ($Cu(NO_3)_2$) (0.97 and 0.90 respectively), indicated also that the local variety of bean (Mamdouh) is a hyper-accumulator plant for Cd and Cu in vegetative stage.

**Table 7 Translocation factor (TF) of Pb, Cu and Cd at different concentrations from root to stem and leaves of two local varieties of bean and faba bean in vegetative stage.**

| Species | Translocation factor (TF) | | | | | |
|---|---|---|---|---|---|---|
| | $TF_{Pb\,C1}$ | $TF_{Pb\,C2}$ | $TF_{Cu\,C1}$ | $TF_{Cu\,C2}$ | $TF_{Cd\,C1}$ | $TF_{Cd\,C2}$ |
| Bean | 0.23 | 0.36 | 1.02 | 0.90 | 0.97 | 0.74 |
| Faba bean | 0.28 | 0.46 | 0.87 | 1.07 | 0.70 | 0.70 |

## 4. Discussion

### *4.1 Effects of Heavy Metals on Plant Growth and Biomass*

To evaluate the potential of two local varieties of bean and faba bean in vegetative stage for phytoremediation of lead, copper and cadmium, Mamdouh and Badii growth was studied under different concentrations of Pb, Cu and Cd. For Mamdouh light increase in plant height was observed with the highest concentration of copper (0.3 mg/l ($Cu(NO_3)_2$), whereas light decrease in plant height was observed with the highest concentration of lead (6 mg/l ($Pb(NO_3)_2$) and the highest concentration of cadmium (0.03 mg/l ($CH_3CO_2)_2Cd$). For Faba bean, Badii, showed light increase in plant height with all the highest concentration of lead, copper and cadmium. This result was later approved by Lui et al. [8], who reported increases in growth and biomass at the Cd level of 30 mg/kg of soil in ornamental plants, Calendula officinal is and Althaea rosea, while growth decreased with higher Cd levels. Also, Hafiz et al. [9], reported that plant growth characteristics and biomass gradually increased under lower metal stress (0.5 and 1.0 mM) as compared to control while decreased under higher metal stress (2 mM). In another study, Amna et al. [10] reported that growth and biomass of Linum usitatissimum gradually decreased with increasing Cd levels in the soil. Increasing addition of Cd in soils enhanced Cd concentration in plants, resulting in decreased plant growth and cell ultra-structural changes in both roots and leaves [11]. In the other hand, Copper is an essential element in plant growth, but excessively high levels of Cu in the substrate can also hamper plant growth [12]. Results obtained from Cd treatment of local variety of bean plants in vegetative stage confirm that Cd affects plant growth, as already reported among others by A. L. Page et al. [13]

*4.2 Metal Uptake and Accumulation*

Results from the metal analysis of bean and faba bean showed that the local variety of bean (Mamdouh), accumulated more Pb and Cd in roots than the local variety of faba bean (Badii), whereas, Badii, accumulated more Cu in roots than Mamdouh. In the other hand, Mamdouh accumulated more Cu and Cd in shoots and stem than Badii, while Mamdouh accumulated more Pb in shoots and stem than Badii (Table 1 and Table 2). Kadukova et al. [14] reported that Tamarix smyrnensis Bunge a salt tolerant species, showed very high tolerance to Cd up to 30 ppm and Pb. Many authors showed that lead was tightly retained by root tissues, these accumulation data clearly demonstrate that Faba bean has an interesting potential, as an efficient lead phytoextarcting species in rhizofiltration setups [15]. The distribution of Cd in plant tissues showed that the total Cd of roots exceeded by about one and two orders of magnitude Cd concentrations in stems and leaves respectively. The roots of bean accumulated higher Pb, Cu and Cd at different concentrations except high concentration of copper than the shoots, and the roots of faba bean accumulated higher Pb, Cu and Cd at different concentrations except low concentration of copper than the shoots because they are the first organ that contacts the metal. This result was later confirmed by Voutsa et al. [16] who reported heavy metals may be a consequence of the preferential accumulation in roots. Plants have developed two strategies of metal tolerance: exclusion and accumulation, and sequestration in vacuoles and complexation by organic ligands [17]. Up to a certain critical limit of metal concentration, some mechanisms of plant uptake regulation take place regarding metal contamination. This was attested by the concentrations, which generally decreased from roots to leaves, and with increasing level of substrate contamination. Nevertheless, metal concentration in plant depends not only on substrate metal concentration level, but also on metal type. The mobility of mineral elements is a limiting factor, and plants exude chelators (external) to mobilize these elements and make them available. This natural system is phytoremediation, including phyto-accumulation and rhizofiltration by the addition of chemical compounds [18]. In the case where the metallique iron enter free in the plant, it is supported by molecules similar to internal chelators, at the entrance of Pb in free iron form in the plant, it is supported by chelators produced by the plant such as glutathione, organic acids and amino acids [19]. Overall, Pb, Cu and Cd were particularly concentrated in roots. Anne et al. [20] reported that metal accumulation is in the order of stem<leaf<root, except for Pb and Cd leaf<stem<root, whereas in our results the order of metal accumulation at different concentrations is in the order of leaf < root for both concentration of Pb and Cd and the lowest concentration of Cu for the local variety of bean (Table 1). For the local variety of faba bean, the order of metal accumulation at different concentration is in the order of leaf < root for both concentrations of Pb and Cd and the highest concentration of Cu (Table 2). It could be deduced that in response to high concentrations of toxic metals, V. faba root cells synthesizes certain phytochelators (PCs)-metal complexes, probably to be PC-Pb complexes. These PC-metal complexes might influence toxic metal transport to the stems and leaves. PC-metal complex was also reported in other plants such as tobacco [21]. Similar data have been reported by Vogel-Mikus et al. [22] in Thalaspi praecox Wulf. The average level of copper found in the studied local variety plant of bean (Mamdouh) in vegetative stage was in the range of 14.02 ± 0.16 mg/kg of dry matter in the roots and in the range of 14.48 ± 0.14 mg/kg of dry matter in the shoots for the highest concentration of copper (0.3 mg/l of Cu(NO3)2), while for the lowest concentration of copper (0.1 mg/l of Cu(NO3)2), the average level of copper was in the range of 15.56 ± 0.21 mg/kg of dry matter in the roots and in the range of 14.12 ± 0.18 mg/kg of dry matter in the shoots (Table 1). For the local variety of faba bean (Badii) in vegetative stage, the average level of copper was in the range of 14.82 ± 0.32 mg/kg of dry matter in the roots and in the range of 13.05 ± 0.19 mg/kg of dry matter in the shoots for the highest concentration of copper (0.3 mg/l of Cu(NO3)2), while for the lowest concentration of copper (0.1 mg/l of Cu(NO3)2), the average level of copper was in the range of 15.35±1.42 mg/kg of dry matter in the roots and in the range of 16.54±0.16 mg/kg of dry matter in the shoots (Table 2). In previous studies, concentration of this metal in A. cepa was reported to be in the range of 44 mg/kg of dry matter [23]. The level of lead (Pb) found in different parts of the local variety of bean (Mamdouh) in vegetative stage was in the range of 3.66±0.47 and 0.98±0.10 mg/l of dry matter respectively in the roots and in the shoots for the highest concentration of lead (6 mg/l of Pb(NO3)2) as clear in Table 1, while for the lowest concentration of lead (3 mg/l of Pb(NO3)2), the level of lead was in the range of 2.45±0.52 and 1.08±0.63 mg/kg of dry matter respectively in the roots and in the shoots, but was several folds higher than the safe limit (0.3 mg/kg) recommended by FAO/WHO (2001) [15]. Pb uptake can also be promoted pH of the soil and the level of organic matter in the soil. Lead is a serious cumulative body position which enters into the body system through air, water and food and cannot be removed by washing fruits and vegetables [24].

## 5. Conclusion

The results of the present study showed that plant growth and biomass of the local variety of bean decreased with higher metal levels of lead and cadmium, but increased with higher metal levels of copper, while for the local variety of faba bean plant growth and biomass showed an increase with all the highest concentration of lead, copper and cadmium, but showed a decrease with the highest concentration of copper. Our results present the order of metal accumulation at different concentrations for bean and faba bean in vegetative stage. For the local variety of bean, the order of metal accumulation is in the order of roots > shoots for higher and lower concentration of lead and cadmium and lower concentration of copper. For the local variety of faba bean, the order of metal accumulation is in the order of shoots > roots, while the order of metal accumulation is in the order of roots > shoots for higher and lower concentrations of lead and cadmium and higher concentration of copper, but for lower concentration of copper, the order of metal accumulation is shoots > roots. The present study provides the evidence that the local variety of faba bean could accumulate and tolerate higher concentrations of lead and cadmium in vegetative stage better than the locale variety of bean which accumulate and tolerate higher concentration of copper which is less danger than Pb and Cd which can affect human health and environment. However, more detailed studies are still needed especially under mixed contamination of heavy metal and at different stage, to better understand the importance of leguminous for the phytoremediation of environmental pollutants.

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