

# Heavy Metals Accumulation in Plant Tissues of *Satureja Cretica* and *Lathyrus Ochrus* Grown in Contaminated Soils

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## Abstract

Soil protection is crucial for the environment and is also beneficial for Human health. Among the pollutants found on contaminated sites, heavy metals contaminate a large portion of the biosphere. They are persistent inorganic elements that accumulate in the soils, contaminate aquifers and pose a threat to all living species. In this study, we examined the growth of the aromatic plant *Satureja Cretica*, and the pale pea *Lathyrus Ochrus* in heavy metal-polluted soils. The plants were exposed to different levels of Cd, Pb, and Ni in a soil pot experiment conducted in a green house. Toxic metal levels were determined in the roots and the leaves of the two plants. Both plants accumulated relatively high amounts of metals in their roots, whereas the aboveground parts exhibited lower accumulation capacity. Regarding the macroscopic effects, the roots of *Satureja Cretica* exhibited significant reduction in length and total mass in the concentration levels of Ni 600 ppm and Pb 2500 ppm, whereas the aerial part was reduced for the contamination level Ni 600 ppm. In the case of *Lathyrus Ochrus*, the root system of the condition Ni 600 also showed similar behaviour, so this level of contamination shows toxicity for both plants.

**Keywords:** Soil contamination, Heavy metals, *Lathyrus Ochrus*, *Satureja Cretica*, accumulation

## 1. Introduction

Heavy metal pollution in the environment is mainly caused by anthropogenic activities (Glick et al, 2003). The majority of the sources resulted from human actions such as metal manufacture and mining industries with storage, disposal and transportation problems (Zhang et al., 2005). Heavy metal toxicity for plants is the consequence of many alterations as a result of physiological processes caused at cellular/molecular level (Rasico and Navari-Izzo, 2011). In addition to the plants that suffer from

phytotoxic effects, there are also plants that tolerate high amounts of heavy metals in their tissues. These plants are able to survive, grow and reproduce on natural metalliferous soils and on sites polluted with heavy metals. The majority of plant species that tolerate high heavy metal concentrations behave as “excluders”, relying on tolerance and even hypertolerance strategies leading to the restriction of metal entrance. They retain and detoxify most of the heavy metals in the root tissues, with a minimized translocation to the leaves whose cells remain sensitive to the phytotoxic effects.

However, some other hypertolerant species, defined as “hyperaccumulators”, exhibit another heavy metal uptake behavior, based on the distribution and the accumulation of metals in the plant. (Rasico and Navari-Izzo, 2011). Phytoremediation is a technology employing the use of plants for the cleanup of contaminated environments. The possibility of using plants in environmental remediation has increased in the past few years, researches have been conducted and the method became an emerging alternative to the restoration of contaminated sites. Cadmium is one of the most toxic heavy metals, this metal present in most of soils with a concentration within the range of 0.1 – 1.0 mg kg<sup>-1</sup>, but due to anthropogenic activities, higher values are reported for many polluted sites around the world. The recommended level for Cd in aromatic plants is 0.3 mg/kg dry weight (WHO, 1998). Nickel is a micronutrient for plants, hence there is no recommended level for this metal in plants. However, Ni can be toxic when it occurs in high concentrations. Lead is considered as a very toxic metal, the highest recommended level of which in aromatic plants is 10 mg/kg d.w. (WHO, 1998). In this study, *Satureja Cretica*, an aromatic plant endemic in the island of Crete, Greece, and the yellow pea *Lathyrus Ochrus*, consumed mainly as a salad in Mediterranean countries, were examined for their heavy metal accumulation capacity. The two plants were cultivated in a pot experiment in a soil contaminated by different

concentrations of Cd, Ni, and Pb. These metals were determined in roots and leaves by ICP-OES. The Bioaccumulation factors (BAF) and Translocation factors (TF) were also determined.

## 2. Material and methods

### 2.1. Plant Material

Two different plant species were chosen. *Satureja cretica* is an aromatic and endemic plant of Crete Greece, and *Lathyrus ochrus*, a leguminous.

The experiment period for *Satureja cretica* was 6 months from 1 October 2016 until 28<sup>th</sup> of March 2017. For *Lathyrus ochrus*, the experiment lasted 4 months between the 6<sup>th</sup> of December 2016 and the 4<sup>th</sup> of March 2017.

Branches from individual plants of *Satureja cretica* were collected in Autumn 2016 from a wild population in the area of Imbros at Lefka Ori mountain, Municipality of Chania, Crete, Greece. The branches were cultivated in small pots with compost for 12 weeks, then transplanted in pots of 1.3 kg of soil, where they grew for 11 weeks. In the case of *Lathyrus ochrus* seeds were provided from the soil Laboratory in The Mediterranean Agronomic Institute of Chania (MAICh), plants were produced from seeds, and sown in a tray with mixture of compost for 4 weeks, then transplanted in pots of 1.3 Kg of soil where they grew for 9 weeks.

The soil used for the cultivation of the two plants was collected from a local area in Chania, Crete. The soil was sieved then divided into 12 piles, then, appropriate volumes of stock solutions of the nitrate salts of Cd, Pb, and Ni were added to each pile. The soil was homogenized thoroughly and left to dry for 2 weeks with occasional mixing. The final total concentrations of the metals in the soil were: Cd 5, 10, 50, 100 ppm, Ni 20, 60, 200, 600 ppm and Pb 50, 250, 500, 2500 ppm. These concentrations were selected according to previous experience of the authors, (Lydakos et al. 2016), in order to avoid lethal effects on the plants. Three repetitions for each condition were prepared for each plant. After their transplantation in the contaminated soil, the experiment lasted 11 weeks for *Satureja Cretica* and 9 weeks for *Lathyrus Ochrus*

The plants were grown under controlled conditions in the glasshouse at MAICh and the irrigation process was done with respect to the water capacity of the soil in order to avoid leaching of heavy metals.

#### a. Metal concentration in plant tissues

Roots and leaves were harvested at the end of the experiment and their fresh weight was measured. Samples were dried at 65°C for 40 hours, then their dry weight was determined. For the digestion, 0.2 to 0.3 g of leaves and roots was ashed in crucibles at 500°C for 4 hours. The ash was dissolved in 10 mL HCl 2 N, and heated at 100°C for 20 min. The acidic solution was

filtered and diluted to 50 mL for both roots and leaves samples. Finally, for the determination of metal concentrations, ICP-OES Atomic Emission Spectrometry (Agilent Technologies, 5100 ICP-OES) was used. For each sample, three measurements were carried out. For the calibration curves, freshly prepared standard solutions of Cd, Ni and Pb were used.

#### b. Soil analysis

The soil used for the experiment was collected from a land far away from any anthropogenic activities. Appropriate volumes of stock solutions of nitrate salts of Cd, Pb, and Ni were added according to the levels of contamination. The final total concentrations of the metals in the soil were: Cd 5, 10, 50, 100 ppm, Ni 20, 60, 200, 600 ppm and Pb 50, 250, 500, 2500 ppm. The soil samples were dried in an air-forced oven at 37° C. Any plant residues and stones were removed. Then the soil was ground and screened through a 2 mm sieve. Soil samples were collected before (control samples) and after (contaminated samples) the addition of heavy metals.

Available heavy metals were determined with the DTPA method for extraction. The Lindsay and Norvell method (Lindsay and Norvell, 1978; Amacher, 1996) was used for the extraction of the plant-available heavy metals by chelation with 0.005 M diethylenetriaminepentaacetic acid (DTPA), in a buffer containing 0.1 M triethanolamine and 0.01 M CaCl<sub>2</sub>, with a pH of 7.3. 20 g of soil were shaken with 40 g of the extractant for 2 h, then filtered and diluted to 50 ml. All the solutions were then analyzed by ICP-OES.

## 3. Results and Discussion

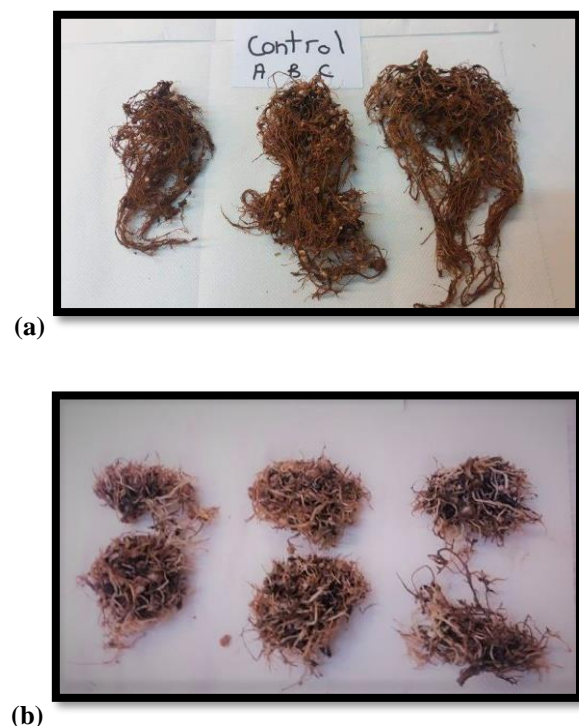
### 3.1. Bioavailability of heavy metals

Plant-availability of heavy metals is the bioavailability of these chemicals to the living receptors (leaves, roots) through direct contact or uptake (Kirkham, 2006). Metal availability in soil, is of main concern, because it is assumed that the available concentration is an indication of the amount available for plant uptake. Therefore, both the available and the total concentration of heavy metals were determined by the means of DTPA extraction and ICP-OES analysis. Table 1 presents the plant-available heavy metals after their determination in the DTPA extraction solutions and the calculated concentrations of the added heavy metals in the soil for both plants *Satureja cretica* and *Lathyrus ochrus*. For control soil, with no heavy metals, note that it contained some amount of the heavy metals in their bioavailable form. Each given value is the mean of three measurements. The total heavy metal content of the soils after the heavy metal additions was determined in the soil digests and a good correlation between the obtained data and the calculated amounts was observed in all conditions (data not shown). This result suggests that the added heavy metals here homogenized in the soil.

### 3.2 Morphological effects of heavy metals on plant tissues

Serious morphological changes were observed in the highest contamination levels of heavy metals, mainly in the root system. Figure 1 shows that in the case of *Lathyrus ochrus*, the highest level of contamination of Ni (600 ppm), caused the reduction of the roots, both in length and mass, in comparison to the control samples. Also, Ni 600 affected the aerial part of the plant: the biomass of the plant was reduced from 5.74 g in the control to 2.73 g (mean values) in the plants cultivated in the soil contaminated with Ni 600, whereas for the highest contamination levels used in this study, Cd 100 ppm and Pb 2500 ppm, *Lathyrus ochrus* showed a moderate decrease of its biomass, as shown in Figure 3.

In the case of *Satureja cretica*, the concentration of Ni 600 ppm also affected the root system by restricting its development, and it was lethal for one sample. However, it is worth mentioning that the three lower Ni levels resulted in higher areal biomass of *S. cretica* in comparison to control (Figure 3). The contamination level Pb 2500 ppm reduced the size of the roots in comparison with the control (Figure 2), however the plant was tolerant for all concentrations of Cd we tested. Again, some lower concentrations of Cd and Pb seemed to affect positively the areal parts of the plants (Figure 3).



**Figure 1.** Roots of *Lathyrus ochrus* (a): control samples, (b): Ni 600 ppm



**Figure 2.** Roots of *Satureja cretica* (a): control samples, (b): Ni 600 ppm, (c): Pb 2500 ppm

### 3.3. Accumulation of heavy metals in plant tissues

#### 3.3.1. *Satureja cretica*

Table 2 and Fig. 4 (A, B, C) present the accumulation of Cd, Ni and Pb, in the roots and leaves of *Satureja cretica*. The major amount of Cd was accumulated in the roots (Fig.4A), whereas Cd was found in the leaves of plants grown only at the two highest levels of pollution (50 and 100 ppm) with an accumulation amount of 1.69ppm and 3.15 ppm, respectively. In the roots, it was observed that the accumulated Cd

increased as concentration of Cd in the soil increased. Similar effects have been observed in the plants studied in Lydakis-Simantiris et al. 2016.

Regarding Ni, the control sample accumulated in the roots 1.2 ppm but no accumulation observed in the leaves. For the contaminated soils, the plants accumulated a significant amount of Ni in both roots and leaves. For the highest level Ni 600ppm, the plant accumulated 351.36 ppm in the roots and 147.1 ppm in the leaves, showing the ability of *Satureja cretica* to accumulate Ni in the roots and to translocate it to leaves, but this reduced the biomass and restricted the development of the root system. The concentrations of Ni in the roots and leaves for the levels 20 ppm, 60 ppm and 200 ppm were also high but had no effect on the plant growth. With increasing the concentration of Ni in soil, the accumulation increased as well, similar uptake of Ni was reported in a study showing the accumulation of Ni in maize seedlings (Nie et al, 2015).

No Pb accumulation was observed in roots and leaves of *Satureja cretica* grown in clean soil and for the first level of contamination (Table 2, Figure 4C). However, for the levels Pb 250 ppm, Pb 500 ppm and Pb 2500 ppm, the plants accumulated respectively in their roots 14.75 ppm, 60.67 ppm and 169.24 ppm, and in the leaves 0.53 ppm, 2.37 ppm and 20.51 ppm. So, accumulation of lead is significantly higher in roots than in leaves and it is increasing as the concentration of Pb increases. The four contamination levels of lead did not cause any toxicity to the leaves and roots of *Satureja cretica*, the plants showed tolerance to this heavy metal. The Bioaccumulation Factor (BAF,  $[\text{metal}]_{\text{tissue}}/[\text{metal}]_{\text{soil}}$ ) and the Translocation Factor (TF,  $[\text{metal}]_{\text{tissue}}/[\text{metal}]_{\text{roots}}$ ) of the heavy metals accumulation in both plants are shown in Table 2. BAF is an indicator of hyperaccumulation if it is higher than 1. In the case of *Satureja cretica*, this factor was less than 1 for the three heavy metals studied which makes it a non-hyperaccumulator plant. Translocation factor was also less than one; this observation shows the limited ability of *Satureja cretica* to translocate heavy metals from roots to shoots.

### 3.3.2. *Lathyrus ochrus*

Figure 4 (D, E, F) illustrates the accumulation of the three investigated heavy metals in the tissues of *Lathyrus ochrus*. Figure 4D presents the accumulation of Cd. In the control samples, no Cd was traced. For the lowest level of Cd 5 ppm, Cd was accumulated only in roots but it was not translocated to leaves. *Lathyrus ochrus* plants cultivated in the soil contaminated with higher concentrations accumulated Cd in their roots much more than they accumulated this metal in their leaves. Cd had no serious toxic effects on

the plants. A study of Panda et al, 2006 showed that a husk of *Lathyrus sativus* was efficient to remove Cd from aqueous solution.

Figure 4E presents the accumulation of Ni; control samples accumulated Ni only in their roots. All the plants grown in the soil enriched with Ni accumulated the metal in both roots and leaves with significant amounts. However, the highest concentration Ni 600 caused the restriction of the growth of the root system. A research conducted on husk of *Lathyrus sativus* as an adsorbent of heavy metals showed that it was successful to adsorb nickel from its aqueous solution (Panda et al, 2007).

Regarding Pb, no accumulation was seen in the leaves except for the highest level Pb 2500 ppm where the plant accumulated 53.73 ppm in its aboveground part. Concerning the accumulation of Pb in the root system, the plants were able to accumulate the heavy metal in their roots and this accumulation increased as the concentration of lead increased in the soil, but it was not translocated to leaves in the case of Pb 50 ppm, 250 ppm and 500 ppm. The highest level of Pb 2500 ppm had an effect on the roots making them very limited compared to the control samples (Figure 3). A similar study investigating the accumulation of lead in *Lathyrus sativus L.* roots showed that this plant was able to accumulate large amounts of lead in its roots and it could be promising as a phytoremediation plant (Brunet et al, 2008).

Bioaccumulation and translocation factors were less than 1 meaning that *Lathyrus ochrus* cannot be considered as an accumulator plant for Cd, Ni and Pb.

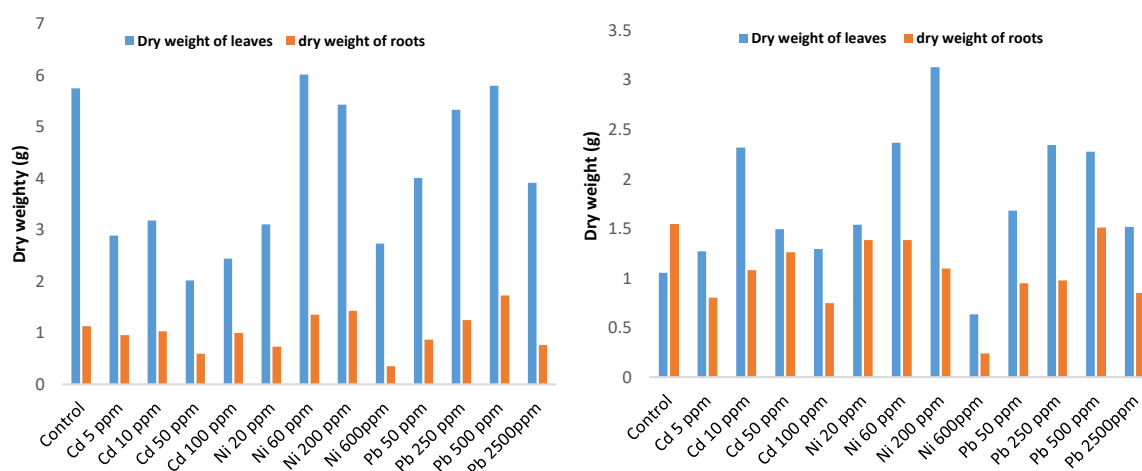
## Conclusions

*Satureja cretica* and *Lathyrus ochrus* cultivated in contaminated soils with heavy metals showed their ability to accumulate Cd, Ni and Pb in their tissues, depending on the level of contamination. However, according to the Bioaccumulation Factor and the Translocation Factor, these two plants were not able to translocate significant amounts of heavy metals to their leaves, so they cannot be considered as potential phytoaccumulators. No serious toxic effects were observed, except for Ni 600 that affected the roots and the biomass. In general, the two plants examined in this study exhibited significant differences regarding their heavy metal accumulation capacity: *Lathyrus ochrus* presented much higher accumulation of all three metals in its roots (per ppm d.w.) compared to *Satureja cretica*.

**Table 1.** Plant-available heavy metals vs. added heavy metals in the soil\* of *Satureja Cretica* (*S.c.*) and *Lathyrus Ochrus* (*L.o.*)

Metal	Added (ppm)	Available (ppm)		Metal	Added (ppm)	Available (ppm)		Metal	Added (ppm)	Available (ppm)	
		<i>S.c.</i>	<i>L.o.</i>			<i>S.c.</i>	<i>L.o.</i>			<i>S.c.</i>	<i>L.o.</i>
Cd	0	0.12	0.12	Ni	0	0.47	0.51	Pb	0	15.48	14.04
Cd	5	3.82	3.98	Ni	20	7.69	8.46	Pb	50	44.77	44.29
Cd	10	7.18	7.55	Ni	60	23.17	24.67	Pb	250	165.14	165.23
Cd	50	37.88	38.73	Ni	200	78.68	84.30	Pb	500	321.14	323.03
Cd	100	83.46	82.09	Ni	600	177.86	196.29	Pb	2500	1586.45	1580.75

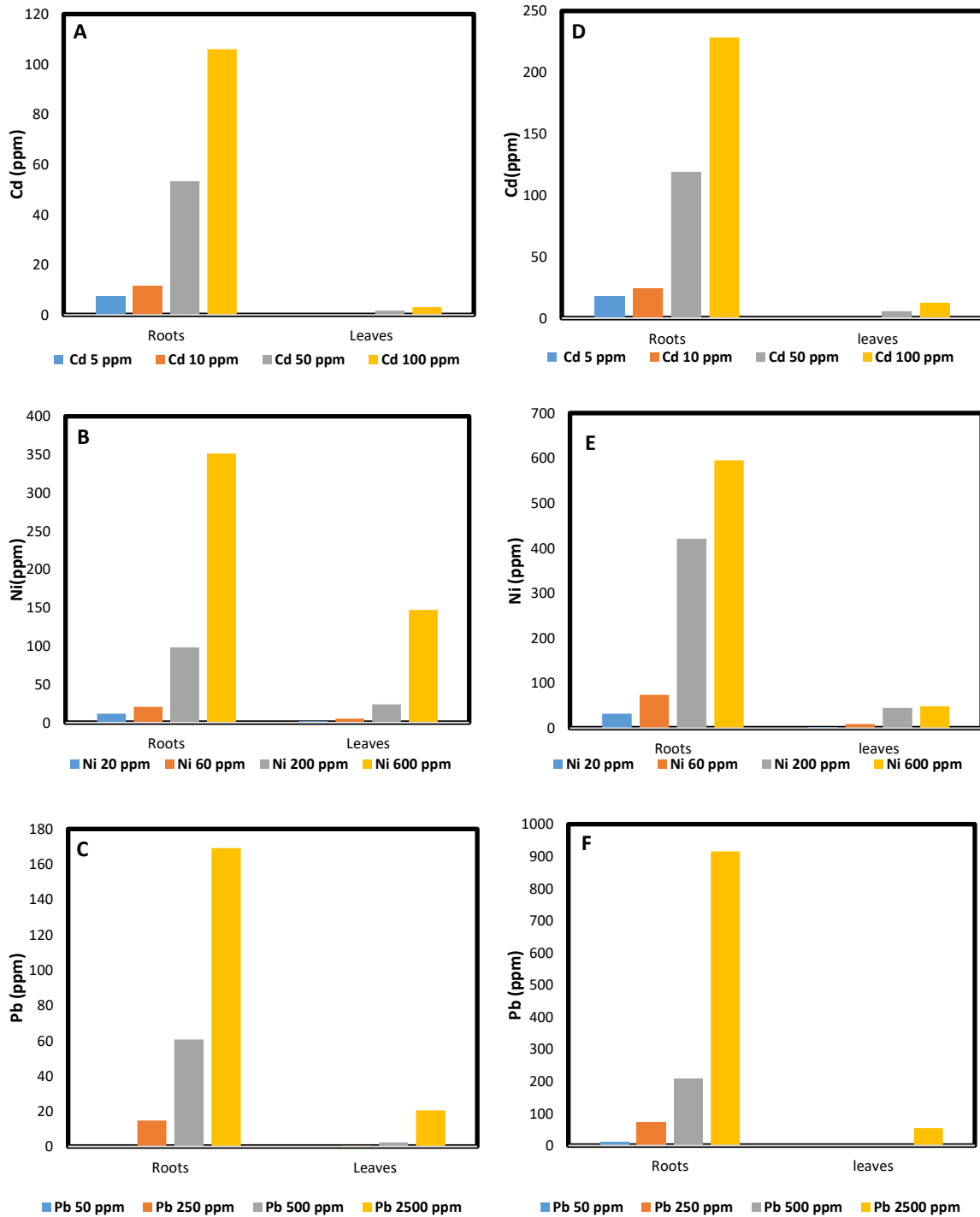
\* The values of the available heavy metals are the means of three measurements.



**Figure 3.** Dry weights of the Tissues of *Lathyrus ochrus* (a) and *Satureja cretica* (b)

**Table 2.** Heavy metal uptake in the roots and leaves of *Satureja Cretica* and *Lathyrus Ochrus* grown in heavy metal-contaminated soils (ppm in dry tissue).

Added (ppm)	metal	<i>Satureja Cretica</i>				<i>Lathyrus Ochrus</i>			
		Roots (ppm)	Leaves (ppm)	BAF Leaves	TF Leaves	Roots (ppm)	Leaves (ppm)	BAF Leaves	TF Leaves
<b>Cd</b>									
0		0	0	0	0	0	0	0	0
5		7.46	0	0	0	17.97	0	0	0
10		11.5	0	0	0	24.33	0.03	0.003	0.001
50		53.31	1.69	0.04	0.03	119.1	5.62	0.14	0.04
100		105.96	3.15	0.03	0.02	228.4	12.51	0.15	0.05
<b>Ni</b>									
0		1.2	0	0	0	6.62	0	0	0
20		11.83	1.9	0.24	0.16	32.07	2.39	0.28	0.07
60		20.59	5.18	0.22	0.25	74.38	8.87	0.35	0.11
200		98.11	23.72	0.30	0.24	420.75	45.03	0.53	0.10
600		351.38	147.1	0.82	0.41	594.51	48.82	0.24	0.082
<b>Pb</b>									
0		0	0	0	0	0	0	0	0
50		0	0	0	0	10.43	0	0	0
250		14.75	0.53	0.003	0.03	71.88	0	0	0
500		60.67	2.37	0.007	0.03	208.69	0	0	0
2500		169.24	20.51	0.01	0.12	915.2	53.73	0.03	0.05



**Figure 4.** Accumulation of Cd (A), Ni (B) and Pb (C) in the leaves and roots of *Satureja cretica*, and the the accumulation of Cd (D), Ni (E) and Pb (F) in the roots and leaves of *Lathyrus ochrus*

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