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Original Article

Cadmium, Nickel, Chromium, and Lead Accumulation in Roots, Shoots, and Leaves of Basil Plants (*Ocinum Basilicum* L.)

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Abstract - *This study aimed to investigate the accumulation of Cadmium(Cd), Nickel(Ni), Chromium(Cr) and Lead(Pb) in roots, shoots, and leaves of basil plants (Ocinum basilicum L.) grown in a greenhouse. for that reason, a) 10 mg L^{-1} $Cd(NO_3)_2 4H_2O$, 20 mg L^{-1} $Ni(NO_3)_2 6H_2O$, 20 mg L^{-1} $Cr(NO_3)_3 9H_2O$ and 20 mg L^{-1} $Pb(NO_3)_2$ respectively, were applied in nine plants per treatment every 2 weeks through irrigation, b) foliar applications were performed with 240 mg CdO, 450 mg NiO, 450 mg CrO and 450 mg PbO per plant respectively, in three plants per treatment and c) nine control plants were irrigated only with tap water. After watering applications, high Cd and Ni concentrations were measured in leaves (257 mg kg^{-1} d.w.) and shoots (762 mg kg^{-1} d.w.) respectively, Cr was accumulated in all organs. in contrast, leaves presented the highest Pb concentration. Foliar applications resulted in high Cd and Ni concentrations in new leaves (3722 mg kg^{-1} d.w) and shoots (5237 mg kg^{-1} d.w) respectively, high Cr concentrations in leaves (1772 mg kg^{-1} d.w), and accumulation of Pb both in leaves and shoots. in conclusion, basil plants can accumulate significant Cd, Ni, Cr, and Pb levels in their shoots and leaves via contaminated water irrigation or foliar deposition of heavy metals.***

Keywords - *Pollution, Contaminants, Aromatic plants, Heavy metals, Phytoremediation.*

I. INTRODUCTION

Basil (*Ocimum basilicum* L.) is an annual plant that belongs to the Ocimum genus of the Lamiaceae family. It is one of the most popular herbs that has been used for centuries as a seasoning and medicinal plant. Due to the high content of its flowers, leaves, and shoots in essential oils, all the above-mentioned plant organs are widely used in traditional medicine and cosmetology, as well as for medicinal and culinary purposes [1]. Moreover, the leaves of basil are commonly used as flavouring ingredients in gastronomy all over the world [2]. in addition, a large number of potted plants are marketed in the international market as ornamental plants.

Basil plants have also been studied for phytoremediation, phytoextraction, and accumulation of Cadmium (Cd), Chromium (Cr), Lead (Pb), and Nickel (Ni), and the results showed that the plants can accumulate and translocate these metals from roots to the aerial parts in different proportions [3]. It is also reported that basil could be considered as hyperaccumulator for Cd, Cr, and Pb [4]. the ability of basil to hyper accumulate toxic metals in its tissues may affect its antioxidant capacity [5] or even make its consumption harmful when plant organs are consumed either raw or dried in foods and infusions [6].

Nowadays, anthropogenic activities like landfills, livestock farming, industrial and urban runoffs, agrochemical applications, constructions, etc., provoke ground, water, and aerial pollution with toxic heavy metal compounds, mainly in urban or suburban environments. This results in the accumulation of heavy metals, either by foliar or root uptake, in plants that are grown in these environments. According to reference [7], the concentrations of Cd in the urban atmosphere of many cities in the USA, Europe, and Japan range from 6 to 360 ng m^{-3}, 2 to 50 ng m^{-3}, and 10 to 53 ng m^{-3} respectively, while Pb concentrations in eastern and western Europe cities atmosphere range from 0.2 to 0.6 mg m^{-3} and <0.1mg m^{-3} respectively. the same author reported that Ni levels in the atmosphere range from 1 to 10 ng m^{-3} in urban areas, but in large cities and near industrialized areas, Ni concentration in the air may reach 170 ng m^{-3}. the Cr concentrations in ambient air in the Netherlands have been reported to range from 2 to 5 ng m^{-3}, while in the UK, average levels of Cr in urban and rural areas ranged from 0.7 to 5 ng m^{-3} [8].

Environmental factors, plant physiological status, and species specificity are crucial factors concerning the responses of plants to elevated concentrations of air contaminants. Plant species, morphological characteristics of the leaf surface (shape, dimensions, hair, and waxes), mechanisms such as endocytosis, phyllosphere organisms, and changes produced by ambient gaseous pollutants can all have an impact on foliar absorption [9]. Heavy metals are absorbed by foliar surfaces via stomata, cuticular cracks, lenticels, ectodesmata, and aqueous pores [10]. the foliar metal transfer appears to be the primary source of pollution, especially when ultra-fine particulates interact with plant leaves [9], with the plant canopy acting as an effective filter for heavy metal emissions in the atmosphere [11]. the processes behind metal absorption transport pathways in plants are currently being studied [9]. Foliar deposition created brownish necrotic zones on basil leaves, presumably as a result of interference with cell metabolism, as well as transfer from directly exposed leaves to freshly formed leaves, according to another study [12]. Metal accumulations have already been linked to leaf necrosis, indicating intense toxicity [13].

Several studies were performed to determine the way that basil plants accumulate different proportions of heavy metals in their organs. Reference [1] reported that Cd is accumulated mainly in roots and mature leaves, while [14] indicated that Cd could be transported via apoplastic and symplastic pathways from roots to the leaves, which are the edible part of the plant. the same reference mentions that basil plants grown in contaminated soil concentrate higher amounts of Ni in plants flowers. It is also reported that long-term consumption of leaves from basil plants grown in soil contaminated with Cd and Pb could be hazardous for human health, even if the concentrations of the toxic metals are below the permissible limits [15]. According to reference [16], the maximum allowed Cd limit in medicinal herbs is 0.3 mg kg^{-1} dry weight (d.w.) while for the foodstuff is about 1 mg kg^{-1} dw [17]. WHO also sets permissible levels for Ni at 1.5 mg kg^{-1} dw and 1.63 mg kg^{-1} d.w. for medicinal and edible plants, respectively, while for Cr, the permissible level for plants is 1.30 mg kg^{-1}. in European Commission Regulation no. 1881/2006, it is preferred that the limit for foodstuff contamination for both Pb and Cd is 0.1 mg kg^{-1} d.w. and in fresh herbs is 0.2 mg kg^{-1} d.w.

This study aimed to investigate the accumulation of Cd, Ni, Cr, and Pb in different plant organs (root, shoots, leaves) of basil plants grown for a short period in a greenhouse after being treated with water solutions or dusted with powders contained in excess these heavy metals.

This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project "Study on the accumulation of heavy metals in plant parts, essential oil and dried aromatic plants cultivated by urban agriculture." (MIS 5048926).

II. MATERIALS AND METHODS

Young basil plants (4 weeks after sowing) were supplied by seedlings producer and were transplanted in PVC pots of 150 mm diameter, which contained peat as substrate. the potted plants (57 in total) were placed for two weeks in the greenhouse for acclimation until the beginning of the experiment. Heavy metal salts ($Pb(NO_3)_2$, Ni $(NO_3)_2 6H_2O$), $Cd(NO_3)_2$ $4H_2O$, and $Cr(NO_3)_3$ $9H_2O$ and oxides (PbO, NiO, CdO and CrO) obtained by Sigma-Aldrich were used for plants watering and foliar application treatments, while nine (9) of basil plants did not undergo any heavy metal treatment and were considered as control plants. the experiment took place in a greenhouse located at the University of Thessaly near Volos, Greece (Velestino: Latitude 39° 22', Longitude 22° 44', Altitude 85 m).

A. Watering Applications

Thirty-six (36) potted plants were used for this treatment, separated into four subgroups of nine plants each. Every two weeks, each subgroup was irrigated with 250 mL of a solution containing the appropriate amount of a heavy metal salt. the first subgroup was irrigated with Cd solution containing 10 mg L^{-1} $Cd(NO_3)_2$ $4H_2O$ (2.8 mg L^{-1} Cd), the second subgroup with Ni solution containing 20 mg L^{-1} $Ni(NO_3)_2$ $6H_2O$ (4.4 mg L^{-1} Ni), the third subgroup with Cr solution containing 20 mg L^{-1} $Cr(NO_3)_3$ $9H_2O$ (2.6 mg L^{-1} Cr) and the fourth subgroup with Pb solution containing 20 mg L^{-1} $Pb(NO_3)_2$ (10.4 mg L^{-1} Pb) [18]. in total, five (5) irrigation events were performed with the above-mentioned solutions at 0, 2, 4, 6, and 8 weeks after transplanting. Roots, shoots, and leaves samples were collected for chemical analysis from twelve potted plants (three of each treatment subgroup) at the 4th, 8th, and 10th week after transplanting.

B. Foliar Applications

Twelve (12) potted basil plants were used in this treatment, separated into four subgroups of three plants each. the surface of all pots was covered with a plastic membrane to prevent soil contamination. Three plants of each subgroup were dusted using a fine paintbrush. the first subgroup was dusted with 720 mg of CdO (240 mg $plant^{-1}$ Cd), the second with 1350 mg of NiO (450 mg $plant^{-1}$ Ni), the third with 1350 mg of CrO (450 mg $plant^{-1}$ Cr), and the fourth with 1350 mg of PbO (450 mg $plant^{-1}$ Pd) [9].

Ten (10) weeks after the CdO, NiO, CrO, and PbO applications, roots, shoots, and new leaves developed 30 days following plant dusting were collected for chemical analysis from all control and treated plants.

C. Control Plants

Nine (9) basil plants were irrigated only with tap water and were used as control plants. Roots, shoots, and leaves samples were collected from three potted plants at the 4th, 8th, and 10th week after transplanting for chemical analysis.

All groups were irrigated every second day with the appropriate amount of tap water to keep the substrate wet. After each irrigation, no drainage occurred. During the experiment period, there was no fertilizers application.

D. Chemical Analysis

The samples from basil plants were washed in running tap water and sorted by roots, shoots, and leaves. After drying for 72 h at 45 °C, the samples were ground, and 0.5 g of each sample were weighed and placed for incineration for 4h at 550°C. Ash of each sample was digested with the use of 2 mL HCl 1:1 (v/v) and 15 mL deionized water. the solution was heated at 90°C for 30 min, and the final volume was fixed to 50 mL with deionized water. Final solutions of the samples were analyzed for Cd, Ni, Cr, and Pb concentration, with the use of a colorimeter (Smart 3, LaMotte). in addition, tap water used for plants irrigation as well as extracts of the substrate used for basil planting were chemically analyzed to determine the concentration of Cd, Ni, Cr, and Pb.

E. Statistical Analysis

The results were expressed as mean values of three replicates ± standard deviation (SD). the data were evaluated statistically using paired samples t-tests and/or Analysis of Variance (ANOVA) followed by Turkey's HSD post hoc test to separate means at a probability level of $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Chemical analysis revealed that Cd, Ni, Cr, and Pb concentrations in tap water used for the preparation of watering solution as well as for irrigation of the control plants, were 130, 200, 240, and 100 μg L^{-1}, respectively.

The permissible limit by WHO guidelines concerning the drinking-water quality is up to 3 μg L^{-1} for Cd [19]. in many European countries as well as in many states in the USA, Ni concentrations in drinking-water were below 10 μg L^{-1}[20], whereas Cr concentration ranged from 1 μg L^{-1} (in the Netherlands) to 14 μg L^{-1} (in Canada) or even up to 60 μg L^{-1} (in the USA) [21]. US EPA and WHO set the limit of Pb in drinking water up to 15 μg L^{-1} and 10 μg L^{-1}, respectively [22]. However, Pb concentration in drinking water in the United Kingdom was measured above 50 μg L^{-1}, whereas according to a 1989 report, the concentration of this heavy metal in drinking water in the USA is on average 2.8 μg L^{-1} [23].

The irrigation of cultivated plants is based on soil water and/or groundwater use. in soil water and groundwater, the average Cd concentration worldwide is up to 5 μg L^{-1} [24] and 1 μg L^{-1} [25], respectively. in leachates taken from landfills and contaminated soils that have occurred from municipal solid waste in European countries, Cd concentrations up to 2700 μg L^{-1} were measured [26], whereas, in the United States, the measured Cd concentrations in wastewater were up to 6000 μg L^{-1} [27].

Reference [8] has also indicated that in tap water, Ni concentration ranges from 0.2 to 540 μg L^{-1}, whereas in polluted areas, it has been measured 100 to 2500 μg L^{-1} of Ni in groundwater and municipal tap water. the same quantity was also found in areas where the natural Ni was mobilized. However, soil pH, human impact, and depth of sampling affect the concentration of Ni in groundwater strongly.

Regarding the Cr, it was reported that salinity plays a key role in its presence in the water. the limits that are recommended for Cr concentration in water are 8 μg L^{-1} for Cr(III) and 1 μg L^{-1} for Cr(VI) [28]. Chromium concentration in wastewater ranges from 0.005 to 525 mg L^{-1} [29] due mainly to industrial activities.

Although Pb is one of the most toxic heavy metals, few studies have been conducted on the groundwater concentration of this element. Reference [30] reported that in 557 surface water and 1661 groundwater samples taken from various US states, the Pb average concentrations were 4.6 μg L^{-1} and 13.4 μg L^{-1}, respectively. the permissible limit of Pb in wastewater and soils used for agriculture are 0.01 and 0.1 μg L^{-1}, respectively [31]. As previously documented, the atmosphere is mostly responsible for Pb emissions in natural waters since it increases the values of the element over 1000 ng L^{-1} into the lakes and rivers, with the major discharges to occur in Europe, North America, and Asia countries [32].

Consequently**,** the tap water used for the irrigation of control plants can be considered as cadmium, chromium**,** and lead**-**contaminated water with characteristics that occur in urban or suburban areas. However, nickel concentration, although relatively high, is similar to that measured in tap water in many non-contaminated areas worldwide. in contrast**,** the substrate water extract had no detectable concentrations of Cd, Ni, Cr**,** and Pb.

A. Control Plants

High accumulation of Cd was measured in the shoots of control plants (Fig 1b), while no detected concentration of this element was measured in the roots and leaves (Fig.1a and c). Reference [33] measured proportional concentration in basil plant shoots that were cultivated in low concentration contaminated soils. It has also been stated that in soils with low Cd concentration where lettuce (*Lactuca sativa*), spinach (*Spinacia oleracea*), cauliflower (*Brassica oleracea*), and oat had been cultivated, Cd concentration was higher in the shoots of the plants in contrast to those of the roots [34]. These results follow other studies where it is reported that one of the mechanisms for Cd accumulation in plant parts is explained by the ion transportation from root to the aerial parts of the plant at the same speed as the water with transpiration [35]. An important proof of this came from a study that showed that stomatal closure induced by ABA reduced the Cd accumulation significantly in shoots of Indian mustard plants [36]. That hypothesis might explain probably the rapid transfer of Cd within the xylem sap flow to the aerial part of the plant. However, during the movement of these elements through the xylem, Cd ions form complexes with ligands of the xylem cell walls of the vessel and are absorbed on them [35]. Several metals were moved in the xylem as complex Aluminum (Al). for example, it was transported in shoots in the form of an Al–citrate complex [37], whereas nickel (Ni) forms a complex with histidine [38]. These agree with the hypothesis that Cd moves in plants' xylem vessels in the form of some type of complex. That procedure concludes to no adequate Cd ions remain to the sap to be transferred to the leaves when this element is in relatively low concentration in the substrate. By Cd binding in xylem cells, walls may affect significantly both the distribution and the storage of this element in the different plants' organs. the permissible Cd limits in plants are up to 0.02 mg kg^{-1} [8].

There was no detected concentration of Ni in the roots of control plants (Fig.2a). This is by a previous study [39] indicating that the sequestration of this element in plants roots is prevented by the presence of high concentrations of histidine in roots tissues. in contrast, the concentration of this element was increased in shoots and leaves (940.94 and 240.00 mg kg^{-1} respectively, Fig 2b and c) that might derive from the presence of Ni in tap water. in another study [30], it is referred that Ni presented high mobility within the xylem and the phloem, something that is strongly affected by histidine at pH 6.5. Reference [40] measured concentrations almost up to 1 mg kg^{-1} in the shoots of maize plants that had grown in a hydroponic system and irrigated with a nutrient solution with no detectible Ni concentration. Increased Ni concentrations were also measured in basil shots compared to those in the root [1]. However, the distribution of Ni in different plants organs strongly depends on the plant species. Reference [41] reported that the accumulation of Ni in ryegrass shoots was 5 to 7 times higher than in maize when these plants were grown in a nutrient solution that contained 20 μM Ni. Since peat moss, the substrate where control plants were grown, has a high adsorption capacity for heavy metals compared to other natural adsorbent materials [42], the concentration of Ni in the root environment of the plants during the experiment period (10 weeks after transplanting) should be far exceeded the 200 μg L^{-1} measured in tap water. This can explain the high accumulation of Ni in plants shoots despite its low concentration in tap water. the permissible Ni limits in plants are up to 10 mg kg^{-1} [8].

Several researchers [30],[43] reported that Cr^{3+} concentrations ranging from 100 to 750 μg L^{-1} have a stimulatory effect on plants, increasing their normal growth. Chromium concentrations measured in plants root, shoot, and leaves of the control plants were 15.00, 18.52, and 13.98 mg kg^{-1}, respectively (Fig 3a, b, c). Reference [44] measured similar or slightly higher Cr concentration in leaves and roots of basil plants which were grown in nutrient solution with 520 mg L^{-1} of Cr, in which all ions are available to be absorbed by the plant. in the present work, a solution with half the Cr concentration was used, and in addition, peat moss (which was used as a substrate for plant growth) should reduce the availability of this element in plants since peat moss can absorb a significant amount of this element [42],[45]. Different climatic conditions can also justify different absorption rates and accumulation of the element in different plant organs. the above mentioned can explain the comparable Cr concentration in the leaves and roots of the plants of the two experiments. the shoots of the control plants had a slightly higher Cr concentration compared to roots and leaves (Fig 3a, b, c). Reference [1] also reported a similar distribution of chromium between the roots, shoots, and leaves of the control plants, which were grown in soil where the concentration of this element ranged from 17.5 to 54.3 mg kg^{-1}. However, several authors reported different root: shoot accumulation proportions of this element in different vegetable species [46]–[47]. Plant species, as well as the Cr concentration in the substrate and the oxidation state of this element, can affect strongly the distribution and translocation of Cr in plants' roots shoots and leaves [7]. Due to its low mobility compared to other heavy metals, Cr concentration in the roots is sometimes a hundred times higher than in shoots [48]. the higher accumulation of Cr in roots may be accrued through a plant's protective mechanism for the sequestration of Cr in root cells vacuoles [49]. in addition, Cr (III) is strongly binding with the cell walls, and thus Cr movement to aerial plants' organs is hindering [50]. the permissible Cr limits in plants are up to 1.3 mg kg^{-1} [8].

High Pb accumulation was measured in the shoots of control plants (Fig. 4b), while no detected concentration of this element was measured in the roots and leaves (Fig 4a and c). Most plant species accumulate Pb in the roots (approximately 95% or more), and only a small fraction of this element is translocated in the shoots and the leaves [51]–[52]. However, hyperaccumulator species exude from their roots substances that can dissolve metals that are present in the soil, increasing their uptake and translocation [53]. After its absorption, Pb mainly moves by apoplast and through the transpiration stream reaches the endodermis [54]. Reference [55] using X-ray mapping measured high Pb deposition in xylem and phloem cells, whereas reference [54] reported that Pb might also be transferred in inorganic form with the same mechanism that Cd is transferred within plants. This is by the results of the present work since the distribution of Pb within control basil plants was similar to those of Cd. According to reference [8], the permissible Pb limits in plants are up to 2 mg kg^{-1}.

B. Watering Applications

During the experiment period, Cd concentration was increased in roots and leaves of the plants, which were irrigated with solution contaminated 2.8 mg L^{-1} Cd, as presented in Figure 1. Cadmium average concentration in the roots of these plants was 4.5 ± 0.7 mg kg^{-1} of d.w., four weeks after transplanting and decreased to 2.0 ± 0.1 mg kg^{-1} of d.w., eight weeks after transplanting. This level remained almost stable until the end of the experiment period (ten weeks after transplanting). Basil leaves accumulated more Cd (185.3 ± 2.0 mg kg^{-1} of d.w.), in comparison to the roots and shoots four weeks after transplanting, while it increased to 285.8 ± 13.0 mg kg^{-1} of d.w. Eight weeks after transplanting, probably because the excess cadmium that could not be sequestrated in shoots was moved by the transpiration current to the leaves where it accumulated. However, by the end of the experiment period, the Cd concentration in leaves slightly decreased to 257.0 ± 1.0 mg kg^{-1} of d.w. Cadmium concentration in the shoots of both the control and treated plants was similar (Fig. 1b), either because this represents the maximum amount of Cd that can be accumulated in shoots tissue of this plant species or because excess Cd was translocated to the veins of the recently formed leaves which are rich in Cd-binding sites [56]. the movement of Cd throughout the plant can be influenced significantly by the cellular sequestration of Cd and thus from the level of the free Cd in the symplast. Cadmium sinks both at the cell and tissue level vary between different plants species. High Cd concentrations have been found in the cell walls of different plant tissues. Several researchers [57]–[58] have found high concentrations of this element in the roots of *Phaseolus vulgaris* seedlings and *Zea mays* plants, whereas references [10] and [59] measured high concentrations in shoots and leaves of *Polygonum thunbergii* and *Brassica napus* respectively. the results of this work are by other studies where it is stated that one of the mechanisms for Cd accumulation in plant parts is explained by the ion transportation from root to the aerial parts of the plant at the same speed as the water with transpiration [35]. Basil plants accumulate high amounts of Cd in their tissues (especially in leaves) when this element is in excess in the substrate's solution. the passive root absorption is followed by the diffusion and translocation through the water movement into the plant via xylem pathways [60]. Moreover, plants that hyper accumulate Cd contains more than 100 mg of the heavy metal per each Kg of their d.w., while the highest concentration of Cd is measured in their aerial parts (shoots, leaves) than in their roots [61]. It has also been reported that apoplastic and symplastic routes involve the Cd transfer through the roots, stems, and leaves [14],[62]. Young basil leaves tend to accumulate less Cd than mature ones. This may be due to the longer period of exposure of the mature leaves to contamination as well as to their more intensive transpiration [63]. Due to the high accumulation of basil in Cd, it is suggested for phytoremediation in highly polluted soils with the additional use of KCl fertilizer as an additional factor [64].

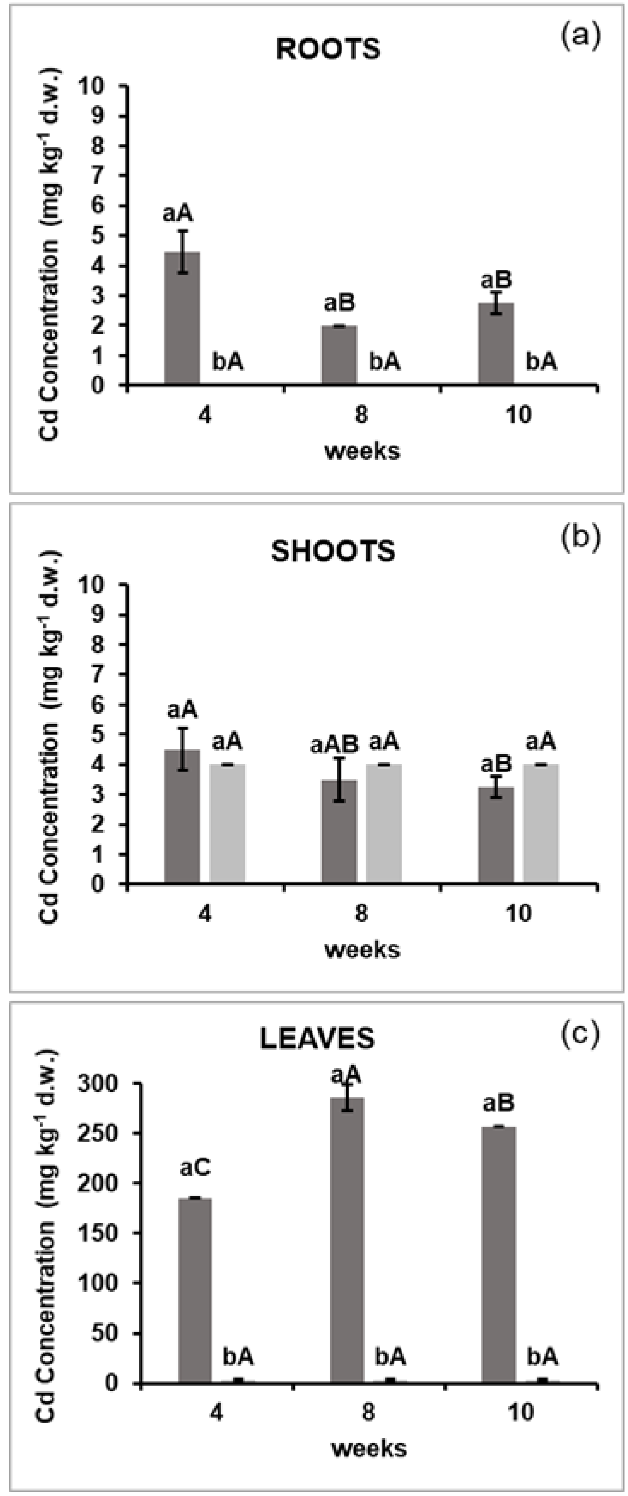

Fig. 1 Cadmium concentration in basil roots, shoots, and leaves dry weight (d.w.) after irrigation with Cd contaminated water (■) and tap water (■) during the experiment period. Different lower-case letters denote statistically significant differences among contaminated and non-contaminated samples of the same week, whereas upper case letters denote statistically significant differences among contaminated and non-contaminated samples between the different weeks, respectively (P ≤ 0,05)

More than 500 plant species have been identified globally as heavy metals hyperaccumulators, the majority of which are Ni hyperaccumulators [61]. Basil shoots had the highest accumulation in Ni, followed by this of leaves when 4.4 mg L^{-1} of this element was added to the irrigation water. Four weeks after transplanting, Ni concentration in the shoots and the leaves of the treated plants was 1658.1 ± 147.5 mg kg^{-1} and 860.3 ± 1.7 mg kg^{-1} of their d.w. Respectively. However, gradually over time during the experiment period, Ni concentration in the shoots and the leaves of the treated plants was significantly decreased at the eighth and tenth week after transplanting, in a level similar to those of the control plants, as shown in Fig. 2. in contrast, in the root tissue, the accumulation of Ni was at a significantly lower level compared to this in shoots and leaves. in specific four weeks after transplanting, the concentration of Ni in roots was 280.0 ± 1.0 mg kg^{-1} of d.w., while it decreased to 225.0 ± 1.7 mg kg^{-1} of d.w. Eight weeks after transplanting. Afterward, there was an increase of up to 360.7 ± 2.1mg kg^{-1} of dry weight at the end of the experiment, probably because some of the Ni that was initially stored in the leaves or stems of the plant moved later to the root. Similar results were reported from references [65] - [66] concerning both downward and upward movement of Ni during phloem translocation in wheat seedlings as well as in lettuce, radish, and bean, respectively. Nickel is an essential element that contributes to plant growth and the processes of photosynthesis [1], [67]. When the amounts of the metal are in excess in the soil or substrate, the transpiration rate and the plant growth are affected through the resulting decrease of the leaf area and the stomata function. This is finally leading to the diminishing of the moisture content [68]. This results in prohibiting the transport of the element to the aerial parts of the plant after a long period of plant cultivation in polluted soil. Despite that leaves are the main organs where Ni is accumulated (in no active sites in leaves, e.g., vacuoles and apoplast), reference [39], reported that when nickel is in excess in the aerial part of the plant, large quantities of Ni are transported through the phloem back to roots and/or soils, while the xylem can hold enough amount of Ni. As in the case of other heavy metals, Ni translocation depends on the plant species [69], while the movement of the element from the older leaves to the younger ones has been observed [70]. the hypothesis that Ni may be efficiently translocated via phloem in hyperaccumulators is reinforced by the fact that reproductive organs (flowers and seeds) are usually considered to have weak xylem transport but strong phloem translocation activities that lead to the accumulation of high concentrations of Ni [71]. the metal transportation is being affected by forming complexes between the Ni ions, histidine, and organic acids, something that is essential for the translocation of the metal within the xylem and the phloem [72].

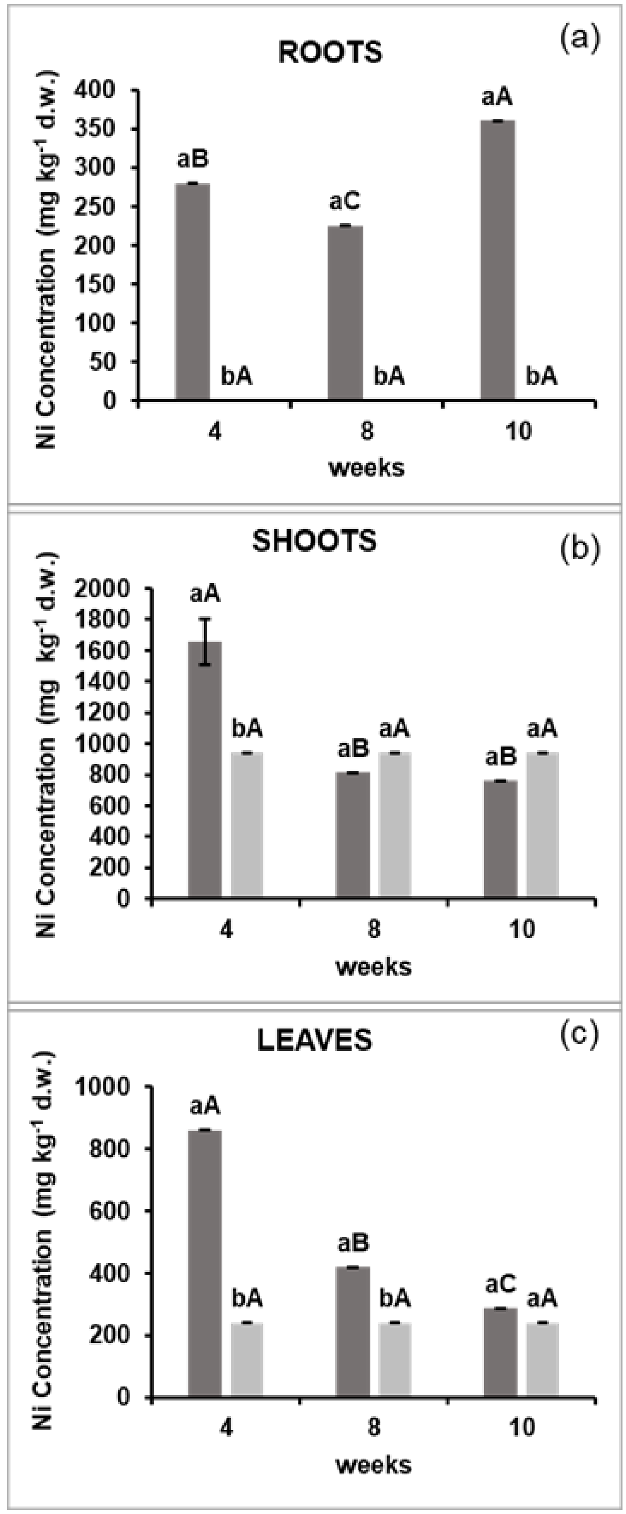

Fig. 2 Nickel concentration in basil roots, shoots, and leaves dry weight (d.w.) after irrigation with Ni contaminated water (■) and tap water (■) during the experiment period. Different lower-case letters denote statistically significant differences among contaminated and non-contaminated samples of the same week, whereas upper case letters denote statistically significant differences among contaminated and non-contaminated samples between the different weeks, respectively (P ≤ 0,05).

Accumulation of Cr in roots, shoots, and leaves of the plants which were irrigated with 2.6 mg L^{-1} Cr, was similar to that of the control plants four and ten weeks after transplanting (Fig 4). During the eighth week, the concentration of Cr in the above-mentioned plant organs raised to levels of 25.0, 28.7, and 31.9 mg kg^{-1} of d..w, respectively. According to several researchers [63], [73], basil plants accumulate major amounts of Cr in their shoots and mainly in roots. Since chromium has attracted significantly less attention than other heavy metals like Cd and Pd, it is still not known exactly how it is absorbed and distributed in the vegetative and reproductive organs of plants [74], while no specific mechanism has been proposed for its absorption [75]. However, reference [74] reported that Cr is transported mainly through the xylem after absorption by the plant. in this sense, transpiration may play a significant role in Cr movement and translocation within plants. the results of the present work showed that the concentration of Cr in the roots of basil plants after four weeks of treatment was similar to those in leaves, whereas, after 10 weeks of treatment, its concentration was higher in roots than in the leaves of the plants (Fig. 5). the accumulation of Cr in the roots and its immobilization in root cells vacuoles, which is mainly observed, probably act as a mechanism for the protection of the photosynthetic apparatus in leaves [76]. Reference [77] observed in experiments which were performed on *Leersia hexandra* that Cr accumulated and sequestrated initially in root cell walls and secondarily in leaf cavities. However, the concentration of this heavy metal in the substrate seems to play a significant role not only in Cr uptake but also in its distribution in different plant organs. in this regard, reference [78] measured higher Cr concentration in the roots of *Capsicum annuum* than in the shoots and leaves, when plants were grown in substrate contained high (65.5 mg Kg^{-1}) and medium (31 mg Kg^{-1}) Cr concentration, whereas when plants grown in a substrate with low Cr concentration, the roots and the leaves of the plants had similar Cr accumulation level. in other experiments performed on wheat (*Triticum uestivum* L.), Cr concentration was measured almost twice in the leaves than in the shoots, when the concentration of this element in the nutrient solution was 0.1 mM [12], while Cr concentration in leaves was two to three times lower than in root when the concentration of the heavy metal in the nutrient solution was lower or greater than 0.1 mm. From another study concerning hyperaccumulating plants, it is indicated that high levels of Cr can be transferred and translocated in the shoots of the plants [79]. Measurements taken during the eighth week of the treatment period revealed probably a rearrangement of Cr distribution in the plant tissues, which may have been caused by the change of Cr concentration in the substrate (Fig 5).

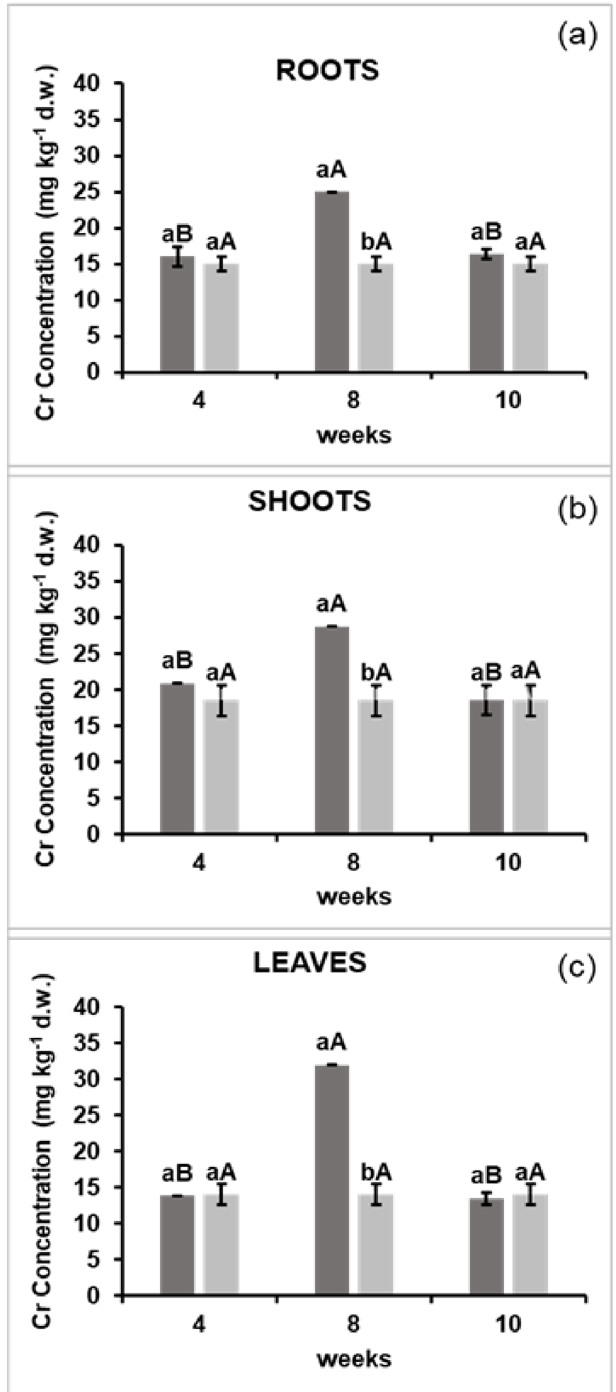

Fig. 3 Chromium concentration in basil roots, shoots, and leaves dry weight (d.w.) after irrigation with Cr contaminated water (■) and tap water (■) during the experiment period. Different lower-case letters denote statistically significant differences among contaminated and non-contaminated samples of the same week, whereas upper case letters denote statistically significant differences among contaminated and non-contaminated samples between the different weeks, respectively (P ≤ 0,05)

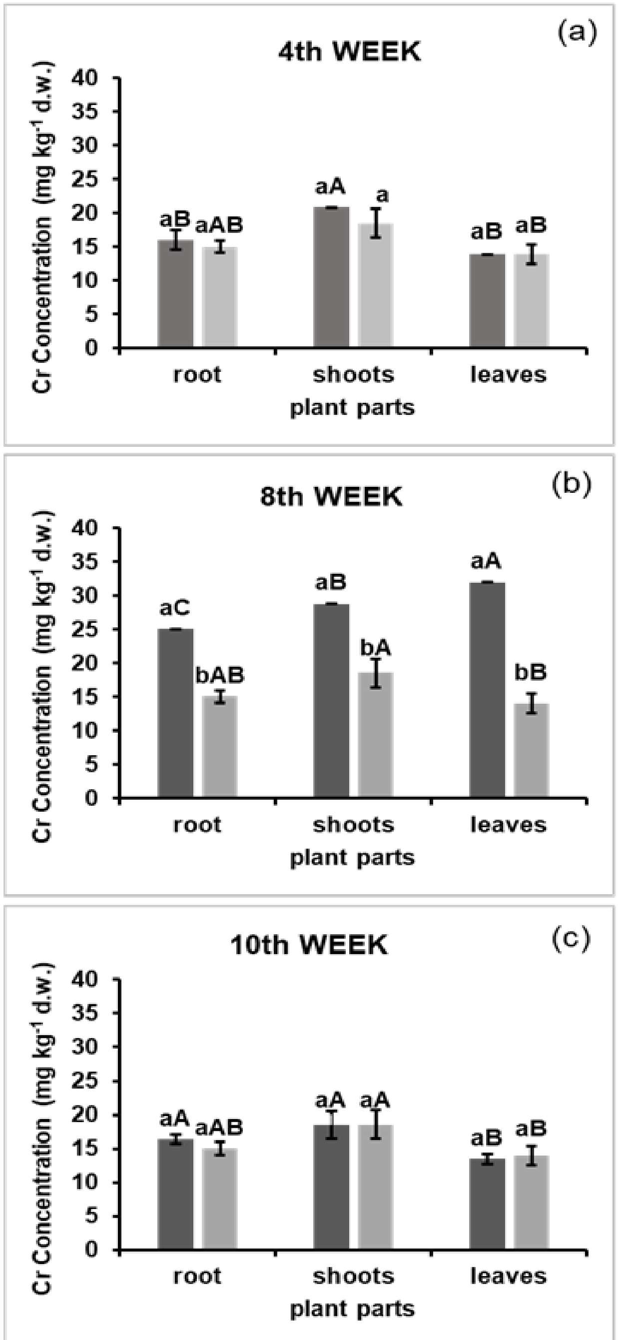

Fig. 4 Chromium concentration in basil roots, shoots, and leaves dry weight (d.w.) after irrigation with Cr contaminated water (■) and tap water (■) by the 4th, 8^{th,} and 10th week of the experimental period. Different lower-case letters denote statistically significant differences among contaminated and non-contaminated samples of the same week, whereas upper case letters denote statistically significant differences among contaminated and non-contaminated samples between the different weeks, respectively (P ≤ 0,05)

Lead concentration was increased rapidly in all plant organs (roots, shoots, and leaves) after plants watering with a solution that contained Pd in a concentration of 10.4 mg L^{-1}. Lead accumulation has already been reported in plant tissues of roots, shoots, and leaves from different species [80]. However, the leaves that contained the higher Pd concentration four weeks after beginning the treatments had no detectable concentrations of this element in their tissue after the 8th week of the experiment period. This suggests a downward translocation of Pd in plants and agrees with unpublished data of the Gardea-Torresdey research group, which indicated that in *Prosopis* sp. plants that were grown in high-Pb concentrations hydroponic solution, accumulated Pb initially in the phloem tissues due to the movement of this element to the leaves through the xylem, while later Pd was returned through the phloem to the plant body [11]. in roots, the Pd concentration reduced gradually from 15.0 to 9.8 mg kg^{-1} of d.w. after eight weeks of treatment, whereas at the end of the experiment period, no detected concentration of the element was found in roots. Consequently, the rate of reduction of Pd concentration in plant roots was significantly lower than that of leaves. This is to be expected since roots are the first natural barrier for Pb translocation to the above-ground plant parts while most of the absorbed Pb remains deposited in the roots [81]. in addition, the prolonged period during which increased accumulation of lead in plant roots was measured may be because lead forms complexes with organic materials [13], and consequently, high levels of its concentration are maintained in the root environment. Many researchers indicated that Pd accumulation increases in direct proportion to exogenous Pb concentrations. in contrast, shoots Pd concentration decreased slightly after four weeks of treatment (from 29.94 to 27.05 mg kg^{-1}) and remained almost stable until the end of the experiment period. According to these results, basil plants accumulated Pb only in shoots while the level of accumulated element in the shoots of the treated plants was similar to that of the control. Reference [11] stated that when high Pb concentrations are performed, the element tends to concentrate in the phloem tissues, and within a movement towards the xylem up to the leaves, it can return to shoot and roots. Despite that, no specific channels for the transportation of Pb inside the plant are reported. As it was mentioned before, Pb moves mainly through the apoplast and reaches the endodermis through transpiration [54]. Pb deposits in the xylem and phloem can be high [55], whereas Pb can also be transferred in an inorganic form via the same mechanism by which Cd is transported in plants [51].

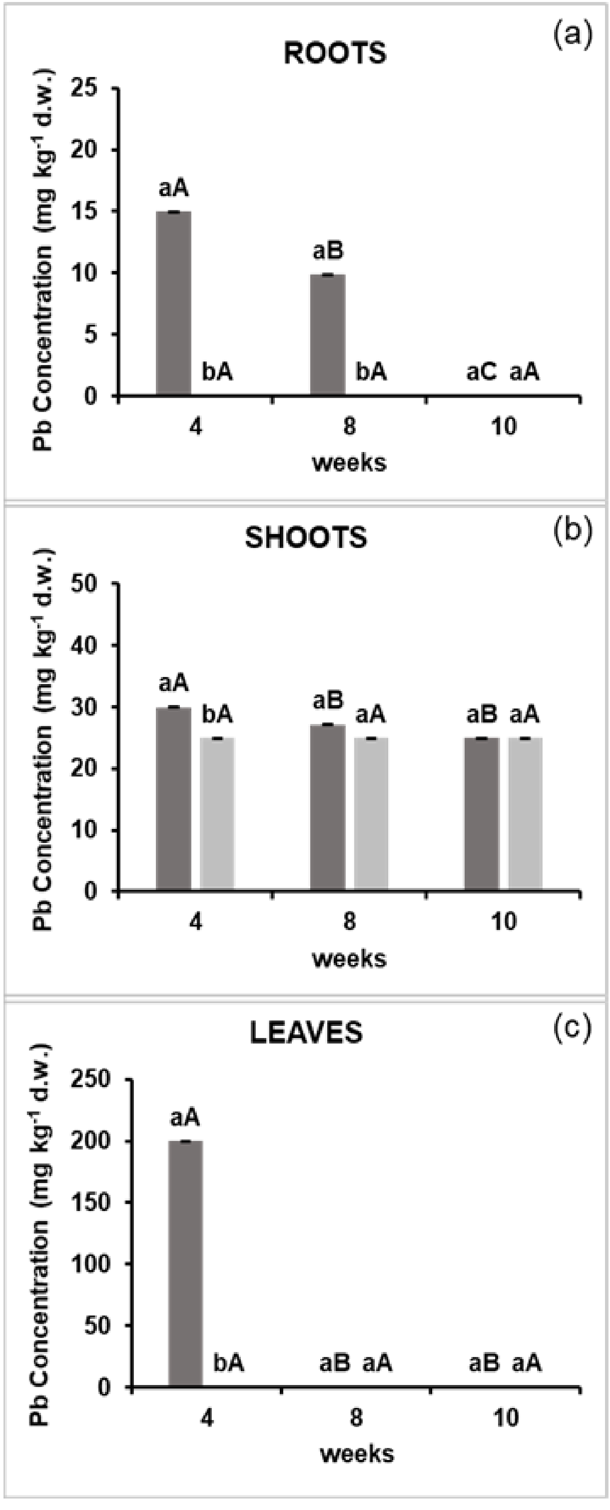

Fig. 5 Lead concentration in basil roots, shoots, and leaves dry weight (d.w.) after irrigation with Pb contaminated water (■) and tap water (■) during the experiment period. Different lower-case letters denote statistically significant differences among contaminated and non-contaminated samples of the same week, whereas upper case letters denote statistically significant differences among contaminated and non-contaminated samples between the different weeks, respectively (P ≤ 0,05)

C. Foliar Applications

Cadmium concentrations increased to 3721.8 mg kg^{-1} of d.w. in new leaves, which were emerged 30 days after foliar application with CdO and sampled at the end of the experiment period (10^{th} week). the concentrations of Cd in the shoots and roots of the plants remained relatively low (1.99 mg kg^{-1} and 6.58 mg kg^{-1} of d.w., respectively), as shown in Table 1. the above measurements indicated that an adequate amount of Cd deposited to the aged leaves was moved and stored to the recently formed leaves. Even though there are few relative references, there is strong evidence that a portion of the Cd deposited in the leaves is transferred to other plant organs via the phloem. Reference [82] concluded that Cd translocation in metal hyperaccumulators might occur in two crucial steps. the first step is probably energy-dependent, while the second step is driven mainly by transpiration. This can explain the fact that although leaves are considered almost certainly irreversible Cd sinks [35], movement of the element can take place through the phloem, in organs where increased transpiration occurs, or in young tissues. However, the intensity of transport and storage of cadmium in younger leaves depends significantly on plant species. Reference [56] showed that when *Noccaea caerulescens* plants were exposed to very low Cd concentrations, the metal was mainly located in the young leaves, while exposure of plants to higher Cd concentrations led to an accumulation of the element in older leaves. in contrast, Cd accumulated both in young and old leaves when willow plants (*Salix viminalis*) were exposed to various Cd concentrations. the results of Cd accumulation in new leaves by foliar application in basil plants are comparable to those of *Lactuca sativa* [83].

Nickel concentrations rose dramatically in all plant organs following NiO deposition on plant leaves. Shoots accumulated the higher Ni concentration (5237.1 mg kg^{-1} d.w.) while new leaves the lowest (2745.4 mg kg^{-1} of d.w.). It is worth noting that the roots of the treated plants accumulate higher Ni concentrations than the newly formed leaves. These results are by several researchers [84]–[85] who stated that Ni could be readily transferred from sources to sinks since Ni is extremely phloem-mobile and can be moved both downward and upward during phloem translocation [65–66]. Reference [39] also reported the downwards movement of nickel to the root of the plant when $^{61}Ni^{2+}$ was applied to the old leaves. However, most of the elements exported from the old leaves (89%) moved upwards to the young leaves. the unimpeded movement of Ni through the phloem to other plants' organs is probably due to the inability to form Ni complexes in the phloem of some plant species. There is no available information concerning Ni complexation in the phloem sap of hyperaccumulators. Although in most of the Ni hyperaccumulator plants, the typical Ni concentration distribution follows the order leaves > stems > roots; Ni partitioning pattern that was transported and accumulated in different plant organs is strongly dependent among others on plant species [86].

Chromium accumulation increased significantly in new leaves and shoots after the deposition of CrO on the leaves of the plants. the highest concentration was observed in new leaves (1772.5 mg kg^{-1} of d.w.) followed by shoots (662.0 mg kg^{-1} of d.w.) (Table 2). in contrast, Cr concentration in the roots was significantly lower (29.5 mg kg^{-1} of d.w.) than that in the shoot and leaves. Many researchers [87]–[88] reported that Cr was carried into the xylem through the symplastic pathway and deposited in the cytoplasm of cortical cells at a relatively moderate rate. However, this way of upwards transportation prevents the element accumulation in the root when it is deposited on the leaves. the relatively high concentration of Cd in basil plant shoots is, in addition, explained from the fact that specific parts of the shoot are used for Cr accumulation to prevent this toxic element from reaching the photosynthetically active tissue [88]. According to the same author's assumption that leaf veins might be Cr accumulation tissue to reduce mesophyll damage from overexposure to Cr, the high concentration that has been measured in new leaves after plant dusting with CrO can be explained. However, the distribution and translocation of Cr within plants depend, among others, upon the plant species [7].

Lead foliar deposition caused a vast increase of Pb concentration in both new leaves and shoots 30 days after the treatment. the highest accumulation was measured in new leaves (4644.5 mg kg^{-1} of d.w.) followed by shoots (2094.2 mg kg^{-1} of d.w.), while the lowest concentration of this element was measured in roots (29.9 mg kg^{-1} d.w.) Foliar exposure to Pb can cause the metal to be internalized via stomata or firmly attached to the leaf surface due to its structure [9], [89]. the presence of PbO particles inside stomatal openings on adaxial and abaxial surfaces has been previously reported [89]. Except for entering the apoplast through stomata, dissolved particles (due to climate conditions such as high humidity) may infiltrate through aqueous pores of the cuticle following the hydrophilic pathway [60], [89]. Phyllosphere microbiota seems to play an important role, too, because of the interactions among microbes and Pb microparticles leading to concentration alterations [60]. Until today, all findings support the concept that Pb compartmentalization and speciation alter as a result of absorption and translocation processes happening in the plant, which differ depending on the mechanism of Pb–plant interaction [90]. However, there is a lack of information explaining the foliar Pd uptake and the mechanisms which are responsible for its foliar absorption. Reference [89] reported that the base of the central vein contained more Pd particles than the other leaf regions. This probably indicates that Pd, after entering the leaf via stomata, is transferred to the phloem cells and finally reaches the other aerial plant organs.

Basil plants contaminated with Ni, Cr, and Pb by irrigation or foliar deposition did not present the highest accumulation at the same plant parts by the end of the experiment, indicating that maybe there are different factors involved.

Table 1. Cadmium and Nickel accumulation in roots, shoots, and leaves of basil plant at the end of the experiment period, after foliar deposition of CdO and NiO

Plant Part	Group	Cd (mg kg^{-1})			Ni (mg kg^{-1})		
		MO	SD		MO	SD	
roots	control	0,00	0,00	b	0,00	0,00	b
	foliar deposition	6,59	0,63	a	4309,32	159,52	a
shoots	control	4,00	0,00	a	940,94	0,00	b
	foliar deposition	1,99	0,00	b	5237,14	222,68	a
leaves	control	3,00	1,40	b	240,00	0,00	b
	foliar deposition	3721,84	399,04	a	2745,37	133,40	a

Different lower-case letters among the columns denote statistically significant differences among contaminated and non-contaminated samples of the same plant part, respectively (P ≤ 0,05).

Table 2. Chromium and Lead accumulation in roots, shoots, and leaves of basil plant at the end of the experiment period, after foliar deposition of CrO and PbO

Plant Part	Group	Cr (mg kg^{-1})			Pb (mg kg^{-1})		
		MO	SD		MO	SD	
roots	control	15,01	0,93	b	0,00	0,00	b
	foliar deposition	29,52	4,79	a	29,91	0,00	a
shoots	control	18,52	2,12	b	24,91	0,00	b
	foliar deposition	661,97	194,30	a	2094,12	566,95	a
leaves	control	13,98	1,44	b	0,00	0,00	b
	foliar deposition	1772,49	257,84	a	4644,47	1351,33	a

Different lower-case letters among the columns denote statistically significant differences among contaminated and non-contaminated samples of the same plant part, respectively ($P \leq 0{,}05$).

VI. CONCLUSION

Shoots of basil plants, irrigated with water that had concentrations of Cd, Ni, Cr, and Pd similar to soil water or groundwater of the contaminated urban and suburban areas, can accumulate high amounts of all the above-mentioned heavy metals. in addition, the leaves of these plants are also organs with a high accumulation of Ni. Nickel is under the detection limits in the roots, while for Cd and Pd, the quantities found are under the detection limits in the roots and the leaves of these plants.

Basil plants irrigated with water that contained high concentrations of Cd, Ni, Cr, and Pd can accumulate high amounts of Cd in roots and leaves. Nickel and Pd concentrations rapidly increased in all plant organs after plants irrigation; however, after a relatively long period, the accumulation of these elements can remain in high levels, mainly in the roots of the plants, especially in the case of Ni. However, plants irrigated with high-concentration chromium water do not appear to accumulate more of this element in their roots and leaves than basil plants that are grown in urban or suburban environments, with heavy metal-laden water. in contrast, high Cr accumulation can occur in the shoots of these plants.

Basil plants that were contaminated with heavy metals due to the air pollution through dust deposition on their leaves can accumulate Cd, Cr, and Pd in young leaves, while the high concentration of Cr and Pd can also be accumulated in shoots. the strong upwards transportation of Cr and the hydrophilic transportation pathway of Pd possibly prevent their accumulation in the roots when they are deposited as pollutants on the leaves. Nickel can be distributed throughout the plant after the contaminant is deposited on leaves and practically can be rearranged in the plant's organs. Nickel concentration distribution followed the order stems > leaves > roots in basil plants whose leaves were contaminated with Ni.

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