

**The influence of toxic metals As, Cd, Ni and Pb on nutrients accumulation in *Mentha piperita***

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**Abstract**

Medicinal plants are, for a considerable part of the population, an important source of treatment for certain diseases. They contain essential nutrients for the human body such as magnesium, iron and zinc. The present study shows the influence of the addition of As, Cd, Ni, Pb on mineral nutrients in different organs of *Mentha piperita*. The experiments were carried out in the laboratory for a period of three months (May-July). *Mentha piperita* plants were AsCd and AsCdNiPb exposed up to final concentrations corresponding to the soil intervention threshold according to Romanian Order no.756/1997 (25 mg/kg As, 5 mg/kg Cd, 150 mg/kg Ni and 100 mg/kg Pb). Simultaneously with these experiments, a control experiment (M) was performed. To evaluate the effect of the addition of AsCd and AsCdNiPb on the accumulation and transfer of Ca, Cr, Cu, Mn, Mo, Fe and Zn, the transfer coefficient (TC), the translocation factor (TF), and the enrichment factor (EF) were calculated. A higher concentration of Ca, Cr, Cu, Fe, Mn, Mo, and Zn was observed especially in the mint root in the experiments in which AsCdNiPb was added compared to those in which only AsCd was added. The AsCdNiPb addition did not influence the translocation of micro and macronutrients from the root to the aerial (edible) parts of the plant. In the case of the AsCd, addition, the translocation of zinc from the root to the aerial parts (leaves and stem) of the plant was increased.

**Keywords:** *Mentha piperita*, metals, transfer coefficient, nutrients

**INTRODUCTION**

For most people, medicinal plants are the fundamental source for treatment of certain diseases. High concentrations of metals in sediments, muds and soils can pollute groundwater and can be transferred to plants, thus affecting animals and humans [1].

Certain metals, such as Cr, Co, Cu, Mn, Mo, Ni, Fe, Se and Zn are necessary for the optimal functioning of biological processes in animal organisms (including humans) being considered essential metals or micronutrients [2].

The bioavailability of metals in soil is an active process related to specific combinations of chemical, biological and environmental parameters. In soils polluted with heavy metals, plant growth can be inhibited by metal absorption. Certain plant species are able to accumulate large amounts of heavy metals, which poses a potential risk to animals and humans [3].

Copper is considered a micronutrient for plants and has an important role for the assimilation of CO<sub>2</sub> and the synthesis of ATP (adenosine triphosphoric acid). Copper is also an essential component of various proteins such as plastocyanin in the photosynthetic system and cytochrome oxidase in the respiratory electron transport chain. Mining and industrial activities have contributed to increasing the copper content in ecosystems. Copper is added to the environment through various

activities including the extraction and smelting of ores containing copper. Excess copper in the soil plays a cytotoxic role inducing stress and causing plant damage, resulting in delayed plant growth and leaf chlorosis. Oxidative stress disrupts metabolism and damages plant macromolecules [4].

Chromium is a heavy metal that causes serious contamination in soils, sediments and groundwater. Cr(VI) is a highly toxic substance, and has been shown to be carcinogenic by the International Agency for Research on Cancer (IARC), the Environmental Protection Agency (EPA) and the World Health Organization (WHO). Chromium toxicity has been studied in many plants. Excess chromium inhibits plant growth, produces chlorosis in young leaves, nutrient imbalance, wilting of the tips and breaking of the roots. Among the toxic effects of chromium in the growth and development of plants are included the alteration of the germination process as well as the growth of roots, leaves and stems. Chromium can have harmful effects on the physiological processes of plants such as photosynthesis, absorption of water and mineral nutrients [5, 6].

Cobalt occurs naturally in the earth's crust as cobaltite (CoAsS), erythritol [ $\text{Co}_3(\text{AsO}_4)$ ], or enamel (CoAs<sub>2</sub>). The increase in soil concentration may be due to the deposition resulting from the burning of fuels, the spreading of sewage sludge and manure. Plants can accumulate small amounts of cobalt in the soil. The absorption and distribution of cobalt in plants depends on the species and is controlled by different mechanisms such as ion-exchange mechanism and metabolism-independent and - dependent processes [7, 8].

Iron (Fe) is considered an essential micronutrient for plant growth. Iron is indispensable for synthesis and other cellular processes, among which such as respiration, chlorophyll biosynthesis and photosynthetic electron transport. Although iron is not considered toxic, its presence is significant due to its interaction with other metals that are toxic. Iron oxides absorb many elements and participate in lowering the level of heavy metals in plants. Excess heavy metals (and especially Mn, Ni and Co) lead to reduced iron absorption and translocation resulting in a decrease in chlorophyll. On the other hand, increased concentrations of iron and its compounds in the soil lead to a decrease in the assimilation of metals [9].

Some plants are tolerant to selenium showing a high ability to accumulate this element without symptoms of toxicity. They are able to convert selenium into bioactive compounds that play an important role in human nutrition and phytoremediation [10].

Depending on its role in the metabolic processes of plants, manganese can be an essential micronutrient but it can also be a toxic element when it is present in excess. In plant matter, manganese enters the structure of proteins and enzymes, insufficient amounts of manganese affecting photosynthesis. The toxicity of manganese is favored by acid soils. With the decrease of pH, manganese is found especially in the form of  $\text{Mn}^{2+}$ . This form of  $\text{Mn}^{2+}$  is available to plants and can be easily transported to root cells and translocated into shoots. Instead, at higher pH values are found mainly Mn (III) and Mn (IV), forms that cannot be accumulated by plants because they are not available. Very high concentrations of Mn in plant tissues can influence certain processes, such as enzyme activity, absorption, and translocation of other minerals (Ca, Mg, Fe and P), leading to oxidative stress [11 -13].

Zinc, although an essential nutrient for plants, can cause phytotoxicity in contaminated soils. High zinc levels in the soil inhibit many metabolic functions of plants leading to their delayed growth. Zinc toxicity can cause chlorosis in young leaves and older leaves can be affected by prolonged exposure to high concentrations of zinc in the soil. Chlorosis may occur in part due to iron - induced deficiency, as  $\text{Zn}^{2+}$  and  $\text{Fe}^{2+}$  ions have similar rays. An excess of zinc can also lead to a decrease in manganese and copper in plant shoots. Another typical effect of zinc toxicity is the appearance of a purple red color in the leaves, which is attributed to phosphorus deficiency.

Root absorption is the main route of metal transfer to the plant. The absorption of heavy metals depends on several characteristics of the soil such as soil pH, water content, organic substances present in the soil and the most important factor is the availability of metals in the soil. Heavy metals exist in soils in various forms, either as free ions or in the form of complexes bound to organic or inorganic substances, or in the form of compounds such as silicates, oxides or hydroxides.

Mint commonly known as aromatic plants of Lamiaceae family, family that includes *Satureja hortensis*, *Thymus serpyllum*, *Salvia officinalis*, *Ocimum basilicum*, etc. Although, not all mint species have therapeutic properties, *Mentha aquatica* (water mint), *Mentha viridis* or *Mentha spicata* (sweet mint) and *Mentha piperita* (peppermint) are recognized as mint species with medicinal properties. Mint is distributed especially in temperate and sub temperate regions and grows best in shady places. Cuttings or stolons with a very fast growth propagate mint.

In Europe, mint came into medicinal use in the mid-eighteenth century, the main symptoms treated being nausea, vomiting and gastrointestinal disorders. In addition to its therapeutic properties, mint also has insecticidal properties [14]. Due to its properties, mint is used in the pharmaceutical industry, food industry and cosmetics. Herbal phyto-therapeutic preparations are used in diarrheal disease, gallbladder diseases, in the fight against nausea and colic (including in the case of irritable bowel syndrome) [15].

In this study, the effect of the toxic metals As, Cd, Ni and Pb on the macronutrients (Ca and Mg) and micronutrients (Cu, Mn, Mo, Fe and Zn) in *Mentha piperita* was monitored in a laboratory study. In our previous study we evaluated the effect of As, Cd, Ni and Pb in *Mentha piperita* [16].

## EXPERIMENTAL PART

Mint seedlings (10 for each experiment) were planted in two pots for each experiment. A quantity of 5 kg of soil was added to each pot (amendment).

Initially, before planting, a physical-chemical analysis was made of both the soil (Table 1) in which the seedlings were planted and the water with which the plants were watered throughout the experiment (Table 2).

Three independent experiments were performed 1) control (M), 2) experiment with AsCd addition and 3) experiment with AsCdNiPb addition. The added metal content was calculated to reach the level of the intervention threshold of Order 756/1997 [17] (25 mg/kg dry weight d.w. As; 5 mg/kg d.w. Cd; 150 mg/kg d.w. Ni and 100 mg/kg d.w. Pb).

For the enrichment of the soil, standard solutions of As, Cd, Ni and Pb in 5% HNO<sub>3</sub> were used. The addition of elements was calculated considering the difference between the metal concentration to be reached and the initial concentration of element of interest in the soil. The enrichment of the soil with the metals of interest was done one week after planting the seedlings. Subsequently, the plants were watered only with tap water. The size of the seedlings before planting was 20-25 cm.

The experiments were developed over a period of 3 months (May-July). Monthly, soil and plant samples were collected (root, stem and mint leaves).

The soil samples were taken at 2 depths (0-6 cm and 6-12 cm), then they were air dried, ground, sieved and homogenized, retaining for analysis the fraction <150 µm according to the standards in force. To determine the total content of metals extracted in the aqua regia solution, approximately 2 g of soil was weighed, a mixture of 7 mL HNO<sub>3</sub> and 21 mL HCl was added and mineralized in an open system until the remaining liquid was clarified. The mixture was filtered, washed with distilled water and the obtained filtrate was collected in a 50 mL volumetric flask (SR ISO 11466/99 - Soil quality. Extraction of water-soluble microelements).

The plants, after harvesting, were washed with tap water and then with distilled water. After washing, the plants were separated into components (root, stem, and leaves) and dried in a lyophilizer (Christ Alpha 1-2 LD lyophilizer). After drying, the samples were subjected to a digestion procedure as follows: approximately 0.5 g of the sample was mineralized with 10 mL of nitric acid and 3 mL of hydrogen peroxide, ultrapure quality reagents. The glasses were covered with watch glass, initially digesting at room temperature for 24 hours to destroy organic matter (cold mineralization). After cold mineralization, digestion was performed in a microwave system with temperature and pressure control. The applied program limited the digestion temperature to 180 °C. The samples were filtered and brought to a 25 mL volumetric flask, the residue was washed with pure water and the resulting water was collected in the volumetric flask.

Metals concentrations in soil and plant samples were determined by inductively coupled plasma optical emission spectrometry using ICP-EOS AVIO 500 Perkin Elmer equipment.

All the analyzes were performed in duplicate, the results represent the average of two determinations.

**Table 1.** The results of the physical-chemical characterization of the soil, mg/kg d.w.

Indicator	Soil	Reference values for soils with sensitive uses [17]		
		Normal value (NV)	Alert threshold	Intervention threshold
Total nitrogen	12055	-	-	-
Total phosphorus	4173	-	-	-
Organochlorine pesticides	<0.01	<0.2	1	2
Triazine pesticides	<0.03	<0.1	1	2
Potassium	1872	-	-	-
Cadmium	<0.08	1	3	5
Cobalt	3.05	15	30	50
Copper	12.3	20	100	200
Nickel	13.4	20	75	150
Lead	4.8	20	50	100
Arsenic	1.29	5	15	25
Manganese	373	900	1500	2500
Zinc	23.55	100	300	600
Chromium	11.05	30	100	300
Calcium	108099	-	-	-
Magnesium	3171	-	-	-
Sodium	132	-	-	-
Molybdenum	1.02	2	5	10
Antimony	<0.18	-	-	-
Selenium	<0.3	1	3	5

Regarding other characteristics than metals, the soil had 6.7 pH value, a conductivity of 475  $\mu\text{S}/\text{cm}$  and 9.3% humus content.

**Table 2.** The results of metal concentration in watering water

Element	Tap water ( $\mu\text{g}/\text{L}$ )	Element	Tap water ( $\mu\text{g}/\text{L}$ )
As	<2.0	Zn	16.3
Cd	<0.4	V	<1.5
Co	<0.85	Mo	<2.0
Cr	<1.3	Ca	41800
Cu	5.1	Mg	4800
Ni	<1.0	Se	<3.3
Pb	<0.75	Sb	<0.9
Mn	2.6	Al	109
Fe	39.3	-	-

The concentration of metals in plants was compared with the normal range in plants found in the literature [18] according to the data presented in Table 3.

**Table 3.** Metal concentration in plants, mg/kg

Metals	Normal range in plants	Metals	Normal range in plants
Calcium	1830 – 2042.5	Iron	640 – 2486
Chromium	0.006 – 18	Manganese	15 – 100
Copper	0.4 – 45.8	Zinc	1 – 160

To evaluate the ability of the plant to accumulate metals in the soil and to translocate them from the root to the aerial parts (stem, leaves, flowers), the transfer coefficient (TC), the translocation factor (TF) and the enrichment factor (EF) were calculated [19, 20]. Plant species with TC / BCF and TF > 1 can be used in phytoremediation.

$$TC = [M] \text{ root} / [M] \text{ soil}$$

Where [M] = metal concentration

TC > 1: the plant accumulates metals

$$TF = [M] \text{ leaves, stem} / [M] \text{ root}$$

TF > 1: the plant translocate metals from the root to the aerial part (stem, leaves and flowers).

$$EF = [M] \text{ uncontaminated soil (plant)} / [M] \text{ uncontaminated soil (plant)}$$

EF > 1 higher availability of metals in contaminated soil

## RESULTS AND DISCUSSION

The results obtained for the physical-chemical characterization of the soil showed that all the analyzed parameters fall within the normal values for sensitive soils according to Romanian Law [17]. A low acid pH and a C/N ratio of 19.2 can be observed, which indicates microbial activity in the soil and the release of nitrogen available for the plants. In irrigation water, small amounts ( $\mu\text{g/L}$ ) of micro and macronutrients Cu, Mn, Fe, Zn, Ca, Mg and Al were observed. The tap water contained 41800  $\mu\text{g/L}$  Ca, 4800  $\mu\text{g/L}$  Mg, 2.6  $\mu\text{g/L}$  Mn, 39.3  $\mu\text{g/L}$  Fe, 16.3  $\mu\text{g/L}$  Zn. No toxic elements were determinate.

The results obtained for the concentration of calcium in mint showed that, in the AsCd experiment, calcium has the same values as in the control experiment. In the AsCdNiPb experiment, in June and July the concentration of calcium in the root was about 2.4 times (June) and 1.5 times (July) higher compared to the AsCd experiment. Although no additional Ca was added in any experiment, excepting de Ca from watering water, the results obtained indicate an influence on Ca accumulation due to the addition of AsCdNiPb. The normal level of Ca (2043 mg/kg) in plants was exceeded in all experiments: in control experiment (June) in AsCd (July) and AsCdNiPb (June, July). In the control experiment, the Ca concentration increases in the order root > leaves > stem, in AsCd the order is leaves > root > stem and in the AsCdNiPb experiment, the order of calcium accumulation in mint is root > leaves > stem (Fig. 1). Ca is an essential element for plant development involved in cell wall structure and stability of membrane and have an essential role in the plant tolerance to the stress conditions such as metal pollution [21]. The need of Ca increased in the period of plant maturity observed in June and July in case of both experiments. The Ca absorption is more efficient in case of experiments compared to the control experiment. This observation could be related with the role of Ca in the reduction of metal toxicity due to the plants metabolic mechanisms to extract specifically the essential elements.

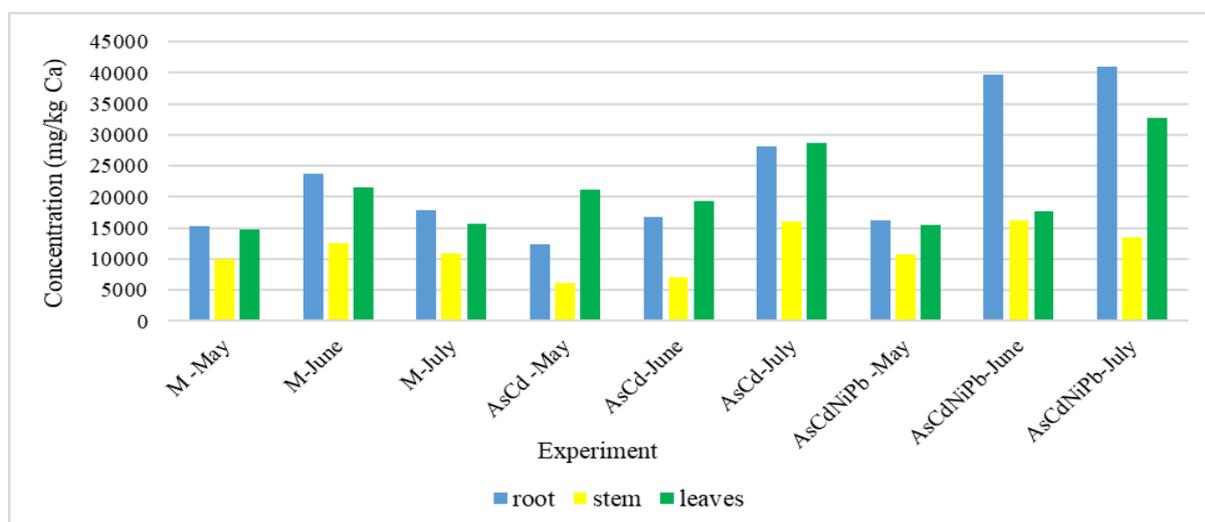
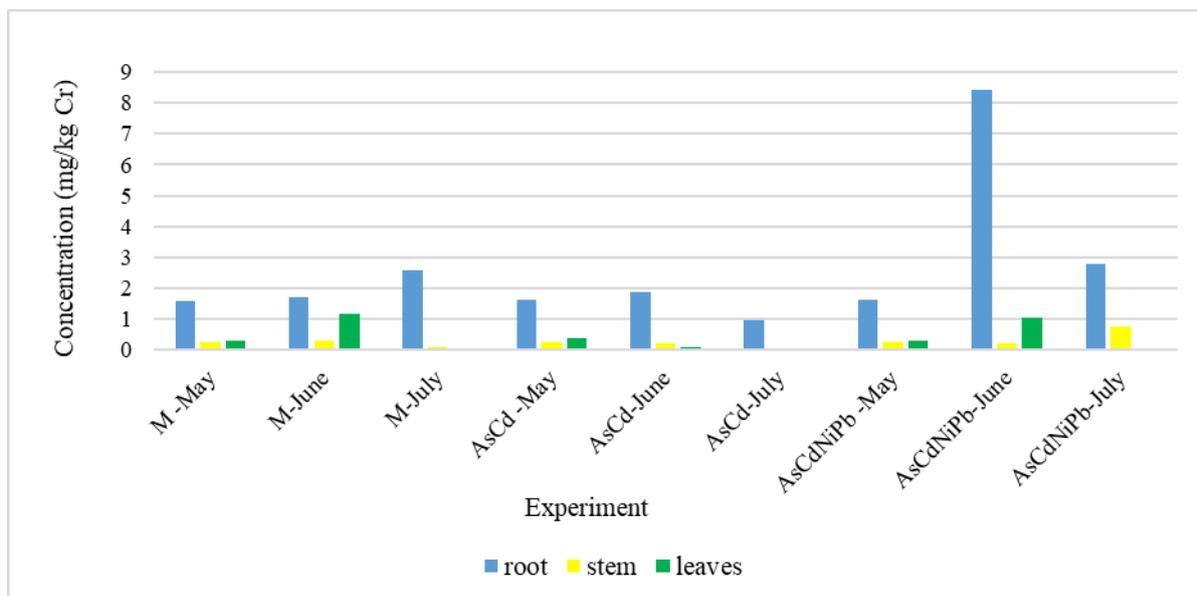


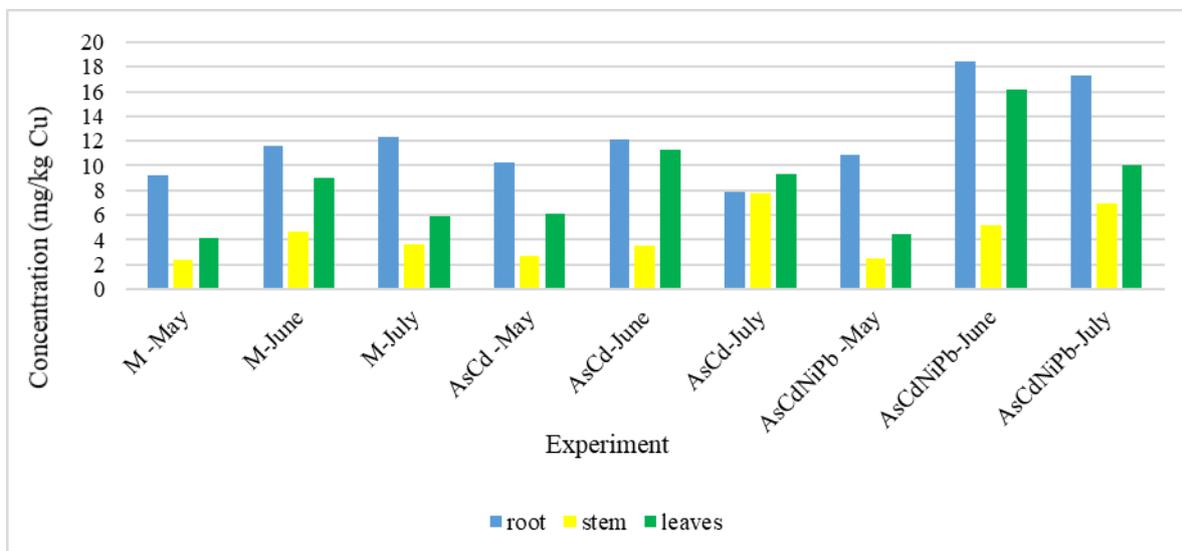
Fig. 1. Ca content in different parts of *Mentha piperita*

The Cr concentration (Fig. 2) recorded a maximum value in June, in the root (8.44 mg/kg) for the AsCdNiPb experiment. It was observed that in all experiments the highest concentration of Cr remained in the root, a very small amount migrating to the leaves or stem.



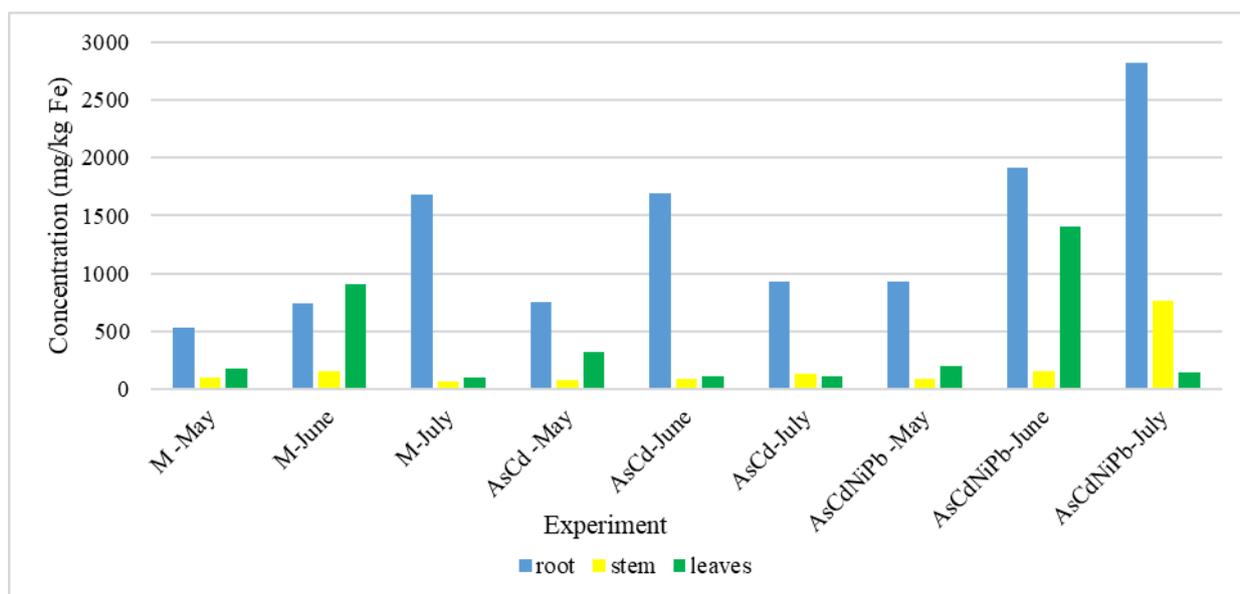
**Fig. 2.** Cr content in different parts of *Mentha piperita*

Copper have the role in the enzymatic activities in plants and for chlorophyll and seed production. The Cu concentration (Fig.3) increased in the order root > leaves > stem in all experiments (M, AsCd and AsCdNiPb). As in the case of Ca, a higher concentration was observed in the root of AsCdNiPb experiment compared to the AsCd experiment.



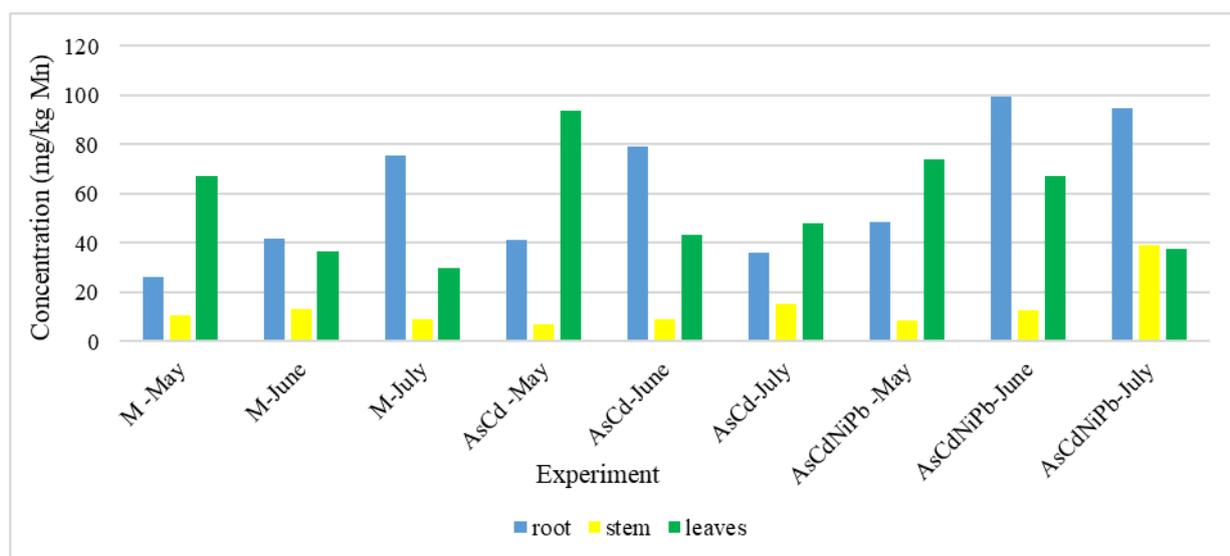
**Fig. 3.** Cu content in different parts of *Mentha piperita*

Iron (Fig. 4), like Ca, Cr and Cu, accumulates mainly in the root compared to the leaves and stem. The highest concentration of iron is in the root, in July, in the case of the AsCdNiPb experiment being 3 times higher compared to AsCd and 1.7 times higher than the control sample. It can be said that the addition of AsCdNiPb influenced the bioavailability of iron. In the leaves, the highest concentration of Fe was in June in AsCdNiPb. In the AsCdNiPb experiment, Fe had a behavior similar to the control sample, the Fe concentration increasing in the root from one month to another (May < June < July); in leaves May < June > July. In AsCd, unlike the other experiments, the maximum value at the root was in June.



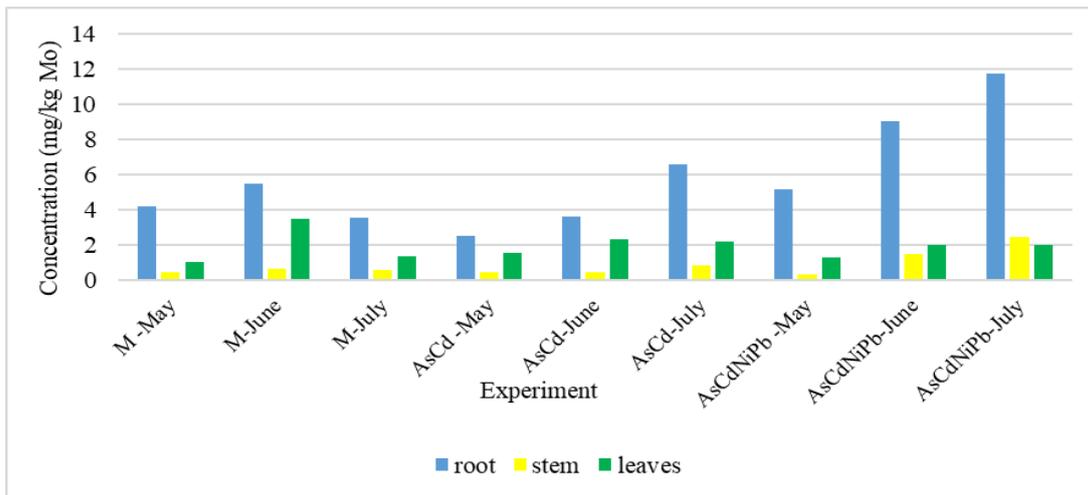
**Fig. 4.** Fe content in different parts of *Mentha piperita*

In the case of manganese (Fig. 5), in May, a higher concentration was observed in the leaves compared to the root and stem at M, AsCd and AsCdNiPb. The normal concentration in plants (100 mg / kg) was not exceeded in any experiment but was very close to it in May (AsCd), in June and July (AsCdNiPb). Although Mn was not added in any experiment, it was observed that this element accumulates differently in the AsCd experiment than in the AsCdNiPb experiment.



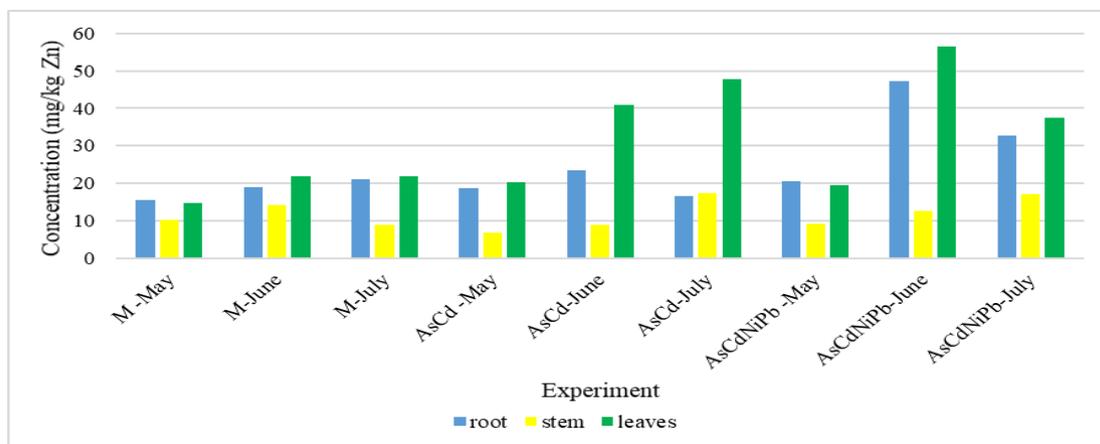
**Fig. 5.** Mn content in different parts of *Mentha piperita*

Molybdenum (as well as Ca, Cr, Cu and Fe) accumulates more in the root compared to the other parts (root > leaves > stem) (Fig.6). The addition of AsCdNiPb influenced the accumulation of Mo in the mint root, the concentration of Mo in the mint root being 2.5 times (in June) and 1.8 (in July) higher in the AsCdNiPb experiment compared to AsCd.



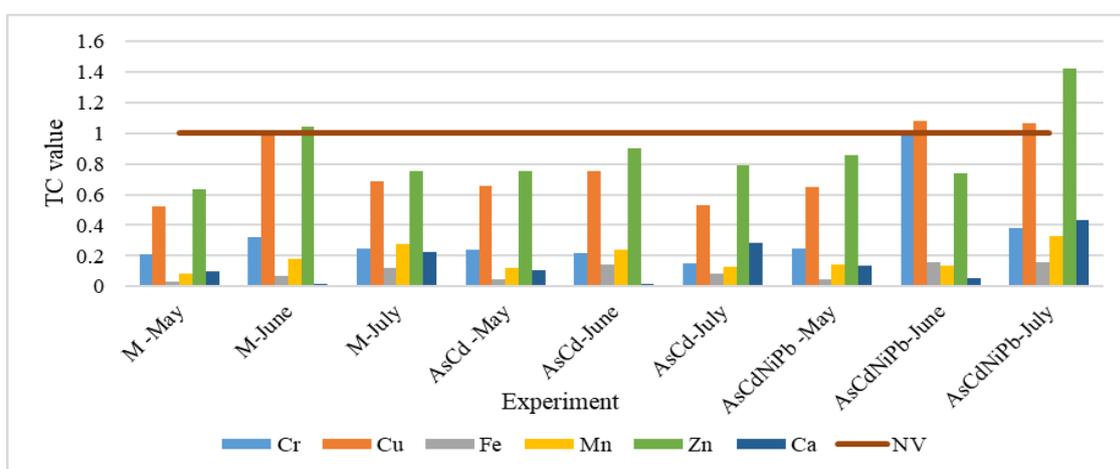
**Fig. 6.** Mo content in different parts of *Mentha piperita*

For zinc, a higher accumulation in the leaves was observed compared to the root and the stem (Fig.7) in the AsCd and AsCdNiPb experiments in June and July.



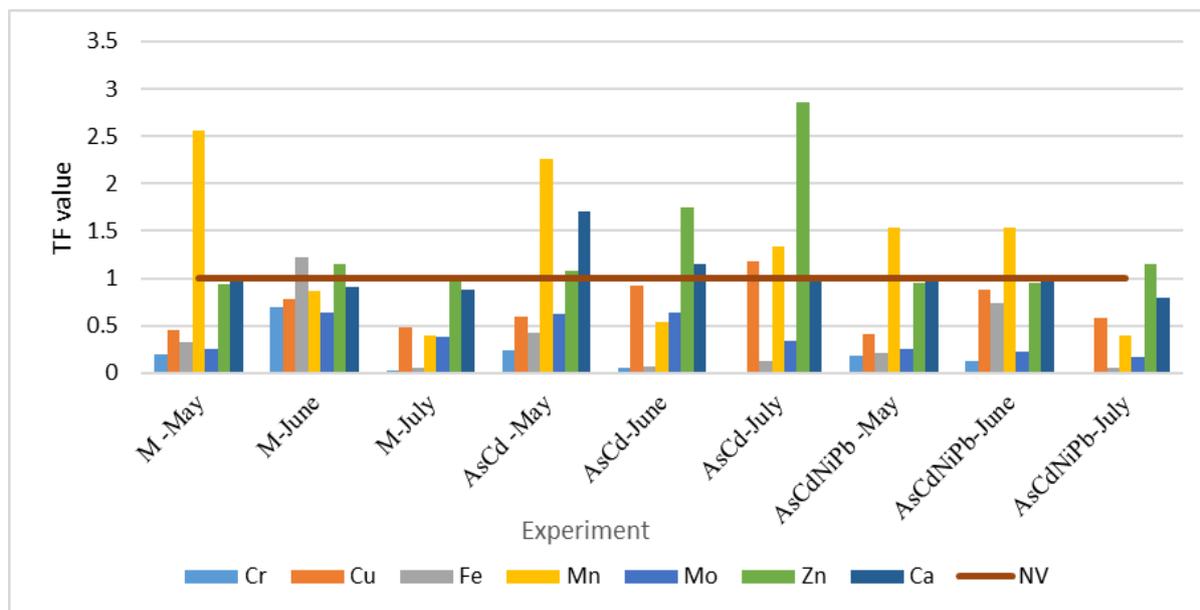
**Fig. 7.** Zn content in different parts of *Mentha piperita*

The transfer coefficient (TC) was represented in Fig.8. The elements with TC values above 1 were Cu (in control experiment in June and in AsCdNiPb in June and July) and Zn (in control experiment in June and in AsCdNiPb in July). Cr showed values between 0.15 and 0.99. For Pb TC had very low values between 0.03 and 0.16. Mn shows TC values between 0.08 and 0.33. Ca, just like Fe and Mn had small values between 0.10 and 0.44.



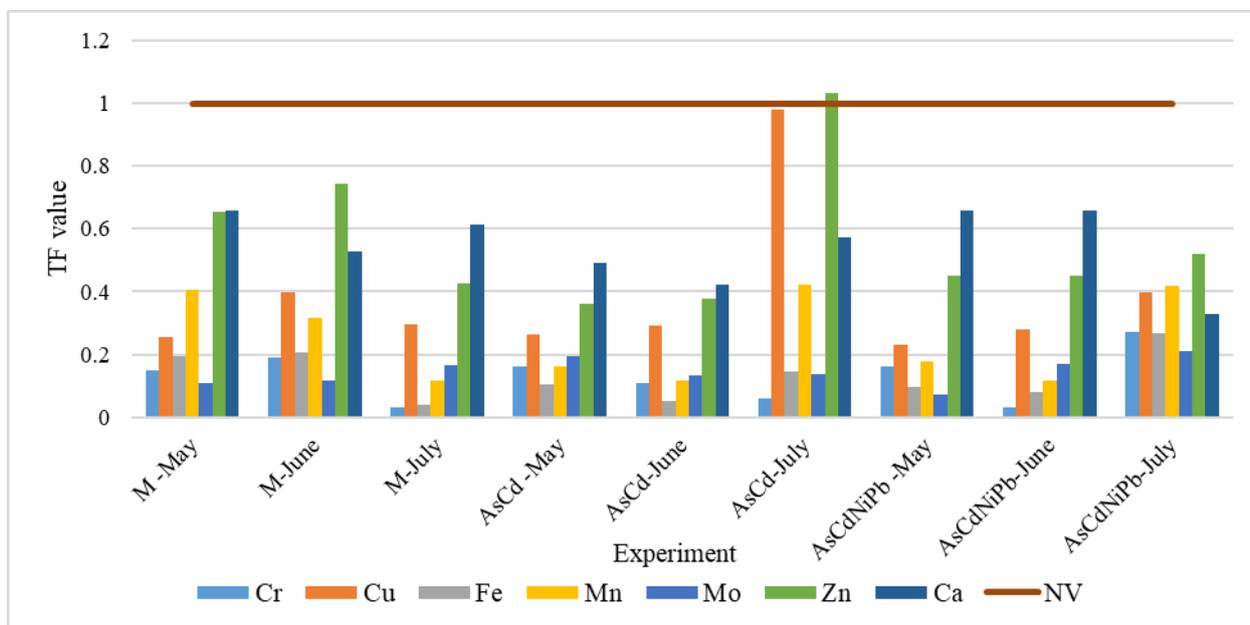
**Fig. 8.** Transfer coefficient of metals from soil to root

The ability to translocate metals from root to leaves (Fig. 9) showed that Mn, Fe, Ca, Cu and Zn accumulated more in the leaves compared to the root (TF values above 1), in certain stages of experiment. Cr and Mo, in the experiments studied, were not translocated from root to leaves.



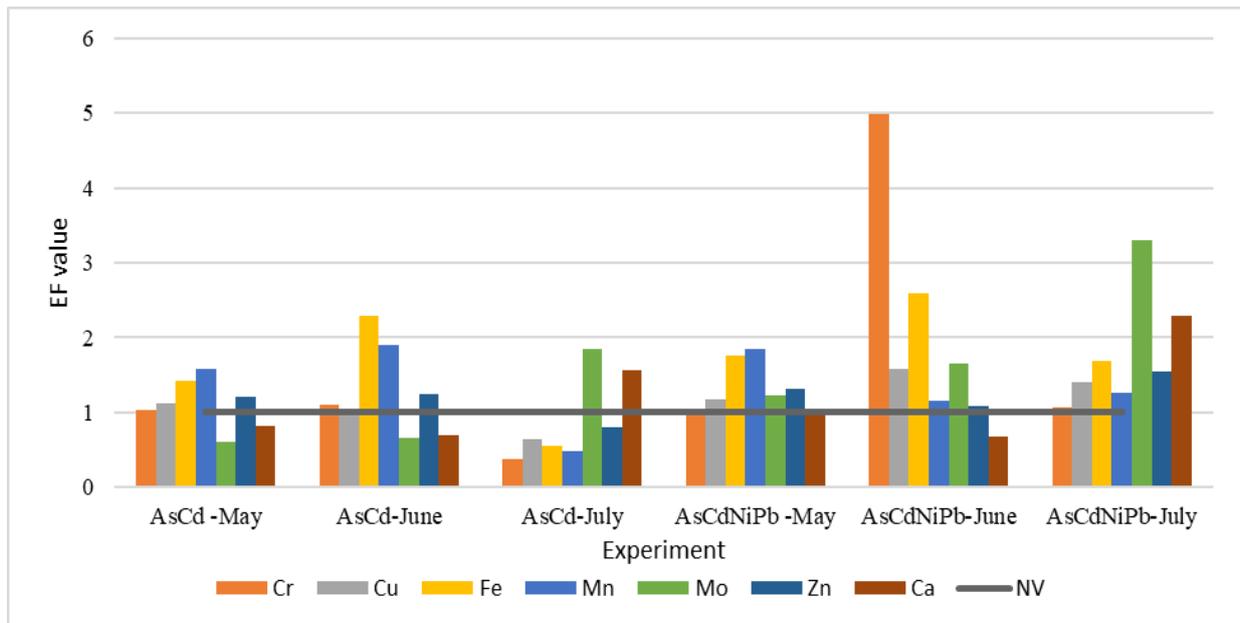
**Fig. 9.** Translocation factor of metals from root to leaves

Unlike root to leaves transfer, in the case of root-stem transfer, only zinc had a value above 1 in the AsCd experiment in July (Fig. 10). Translocation of metals from root to aerial parts of the plant (stem, leaves) was not influenced by the addition of AsCdNiPb. When only AsCd has been added to the soil, an increase in the transfer of zinc from the root to the leaves and stem was observed.



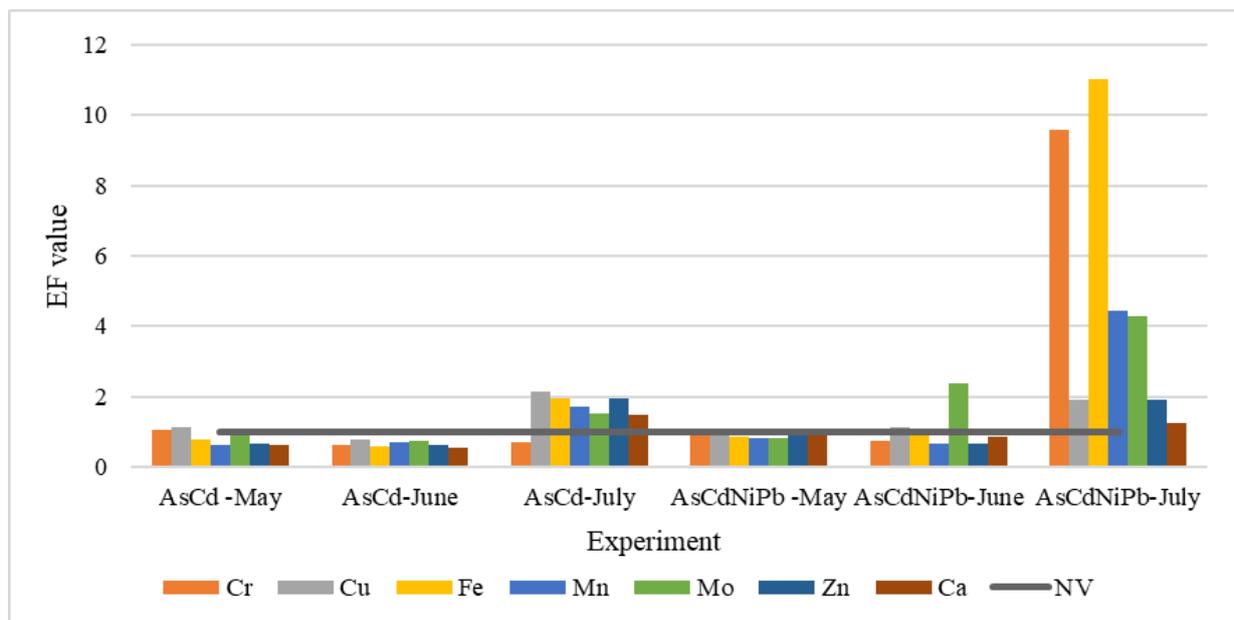
**Fig. 10.** Translocation factor of metals from root to stem

Enrichment factor (EF) was used to assess the level of accumulation of metals in the contaminated plant compared to plants grown on unpolluted soil. EF in root showed the maximum value for Cr in AsCdNiPb experiment in June (Fig. 11). From Fig.11 it was observed that all the essential elements studied showed values of EF in the root, higher than 1.



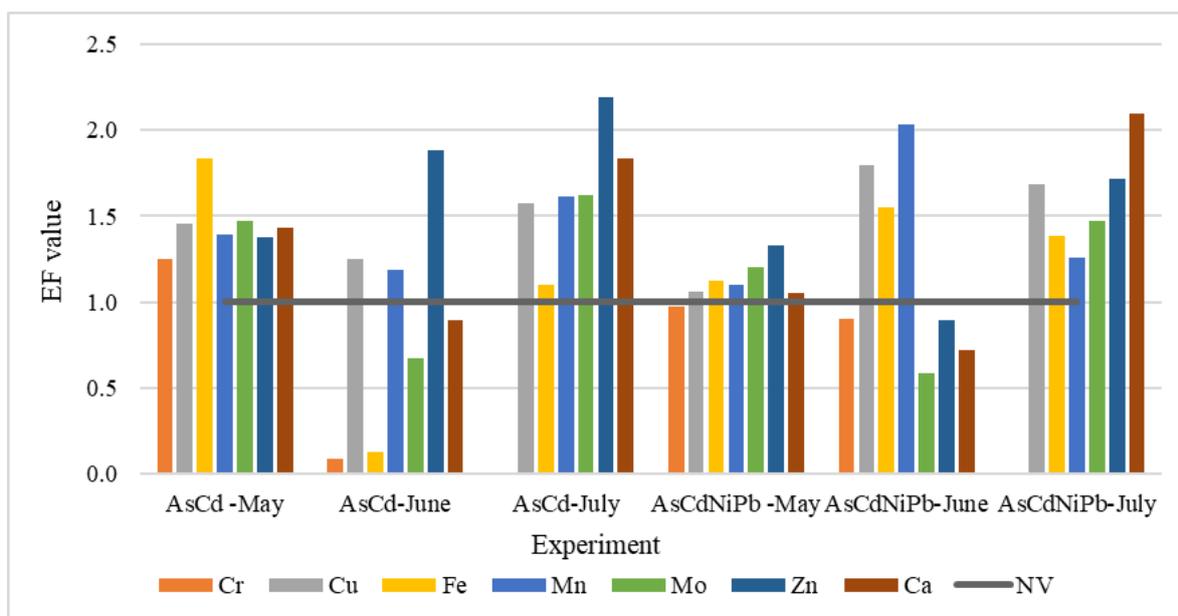
**Fig. 11.** Enrichment factor of metals in root

EF in stem (Fig.12) had the maximum value for Fe in AsCdNiPb in July. In July, in AsCdNiPb experiment all studied elements in stem were presented higher values than 1. In this study, the addition of AsCdNiPb influenced the enrichment factor in stem for the elements Cr, Cu, Fe, Mn, Mo and Zn.



**Fig. 12.** Enrichment factor of metals in stem

EF in leaves, as well as EF in root showed values above 1 for all the elements studied. The maximum value of EF in leaves was 2.19 for Zn in AsCd in July.



**Fig. 13.** Enrichment factor of metals in leaves

There were no significant differences in the enrichment factor of the essential metals in the leaves in the case of the addition of AsCd compared to AsCdNiPb. Mint have developed defense mechanisms against toxic elements through the specific absorption of nutrients that compete with them. It is observed the concentration of these nutrients at the level of roots and leaves that are the main centers of physiological processes. Certain metallic nutrients (Zn, Ca, Mg and Mn) are detected in higher concentrations in plants subject to Cd pollution for example indicating an antagonistic or concurrenial behavior and Cd toxicity limiting [22].

## CONCLUSIONS

The addition of AsCd in the soil on which mint seedlings were planted did not influence the accumulation of essential nutrients (Ca, Cr, Cu, Fe, Mn and Mo) in the plant. In the case of zinc, the concentration was 2 times higher in AsCd compared to the control sample. Another influence of the addition of AsCd was to increase the translocation of zinc from the root to the aerial parts of the plant (leaves, stem).

For the addition of AsCdNiPb, an increase in the accumulation of Ca, Cr, Cu, Fe, Mn, Mo and Zn was observed compared to the control sample. The addition of AsCdNiPb did not influence TF and TC, but increased the enrichment factor in the stem for Fe, Cr, Mn and Mo.

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