Behavior of Cd Accumulation in Sinapis alba L. in the Presence of Essential Elements (Ca, Mg, Fe, Zn, Mn, Cu, Ni)

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Abstract. Heavy metal toxicity in plants is well known due to their severe phytotoxic effects and also because of their capability to accumulate in vegetables. The use of aromatic plants in remediation techniques has increased in the context of environmental pollution issues, including metal soil contamination. Cadmium is knowed as a toxic and bio accumulative element provided by natural or anthropic sources. In this context, the paper presents a laboratory experimental study aiming to evaluate the accumulating and transfer behavior of Cd in the plant organs of Sinapis alba L. (white mustard) and in the presence of essential elements (Ca, Mg, Fe, Zn, Mn, Cu, Ni). The study involves a comparison between white mustard cultivated in unpolluted soil and in two Cd polluted soils at values above the alert (2.8 mg/Kg), respectively intervention threshold for soils with sensitive use (5.6 mg/kg) according to the legislation in force in Romania. While Cd accumulated predominantly in roots (TC values 1.46 and 2.22), its transfer to the stem and leaves was observed too, the TF values for the aerial part of the plant being greater than 1. Moreover, the study showed that certain elements (Zn, Ca, Mg, Mn) were found in higher concentrations in plants subjected to Cd pollution than in the control sample indicating antagonistic effects and Cd toxicity limiting. Other essential metals, such as Cu, Fe and Ni were found in lower concentrations in intoxicated plants compared to control plants. Their translocation from soil in plant organs could be reduced by the Cd toxicity. Contrariwise, the mobility of these elements from roots to leaves could support the tolerance effect of plants to Cd stress. The study allows us to consider that Sinapis alba L. aromatic plants are suitable for soil phytoremediation technologies used in Cd decontamination.

Keywords: Sinapis alba, mustard, cadmium, translocation factor, bioaccumulation, soil

1. Introduction

Natural processes, such as volcanic eruptions, continental dusts and anthropic activities namely mining, fossil fuel burning, fertilizer production, military activities, metal processing industry, etc. lead to emissions and accumulation of heavy metals in the ecosystem [1, 2]. Since the beginning of the industrial revolution, the biosphere pollution with toxic metals has increased dramatically. The widespread application of pesticides and fertilizers pose a serious problem for food production, and, last but not least, it represents a potential threat both for agriculture and life globally [3].

The earth's surface contaminated by large amounts of metal elements such as lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), in different combinations and concentrations, adversely affects the health of millions of people worldwide. While some metals are necessary for life (microelements), excessive accumulation of others in living organisms, such as heavy metals, is toxic. The most aggressive and irreversible heavy metals toxic effects are DNA damages or carcinogenic effects on animals and humans, due to their mutagenic potential [4].

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A real threat is that heavy metals are not degradable without outside intervention, as they remain in soil for centuries. Heavy metal contamination has reached toxic levels in air, soil and water in many parts of the world, and their removal has become an urgent problem in order to minimize the entering of toxic elements into the food chain. Conventional remediation techniques (other than bioremediation) used for in-situ and ex-situ remediation are usually costly and destructive. These include solidification and stabilization, soil washing, electro-kinetics, chemical reduction/oxidation, low-temperature thermal de-sorption, incineration, vitrification, pneumatic fracturing, excavation/recovery, storage and disposal [5].

In recent years, phytoremediation has been studied in particular due to the fact that it is an economically efficient soil detoxification method and it is in the same time an ecological method because it uses plants which hyper accumulate metal to extract heavy metals from polluted soils [6]. Generally, in phytoremediation studies, the soil contamination with metals was evaluated based on the plants ability to extract the metals from soil and transport them to their organs, quantified as transfer coefficients or translocation factors. The transfer or bioaccumulation coefficient (TC) is given by the ratio between the metal concentration in the root and the metal concentration in the soil, results expressed in mg/kg. A value greater than 1 indicates metal bioaccumulation in the root. The higher this coefficient, the more the plant extracts the metal and fixes it to the root [7].

The translocation coefficient (TF) represents the ratio between the metal concentration in the aerial components (stem, leaves, or inflorescence) and the metal concentration in the plant root. In this case, a TF value greater than 1 also indicates the bioaccumulation in that part of the plant and this should be taken into account when assessing the risk of using the plant for therapeutic purposes [7].

Numerous aromatic plants such as Salvia officinalis, Matricaria chamomilla, Ocimum basilicum, Lepidium sativum, Calendula officinalis were used as biological target for heavy metals phytoremediation studies [8].

More research has shown that plants from the Brassicaceae family (including Sinapis alba) can be used as heavy metals accumulators [9] due to their specific tolerance. Besides phytoremediation, white mustard provides antimicrobial activity and it could be used in order to improve crop yield and soil quality, acting as a natural fungicide and bacteriostatic [10].

Several studies regarding copper (Cu), nickel (Ni) and zinc (Zn) accumulation [10-12] have concluded that, for a moderate level of contamination, white mustard could be used for remediation of contaminated soil. A study on germination and development of mustard seeds in Cr-polluted soil indicated that white mustard is tolerant to high Cr levels and has hyper accumulative abilities [13]. Another study that looked at the effect of aluminum (Al) whose toxicity can inhibit mustard germination and development in acidic soils indicated that it does not accumulate in plant matter, but productivity may be impaired [14].

Cadmium (Cd) is an environmental contaminant introduced into the soil through anthropogenic activity. Under the stress due to Cd, hyper or non-hyper accumulative responses of plants differ depending on their morphological responses and physiological processes, such as photosynthesis and respiration, transport, absorption and assimilation of minerals and nitrogen, as well as water absorption and transport, all these contributing to the ability of plants to accumulate and detoxify Cd [15]. The cadmium in the soil can affect the plants that have reached maturity either from spontaneous or cultivated flora. As the soil has a more acidic pH, the mobility of metals increases, so they can be found either in the roots, in the aerial or inflorescence part of some plants [7].

Another study regarding the effect of Cd on mustard plants showed that it has an inhibitory effect on root development without influencing the growth and development of the plant [16]. The toxic effects of Cd on plant roots can be reduced by applying organic amendments to the soil, which lead to an increase in soil pH, lowering the solubility of Cd, while adsorption and complexation reactions reduce its bioavailability for vegetation [17]. The World Health Organization (WHO) imposed a limit of 0.3 mg/kg for Cd accumulation in medicinal herbs [18]. In addition, the European Commission established the limit of Cd in food supplements of about 1 mg/kg [19].
This paper presents a laboratory experimental study aiming to evaluate the accumulating and transfer behavior of Cd in the roots, stems and leaves of white mustard plants grown also in the presence of essential elements (Ca, Mg, Fe, Zn, Mn, Cu, Ni). The study makes a comparison between white mustard cultivated in unpolluted soil and in two Cd polluted soils at values above the alert, respectively intervention threshold for soils with sensitive use according to the legislation in force in Romania [20].

2. Materials and methods
2.1. Materials and reagents
Biological materials were mustard seeds, Sinapis alba L., batch number SIA230218 and SIA100715 provided by MicroBiotests Belgium. A universal substrate for plants, type Agro CS (40 L), was used for seeds planting. To assure the results validation, a certified reference materials (CRM) type Multi-element 21 (Ca, Cd, Cu, Fe, Mg, Mn, Ni, Pb, Zn), 100 mg/L (traceable to NIST, Merck) was used for metal calibration curves. A solution of 1000 mg/L Cd (CRM, Certipur, LGC) was used to enrich the substrate.

2.2. Equipment
A Mortar Grinder RM 200 - Retsch, a Lyophilizer Christ Alpha 1-2 LD (Martin Christ GmbH, Germany) and an Ethos Up Microwave Digestion Systems - Milestone were used for soil or plants samples preparation before metals determination. The Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) with an Optima 5300 DV Perkin Elmer spectrometer was used for determination of the metals.

2.3. Experimental conditions
The start of the experiment - Polluted soil grown with mustard (Sinapis alba L.)
Mustard seeds and universal substrate composed of peat soil and humus for garden plants were used. The substrate had the following characteristics mentioned by the producer: pH between 5.0 to 7.0; N total 1.9%, P total 0.5%, K total 0.9%, conductivity: 1.2 µS/cm and water humidity: 65%.
In order to establish the reference point of the experiment, the mustard seeds, the soil and the water used for watering were analyzed from a physical-chemical point of view. The determinations were performed on two replicates for each type of test, the average value (\( \bar{x} \)) being considered the reference value.

The metal content of mustard seeds (Table 1) indicates an initial Cd concentration of 0.13 mg/kg. The watering tap water (Table 2), as well as the soil used to grow mustard seeds (Table 3), did not presented an initial Cd content.

| Table 1. Mustard seed analysis, mg/kg |
|-------------------------------------|
| Element | Sample 1 | Sample 2 | \( \bar{x} \) | Element | Sample 1 | Sample 2 | \( \bar{x} \) |
| Ca | 4.088 | 4.106 | 4.097 | Mg | 3.158 | 3.153 | 3.156 |
| Cd | 0.13 | 0.14 | 0.13 | Mn | 21.5 | 21.5 | 21.5 |
| Cu | 5.75 | 5.71 | 5.73 | Ni | 1.87 | 1.12 | 1.50 |
| Fe | 62.2 | 77.9 | 70.0 | Zn | 49.3 | 42.3 | 45.8 |

| Table 2. Analysis of tap water used for watering the mustard plants, µg/L |
|-----------------------------|
| Element | Tap water | Element | Tap water | Element | Tap water |
| Ca | 41.8 | Fe | 39.3 | Ni | <1.0 |
| Cd | <0.4 | Mg | 4.8 | Zn | 16.3 |
| Cu | 5.1 | Mn | 2.6 |
The soil used in the in translocation and bioaccumulation experimental tests, the same as the one used in our similar design study, but made for the chamomile plant [21], has a pH around 6.4, with a C<sub>total</sub>/N<sub>total</sub> ratio of 17.52, indicating moderate mobility of nitrogen transfer to the plant. The metal concentration in substrate indicated normal values [20].

### 2.4. Experimental bioaccumulation and translocation tests

The experiment began in mid-April and finished two months later, in June. The approach of the experiment was designed to highlight the Cd effect on the germination and growth process of the mustard seeds by polluting the soil with two different concentrations of Cd (2.8 mg/kg and 5.6 mg/kg), concentrations situated at the alert threshold for sensitive use, respectively above the intervention threshold for sensitive use [20].

The soil used in the experiment was manually cleaned of solid materials, and the larger pieces of soil were manually crushed. About 800 g of soil were weighed for each experiment, with an initial humidity of about 69%.

The first experiment was considered as the control sample and a number of 33 mustard seeds were added to 560 g of dry soil for germination, the experiment being noted with M<sub>1</sub>. In order to verify the translocation and bioaccumulation of Cd in the mustard plants within the two tests for 2.8 mg/kg and 5.6 mg/kg Cd concentrations (M<sub>2</sub> and M<sub>3</sub>), the same amount of soil and seeds was used as in control test. All experiments were conducted at an ambient temperature in the range of 20°C - 25°C, from April to June, using natural light for about 12 hours/day.

### 2.5. Preparation of soil and plant samples for determining the metals concentration

The collection of soil and plant samples (whole plant with root, stem and leaves) was performed after each month of experiment (at 30 days and at 60 days).

The soil samples were air dried, grinded in the mortar, sieved and the fraction of less than 150 µm was retained for determining the total metal concentration. After harvesting, the plants were washed with distilled water, measured and dried in a lyophilizer to determine the metal content.

The metal content of the soil was determined after extraction in aqua regia (15 mL of 37% HCl and 5 mL of 65% HNO<sub>3</sub>) from approximately 1.0 g of sample. Afterwards the samples were filtered and diluted with ultrapure water to a constant volume of 50 mL and Cd amount was determined using the ICP-EOS technique [22].

Regarding the vegetation samples, after drying in the freeze dryer, each sample was minced, a quantity between 0.12 g and 0.32 g was weighed and mineralized with a mixture of suprapure HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in a ratio of 10:3 (v/v). The samples were initially mineralized at room temperature for 24 h to destroy organic matter (cold digestion), followed by a microwave digestion program according to the data presented in table 4 [23 - 25].
Table 4. Microwave program

| Stage | Power | Time     | Temperature       |
|-------|-------|----------|-------------------|
| Stage 1 | 1800 W | 15 minutes | up to 180° C |
| Stage 2 | 1800 W | 15 minutes | 180° C         |
| Stage 3 | -     | 10 minutes | cooling          |

After the mineralization was completed, the samples were filtered and diluted with ultrapure water to a constant volume of 14 mL. The metals determined both from soil and plant samples were the target metal (Cd), but also the metals playing a role in the plant nutrition and development, namely Cu, Fe, Mn, Ni, Zn, Ca, Mg.

3. Results and discussions

3.1. The translocation and bioaccumulation factors for Cd

Excess concentrations of Cd in soil lead to decreased germination [26]. This idea was confirmed by our tests. The germination of the mustard seeds was differentiated in the control soil sample (Figure 1) compared to the Cd contaminated tests. In the case of the M3 experiment, a percentage of 57.6% of the total planted seeds germinated (Figure 3), whereas in the case of the M2 experiment about 78.8% of the seeds (Figure 2) germinated.

It should be mentioned that in the control sample the germination percentage was 84.8%. Germination in the M2 test was 10% lower than in the control sample, respectively 32% lower in the case of M3 test compared to the control sample.

Figures 4 and 5 show the evolution of Cd concentrations in soil and plants within the two experiments compared to the control sample.

The Cd content in the plants from the M2 test has increased during the 60 days of the experiment, the final value being 10.2 mg/kg, value at the limit of the phytotoxic concentration (10 mg/kg)[27], with a value of the transfer rate of 3.15 for the whole plant (from the soil to the root and the aerial part).

As presented in Figure 5, after the first month the Cd values in mustard from blank M1, M2 and M3 samples were around the normal value in plants (<0.1 ÷ 1 mg/kg) [28], but after 60 days, at the second harvest, the total concentration exceeded the phytotoxic value of 10 mg/kg. The value recorded in experiment M3 is two times higher than that in plants from the M2 experiment.
Comparing the bioaccumulation model of Cd from the control sample (M₁) with that of the M₂, respectively M₃ contamination experiments, it can be observed that this toxic metal accumulates predominantly in the roots at values of the bioaccumulation coefficient ranging from 1.46 to 2.22. Nevertheless, the Cd translocation to the stem and leaves can be noted, the TF values for the aerial part of the plant being over 1. It should also be noticed that the values of the concentrations in the stems (3.39 mg/kg for M₂ and 9.98 mg/kg for M₃) and leaves (3.57 mg/kg for M₂ and 5.87 mg/kg for M₃) did not exceed the value of the phytotoxic concentration (10 mg/kg), but the stems of the mustard samples selected for the analysis in the M₃ experiment reached this phytotoxic value (Figure 6).

In most plant species, Cd mainly accumulates in roots, the concentration being about ten times higher than the concentration in the stems. Cd inhibits root and stem growth, affects nutrient extraction and it frequently accumulates in agricultural crops. It is an inhibitor of photosynthesis, as an effect of reducing the amount of chlorophyll and other pigments, but also by closing the stomata. The accumulation of Cd in the different parts of the plant is generally done in the following order: roots > stems > leaves > seeds [26, 29, 30].
expression, in the biosynthesis of proteins, nucleic acids, growth substances, chlorophyll and secondary metabolites as well as in carbohydrate and lipid metabolism [31, 32].

In the processes of germination and development of plants subjected to the stress of metal pollution, mechanisms of self-defense/resistance/adaptation of the plant to the metallic elements are activated. The presence of anti-stress factors, respectively high content of Ca, Zn and Mg, microelements necessary for the growth/development and the functioning of photosynthesis processes allowed the development of plants even in conditions of exceeding the value of the intervention threshold for sensitive uses in soil for Cd [33].

3.2. The translocation and bioaccumulation factors for Zn in the Cd experiments

Zinc is considered an essential metal for plant development, the normal concentration in plants being in the range: 15 ÷ 150 mg/kg [28]. Unlike the control test in which Zn bioaccumulated in the root, in the two experiments of Cd contamination, Zn bioaccumulated in the stem and leaves, a fact proven by the high values of TF (Figure 7).

Thus, in the first experiment with Cd, the translocation coefficient of Zn from root to leaf (TF = 3.01) is higher than that from root to stem (TF = 2.53). In contrast to this experiment, in the Cd contamination test at a concentration value of 5.6 mg/kg (value above the intervention threshold for sensitive use), Zn accumulated predominantly in the stem (TF = 3.11), compared to leaves (TC = 1.91), at values that also indicated bioaccumulation.

The cumulative concentration of Zn in the root, stem and leaves for the control sample (33.1 mg/kg) was much lower than the total value for the mustard plants used in the M2 experiment (76.7 mg/kg) and that in the M3 experiment (113.7 mg/kg). The more stress the plant undergoes due to the toxic concentration of Cd, the higher the concentration of Zn extracted. This element is chemically similar with Cd and have antagonistic effects, limiting Cd accumulation [34]. The total values of Zn from mustard plants in both experiments with different Cd concentrations were below the phytotoxic concentration value (200 mg/kg) [27].

3.3. The translocation and bioaccumulation factors for Ca and Mg in Cd experiments

Calcium is used by the plant for growth and development, being predominantly accumulated in the leaves and stem and less in the root. While the control sample showed a concentration of 118,000 mg/kg after 60 days, the samples collected from the M2 experiment showed a concentration of 390,000 mg/kg in the whole plant (root, stem and leaves), while the plants from the M3 test have presented a concentration of 488,000 mg/kg. The greater the stress due to the toxicity, the higher the amount of Ca extracted to compensate for the toxic effects (Figure 8). The Cd could be limited by Ca blocker and other protective proteins. In the situation of Ca and Fe deficiency, the Cd could be much easily uptaken in plants [15].

In the case of the M3 experiment it can be observed that the mustard plants accumulated more Mg in the stem (TFstem/root = 3.54) than in the leaves (TFleaf/root = 1.45), which represents a different behavior from the control test and from the M2 experiment (Figure 9). Within the M2 experiment, the bioaccumulation order is: TFleaves/root = 3.67 > TFstem/root = 3.37, the coefficients values being higher than in the control sample (TFleaf/root = 3.29 > TFstem/root = 2.27). Mg remains in the root in a low concentration directing itself mainly towards the stem and leaves. In the mustard samples from the control test, Mg was found in a concentration of approximately 1900 mg/kg, while in the contaminated tests the Mg values were significantly higher: 6,500 mg/kg in M2, respectively 7,000 mg/kg in M3. Therefore, the same observation as in the case of Ca is also valid for Mg.
3.4. The translocation and bioaccumulation factors for Mn and Fe in Cd experiments

A number of essential metals or micronutrients such as Cr, Co, Cu, Mn, Mo, Ni, Fe, Se and Zn are required for optimal functioning of biological and biochemical processes in plants [34]. In the case of Mn, it is observed that the translocation model for the control sample is also valid for the contaminated tests samples, namely: TF_{leaf/root} > TF_{stem/root} > TC_{root/soil}. Mn does not remain in the root; it goes mainly towards the leaves and less in the stem. Regarding the total Mn concentrations in the plant at 60 days, the Mn concentrations are not significantly different: 37 mg/kg Mn in the M2 test, respectively 40 mg/kg Mn in the M3 test compared to the control sample (35 mg/kg Mn). The translocation indices are greater than 1, indicating the accumulation of Mn in the stem and leaves. In M2 test: TF_{leaf/root} = 9.4 > TF_{stem/root} = 1.92; for the M3 test the values of the translocation coefficients have the following order: TF_{leaf/root} = 3.69 > TF_{stem/root} = 1.50 (Figure 10).

Other studies showed that Mn is a competitor for Cd using the same mechanism for transfer in plant [33].

In the case of Fe, the concentrations extracted from the mustard plants did not indicated bioaccumulation in any of the polluted and control tests (Figure 11). Fe directs itself to the plant especially in the stem and leaves. Comparing the values extracted from the control mustard plants (231 mg/kg), it is observed that in both Cd contamination tests, the plants extracted a total Fe value of about 190 mg/kg, representing a 17% decrease in the Fe concentration. Fe is an essential metal for chlorophyll production. These results proved that Cd contamination could have effects on the normal Fe accumulation that leading to photosynthesis inhibition [35]. After 60 days of Cd exposure, the
transfer factors are two times higher than in control test but lower than 1, indicating adaptive or tolerance process.

3.5. The translocation and bioaccumulation factors for Cu and Ni in Cd experiments

Cu extracted from the mustard plants grown in Cd pollution experiments recorded a value of the translocation coefficients from root to leaves greater than 1: $TF_{\text{leaf/root}} = 1.72$ in the case of the control sample; $TF_{\text{leaf/root}} = 2.19$ for M$_2$ experiment and $TF_{\text{leaf/root}} = 1.47$ for the M$_3$ experiment. It can be concluded that Cu goes mainly to the leaves then to the stem and less to the root.

From Figure 12 and Figure 13 it can be noted that the plant extracted the Cu and Ni nutrients that it needed from the soil. The total Cu concentrations in plants are in the normal concentration range, the values being between 8.61 ÷ 9.42 mg/kg, but below the value of the control test (15.3 mg/kg) and much below the phytotoxic value of 20 mg/kg [27]. The studies showed that Cd could reduce or increase the Cu uptake in plants organs [33]. In M$_3$ test, after 60 days, transfer factors were lower than M$_2$, indicating a reduced ratio of Cu mobility in mustard plants inversely proportional to the Cd concentration. The results showed also insignificant increases or decreases of Cu transfer comparing to the control test indicating an adaptive plant response to Cd toxicity.

Compared to Cd, which has no physiological function in plant development, Ni is essential for various metabolic process including photosynthesis [36]. Our study showed that Cd toxicity reduces Ni uptake through plants roots. Thus, total Ni concentrations in contaminated plants (6.6 - 6.8 mg/kg) are 30% lower than in the control sample (9.50 mg/kg), slightly above the normal maximum value in plants, 5 mg/kg [28]. The Ni mobility from roots to leaves indicates a tolerance mechanism to Cd toxicity.

4. Conclusions

In this paper, the bioaccumulation and transfer factors from a soil contaminated with Cd to the white mustard plant (*Sinapis alba L.*) have been studied in correlation with several essential metals (Ca, Zn, Mg, Mn, Cu, Fe and Ni). Cd predominantly accumulated in roots at values of the bioaccumulation coefficient of 1.46 (M$_2$) and 2.22 (M$_3$). These values increased as the initial Cd concentration added in the growing soil also increased. Cd translocation to the stem and leaves was observed too, the TF values for the aerial part of the plant being greater than 1. The values of the concentrations in the stems (3.39 mg/kg for M$_2$ and 9.98 mg/kg for M$_3$) and from the leaves (3.57 mg/kg for M$_2$ and 5.87 mg/kg for M$_3$) did not exceed the level of phytotoxic concentration (10 mg/kg), but the stems of the mustard samples selected for analysis from the M$_3$ experiment reached this phytotoxic value. Mustard plants have phytoaccumulation capabilities for Cd but their utilization as
food is not recommended because of Cd concentration exceeding the limits for aromatic or medicinal products.

The presence of essential metals at values within the normal range for soil increases the adaptation and tolerance of mustard plants to Cd toxicity, through competitive or antagonist processes. The study showed that certain metals (Zn, Ca, Mg, Mn) were found in higher concentrations in plants subjected to Cd pollution than in the control sample indicating antagonistic effects and Cd toxicity limiting. The greater the stress due to the toxicity, the greater the amount of extracted metal to compensate for the toxic effects (significantly higher values in the M₃ test compared to the M₂ test). Other essential metals, such as Cu, Fe and Ni were found in lower concentrations in intoxicated plants compared to control plants. Their translocation from soil to plant organs could be reduced by the Cd toxicity. Contrary, the mobility of these elements from roots to leaves could support the tolerance effect of plants to Cd stress. The study allows us to consider that Sinapis alba L. aromatic plant is suitable for soil phytoremediation technologies used in Cd decontamination.

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