

The effect of toxic metal As on the *Matricaria Chamomilla* L. medicinal plant

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*The paper presents an experimental laboratory study of the bioaccumulation of the toxic metal arsenic in the medicinal plant chamomile (*Matricaria Chamomilla* L.).*

The study makes a comparison regarding the bioaccumulation capacity of the chamomile plant in which arsenic is found as unique contaminant, as well as in mixtures of 2, 3 or 4 toxic metals (Cd, Ni and Pb) on a natural soil enriched with metals, compared to the chamomile plant developed on an unpolluted substrate. The tests followed the effects of soil pollution with metals on the germination and development of chamomile. The experimental results indicated that arsenic does not bioaccumulate in the chamomile plant, remaining in the soil. The experiment that was an exception is the one with arsenic as the only contaminant (E_1) in which at 90 days, the arsenic content in the chamomile plant was 3.58 mg/kg arsenic, the value that is within normal limits, below the phytotoxic value of 5 mg/kg, but was higher than that determined in the plant from the control test experiment (<0.75 mg/kg). The bioaccumulation factor (BCF) after 90 days, in all experiments, either by combination of metals or single contaminant had values lower than 1, indicating that the plant does not accumulate arsenic. The total chlorophyll from the results obtained indicates that the toxicity in the E_1 experiment is higher than in the metal mixture.

Keywords: *Matricaria Chamomilla* L., As, bioaccumulation coefficient (BCF), phytoremediation

INTRODUCTION

Contamination of soil, water and air with toxic metals as a result of anthropogenic activities, such as industrialization, urbanization, traffic, mining and agricultural activities is a threat to the environment [1-3].

In the context of ecosystem pollution, toxic metals require special attention, as they can generate hazards and risks to human health [1-6]. The level of contamination of air, water and soil with metal ions is a very important problem for human health, because on populations exposed to them in the long term can generate carcinogenic, mutagenic effects, etc., causing various diseases. On the environment, contamination with metal ions can cause the death of plants and trees by exceeding the phytotoxic concentration and, consequently, the depreciation of green areas in urban areas and agricultural areas. Under these conditions, simple ways of signalling the increase of metal ion concentrations above a certain threshold, and decontamination techniques are needed to minimize the risk and to avoid the real major consequences [2, 7, 8]. Agricultural areas are contaminated with toxic metals as result of pesticides (insecticides), fertilizers spreading and mismanagement of waste [9, 10]. The insecticides used are one of the forms of release of arsenic in the environment; it falls into the category of pollutants with high risk on the environment, being widely distributed in the environment.

Arsenic in the environment is found in the following soluble forms, inorganic form: respectively As (III) - arsenite (arsenic acid - H_3AsO_3 and its salts) and As (V) - arsenate (arsenic acid - H_3AsO_4 and its salts) and organic form: monomethyl arsenic acid (MMAA) and dimethyl arsenic acid (DMAA), organic compounds are less toxic than inorganic compounds [2, 10-13]. Inorganic arsenic is found in herbicides, insecticides, applied to various crops. They are pesticides that are produced by introducing arsenic in the composition, used to eradicate pests of crops [1, 10-16].

The toxic effects of arsenic on long-term exposure of the human body place it in group I carcinogenicity (cancer of the skin, lung, liver and bladder), says the International Agency for Research on Cancer (IARC), and acute arsenic poisoning causes damage to central nervous system, sometimes coma or even death [1, 2, 17].

Arsenic is not an essential metal for plant growth and development, but it can accumulate in plants at toxic levels. Arsenic contamination of the human food chain is a global concern. Food has been identified as a major source of contamination of the population with arsenic, along with the consumption of contaminated drinking water [18].

Metals persist for a long time in the soil because they are non-biodegradable, they cannot be degraded either by microbial action or by chemical action. Toxic metals are fixed in the soil depending on the composition (nitrogen and phosphorus concentration) and pH of the soil. In contaminated areas, the areas are decontaminated by biological methods. Phytoremediation is a biotechnology that uses various plant species to clean soils contaminated with metals. Depending on how the plant reacts to the contaminant, there are three phytoremediation techniques, namely by phytoextraction, phytostabilization and phytovolatilization. Depending on the plant's response to metals, plants are classified into three types: exclusion plants, bioindicator plants and accumulating or hyper-accumulating plants that can take over or store significant amounts of metals in their underground and aerial parts, being used extensively in remedying contaminated soils [5, 7, 8, 18-23].

Phytoremediation represent the ability of plants to absorb metals from the soil through the roots and transport them to the aerial parts of the plant. The parameters that indicate the efficiency of this biotechnology are the bioaccumulation (BCF) or transfer coefficient (TF). BCF represents the ratio between the metal concentration in the root and the metal concentration in the soil. TF is given by the ratio between the metal concentration in the aerial components (stem, leaf or inflorescence) and the metal concentration in the root. Both indices indicate bioaccumulation, respectively translocation when the values are greater than 1 [5, 8, 13, 18-20, 23].

The consumption of herbal medicines is used more and more global, conducting numerous studies on the quality and safety of medicinal plants, so the WHO has imposed the determination of toxic metals in plant species used for medicinal, aromatic and food use [24-26]. Chamomile (*Matricaria Chamomilla* L.) is one of the most appreciated medicinal plants in terms of biologically active components, which is part of the Asteraceae family and is represented by two common varieties, the German chamomile (*Chamomilla recutita*) and the Romanian chamomile (*Chamomilla nobile*) [27-29].

The aim of the paper is to carry out a study to evaluate the accumulation capacity of arsenic metal in the medicinal plant of chamomile based on the bioaccumulation factors (BCF) and translocation factors (TF). The study makes a comparison regarding the bioaccumulation capacity of the chamomile plant in which arsenic is found as unique contaminant, as well as in mixtures of 2, 3 or 4 toxic metals (Cd, Ni and Pb) on a natural soil enriched with metals, compared to the chamomile plant developed on an unpolluted substrate.

MATERIALS AND METHODS

Chemicals and reagents

Chamomile seeds (*Matricaria Chamomilla* L., AGROSEL producer) and universal plant substrate (Agro CS producer), enriched with nutrients N and P [5], were used.

The calibration curve was drawn with a Certified Reference Material (MRC) type Multielement 21, 100 mg/L (Sigma-Aldrich) for the metals tracked and a Reference Material (RM) Quality Control Standard 21, 100 mg/L (LGC) were used.

The metal enriched soil from the experiments was produced using Certified Reference Materials (MRC) of As, respectively of Cd, Ni, Pb, of 1000 mg/L (LGC).

For the determination of metals all, the following reagents and chemicals were used: hydrochloric acid 37% (Sigma-Aldrich); nitric acid ultrapure grade 69% (Merck); hydrogen peroxide suprapur 30% (Merck); ultrapure water.

Equipment

For pretreatment of soil and chamomile plants were used Lyophilizer Christ Alpha 1-2 LD (Martin Christ GmbH, Germany), Mortar Grinder RM 200 (Retsch) and Ultrapure water system Elix Technology Inside with an Quantum ICP Polishing Cartridge (Milli-Q, France).

Determination of the metal content in soil and chamomile samples was performed on an inductively coupled plasma optical emission spectrometer (ICP-OES) type AVIO 500 Perkin Elmer.

Experimental design

The soil was contaminated with arsenic (a unique contaminant and a mixture of metals) and cultivated with chamomile (*Matricaria Chamomilla* L.) to follow the effect of pollution.

Six laboratory experiments were performed in pots, two control samples (M_1 and M_2), and four experiments following the behavior of chamomile under arsenic pollution stress, either as the single pollutants (E_1), or in combinations with other metals (E_2 , E_3 and E_4) in order to assess the migration of metals from soil to the plant.

The nominal concentrations tested were 20 mg/kg dry weight (d.w.). As (E_1), 20 mg/kg d.w. As+6 mg/kg d.w. Cd (E_2), 20 mg/kg d.w. As+6 mg/kg d.w. Cd+165 mg/kg d.w. Ni (E_3), respectively 20 mg/kg d.w. As+6 mg/kg d.w. Cd+165 mg/kg d.w. Ni+105 mg/kg d.w. Pb (E_4).

Chamomile seeds, soil and tap water used to irrigate plants were analyzed. The concentration of toxic metals (As, Cd, Ni, Pb) and the essential ones needed for plant growth and development were determined, the detailed concentrations were founded in our previous paper [5]. The initial conditions of the experiments were identical, they had the same amount of soil (750 g for pots), seed (0.2 g for pots), humidity (69%), irrigation regime, temperature, brightness (between April and July).

Germination experiments

Initially the quality of chamomile seeds was tested by checking their germination on a reference soil, an artificial soil with a composition indicated in the Phytotoxkit microbiotest (lot no. OERS050419). Chamomile germinated in an estimated 100% after about 2 days of incubation and formed roots.

For the evaluation of the soil quality, the legislation provides values considered alert thresholds and intervention thresholds for sensitive uses, respectively less sensitive for several specific indicators both organic and inorganic [30].

The concentrations of initial metals found in the soil used in laboratory experiments are:

As 1.8 mg/kg d.w., Ni 13.7 mg/kg d.w. , Pb 4.74 mg/kg d.w., and Cd below the limit of determination of method (<0.08 mg/kg) and for the simulation of a polluted soil it was necessary to enrich it with additions of metals of interest, to determine the total content of metals in the soil at the end of the experiments, at the level of the alert threshold for sensitive use (As 15 mg/kg d.w., Ni 75 mg/kg d.w., Pb 50 mg/kg d.w., and Cd 3 mg/kg d.w.), respectively of the intervention threshold for sensitive use (As 25 mg/kg d.w., Ni 100 mg/kg d.w., Pb 100 mg/kg d.w., and Cd 5 mg/kg d.w.), according to legislation [30], and the phytotoxic value of arsenic in plants being 5 mg/kg, enrichment solutions were applied by spraying [5; 24-25].

Sampling of soil and chamomile plants and chemical analysis

Determination of the total content of metals in the soil was performed from a sample amount of 1.5 g after extraction in aqua regal with HCl 37% and HNO₃ 65% in a ratio of 9:1 (v/v) and was analyzed by ICP-EOS spectrometer [32]. The samples before being dissolved in aqua regal, were air dried, ground, sieved and harvested in double sample [5], after extraction were filtered and brought to a constant volume of 50 mL.

The determination of the total metal content of the chamomile plant samples it was done from a sample amount of 0.5 g, washed with ultrapure water, dried in lyophilizer, chopped and harvested in duplicate [5]. Samples were harvested at the end of each month. The extraction was performed by an open system digestion method consisting of a mixture of HNO₃ 65% and H₂O₂ in a ratio of 10:3 (v/v) for 24 hours at room temperature, then filtered, brought to a volume constant of 25 mL and analyzed by ICP-EOS technique [5, 31, 32].

Chlorophyll measurement

The analysis of the chlorophyll content was performed only after the first month of the experiment (30 days). They were analyzed using UV-VIS VWR UV-6300PC spectrometer at 662 nm and 645 nm [5].

The way in which the contamination of the all experiments with As by irrigation of the chamomile plants corresponding to each month of monitoring was performed is presented in table 1.

Table 1. Irrigation mode of experiments

Experiment	Metals	Irrigation		
		0-30 days	31-60 days	61-90 days
E ₁	As	mixture of metals in tap water	Tap water*	mixture of metals in tap water
E ₂	As+Cd	mixture of metals in tap water	Tap water	mixture of metals in tap water
E ₃	As+Cd+Ni	mixture of metals in tap water	Tap water	mixture of metals in tap water
E ₄	As+Cd+Ni+Pb	mixture of metals in tap water	Tap water	mixture of metals in tap water

*Tap water it means As free

RESULTS AND DISCUSSIONS

Experimental bioaccumulation tests for As from chamomile plants

The As experiment was designed to highlight the separate effects of the toxic metal on the process of germination, development and bioaccumulation, as well as the effects due to the use of several mixtures metals (Cd, Ni and Pb) on the same processes, on a natural soil enriched with metals, compared to the chamomile plant sown in an unpolluted soil (control test). Six laboratory experiments were performed in pots, two of which were not contaminated, being considered control samples (M₁ and M₂), and the other four experiments followed the behavior of chamomile under the stress of arsenic pollution, either as the only contaminant (E₁), or by combinations of metals (E₂, E₃ and E₄) regarding the migration of metals from the soil to the plant.

Analytical concentrations determined in solution watering water and soil

The concentrations of As added to the soil by irrigation during the experiments are presented in table 2, highlighting the final concentrations in the soil corresponding to each monitoring month. The same concentration of arsenic was added in all experiments (E₁, E₂, E₃ and E₄), the water enriched with arsenic having a concentration of 19.8 mg/kg d.w. of As in the soil in the first 30 days. At the end of the 30 days, the total concentration of arsenic in the soil and in the plant is 21.6 mg/kg. Concentration of approximately 22 mg/kg d.w., I would keep it during the second month of the experiment, because the irrigation was done only with water. At the end of the second month of

the experiment (60 days) it was additionally irrigated with arsenic enriched tap water representing a concentration of 6.6 mg/kg d.w. The total arsenic concentration at the end of the experiment after 90 days of development was around 28 mg/kg.

Table 2. Analytical concentration of As added during the experiments, mg/kg d.w.

Seed concentration n	Initial soil concentration 0 days	Concentration in watering water		
		up to 30 days	60 days	90 days
<0.75	1.80±0.26	19.82±2.83	tap water As free	6.60±0.94
Final concentration in the soil				
		30 days	60 days	90 days
		21.61±3.09	19.63±2.80	28.22±4.03

The addition of arsenic added by irrigation aimed to highlight the effects on germination and development of chamomile in a soil periodically enriched with surplus As, which in real conditions can come in an area polluted by the contribution of rainfall that washes areas contaminated with arsenic (mining waste storage dumps or even a mining area).

Accumulation of metals in soil and chamomile plant

Arsenic concentrations in soil and chamomile plants developed in experiments E₁:As, E₂:As+Cd, E₃:As+Cd+Ni, E₄:As+Cd+Ni+Pb and in the control tests are represented graphically in the figures 1 and 2.

In the first month of the experiment, the concentration of arsenic accumulated more in the plant in the experiment in which As was the only contaminant or where the metal mixture was composed of fewer metals, the order of accumulation in the plant being: E₁> E₂> E₃ > E₄, represented graphically in figure 1.

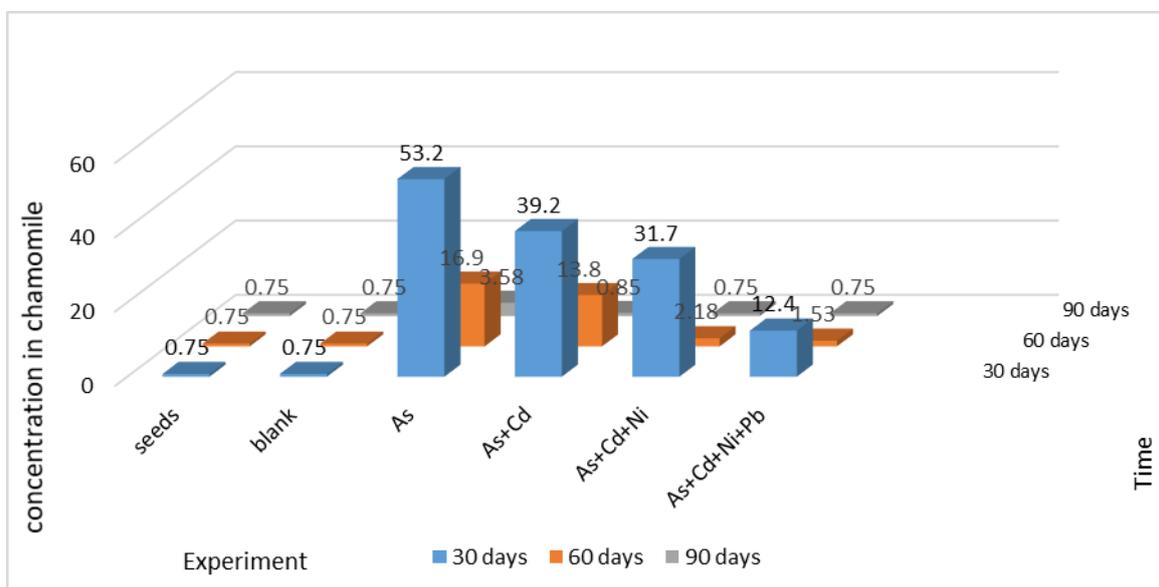


Fig. 1. The concentration of As in chamomile plants in all experiments with arsenic pollution, mg/kg

Thus, the added arsenic content was above the alert threshold for sensitive use (15 mg/kg d.w.), after the first and second month of the experiment, and in the third month of the experiment it exceeded the intervention threshold for sensitive use, which is same as the alert threshold for less sensitive use (25 mg/kg d.w.).

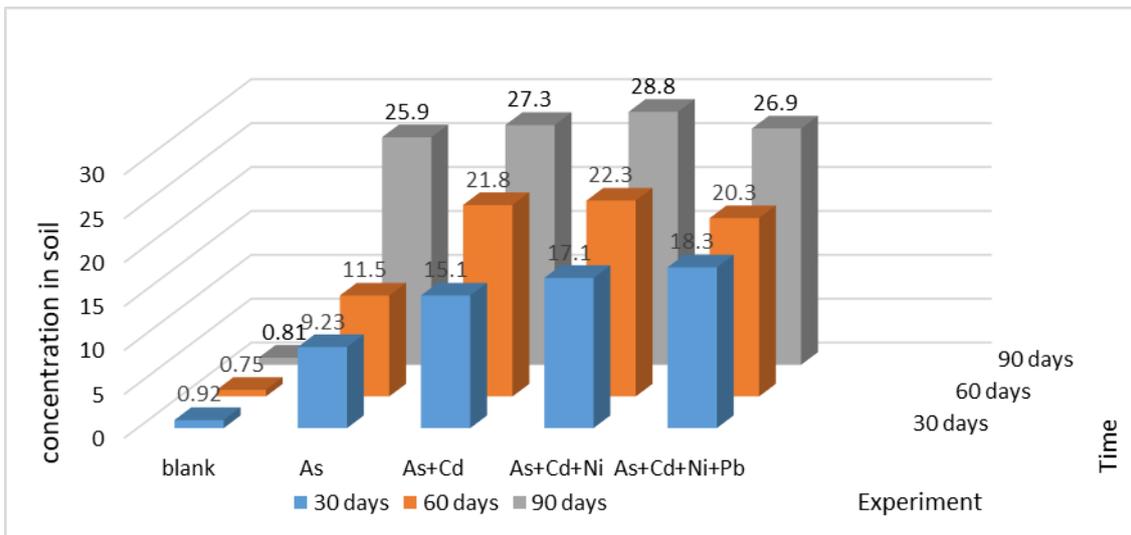


Fig. 2. The concentration of As in the soil in all experiments with arsenic pollution, mg/kg d.w.

Biometric and biological data

During the experiment, it was noticed that plants irrigated only with water (M_1 and M_2) germinated and grew faster than those irrigated with a mixture of metals. After the first month of metal irrigation of the 4 experiments (E_1 - E_4), a low number of seeds germinated, and the plants had a lower development than the control ones. In the second month of the experiment, the 4 experiments showed an improvement in the germination process, an increase in the number of germinated plants and their development. Table 3 shows the dimensions of the plants harvested for analysis. It is observed that the plants in the control samples (M_1 and M_2) have larger size ranges of the whole plant (root, stem, leaves) than most contamination experiments. The short exposure of the plants to the metallic stress showed a positive impact on the biometric parameters

Table 3. Biometric data for monitoring the length of harvested plants for analysis, cm

Period	M_1	M_2	E_1	E_2	E_3	E_4
30 days	2-7	4.5-7.5	7-8	4-7	2-4.5	1.5-4
60 days	7-14	10-15	12-17	8-15	7-17	8-14
90 days	16-22.5	16-22	18-21	16-19	18-20	15-17

The content of total chlorophyll, ρ chlorophyll a and ρ chlorophyll b after the first 30 days from the beginning of the experiments is represented in figure 3.

The total chlorophyll from the results obtained indicates that the toxicity in the E_1 experiment (30.5 mg/mL) is higher compared to the other E_2 , E_3 and E_4 experiments. High content of microelements necessary for the growth, development and functioning of photosynthesis processes found in the soil Ca, Mg, K, Zn, Fe, Mn etc. allowed the development of plants even in the presence of stress factors.

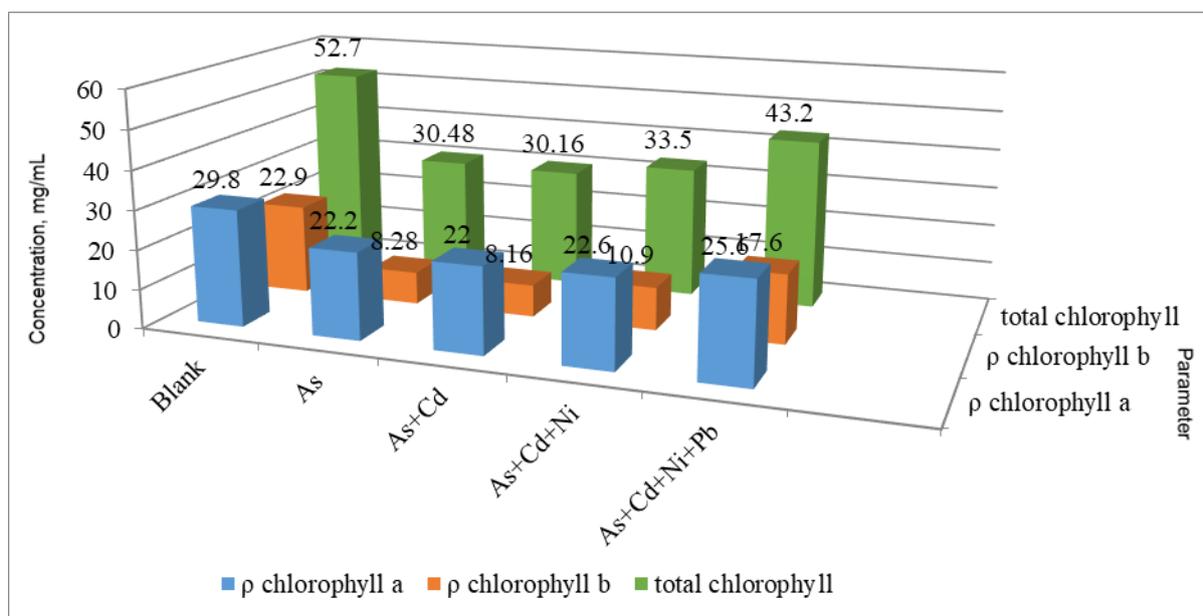


Fig. 3. Chlorophyll content of all experiments - 30 days, mg/mL

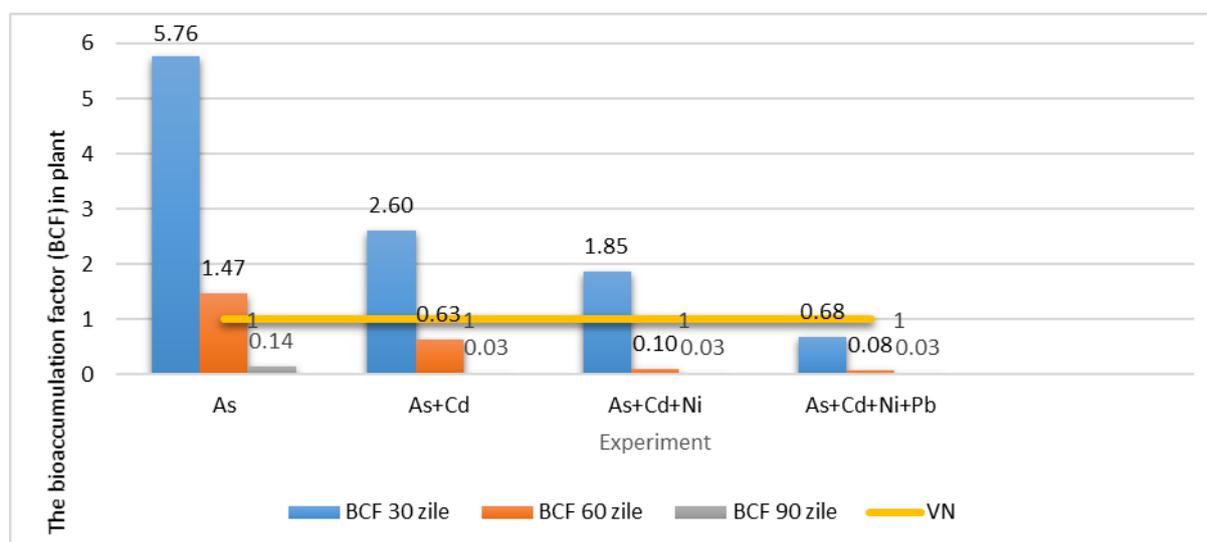


Fig. 4. Soil to plant the bioaccumulation factor (BCF) for arsenic experiments

As complex mixtures of metals are added, the assimilation of arsenic by the plant decreases due to the competitiveness of the metals.

In months 2 and 3 of the experiment, there is a drastic decrease in the arsenic content in the plant, in all experiments, it remains in the soil, by binding to less accessible structures in the soil, such as: organic matter, iron oxides and manganese [5]. This is confirmed by the transfer coefficients calculated by relating the concentration of arsenic in the plant to the concentration of arsenic in the soil. From figure 1 it can be seen that, although at 30 days the arsenic exceeds the normal value of 5 mg/kg d.w. and in all experiments, it decreases in time, so that at the end of the 90 days only in experiment E₁ is still found As in the plant, but within normal limits.

After reporting the results, the bioaccumulation factor (BCF) was calculated and observing the values obtained for BCF in the first month of the experiment (figure 4), it is concluded that the order of bioaccumulation of arsenic is: E₁ (BCF = 5.76) > E₂ (BCF = 2.60) > E₃ (BCF = 1.85) > E₄ (BCF = 0.68) the explanation being given by the competitiveness of arsenic in relation to other metals regarding the bioavailability and absorption processes. In the second month of the experiment, the BCF values vary in order:

E_1 (BCF = 1.47) > E_2 (BCF = 0.63) > $E_3 \sim E_4$ (BCF = 0.1), the concentration of arsenic in the plant decreasing very much. In the third month of the experiment, the concentrations of arsenic in the plant in all 4 experiments indicate that the developed plant does not accumulate arsenic.

In the first month, the added arsenic fails to bind to the organic matter in the soil, remaining in a bioavailable form for the plant, which is reflected in the results of arsenic concentrations obtained in the plant in experiments E_1 - E_4 (figure 1).

As a result of the stabilization reactions in the soil, the arsenic may be related to less available forms, so that in months 2 and 3 the arsenic accumulates in the soil. In the soil, arsenic remains around the maximum added value (at 60 days, respectively 90 days), figure 2.

Toxicity, in the case of experiments with a single metal is higher, than in the case of experiments in which a mixture of metals was used, conclusion deduced from the content of chlorophyll a and chlorophyll b, so if a mixture of metals is added, the total value of chlorophyll increases in the order $As < As+Cd < As+Cd+Ni < As+Cd+Ni+Pb$ approximately equal to the control sample (M_1 and M_2). Although irrigation with a mixture of metals inhibits the sowing process, the germinating plants are more vigorous, having a chlorophyll content similar to that of the control sample.

The only experiment in which arsenic accumulates in the plant at the end of the 90 days is the E_1 experiment, in which a concentration of 3.58 mg/kg d.w. is obtained, a lower value than the normal one in the plant (5 mg/kg d.w.).

The translocation coefficients (TF) determined from the concentration of metal in the root of the plant and the aerial components (stem, leaves or inflorescence) of the chamomile plant could not be determined because the experiment did not run until the inflorescence.

Stimulation of the photosynthesis process under the action of metal mixtures (in high concentrations) can be caused by the activation of adaptive and compensatory mechanisms with the role of maintaining normal assimilation processes.

CONCLUSIONS

The study aimed at the bioaccumulation of the toxic metal As in the chamomile plant (*Matricaria Chamomilla* L.) following germination and development tests on a natural soil enriched with metals, added to the soil separately, or by a combination of several metals (Cd, Ni, Pb).

Regarding arsenic experiments, as the only contaminant or by combination of metals, the values of arsenic accumulated at 30 days were higher than the normal value of arsenic in the plant (5 mg/kg), decreasing to 60 days, as at the end of the experiments to reach below the detection limit of the applied method. The only experiment that was an exception was the one with arsenic as the only contaminant (E_1) in which at 90 days, the arsenic content in the chamomile plant was 3.58 mg/kg d.w. arsenic, the value that is within normal limits (5 mg/kg d.w.), but higher than that determined in the control plant (<0.75 mg/kg d.w.).

The bioaccumulation factor of all tests performed by combination of metals had values lower than 1, which indicates that no arsenic accumulates in the plant.

The total chlorophyll determined confirms that the toxicity in the single metal experiment is higher than in the metal mixture. Even if in experiments, due to the small amount of plant sample, it was not possible to determine the arsenic contents on parts of the plant (root, stem, leaves), we can conclude that the type of soil used and the added arsenic contents, chamomile does not accumulate arsenic above normal values in the plant. In terms of arsenic content chamomile can be used for pharmaceutical and medical purposes and has no applicability in phytoremediation processes of soils contaminated with arsenic.

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