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Effects of Toxic Metals Cd, Ni and Pb on *Matricaria Chamomilla* L. Growth in a Laboratory Study

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Abstract: *The paper presents a comparative bioaccumulation study between the growth of chamomile (Matricaria Chamomilla L.) exposed to toxic metals (Cd, Ni and Pb) and the growth of same plant species unexposed to metals. The soil was contaminated within three independent experiments with Cd, Ni and Pb at the intervention threshold value for sensitive use. Each of the toxic metal was added by watering the seeds, and subsequently the germinated plants. The experiments targeted the effects of soil pollution on the growth of chamomile during three months period. The results showed that in the first month of the study, all three metals accumulated in plants. After the seeds germination and plant growth, high Cd concentration in chamomile plants was detected. Moreover, Ni and Pb were detected in soil in the percentage of 96%. Overall, it was observed that chamomile plants were Cd accumulators at the tested concentrations of 6 mg/kg with no phytotoxic effects. Matricaria Chamomilla L. could be used in phytoremediation of polluted soils, with limitations of use for human consumption, except for the extracts.*

Keywords: *Matricaria Chamomilla, Cd, Ni, Pb, transfer index*

1. Introduction

Contamination of the environment with various inorganic and organic pollutants is one of the most significant dangers in terms of human health. The mining areas, by processing the extracted ores, represents a source of contamination both of the soil and the water, respectively due to the improper waste management, leading to the release of toxic elements in the environment [1,2]. Toxic metals have become one of the main stress agents for living organisms. Compared to other pollutants, they cannot be degraded and are easily bioaccumulated in the food chain, thus becoming a long-term threat to ecosystems (flora, fauna and humans) [2, 3]. Some metals can be considered as essential micronutrients of plants, while others have no beneficial physiological function to plants [4]. Thus, some medicinal and aromatic plants can absorb and accumulate metallic contaminants in their vegetation (roots, stem, leave or inflorescences), such plants having the capacity of being a feasible alternative for the phytoremediation of contaminated soils by [1].

The toxic metals reaching the soil can be found in the dissolved form, available for plants and migration towards the groundwater, fixed on soil particles (fixed by organic matter, clay minerals, silicates) or, a part of them can be transformed by different reactions into other compounds (hydroxides, organometallic compounds).

Metal binding depends on the pH and the composition of the soil, so that the sandy soils fix the metals very weakly, while the clay soils rich in organic matter fix the metals most strongly. Metals dissolved in soil and weakly bounded in different compounds are available for extraction by plant roots, and so can reach trophic chains and produce toxic effects. This represent the bioavailable part of the metals in soils, which is responsible for the toxic effects. It is known that at the freshwater / groundwater levels, the metals such as cadmium, lead and nickel can reach through the discharge of

**email: gabriela.vasile@incdecoind.ro* *# Author with equal contribution* contaminated wastewater from industrial (electrochemical, hydrometallurgical, galvanic coatings, extractive activities, pigment manufacturing, etc.) activities, including acid mine leakage.

In areas where soils are contaminated with heavy metals, the plants develops tolerant or resistant effects to the toxicity of heavy metals, using exclusion and accumulation as defense mechanisms. The exclusion mechanism by which heavy metals extraction and transport is restricted to the root could triggers plant tissues to exhibit very low metal contents. The mechanism consists of metals accumulation in plant in a non-toxic form, leading to a high metal content in the plant tissues.

Aromatic and medicinal plants play an important role in the agricultural production, mainly due to their increased use as a raw material in the pharmaceutical and food industry [4, 5].

The chamomile plant (*Matricaria Chamomilla* L.) belongs to the Asteraceae / Compositae family of flowering plants, being one of the oldest medicinal plants. The cultivation of chamomile (*Matricaria Chamomilla* L.) covers a period of 3-6 weeks during March-April on any soil type [4].

Chamomile has anti-inflammatory, antioxidant, antimicrobial, analgesic, antiseptic, antispasmodic and sedative properties, being used in the treatment of anxiety, because it is a good antidepressant [6-10]. Chamomile is used to heal wounds, skin irritations, eczema, varicella, neuralgia, rheumatic pain, gout, hemorrhoids and foot ulcers [11], while its essential oils are widely used in cosmetics and aromatherapy [12-15].

The biological activity of chamomile is mainly due to biologically active chemical substances, such as chlorogenic acid, caffeic acid, polyphenols and flavonoids: apigenin, luteolin, quercetin, patuletin and essential oil constituents such as α-bisabolol oxides, α-bisabolol A and B and its azulenes [11, 6-8]. Chamomile also contains other active substances such as tannins, anthemic acid, choline, polysaccharides and phytoestrogens [11].

The purpose of this study was to highlight the toxic effects of some metals (Cd, Ni, Pb) on the germination and growth of chamomile, a medicinal plant with wide medicinal uses in Romania. The soil was enriched at concentrations corresponding to the intervention limit for sensitive use land in order to simulate a polluted mining soil. The metals content both in soil and in plant, respectively, was determined using the ICP-EOS technique (AVIO 500 Perkin Elmer) and bioaccumulation indices were calculated, highlighting the behavior of the *Matricaria Chamomilla* L. species under such conditions.

2. Materials and methods

Chamomile seeds (*Matricaria Chamomilla* L., 2 g, producer: AGROSEL) and universal substrate (producer: Agro CS, 40 L) consisting of peat and humus for garden and balconies plants cultivation - soil enriched with nutritive substances, were purchased.

In order to establish the starting point of the experiment the chamomile seeds, the water used for watering as well as the soil were analyzed from a physical-chemical point of view (Tables 1 ÷ 4). The analysis were performed in replicates, and the average value was considered the reference value.

Table 1. Chamomile herb seed analysis (mg/kg)

Element	Sample 1	Sample 2	Mean value	Element	Sample 1	Sample 2	Mean value
As	<0.75	<0.75	<0.75	Zn	53.22	51.36	52.3
Cd	0.549	0.605	0.580	Fe	77.31	76.04	76.68
Co	<0.04	<0.04	<0.04	Mo	1.329	1.389	1.360
Cr	<0.07	<0.07	<0.07	Ca	5486	5328	5407
Cu	7.11	7.24	7.18	Mg	3376	3312	3344
Ni	1.04	1.07	1.06	Se	<0.3	<0.3	<0.3
Pb	<1.5	<1.5	<1.5	Sb	0.246	0.213	0.230
Mn	63.15	61.33	62.24				

Table 2. Analysis of tap water used for chamomile herb watering (µg/L)

Element	Tap water	Element	Tap water	Element	Tap water
As	<2.0	Pb	<0.75	Ca	41,8
Cd	<0.4	Mn	2.6	Mg	4,8
Co	<0.85	Fe	39.3	Se	<3.3
Cr	<1.3	Zn	16,3	Sb	<0.9
Cu	5.1	V	<1,5	Al	109
Ni	<1.0	Mo	<2,0		

Table 3. Metal content in soil, mg/kg dry matter (dm)

Element	Sample 1	Sample 2	Mean value	Element	Sample 1	Sample 2	Mean value
As	1.35	2.24	**1.80**	Pb	4.48	4.99	**4.74**
Cd	<0.08	<0.08	**<0.08**	Se	<0.3	<0.3	**<0.3**
Co	3.12	3.17	**3.15**	Sb	<0.18	<0.18	**<0.18**
Cr	11.77	10.87	**11.32**	V	16.14	15.35	**15.74**
Cu	12.81	12.12	**12.46**	Zn	24.94	23.72	**24.33**
Mn	362	352	**357**	Ca	107102	109357	**108229**
Mo	1.15	1.04	**1.09**	Mg	3172	3138	**3155**
Ni	13.80	13.58	**13.69**				

Table 4. Characteristics of the soil used in experimental studies

No.	Parameter	MU	Sample 1	Sample 2	Mean value
			6.2	6.5	**6.35**
2.	Conductivity	µS/cm	549	560	**554.5**
3.	Organic Carbon	% dm	12.37	11.59	**11.98**
4.	Total Carbon	% dm	22.27	24.69	**23.48**
5.	Humidity	%	39.45	40.28	**39.9**
6.	Total Nitrogen	mg/kg dm	13855	12957	**13406**
7.	Total Phosphorus	mg/kg dm	2622	3008	**2815**
8.	Organochloride Pesticides	mg/kg dm	<0.01	<0.01	**<0.01**
9.	Triazine Pesticides	mg/kg dm	<0.03	<0.03	**<0.03**

Five experiments were performed, of which two were used as control samples (M_0 and M_1), while the others were used to assess the bioaccumulation of metals in the chamomile herb (E_1 – Cd, E_2 – Ni, E_3 – Pb). All experiments were conducted at an ambient temperature in the range of 20°C - 25°C, from April to July 2019, in natural light for approximately 12 hours/day. 750 g of soil and 0.2 g chamomile seed were used for all testing recipients. The toxic metals were added by watering. The metal concentrations (Cd, Ni, Pb) were added to the tap water, considering: a) the initial soil concentration, b) the amount of soil used, c) the soil moisture and d) the final concentration to be reached. Table 5 presents the watering regime and the concentrations of metals added during the 3 months of the experiment, each bioaccumulation experiment containing only one pollutant.

Table 5. Watering regime and metal concentrations in pots (mg/kg dry matter)

Experiment	**Watering regime**		
	0÷30 days	30÷60 days	30÷60 days
E_1–Cd	Tap water enriched with 4.5 mg Cd	Tap water	Tap water enriched with 1.5 mg Cd
E_2–Ni	Tap water enriched with 112.5 mg Ni	Tap water	Tap water enriched with 37.5 mg Ni
E_3–Pb	Tap water enriched with 75 mg Pb	Tap water	Tap water enriched with 25 mg Pb

Metal concentrations in pots					
Experiment	Metal	Initial concentration	30 days	60 days	90 days

E_1–Cd	Cd	<0.08	4.60	4.60	6.10
E_2–Ni	Ni	13.7	126	126	164
E_3–Pb	Pb	4.74	80	80	105

The metal concentrations in soils at the end of the experiments were above the intervention threshold for sensitive use (IT), such as agricultural and residential areas, according to in-force legislation [16].

Table 6. Metal concentrations in soil and plants (mg/kg)

Metal	Soil			Plant	
	Normal value	Alert Threshold	Intervention Threshold	Normal value	Phytotoxic value
Cd	1	3	5	<0,1÷1	10
Pb	20	50	100	0,1÷5	30
Ni	20	75	150	1÷5	20
Cu	20	100	200	3÷15	20
Co	15	30	50	0,05÷0,5	30÷40
Cr	30	100	300	<0,1÷1	2
Zn	100	300	600	15÷150	200
Mo	2	5	10	-	-

Each soil and plant sample was collected as a replicate. The soil samples were air dried, grinded in the mortar, sieved and the fraction of less than 150 µm was retained in order to determine the concentration of pseudo-total metals (dissolved in aqua regia). After harvesting, the plants were washed with distilled water, measured, photographed and either dried in the lyophilizer (type ALPHA 1-2 LD_{plus}, CHRIST) for metal content analysis or freezed for chlorophyll pigment analysis.

In order to quantify the metal content in soil, a mass sample of approximately 1.5 g was extracted in aqua regia (15 mL 37% HCl: 5 mL 65% HNO_3) [17] and then the extracts were analyzed by inductive coupled plasma optical emission spectrometry [18].

The plant samples (0.2 to 0.4 g) were mineralized with a mixture of ultrapure HNO_3 and H_2O_2 in a ratio of 10:3 (v/v) at room temperature for 24 hours to destroy organic matter (cold digestion) [19, 20]. Subsequently, a 3-stage microwave digestion program was carried out on a Milestone Ethos Up type oven as follows: 15 minutes at 1800 W with temperature increase up to 180 °C; 15 minutes maintaining the temperature at maximum 180 ºC; 10 minutes of cooling [21]. The acid extracts were filtered and diluted with ultrapure water to a constant volume of 25 mL. The metals determined both from soil and plant, respectively were the target metals, Cd, Ni and Pb and also: As, Co, Cr, Cu, Mn, Zn, V, Mo, Ca, Mg, Na, K, Ti, Se and Sb.

3. Results and discussions

Considering the N total content (> 1.20%), the soil used was a clay soil, rich in organic matter. The high phosphorus level in the soil was above the concentration range commonly encountered in soils (0.02 - 0.15% P), the soil having a total phosphorus concentration of 0.28%. The soil used had a C_{total} / N_{total} ratio of 17.52, indicating a moderate mobility of nitrogen transfer to the plant, which could be associated to the vegetative stage of the plant [22].

The analysis of chamomile seeds showed Cd and Ni content. The concentrations of toxic metals: Cd, Ni and Pb in control plants and chamomile samples growth during the experiments E_1-Cd, E_2-Ni and E_3-Pb were represented in Figures 1a, 1b and 1c. The bioaccumulation of Cd, Ni and Pb in plants was influenced mainly by the exposure period. In all the experimental tests performed at the end of the first month of development, the metals recorded very high concentrations in the plant (19.5 mg/kg Cd, 17.8 mg/kg Ni, 155 mg/kg Pb) above the accepted range, exceeding even the phytotoxic value, but during following months of the experiment the concentrations decreased significantly (5.96 mg/kg Cd, 15.2 mg/kg Ni, 33 mg/kg Pb), so that after 90 days from the beginning of the experiment the smallest value was recorded in all 3 experiments (4.7 mg/kg Cd, 5.9 mg/kg Ni, 5.7 mg/kg Pb).

As explanations of this behavior we suggest: the stabilization of the metals addition to the soil at the end of the first month of the experiment, which allowed the toxic metals to bind in less bioavailable combinations, such as organic matter, iron and manganese oxides, respectively the phytostabilization of the metals at the ground level through the roots.

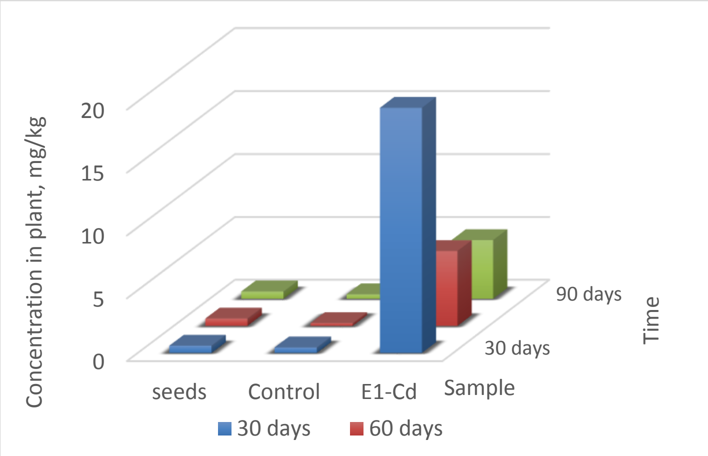

Figure 1a. Cd content in seeds, control plant and Cd polluted plants

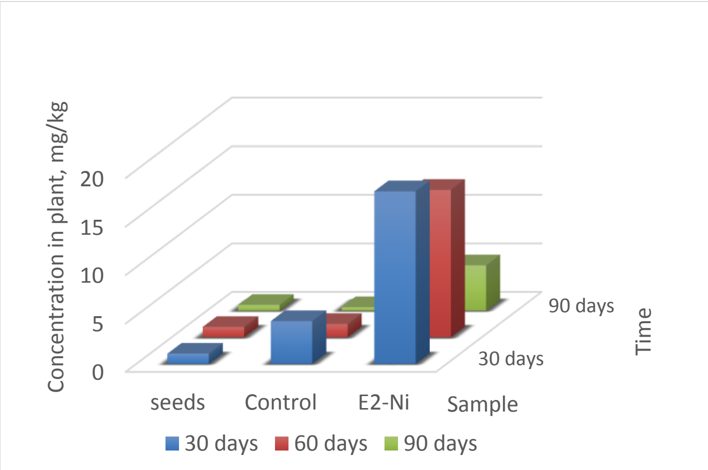

Figure 1b. Ni content in seeds, control plant and Ni polluted plants

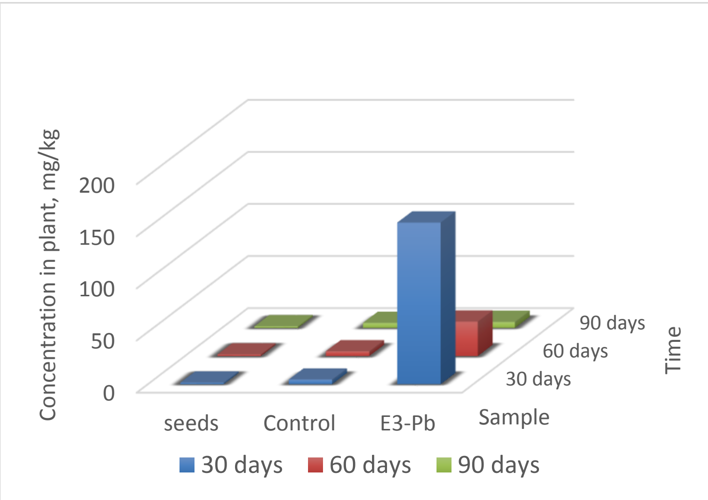

Figure 1c. Pb content in seeds, control plant and Pb polluted plants

The transfer index, calculated as a ratio of the specific metal content in the plant to the concentration of the same metal in the soil, is represented in Figure 2. Values greater than 1 indicate higher absorption of metal from soil by the plant and higher suitability of the plant for phyto-extraction and phytoremediation. In all three experiments, the highest value was recorded at 30 days (E_1-Cd: TI = 9.29 > E_3-Pb: TI = 6.05 > E_2-Ni: TI = 0.71). The values of the transfer index decrease over time, so that at the end of the experiment was observed that none of the indices exceeds the value of 1 which indicates that plants exclude the metals from uptake.

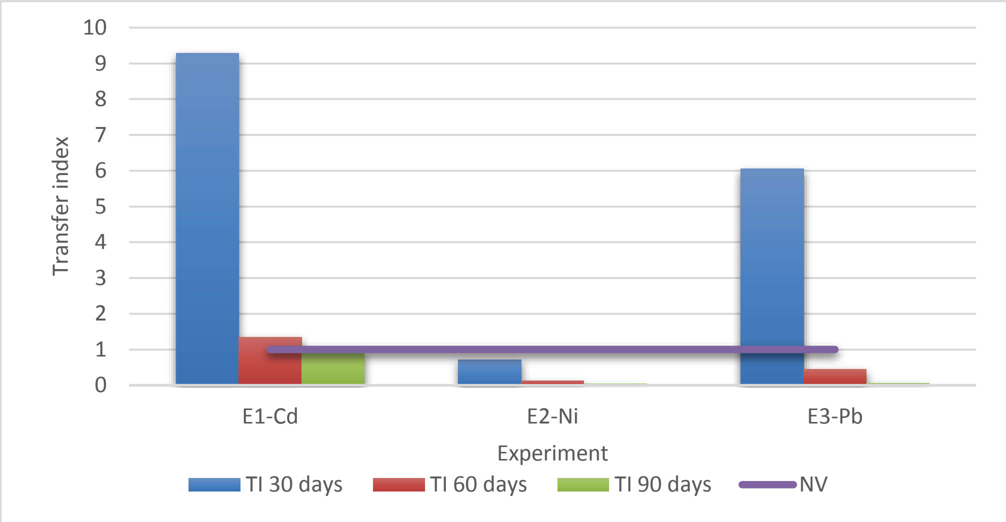

Figure 2. Transfer index for Cd, Ni and Pb in polluted plants

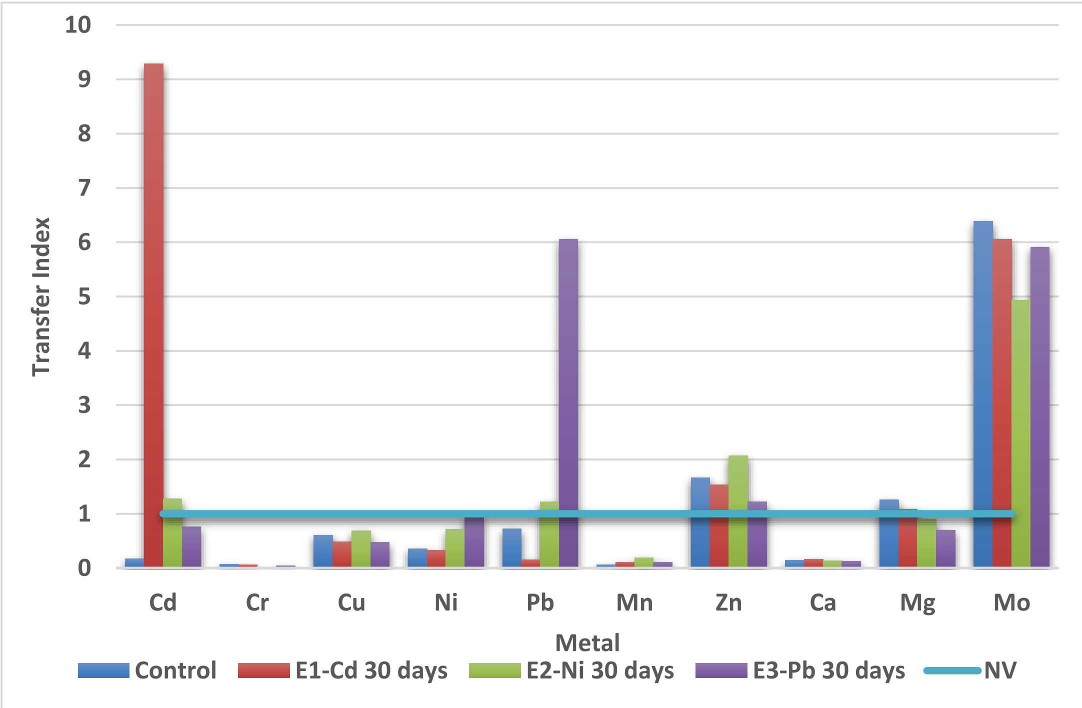

Figure 3. Transfer index for other metals at 30 days

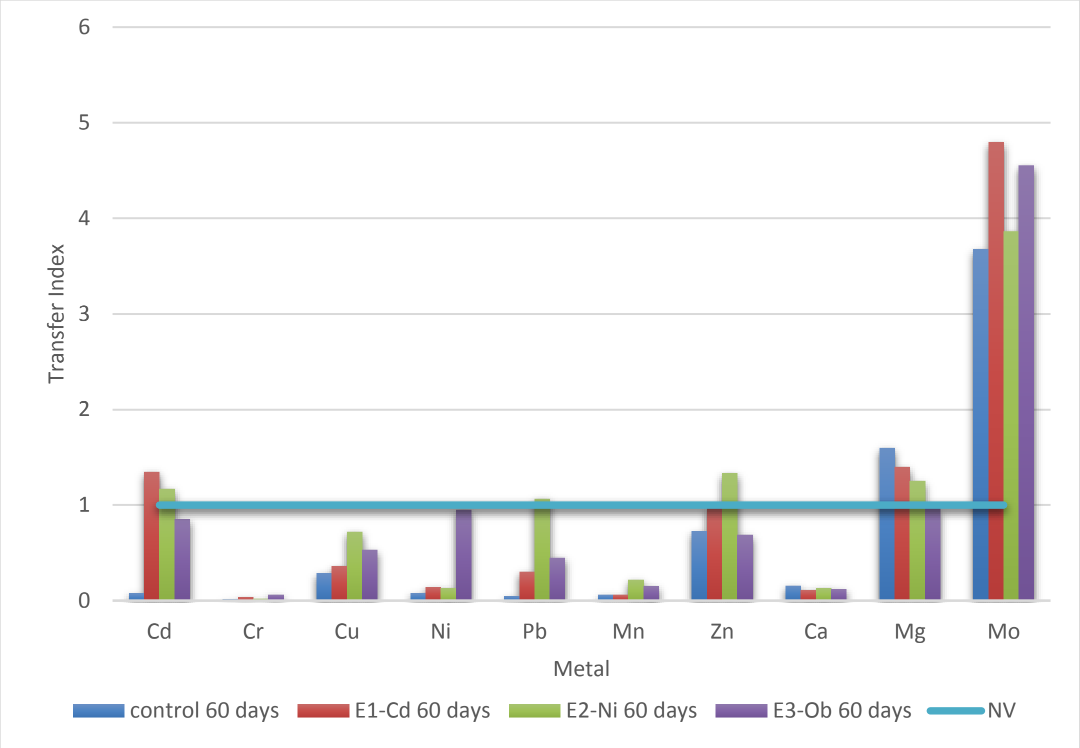

Figure 4. Transfer index for other metals at 60 days

In most plant species, cadmium mainly accumulates in roots, the concentration being about 10 times higher than the concentration in the stems. Cadmium inhibits root and stem growth, affects nutrient extraction and accumulates frequently in agricultural crops. It is an inhibitor of photosynthesis, as an effect of reducing chlorophyll and other pigments, but also by closing the stomata. The accumulation of cadmium in the different parts of the plant is generally done in the following order: roots > stems > leaves > seeds [5, 23, 24]. Generally, it is accepted that the normal Cd concentration in plants are between 0.2-0.8 mg/kg [25].

The Cd content in the plants from the E_1-Cd test decreased during the 90 days of the experiment, the final value being of 4.7 mg / kg, value above the normal range, but below the phytotoxicity value (Table 6), with a value of the transfer index of 0.96, very close to 1, the highest value recorded at 90 days. Both the chlorophyll content (Figure 5) and the data on the increase in plants length in the E_1-Cd test (Table 7) indicated an accumulation of Cd mainly in the root. A high chlorophyll content was recorder in the contaminated samples (49.8 mg/mL) (only 5 % lower than the control sample). The non-zero values of the transfer index for Cd in the E_2-Ni and E_3-Pb experiments are due to the Cd content of the chamomile seeds (Figures 3 and 4).

Nickel was taken up by plants through roots once it becomes available in the soil. It is an essential nutrient for the normal growth and development of plants and it is necessary for the activation of some enzymes. Nickel is directly coordinated by protein and plays an important role in a number of physiological processes, such as seed germination, vegetative and reproductive growth, photosynthesis and nitrogen metabolism. Thus, plants cannot carry out their life cycle without an adequate supply of nickel [26]. This observation was also confirmed by the tests performed during the E_3-Pb experiment, in which was observed that both at 30 days and at 60 days the chamomile plant polluted with Pb extracts Ni from the soil at a double concentration than normal, accumulating it for use in further development processes (8 ÷ 9 mg / kg, TI approximately 0.95).

However, excessive nickel concentration can cause phytotoxicity in plants by inducing the production of reactive oxygen species that affect numerous physiological and biochemical processes, such as photosynthesis, transpiration and mineral nutrition. Nickel toxicity causes changes in enzymatic activities, metabolic disturbances by inducing oxidative stress, disrupted photosynthesis and eventually causing growth inhibition [27].

From the monitoring data of the E2-Ni experiment it can be observed that the total chlorophyll content is 44% lower than the control sample (Figure 5), indicating that the excess nickel that the plant absorbed in the first month disrupted the photosynthesis process, with consequences on the grown process (smaller plant sizes, Table 7).

The mineral component of the soil bound the lead, in particular, on the clay minerals, while the organic component of the soil bound the lead of humic acids (the binding bonds on humic acids are very strong), being efficient in moving the different cationic metals. As a result, most of the lead in the soil was bounded (generally over 80% of the total lead in the soil is fixed) and only a small part remains bioavailable for plants. This explains why in lead polluted soil the toxicity of plants was not proportional to the total lead content [28]. This finding also applies to chamomile tested on soil rich in organic matter, as at the end of E_3-Pb experiment 96% of the added Pb content remains in the soil.

Lead inhibits metabolic processes such as nitrogen uptake, photosynthesis, respiration and water absorption. This fact was confirmed by the low chlorophyll content recorded in Pb intoxicated plants (24.9 mg/mL) where the value was 53% lower than in the control samples (Figure 5) and by the lower increase in length of the plants in the E_3-Pb experiment compared to the control samples M_0 and M_1 (Table 7).

In the process of germination and growth of plants subjected to the stress of metal pollution, mechanisms of self-defense / resistance / adaptation of the plant to the metallic elements were activated. The presence of anti-stress factors, respectively high content of Ca, Zn and Mg, microelements necessary for the growth / development and functioning of photosynthesis processes allowed the development of plants even in conditions of exceeding the value of the intervention threshold for sensitive uses soil for toxic metals Cd (E_1- Cd), Ni (E_2-Ni), and Pb (E_3-Pb), respectively.

The transfer rates from soil to the plant at 30 days for Zn (E_2-Ni = 2.07 > Control = 1.66 > E_1-Cd = 1.54 > E_3-Pb = 1.22) were greater than 1 in all experiments (Figure 3), the plants extracting Zn from the soil due to their need to grow. At 60 days (Figure 4) the amount of extracted Zn was lower (E_2-Ni = 1.33 > E_1-Cd = 0.98 > Control = 0.73 > E_3-Pb = 0.69, but on the other hand the plant extracts more Mg. Thus, while during the tests at 30 days, Mg accumulates only in the control sample (TI = 1.26) and in the E_1-Cd sample (TI = 1.08), at 60 days the content of Mg extracted from the plant increases and accumulates in the plants of chamomile from the other experiments: Control = 1.60 > E_1-Cd = 1.40 > E_2-Ni = 1.25 > E_3-Pb = 0.96 (Figure 4).

A number of essential metals or micronutrients such as Cr, Co, Cu, Mn, Mo, Ni, Fe, Se and Zn are required for the optimal functioning of biological and biochemical processes in plants [29]. From Figures 3 and 4 was observed that the plant extracts from the soil the nutrients it needs: Cu, Ni, and Mo. Cu concentrations are in the normal concentration range (3.4 ÷ 5.4 mg/kg), while the extracted nickel values were higher (2.5 ÷ 17.8 mg/kg) but below the phytotoxic value of 20 mg/kg. Higher concentrations were observed during the Ni pollution test. Regarding the Mo content, it was observed that in all experiments, either for control or polluted samples, the soil transfer index in the plant for Mo has high values (higher than 4.93 at 30 days, respectively higher than 3.68 at 60 days). Mo concentrations in the plants were in the range of 2 to 6 mg/kg, values considered acceptable for plant growth.

Table 7. Monitoring data of the length of the plants collected for analysis, cm

Period	M_0	M_1	E_1-Cd	E_2-Ni	E_3-Pb
30 days	2-7	4.5-7.5	4-7	3-6	2.5-5
60 days	7-14	10-15	8-15	9-12	8-11
90 days	16-22	16-22	16-19	14-17	12-16

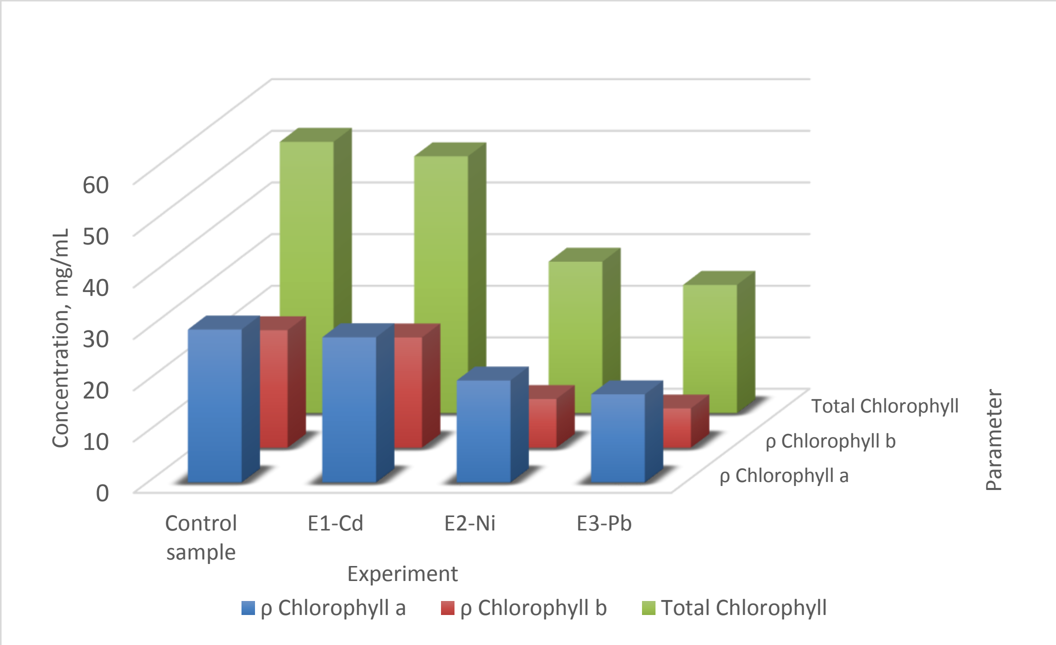

Figure 5. Chlorophyll content in the chamomile plant at 30 days

4. Conclusions

The study emphasized the accumulation of toxic metals Cd, Ni and Pb in the chamomile plant (*Matricaria Chamomilla* L.). The accumulation of metals in chamomile experiments were performed in soil, the toxic concentrations being added by plant watering with tap water enriched with metals. The exceeding of the intervention threshold for sensitive use soil was studied, the tests being carried out in laboratory condition over a three months period (April-July 2019). During the experiments in which nickel and lead were used, it was observed that these metals do not accumulate in the plant at the end of 90 days of experiment, as the additional nickel (150 mg/kg) and lead (100 mg/kg) remained in the soil. Thus, it can be stated that under the given experimental conditions, chamomile do not accumulate nickel and lead.

During the cadmium contamination test, it was observed its accumulation in the chamomile since the first month of the experiment, along with its development (60 days) and at the end of the experiment (90 days). The transfer coefficients from the soil to the plant are high, the final value being close to the value 1, which indicates that the chamomile plants are Cd accumulators at the tested concentrations (6 mg/kg), without showing phytotoxic effects. The final value concentrated in the plant of 4.7 mg/kg, close to toxic concentrations of Cd, defined as 5-30 mg/kg [25] is also dangerous for human health.

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