CASE STUDY CONCERNING THE HEAVY METALS PHYTO-EXTRACTION FROM THREE DIFFERENT SOIL TYPES BY SPECIES FROM *ASTERACEAE* FAMILY

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Abstract: *The purpose of this work is evidencing the phyto-extraction of heavy metals from soil potential with the help of some species from Asteraceae family. This study is determining the physic-chemical characteristics for three soil types and some features of plant chemical composition. These soils are characterised by increased contents in copper, nickel, chrome and lead, and after the analyses realised with the help of a CONTRAA 300 atomic absorption apparatus, produced by Analytikjena. We can notice that the plant species from Asteraceae family have extracted these metals from soil in quantities that are over the normal limits from plants and they haven't shown visible toxicity signs.*

Key words: *phyto-extraction, soil, plant,* heavy metals, *Asteraceae family.*

Introduction

Humankind is depending by the thin and fertile layer from the Earth's surface this being extracted all the resources necessary for living. One of the greatest paradoxes is that the humans tend to endanger the life spring because of his lack of knowledge, greed, negligence or other causes [Butnariu et al. 2004; White et al. 2002]. These facts are leading to the degradation of the soils that were fertile in the past, and now are becoming improper for cultivation, even the modern techniques are helping the humans to introduce in the productive circuit millions of hectares of land. The plants from the spontaneous flora can become important cultivate plants in every moment, a good example being the fodder beet this being cultivated from XVIII century, or penicillin mould cultivated from XX century, and other numerous medicinal plants [Goncharova 2001; Broadley et al. 1999].

When a certain plants category can grow and develop in the presence of some certain toxic elements the literature says that the species is tolerant, indicator or hyper-accumulator [Broadley et al. 1999; Watt 2002].

In the last time there is an increased interest among the scientists that are working with the nature protection concerning the identification of the ecosystems state with the help of these tolerant, indicator or hyper-accumulator plants species. These methods can be used to monitor the entire landscape. The indicator species and the hyper-accumulator too are tolerant species the researches showing the presence of some genetic mechanisms responsible for this thing [Anderson et al. 1993; Aksoy et al. 2000]. Examples of species tolerant for metal are *Holcus lanatus, Agrostis capillaries, Mimulus guttatus* and *Silene vulgaris*. The indicator plants species have great biochemical perspective because these species are absorbing great amounts of toxic substances, usually metals or metalloids during growth and development processes [Lasat et al. 1998;

Assunção et al. 2003]. In a Brooks' (1977) study concerning the hyper-accumulation he defines the nickel hyper-accumulators as plants species that are accumulating metal amounts greater then 1000 Ni µg/g from the dry matter. Later Baker and Brooks (1989) are defining the concentrations for other metals 100 µg /g Cd, 1,000 µg /g Ni, Cu, Co, Pb, and 10.000 µg /g Zn and Mn all reported to the dry matter [Baker and Whiting 2003]. The concentrations enumerated here are great in comparison with other species that aren't accumulating these elements. Hyper-accumulator species are from 45 botanical families [Barnett et al. 1997; Bowman et al. 2003].

The soils with high metal amounts are releasing toxic metals. In the same time, the soils polluted with metals are leading to the death of the most plants species. But there are plants as is *Thlaspi caerulescens* that are from the hyper-accumulator plants group (about 400 species) that can absorb about 200 % zinc and 0.3% cadmium without toxicity signs, there growing and flowering naturally on these soils [Brun et al. 2003; Butnariu et al. 2005a]. The researchers have the intention to cultivate this plant for the soil treatment in the case of the soils polluted with zinc and cadmium from the areas placed in the vicinity of the old metallurgic centres [Butnariu et al. 2003a]. Transformed in a soluble form the metals can enter in root through the plasmatic membrane level of the roots endodermic cells or through apoplasts. Thus they can rich through xylem in the leaves cells [Butnariu et. Al. 2003 b; Ciulei et al. 1993]. Hyper-accumulator plants can be used for phyto-extraction and they must to develop a great biomass amount, they must to be easy cultivated and harvested, preferably few times in a year [Cosico 2004; Frey 2000]. The plants cultivated there will absorb the metals, and when they rich the maturity phase they will be harvested, dried and incinerated, the ash being recuperated and the soil becoming fertile again [Zhao et al. 2003; Kupper et al. 2000].

Soil resistance to the pollution with heavy metals is depending by the tampon capacity of it. The soils with great absorption capacity (great amount of clay and organic matter) can retain these elements in the superior layers. In these soils the amount of toxic compounds of the heavy metals that can be absorbed by plants or leached in the groundwater is greater in comparison with the acid or sandy soils. The sandy soils are retaining weak the most of the heavy metals [Papagiannis et al. 2004; Tolra et al. 1996].

Every of this metals are entering in the trophic network and are exposed to some specific processes. In the trophic circuit of the heavy metals these are meeting different biologic barriers and because of them there takes place a selective bio-accumulation with the purpose to defend the living organisms against the excess of these elements [Wang and Greger 2004].

The action of the biologic barriers is limited because there is found a concentration of the heavy metals through accumulation in the most of the cases. In comparison with other organic molecules the toxic metals cannot be degraded by plants, they can only to be extracted by the plants [Zhao et al. 2000].

Materials and methodes

Researches are developed on some species from *Asteraceae* family from vegetation communities from Beba Veche (Timis County, Romania), Brebu Nou asnd Gradinari (Caras-Severin County, Romania). There are collected soil and plants samples and are registered the vegetation data.

The soil samples are collected in conformity with *STAS 7184/1-84 – Soil sampling for pedological and* *agrochemical studies* from 0-20 cm soil layer. Heavy metal analyses are realised in conformity with *RS ISO 11047 from July 1999 – Soil quality. Cadmium, chrome, cobalt, copper, lead, manganese, nickel and zinc determination from soil extracts in nitromuriatic acid – Methods through spectrometry in atomic absorption in flame and electrotermic atomisation.*

The determination of the heavy metals from a soil extract in nitromuriatic acid is made in conformity with *ISO 11466* and *STAS 7184/1-84*.

Soluble heavy metal extracted with nitromuriatic acid. For heavy metal determination there was weighted about 1.5 g from soil sample with a 0.0001 g exact in a 100 ml reaction pots. It was wetted with approximately 0.5- 1.0 ml water and there was added during stirring 10 ml of hydrochloric acid, then was added 5 ml nitric acid drop by drop to reduce the foaming. The mixture obtained is left for 16 hours at room temperature for easy oxidation of the organic fraction of the soil. After this time the solution mixture is boiled until drying. Nitromuriatic acid extraction must be realized under a well-ventilated hoot. To avoid violent boiling and solution loss is important to add boiling moderator granules in samples. Then the reaction pots are cooling because in soil samples is added distillate water, and then is filtered with filter paper and then is mixed with 50 ml distillate water. Solutions obtained are prepared to determinate nickel, zinc, iron, copper and manganese.

Metals extracted in nitromuriatic acid can't be considered total fractions or bioaccessible fractions, because the extraction process is too powerful to represent a biological process.

Soil pH determining. Soil suspension in distillate water is prepared in a soil-water report 1:2.5 mass/volume. Having in view to determinate pH of the soil suspensions and tampon solutions for electrodes calibrating those samples must to be kept to the same temperature. Soil suspension with potassium chloride 0.1 n is prepared in the next proportion soil: potassium chloride 0.1 n of 1:2.5 mass/volume. The method is adapted after *STAS 7184/13-79*.

Carbon, hydrogen and nitrogen are determined with CHN 2000 Elemental Analyzer LECO and heavy metals are determined with CONTRAA 300 atomic absorption apparatus, produced by Analytikjena.

Granular analysis: under the influence of the organic matter amount from the soil samples there are used two pre-treatment methods. In the case of the samples with more then 5% organic matter this is oxidized with 6% oxygenated water, and then is realised the dispersion with a solution of potassium hexametaphosphate 10% or sodium hydroxide solution for every boiling after Kacinski method. In case of the samples with organic matter less then 5% there is realised dispersion with potassium hexametaphosphate 10% solution. Determining of the granular fractions is made with the pipetting method for the fractions <0.002 mm (clay 2), and through humid sifting in case of the fractions between 0.02 mm and 0.2 mm (clay 1), and dry sifting for the fractions greater then 0.2 mm.

Mineral composition of the clay fraction (<0.01 mm) is determined through the X ray diffraction on oriented samples saturated in calcium and glycolate.

Mineral composition of the coarse fraction is determined with the help of the polarising microscope, the sand granules being fixed with Canada balsam.

Vegetation is analysed with geobotanic and square meter method of Daget and Poissonet (1971). The second method allows the determination of some vegetation quality indexes of the grassland as are pastoral value and specific frequency, the last parameter being dependent by the species multiplying mode. This method facilitates also the calculation of some ecological indexes of the vegetation as are Shannon-Weaver biodiversity ... index ... and dominance index Simpson. [Coste and Arsene 2002; Moisuc and Dukic 2002; Moisuc et al. 1995; Sărățeanu and Moisuc 2004]. Also, there are represented the autoecologic indexes of the species for soil reaction, temperature and humidity after Ellemberg indexes cited by Kovacs (1979).

Results and discussions

For the monitoring of the soil quality there are collected soil samples from the superior layer between 0-20 cm. The soil samples are prepared after the standards. There is determined the heavy metal content from the samples. The physico-chemical features of the soils analysed are presented in table 1.

The analyses concerning the concentration of heavy metals in soil are realised with the help of the atomic absorption apparatus CONTRAA 300 produced by Analytiljena, and from the table can be seen that the average content is greater in the case of the most of the heavy metals analysed there.

In the grassland from Beba Veche the vegetation shows an average biodiversity from the point of view of Shannon-Weaver index that is 3.72. Concerning the species dominance in the vegetation carpet the Simson index is 0.99 this value showing that the vegetation has numerous species with similar populations.

Table 1 Physico-chemical features of the analysed soils

Analysed physico-chemical feature	Beba Veche	Brebu Nou	Grădinari
pH	7.54	6.08	5.13
Carbonate $CaCO_3$%	1.44	-	-
Sand (2.0-0.2 mm)%	0.5	2.1	21.9
Fine sand (0.2-0.02 mm)%	46.8	43.2	42.5
Dust (0.02-0.002 mm)%	16.0	25.0	17.5
Clay 2. under 0.002 mm%	36.7	29.7	18.1
Physical clay. under 0.01 mm%	45.7	42.4	30.0
Humus %	3.63	9.30	9.49
P ppm	84.6	5.4	7.0
P ppm calculated	58.54	5.4	7.0
K ppm	155	147	103
N total %	0.60	0.71	0.98
Cu (ppm) (average content 20 ppm)	23.92	30.76	21.11
Zn (ppm) (average content 100 ppm)	125.8	109.73	112.05
Ni (ppm) (average content 20 ppm)	26.2	30.51	25.6
Pb (ppm) (average content 20 ppm)	15.1	24.05	28.99
Co (ppm) (average content 15 ppm)	16.27	19.54	17.88
Cr (ppm) (average content 30 ppm)	31.99	33.33	46.05

The *Asteraceae* species analyzed from Beba Veche grassland are *Cichorium intybus* and *Ambrosia artemisiifolia.*

Analysing the biodiversity index of the vegetation in the case of the grassland from Brebu Nou we are we can say that there the vegetation has a low biodiversity because the value calculated is 2.45. In the case of the Simpson index, or dominace index the situation is similar with Beba Veche, the value obtained being 0.06.

The *Asteracea* species analysed from Brebu Nou grassland are *Achillea millefolium* and *Leucanthemum vulgare*.

Other vegetation community analysed here is from Gradinari. There the biodiversity index (Shannon-Weaver

index) is 4.68 and shows a great diversity of the vegetation. Also, the Simpson index has the same value as in Brebu Nou grassland, showing the same situation.
Analysed species from the grassland from Gradinari are *Tanacetum corymbosum* and *Cirsium undulatum*.

The species mentioned for these grasslands are harvested and analysed concerning their chemical composition and the heavy metal content the data being represented in table 2.

Table 2 Chemical composition and the heavy metal content of some species from *Asteraceae* family

No	Species	C % d.m.	H % d.m.	N % d.m.	P % d.m.	Cu ppm	Zi ppm	Ni ppm	Pb ppm	Co ppm	Cr Ppm
1	*Cichorium intybus*	42.91	5.62	1.72	0.412	32	119	22	18.8	7.9	1.96
2	*Achillea millefolium*	45.27	5.58	1.71	0.424	21	137	29	13.6	8.7	1.76
3	*Leucanthemum vulgare*	48.42	5.67	1.93	0.324	27	97	33	19.8	9.5	1.85
4	*Ambrosia artemisiifolia*	42.08	5.36	2.17	0.411	30	85	25	17.7	7.6	1.60
5	*Tanacetum corymbosum*	44.72	5.47	1.77	0.393	34	99	35	18.0	7.5	1.94
6	*Cirsium undulatum*	45.58	5.92	1.53	0.315	37	108	20	19.2	8.4	2.4

The average copper content in plants is 5-20 ppm, most of the plants contenting less then 10 ppm. In the plants leaves the copper content when is manifesting the toxicity usually is 20 ppm. The zinc amounts in plants when the toxicity is present are among 300-500 ppm. Nickel toxicity in plants is manifesting when this metal has values over then 100 ppm. Usually the plants are absorbing chrome amounts among 0.1-1 ppm.
From table 2 can be noticed that those six *Asteraceae* species analysed have increased amounts in heavy metals, but the plants aren't presenting visible signs of toxicity to confirm the toxicity of these elements for the plants analysed here.

Conclusions

The study and the analysis of the pollution degree with heavy metals of the soil according with the present legislation leaded to the next conclusions:

- the soil from Beba Veche contains nickel and chrome over the average limits:
- the soil collected from Brebu Nou has nickel, lead and chrome amounts over the medium admitted level;
- in the soil collected from Gradinari the values over the average limit admitted are in case of copper, nickel, lead and chrome.

After the determinations realised we can affirm that in all the samples analysed here the heavy metal content is higher, but there are not found areas to be declared as polluted.
The *Asteraceae* species analysed in this work haven't shown toxicity symptoms even the soil where they were growing contains heavy metals over the average values admitted for soil.

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