SOIL COBALT BIODISPONIBILITY FOR THE PLANTS FROM A GRASSLAND VEGETATION COMMUNITY

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Abstract

This biodisponibility study is based on the evaluation of cobalt from the plants that grow on a soil with increased cobalt content (31.37 ppm). This study establishes the influence of the increased cobalt quantity in soil on the chemical composition of the plants. Cobalt and copper, zinc, nickel, lead, chrome, and cadmium from soil and plants are determined using a CONTRAA 300 atomic absorption apparatus, produced by Analytikjena. Increased cobalt quantities from soil have determined increased cobalt quantities in plants, without visible toxicity symptoms, but with influences on the chemical composition of the plants.
Keywords: *biodisponibility, cobalt, soil, plant*

Introduction

Soil is represented by the superficial part of the terrestrial crust and is formed on the mineral background of it following a complex of mechanical, physical, chemical and biological processes developed during long time periods [Ianos and Goian 1995].

Cobalt concentration from soil is usually dependent by the bedrock type. Cobalt is more abundant in basal rocks its content in cobalt being usually 8 ppm. Cobalt level in soil is important concerning its concentration in plants [Butnariu et al. 2004; Ianos and Goian 1995].

Cobalt enters in a great number of minerals, usually associated with nickel, these two elements being related from agrochemical point of view. In the superior lithosphere cobalt has a great affinity for calcium. Cobalt is found in small amounts in ferromagnesian minerals structure where can substitute partially the magnesium. Cobalt content in rocks varies under the influence of genesis conditions. In the volcanic rocks the cobalt amount varies from 1 ppm to some hundreds of ppm, the greatest cobalt concentrations being registered in the ultrabasic rocks (that are containing ferromagnesian minerals rich in magnesium, and having a cobalt content of 100-300 ppm, and the report Ni:Co is 20) [Butnariu et al. 2005].

In the basic rocks the cobalt amount in comparison with the amount from the acid rocks is 1-10 ppm, and the NI:Co report is close to 2 [Butnariu and Goian 2005].

Cobalt is a heavy metal found in soil in different forms associated with mineral and organic constituents of the solid phase, cobalt from the soil solution being a very small fraction. From the mineral constituents, clay minerals have an important role in cobalt retaining [Markert and Weckert 1989].

In these structures Co^{2+} ion (with the ionic ray of 0.82 Å) is occupying octahedral p0ositions replacing Mg^{2+} and Fe^{2+} isomorphic these having similar ionic rays (0.78 Å and 0.83 Å). Cobalt adsorbed in this way is unchangeable being released only through alteration process. [Ianos and Lacatusu 1994; Wettlaufer 1991].

The average cobalt amount from the Earth soils is estimated to be 8 ppm, but there can appear important variations in report with the chemical and mineral type of the bedrock. The total cobalt amount from the superior layer of the main soil types varies among 1.5-15.9 ppm having 6.5 ppm average value [Christl and Kretzschmar 1999].

The lowest cobalt amounts were determined for sandy soils poor in colloidal fractions, and the alluvial soils there being present clay leaching processes in depth [Wild and Jones 1992].

Relative greater cobalt amounts are found in the clay alluvial soils with clay content smaller then 2µg. Cobalt amounts of 10 ppm are determined also in the soils with fine texture [*Ontario Ministry of the Environment and Energy* 1996].

After the numerous researches realised and taking in account the conditions offered by the different soil types there is determined a direct relationship between the total cobalt content and clay [Anderson et al. 1996].

Cobalt enters also in the ferromagnesian structure of the minerals these being present in the coarse fractions of the soil (sand and clay) because of the instability of these minerals. A great relative part of the cobalt from soil is associated with Fe and Mn oxides and hydroxides, the cobalt retention under this form being made through surface absorption or co-precipitation [Ianos and Goian 1995; *Ontario Ministry of the Environment and Energy* 1996].

Cobalt, as other heavy metals has great affinity for the organic matter forming complexes with different ligand types, combinations with great importance for plants nutrition. In complex combinations as kelate type cobalt is situated in the next order concerning the stability: **Mn < Fe < Co < Ni < Cu < Zn**. A small fraction of the cobalt from soil and is found adsorbed on the surface of the mineral and organic colloids as Co^{2+} or $[Co(OH)^{+}]$ [Ianos and Goian 1995; *Ontario Ministry of the Environment and Energy* 1993].

The report among the ionic species that are influencing the cobalt mobility vary depending by acidity in the acid soils being dominant Co^{2+} retained relatively heavy, while in the alkaline soils is dominant the $[Co(OH)^{+}]$ form which dissociates heavy. After the retaining power cobalt is situated in the next order in comparison with other metals: **Cu < Ni < Co < Zn** [Ianos and Lacatusu 1994].

Usually the plants, especially the superior plants, in comparison with the animals are less sensitive for cobalt insufficiency. In the experiences realised with nutritive solutions is noticed that 10 ppm of cobalt are enough for the nitrogen fixation process in the case of the bacteria from the nodosities of alfalfa. Lower cobalt amounts, less then 1 ppm, are mentioned for *Rhizobium* from the soybean nodosities [http://www.npi.gov.au/database/substance-info/profiles/26.html, accessed in 22 March 2007] even the cobalt amount necessary for the nitrogen fixating bacteria can be provided also in the condition of the soils with relative low cobalt amounts, there existing researches that are showing the stimulator role of the cobalt [Ianos and Goian 1995; Butnariu and Goian 2005].

Cobalt deficiency for the leguminous plants is evidenced in experiences with nutritive solutions there being noticed the inhibition of the leghemoglobine formation and implicitly the fixation of the atmospheric nitrogen, these plants becoming dependent by the nitrogen from fertilizers. Growth decrease is noticed in the case of the species without nodosities with cobalt deficiencies (tomatoes and subterranean clover without nodosities). In the case of the acide soils non-limed can be noticed favourable effects of the cobalt applied in the case of some species (clover, linen, rye, barley, sugar beet), the effects being noticed in the case of the generative organs, these being concretised through the early flowering, the increase of the seed yield and of the seed mass [Butnariu and Goian 2005; http://www.npi.gov.au/database/substance-info/profiles/26.html, accessed in 22 March 2007].

Experimental

Researches are developed on a vegetation community placed in Saravale (Timis 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

In this work is analysed a grassland from Saravale (Timis County, Romania). The vegetation of this grassland is low productive concerning the aerial biomass yield. The vegetation carpet is dominated by *Puccinelia limosa, Achillea setacea* and *Artemisia santonicum* these forming the main conenoses taxonomic units.

The physico-chemical features of the grassland soil where is placed the experimental plot, these being represented in table 1.

Table 1: Physico-chemical features of the soil from Saravale

Analysed physico-chemical feature	Value
pH	7.74
Carbonate $CaCO_3$%	1.39
Sand (2.0-0.2 mm)%	0.5
Fine sand (0.2-0.02 mm)%	45.9
Dust (0.02-0.002 mm)%	17.0
Clay 2. under 0.002 mm%	33.5
Physical clay. under 0.01 mm%	47.2
Humus %	2.68
P ppm	84.6
P ppm calculated	58.54
K ppm	155
N total %	0.60
Cu (ppm) (average content 20 ppm)	13.92
Zn (ppm) (average content 100 ppm)	105.8
Ni (ppm) (average content 20 ppm)	26.2
Pb (ppm) (average content 20 ppm)	15.1
Co (ppm) (average content 15 ppm)	31.27
Cr (ppm) (average content 30 ppm)	31.99
Cd (ppm) (average content 1 ppm)	Undetectable concentration

Cobalt content from soils after the article 756 from 3.11.119 is 15 ppm and alert level is considered 30 ppm [*Romanian Official Monitor*, 1997]. Soil properties as is pH, texture, humus and carbonates content, but also the nitrogen, phosphorus and potassium quantities are determined with the purpose to evaluate the role of cobalt in soil systems and flora type that is tolerating these conditions. After we noticed from table 1, the greatest cobalt quantity in soil is followed by high zinc quantities (105.8 ppm), nickel (26.2 ppm) and chrome (31.99 ppm).

Vegetation community studied in Saravale is low productive and the vegetation features are represented as synthetic vegetation tables (table 2).

The vegetation has 92.4% coverage on soil surface. The dominant species in vegetation carpet is *Puccinelia limosa*. Also, there we find in high proportion the next herbaceous species: *Bromus hordeaceus, Poa bulbosa var. Vivipara, Trifolium striatum,* and *Camphorosma annua.*

Table 2: Vegetation and ecologic indexes for temperature, humidity and soil reaction form grassland from Saravale (Timis County, Romania) (altitude 91m)

No.	Species	Autoecologic index			Abundance-dominance (1-5)	Coverage (%)
		T (1-9)	U (1-10)	R (1-9)		
	Poaceae (Gramineae)					
1	*Puccinelia limosa*	3.5	0	5	5-3	75.5
2	*Bromus hordeaceus*	0	3	0	±1	10.5
3	*Poa bulbosa var. Vivipara*	2	3.5	4	±1	0.5
4	*Cynodon dactylon*	3.5	3.5	0	+	0.3
	Fabaceae (Leguminosae)					
5	*Trifolium striatum*	1.5	3	4	±1	1.5
	Species from other botanical families					
6	*Camphorosma annua*	4	4	5	±1	1.5
7	*Chamomila recutita*	3	3.5	0	+	0.3
8	*Ranunculus pedatus*	3	3	4	+	0.3
9	*Thlaspi arvense*	3	3	4	+	0.3
10	*Althea officinalis*	3.5	3.5	5	+	0.3
11	*Limonium gmelini*	3.5	4	4	+	0.3
12	*Artemisia santonicum*	2.5	4	0	+	0.3
13	*Plantago schwarzenbergiana*	3.5	4	5	+	0.2
14	*Echium vulgare*	3	3	5	+	0.3
15	*Cerastium dubium*	3	3	0	+	0.2
16	*Aster tripolium*	0	0	5	+	0.1

The vegetation carpet of this grassland is composed from 16 vegetal species from that 25% are grasses, 6% legumes, 6% *Cyperaceae* and *Juncaceae*, and 69% are represented by the species from other botanical families.

Althea officinallis, Echium vulgare and *Artemisia santonicum* are concentrating the greatest cobalt amounts from the vegetal species that are growing in this grassland as is shown in table 3.

Table 3: Cobalt content in some plant species from Saravale grassland

No.	Species	Cobalt content (ppm)
1	*Althea officinalis*	7.72
2	*Echium vulgare*	7.165
3	*Artemisia santonicum*	8.645

Cobalt from soil is assimilated in plants in increased amounts in plants but isn't determining toxicity effects. With the nitrogen, potassium, phosphorus and carbon content there are determined also the calcium, magnesium and copper content in these tree plants species and the values obtained there are presented in table 4.

Table 4: Chemical composition of the analysed species from Saravale grassland

Species	CaO % d.m.*	MgO % d.m.	K_2O % d.m.	P_2O_5 % d.m.	C % d.m.	N % d.m.	Zn ppm	Ni ppm	Cu ppm	Cr ppm
Althea officinalis	18.10	6.92	25.67	0.486	42.14	2.46	60.7	30.45	20.4	2.09
Echium vulgare	24.14	7.21	21.37	0.318	46.27	1.59	59	28.29	18.8	1.88
Artemisia santonicum	22.64	6.26	26.18	0.302	39.79	2.85	54.7	23.53	20.1	1.20

(*d.m. – dry matter content)

The cobalt content from soil in the grassland analysed from Saravale is 31.27 ppm, this value passing over the alert level considered for Romanian regulation (30 ppm). This increased amount of cobalt in sol leaded to the accumulation of this element increase for the vegetal species presented above. Thus, *Althea officinalis* is containing 7.72 ppm cobalt, *Echium vulgare* is containing 7.165 ppm cobalt, and *Artemisia santonicum* has 8.645% cobalt. These plants show increased value contents for other heavy metal too but they aren't presenting visible toxicity effects.

Conclusions

This study determinates the cobalt and other heavy metals from soil and from the plants that are growing spontaneous in that place. It is well known that the plants are more sensitive to increased cobalt amounts in comparison with humans and animals.

Toxicity effects in plants are manifesting in case of soil cobalt concentrations greater then 40 ppm (this value varying in the same time with soil properties and vegetal species).

The soils with alkaline pH and great cationic exchange capacity have increased cobalt contents, these being available for plants. The great cobalt content found in the soil from Saravale grassland is associated with increased content in other trace elements as are zinc, nickel and chrome.

Increased cobalt content from soil leaded to the increase of the cobalt content in plants, but they aren't manifesting visible toxicity symptoms, but are determining some changes in plants chemical composition.

In the case of the studies plants species haven't shown visible toxicity symptoms and or mineral deficiencies even the cobalt amount in soil is great.

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