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MACROMYCETES CONTAMINATION WITH HEAVY METALS IN DUMP PLANTATIONS FROM CĂLIMANI NATIONAL PARK (ORIENTAL CARPATHIANS)

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Abstract: The paper deals with the distribution of dump heavy metals in the circuit soil-macromycetes. Soil samples and macromycetes from dump plantations situated at 1600–1800 m altitude have been analyzed. The investigated dumps resulted due to the exploitation of iron and sulfur ore from Călimani National Park (Oriental Carpathian). In order to realize an ecological reconstruction in the dumps, *Picea abies* (L.) Karst., *Pinus mugo* Turra, *Pinus cembra* L., *Larix decidua* Mill. and *Sorbus aucuparia* L. have been planted. Metal content has been determined by atomic spectrometry, and the results have been performed using Microsoft Excel, Origin and SPSS programs. Our results evidence macromycetes flexible capacity to accumulate various categories of metals. The distribution of the heavy metals in the soil varies from one region to another and depends on the sampling depth.

Key words: contamination, heavy metals, macromycetes, dumps, atomic spectrometry

Introduction

In the regions with miner deposits extraction, the soils degrade structurally and functionally, because of the contamination with various pollutants: nitrites, sulfurs, heavy metals, radioactive sediments, carbohydrates etc. [4, 10, 12].

Beside some microorganisms categories (bacteria, algae, filamentous micromycetes), the macromycetes species play an important role in the detoxification of the contaminated medium, by the process called microremediation [5, 9, 11].

The paper studies the distribution of dump heavy metals in the circuit soil-macromycetes. Soil samples and macromycetes from dump plantations situated at 1600–1800 m altitude (47 06' N; 25 13' E) have been analyzed.

The investigated dumps resulted due to the exploitation of iron and sulfur ore from Călimani National Park (Oriental Carpathians). In order to realize an ecological reconstruction in the dumps, *Picea abies* (L.) Karst., *Pinus mugo* Turra, *Pinus cembra* L., *Larix decidua* Mill. and *Sorbus aucuparia* L. have been planted.

Our observations confirm the fact that the macromycetes species are toxicological bioindicators in regions with chemical pollution in the soil. The results evidenced the fact that the accumulation of various categories of metals might have been influenced by the species, development degree, climatic conditions and soil category. There has been also evidenced that the presence of the metals in the soil influences the structure of the fungi groups by disminuting the density of the sporiferous body and the diversity of saprotrophic and mycorrhizic species [1, 3, 6, 14].

Material and Method

Macromycetes species and soil samples have been collected from dump plantations belonging to the mine exploitation, while the blank samples came from the dump's surroundings.

The samples with macromycetes species have been weed out of the ground and vegetal rests (leaves, roots), transported in paper bags and conserved at 4^{o} C, 24 hours. When the preserving conditions are improper (high temperature and humidity) the protein content can suffer modifications. Soil samples have been prelevated from the basis of the macromycetes. In the laboratory, the samples have been dried in Petri boxes at room temperature.

The content of heavy metals (Zn, Cu, Fe, Co, Mn and Cd) has been determined by flame atomic absorption spectrometry (FAAS), the analysis involving weighing of about 1-2 g of solid samples, disintegrated at warm (~ 80 °C), over night, with a mixture made of 20 mL of concentrated HNO_3 and 1 mL HCl, in an Erlenmeyer flask. After complete evaporation of the solvent, 1 mL of distilled water was added, followed, once again, by complete drying. In a subsequent stage, the solution was boiled for a short time with a mixture of 1 mL concentrated HNO_3 and 5 ml of distilled water. After filtration, the solution was completed up to 25 mL with distilled water [7].

For some of the elements in the sample, additional dilution is possible, for attaining the optimum concentration level for analysis. Prior to readings of the atomic absorption device, the optimum working conditions were established, according to the recommendations of the producer (which involves settlement of the wavelength characteristics to the metal subjected to analysis and adjustment of the two gases (air, acetylene) flows. There follows preparation of a set of standard solutions, over the linear domain of concentration indicated by the manufacturer.

Checking on whether reading, on the device, of the most diluted calibration sample follows, after which the device is calibrated through successive readings of the standard solutions, in increasing order of their concentration values, and the samples are read, after their preparation, as described above. The installation used is a Perkin Elmer 3300 flame atomic absorption spectrometer. The results were performed using Microsoft Excel program.

Results and Discussions

Physical and chemical analysis of the dump samples evidenced a severe contamination of the soil, caused by the high concentration of metals, higher than the maximal concentration admitted.

The comparative analysis of the macromycetes samples evidenced similarities and differences between the species, and the possibility of some contaminant agents to be present in the samples. Metal content in the samples is confirmed using atomic spectrometry.

In the pit area, the exploitation works of the sulfur ore included digging of the existing soil layer, dislocation of the rocks, sorting according to the sulphur content, processing of the ore with higher sulfur content and depositing of the resulting waste in dumps.

This working method explains the general aspect of the pit and dumps and also its deepening southwards, where the sulfur content in higher. The zone of the former peak (Negoiu Românesc) remained relatively not exploited, as due to the lower sulfur content and to the presence of the iron.

At the level of such antropogenic forms of relief, the occurrence of an edaphic cover as such is excluded, so that the external layer from the surface of the waste dumps may be included, from a taxonomic perspective, to the Entiantrosol type, rudic sub-type of soil, with skeletal material.

In the opinion of the authors, this is a soil now under development, formed on antropogenic parental materials, with a minimum thickness of 50 cm, evidencing no diagnosing horizons or showing an incipient A horizon, the presence of which may be explained by the harsh climatic conditions and, especially, by the young age of the waste dumps in the area under investigation.

The ecological reconstruction of this soil unit should take into consideration the nature of the deposited materials, their thickness and chemical composition, their reaction, the presence of some polluting substances etc.

Our results evidence macromycetes flexible capacity to accumulate various categories of metals. The distribution of the heavy metals in the soil varies from one region to another and depends on the sampling depth.

Macromycetes capacity to accumulate heavy metals depends on the following factors: the ecological category (mycorrhizant or saprotrophic), the type of soil, the period of collecting, the altitude, the maturity of the sporiferous body, the initial concentration, and the interactions with other chemical elements. Atomic spectroscopy analysis evidenced metals distribution in the circuit soil-macromycetes.

The heavy metals of the soil are chemical indicators which let us appreciate its contamination degree.

Standard conditions used in this paper are present in Table 1. The results of the analytical qualitative control evidence the fact that the procedure is well applied, taking into account the loose of elements during all work steps. On the other hand, the value 100±10% is accepted, indicating a quantitative recuperation and no effect related with the matrix, in the presented experiment. Besides, the values 100±30% are good, depending on the objectives of the analysis.

Table 1: FAAS STANDARD CONDITIONS USED IN THE PAPER

Metal	**Zn**	**Cu**	**Fe (x 100)**	**Mn**	**Cd**
λ (nm) used	213.9	324.8	248.3	279.5	228.8
linear up to (μg/g)	1.00	5.00	5.00	1.00	2.00
detection limits (ppm)	0.032	0.040	0.124	0.049	0.036
conc. stand. sol. for soil analyses (ppm)	0.97	1.02	1.12	1.03	0.94
recovery factor for soil (%)	96	101	111	102	93
conc. stand. sol. for soil analyses (ppm)	0.95	0.97	1.01	0.99	0.93
recovery factor for macromycetes (%)	94	96	100	98	92

The distribution of the heavy metals in the soil varies from one region to another and depends on the sampling depth. The distribution on the analyzed metals is in concordance with similar results obtained by other authors in other dump regions [2, 8, 13].

Metal content is presented according to the analyzed samples represented by the macromycetes' substratum and their sporiferous body. Cobalt has been evidenced, too, in the soil samples.

Most of the macromycetes species have been collected from the dumps that are why an equal number of soil samples have been analyzed. Figure 1 presents a quite constant distribution of the metals in the soil samples. Manganese abounds in the samples 1, 8, 11 and 13. The sample 14 presents a lower content of metals than the other samples, but a higher content of organic substances. The evaluation of metal content has been done for each macromycetes species, collected from various regions. Figure 2 presents the metal content in the samples of soil for *Lyophyllum connatum* species.

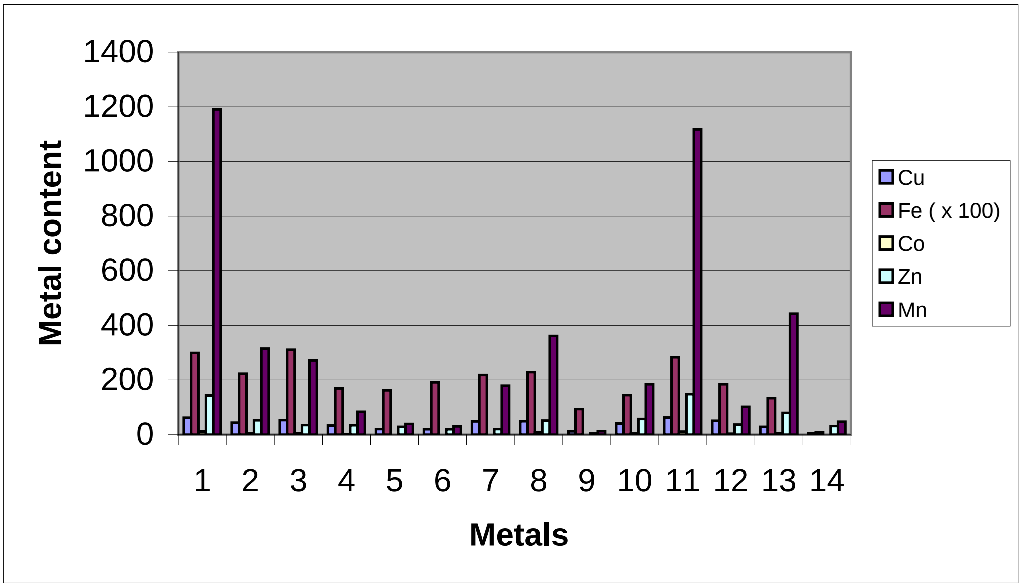

Fig. 1: The distribution of metal content in soil samples for macromycetes species: 1 – *Suillus piceinum* (Ilva Dump); **2** – *Lyophyllum connatum* (*Pinus cembra* Reservation); **3** – *Lyophyllum connatum* (Dumitrel Dump); **4** – *Suillus piceinum* (Pine Dump); **5** – *Suillus variegatus* (Pine Dump); **6** – *Suillus luteus* (Pine Dump); **7** – *Suillus luteus* (Dumitrel Dump); **8** – *Suillus luteus* (Ilva Dump); **9** – *Suillus grevillei* (Pine Dump); **10** – *Suillus variegatus* (*Pinus cembra* Reservation); **11** – *Laccaria laccata* (Ilva Dump); **12** – *Laccaria laccata* (*Pinus cembra* Reservation); **13** – *Laccaria laccata* (dump); **14** –*Leccinum piceinum* (*Pinus cembra* Reservation)

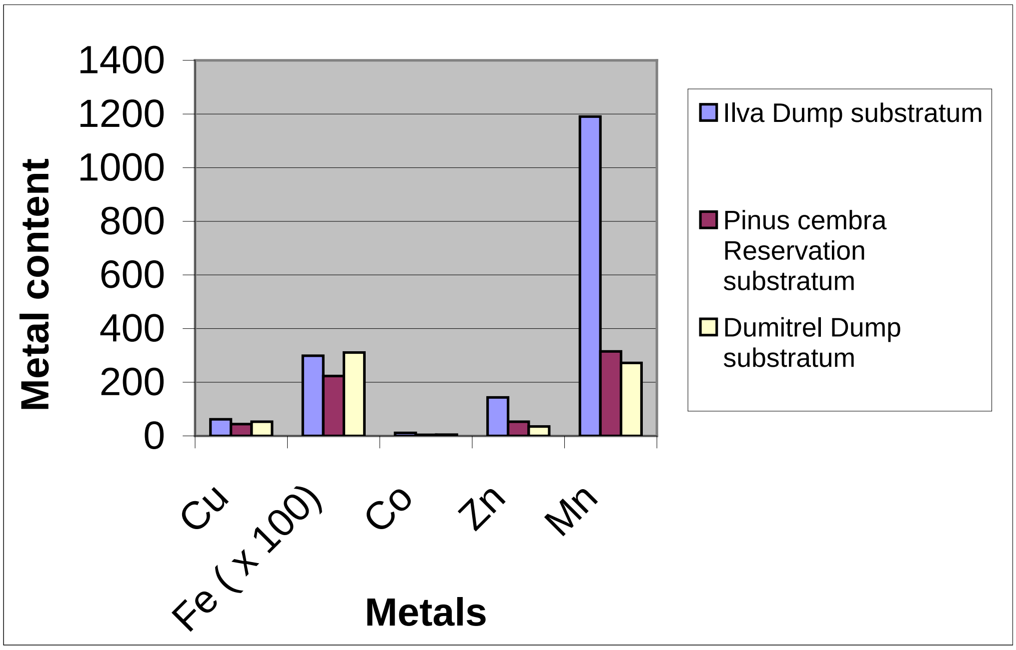

Fig. 2: Distribution of metals in the samples of soil for *Lyophyllum connatum* species

Figure 3 presents the distribution of metals in the samples of soil for *Suillus luteus*, while Figure 4 presents the distribution of metals in the samples of soil for *Lacaria laccata*.

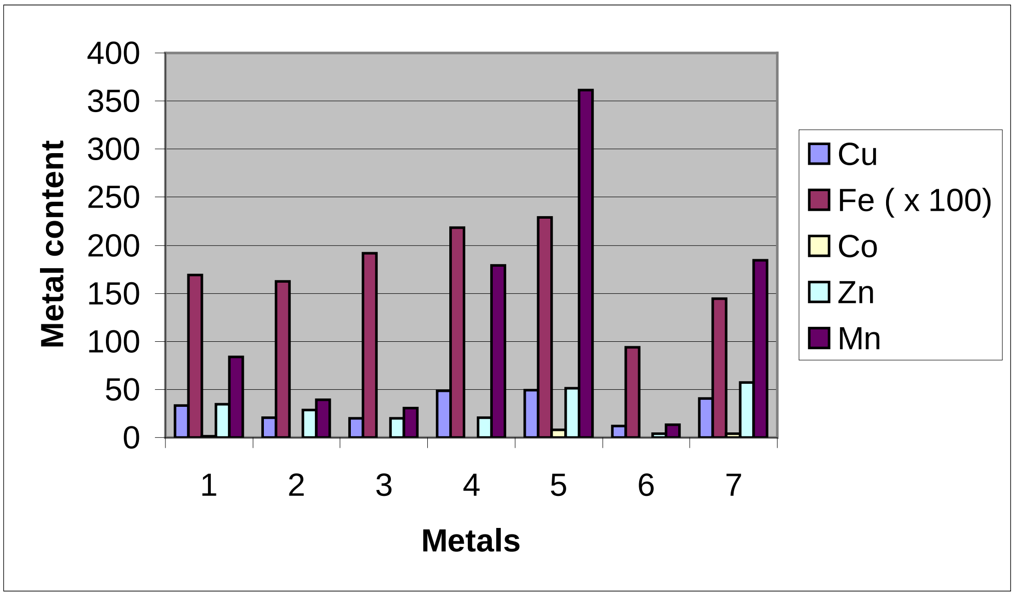

Fig. 3: Distribution of metals in the samples of soil for *Suillus luteus* collected from various dumps and reservations: **1** –Pine Dump (**I**); 2 –Pine Dump (**II**); 3 –Dumitrel Dump (**I**); **4** –Dumitrel Dump (**II**); 5 –Ilva Dump (**I**); **6** –Ilva Dump (**II**); **7** – the reservation with *Pinus mugo* and *Pinus cembra*

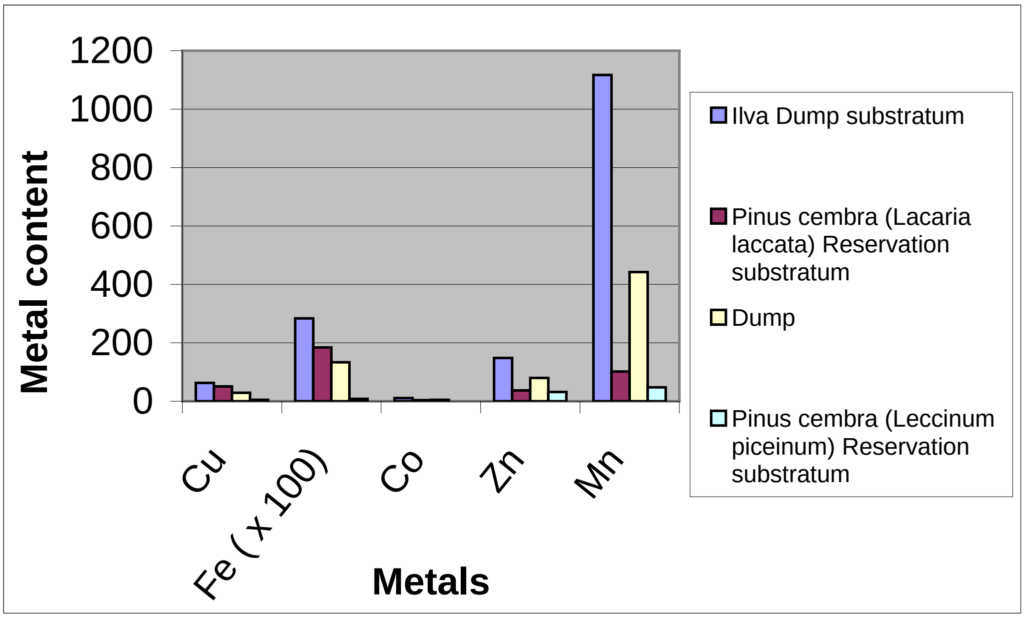

Fig. 4: Distribution of metals in the samples of soil for *Lacaria laccata* and *Leccinum piceinum* from various dumps

The results presented in Figures 2-4 evidence a higher content of manganese in the soil for *Suillus piceinum* prelevated from Ilva Dump. The Mn content presents higher values in the soil samples for *Suillus luteus* and *Laccaria laccata*, collected from Ilva Dump, too. These results evidence a generally higher content in manganese in the samples collected from Ilva Dump. In comparison with the soil samples, the cobalt presented lower values, under the detection limit of the engine, in the macromycetes samples.

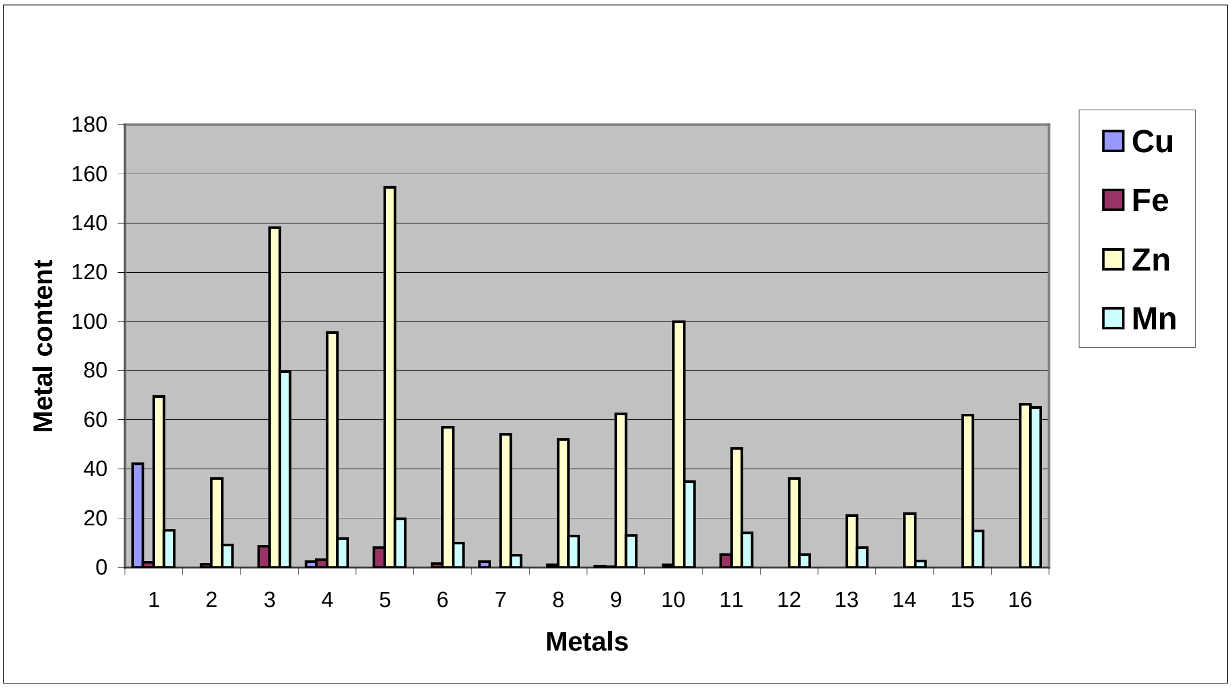

Fig. 5: Metal distribution in the samples with macromycetes: **1** – *Laccaria laccata* (Ilva Dump); **2** – *Lyophyllum connatum* (Ilva Dump); **3** – *Suillus luteus* (Ilva Dump); **4** – *Suillus luteus* (Dumitrel Dump); **5** – *Laccaria laccata* (Dumitrel Dump); **6** – *Lyophyllum connatum* (Dumitrel Dump); **7** – *Leccinum piceinum* (*Pinus cembra* Reservation); **8** – *Suillus grevillei* (*Pinus cembra* Reservation); **9** – *Suillus luteus* (*Pinus cembra* Rezervation); **10** – *Lyophyllum connatum* (*Pinus cembra* Reservation); **11** – *Suillus variegatus* (*Pinus cembra* Reservation); **12** – *Suillus variegatus* (Pine Dump); **13** – *Leccinum piceinum* (Pine Dump); **14** – *Suillus grevillei* (Pine Dump); **15** – *Suillus luteus* (Pine Dump); **16** – *Laccaria laccata* (Pine Dump)

The doubtful values presented in the Figures 1-5 represent the accumulated error (analysis error, errors of the replicates and the statistical error 2σ). Their detection limits (expressed in μg/g) are, as follows:

Element	DL
Cu	0.080
Fe	0.077
Co	0.098
Zn	0.037
Mn	0.015
Cd	0.046

Conclusions

Our results indicate the fact that each macromycetes species concentrates various metals.

Our determination evidenced a generally higher content of manganese in the samples collected from Ilva Dump, for *Lyophyllum connatum*, *Suillus luteus* and *Laccaria laccata*.

Maximal values in the macromycetes species have been registrated for Cu (42.09) in *Laccaria laccata*, collected from Ilva Dump and for Zn (154.57) in the samples collected from Dumitrel Dump. The highest content of Fe (8.58) and Mn (79.65) has been identified in the samples of *Suillus luteus*, collected from Ilva Dump. We consider that the analyzed macromycetes species accumulated quantities of metals comparables with those obtained in similar studies, so they have mycoremediation potential for the soils degraded by mine activities.

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POLUAREA CU METALE GRELE A SPECIILOR DE MACROMICETE SEMNALATE ÎN PLANTAȚII DE PE HALDE MINIERE DIN PARCUL NAȚIONAL CĂLIMANI (CARPAȚII ORIENTALI)

(Rezumat)

Scopul acestei lucrări vizează distribuția metalelor grele existente în haldele miniere în circuitul substrat–macromicete. Pentru aceasta au fost analizate probe de substrat şi de macromicete care provin din plantații de pe halde miniere situate la altitudini de 1600–1800 m. Haldele investigate s-au format în urma exploatării minereurilor de fier şi sulf din Parcul Național Călimani (Carpații Orientali). În scopul reconstrucției ecologice aceste halde au fost plantate cu *Picea abies* (L.) Karst., *Pinus mugo* Turra, *Pinus cembra* L., *Larix decidua* Mill. şi *Sorbus aucuparia* L. Conținutul de metale a fost determinat prin spectrometrie atomică, iar rezultatele au fost prelucrate cu programul Microsoft Excel. Rezultatele obținute evidențiază capacitatea extrem de flexibilă a unor specii de macromicete de a acumula metale. Distribuția metalelor în probele de sol variază de la o zonă la alta şi depinde de adâncimea de la care s-a prelevat proba.

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