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MOSSES ACCUMULATE HEAVY METALS FROM THE SUBSTRATA OF COAL ASH

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Abstract - Plants that are able to accumulate and tolerate extraordinarily high concentrations of heavy metals (hyperaccumulators) can be used for phytoremediation (removal of contaminants from soils) or phytomining (growing a crop of plants to harvest the metals). Two moss species, *Bryum capillare* Hedw. and *Ceratodon purpureus* Hedw., were tested as potential phytoremedies under *in vivo* conditions on a coal ash disposal site in the surroundings of Obrenovac (NW Serbia). The content of various heavy metals (iron, manganese, zinc, lead, nickel, cadmium, and copper) in the mosses and substrata were investigated over a period of three years. Iron and zinc were found to have the highest concentration in the mosses.

Key words: Mosses, heavy metals, hyperaccumulators, coal ash, Serbia

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INTRODUCTION

The use of plants to extract heavy metals from substrata (phytoextraction) has recently received much attention due to the possibility of decontaminating some of the earth's ever-increasing burden of polluted soils (phytoremediation) (S a l t *et al.* 1995; R o b i n s o n *et al*. 1999). In phytoextraction procedure, a crop of plants is grown in soil containing elevated concentrations of one or more trace metals. The plants accumulate the metals spontaneously, or they are induced to do so by some substratum amendments. When mature, the plant hyperaccumulators are harvested, removed, and burnt. Most of the burnt material seeks deposition in some smaller areas, but the wind can cause damage to surrounding soil surfaces. This procedure is not always useful, considering that many plants are not appropriate for growing in great biomass and certainly not in such contaminated soil. Moreover, in most of the attempts performed so far, vascular plants were used.

The use of some cryptogams like bryophytes to cover ash (which contains heavy metals) and perform "bryomining" of heavy metals from the ash, enabling vegetation to grow on these useless and potentially dangerous sites, has also been investigated.

Cryptogamic species are important members of various ecosystems. They contribute to soil stability in the face of wind and water erosion and increase the rate of infiltration of water through the soil.

Mosses have little or no developed cuticle. This is the reason why ions from the surface have direct access for cationic exchanges in the cell membranes. They have a great capacity for trace element retention (G s t o e t t n e r and F i s h e r, 1997; F e r n á n d e z *et al.* 1998). However, not all species have the same capacity for all elements or the same elements (B r o w n , 1984). Mosses have been used to monitor pollutant input in both aquatic (B r u n s *et al* 1995, 1997; S i e b e r t *et al.* 1996 etc.) and terrestrial (F e r n á n d e z *et al.* 1998, 1999; P e a r s o n *et al.* 2000; etc.) ecosystems.

The investigations show bryophytes to be suitable indicators. Certain features they have, like the lack of roots, unistratose leaves, ion-exchange capacity, and uptake of nutrients from the atmosphere make possible bryophyte use for monitoring of regional and local patterns of deposition owing to the high accumulation capacity of these cryptogams (Burton, 1990). Chemical analyses of contaminants in bryophyte samples reflect the state of environmental contamination (Ganeva, 1998).

MATERIALS AND METHODS

Study site

Serbia (88.361 km^2) has various coal ash disposal sites, of which TENT A and B account for 67% of all Serbian coal ash (Gavrić and Mihajlov, 2002).

The TENT B power plant (or Nikola Tesla B Thermoelectric Power Plant, to use its full name) is situated in western Serbia by the town of Obrenovac, on the right side of the Sava River. It uses coal from the Kolubara surface excavation site, which yields a low-calory lignite. The power plant produces electricity and many secondary nuss products like dust, ash, and smoke with high pollutant contents.

The ashes are mixed with water in a ratio of 1:17 and transported to three deposit sites (each 400 ha), which are kept wet during the inactive period to prevent wind blowing of ash. The surface of the deposit site stays bare for long periods of time, and biotechnical measures are needed to establish initial vegetation. However, some colonist moss species (***Bryum capillare*** Hedw., ***B. argenteum*** Hedw., ***Ceratodon purpureus*** Hedw., and ***Funaria hygrometrica*** Hedw.) occur spontaneously. In addition, vascular plants (***Erigeron canadensis*** L., ***Luzula pilosa*** L., ***Poa pratensis*** L., and ***Stenactis annua*** L.) also appear from time to time, but very seldom and with small abundance. The aims of the present study were:

(1) to identify the moss species suitable for growing on ash;
(2) to establish if these species are hyperaccumulators;
(3) to estimate the potential of these mosses for use in phytoremediation, phytoextraction, phytomining, and extensive recovery of ash deposit sites.

Sampling procedure

Two bryophytes (*Bryum capillare* and *Ceratodon purpureus*) found to be the most widespread on the TENT B power plant's coal ash disposal site were collected and examined for heavy metal content.

They were collected during July and August of 1999, 2000, and 2001. Surface samples of ash and moss species were collected on an area of approximately 400 ha of partition II (TENT B). Four samples of moss species and ash were taken from each of 60 localities. Every sample consisted of 6-8 moss patches or ash specimens, and only young shoots were used for analyses, to ensure the yearly accumulation.

Moss samples preparation

The moss samples were collected in paper bags coated with PVC. Right after collecting, they were cleaned of ash remains and rinsed in deionized water for 1-2 minutes. After drying on filter paper, the samples were homogenized and fresh weight was measured. Only young shoots were used for analyses, to ensure the yearly accumulation.

This procedure takes ca. 3 minutes and was performed in a chamber with 70% air humidity chamber to prevent water loss from the plants. The samples were then dried in a dry sterilizer on 55°C until constant dry weight (dw) was achieved. They were then prepared for AAS (atomic absorbance spectrophotometry) using a three-step combined wet and dry dissipation method. The samples were burned in boron-silicate glass vials at 500°C for 7 hours. After that, the remains were digested in a mixture of nitric and perchloric acid in a ratio of 1:3 until dry. The samples were then burned again in an oven at 300°C for 2 hours. The dry ash remains were dissolved in a 5N nitric acid solution and measured on an atomic absorbance spectrophotometer.

Ash sample preparation

Ash samples were collected in glass vials from the same location from which the plant samples were taken. They were prepared in the same way except for the fact that the whole three-step process was repeated twice to ensure higher success of extraction. Concentrations of zinc, nickel, manganese, lead, iron, cadmium, cobalt, and copper were analyzed on a Pye Unicam SP9 atomic absorbance spectrophotometer.

RESULTS AND DISSCUSION

Figures 1-7 show content of all metals examined in

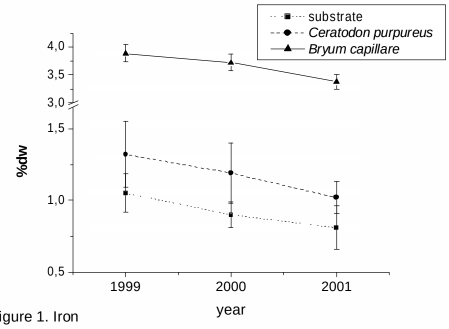

Figure 1. Iron

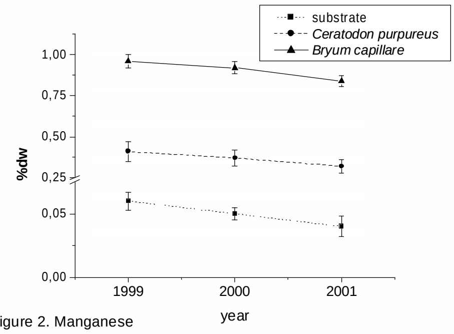

Figure 2. Manganese

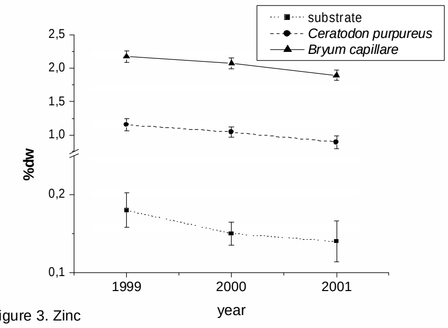

Figure 3. Zinc

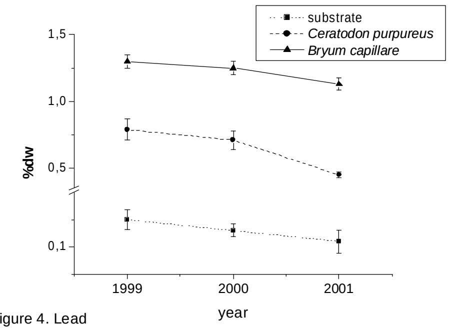

Figure 4. Lead

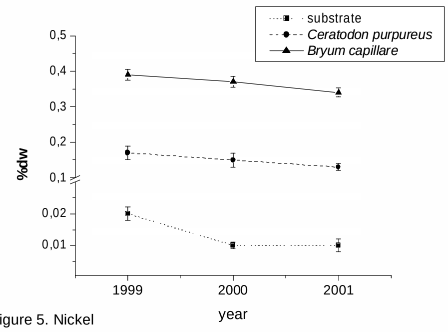

Figure 5. Nickel

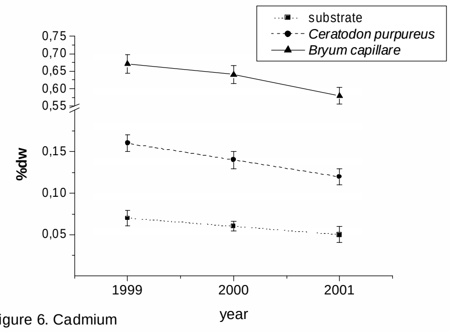

Figure 6. Cadmium

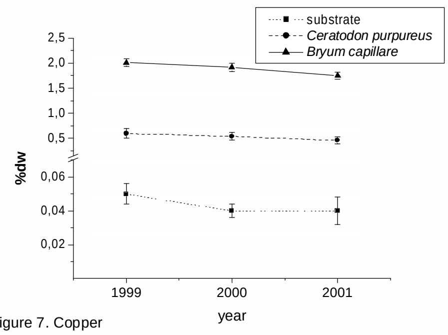

Figure 7. Copper

Figs.1-7. Contents of heavy metals in mosses and substrate in three years period.

bryophytes and the substratum during a three-year period (1999 - 2001). Also, Fig. 8 gives average content of each metal analyzed in both mosses and substrata.

Our results indicate that *Bryum capillare* and *Ceratodon purpureus* are hyperaccumulating species for heavy metals, especially for iron, lead, copper, manganese, and zinc, as shown in Fig. 8. The order of average values of heavy metal accumulation (Fig. 8) was as follows in the mosses:

Bryum capillare: Fe>Zn>Pb≥Cu>Mn>Ni>Cd
Ceratodon purpureus: Fe>Zn>Pb>Cu>Mn>Ni>Cd
while in the ash substrate analyzed it was: Fe>Pb≥ Cd>Zn≥Mn>Ni.

Both species have a great affinity for Mn, Pb, Cu, and Zn, and the same heavy metal accumulation sequence. They can be used as hyperaccumulator species (accumulating more than 0.1% dw) species (Salt *et al.* 1998) for heavy metals, considering that values exceeding 0.1% dw are recorded even for the less accumulating metals (Ni and Cd). Lisboa and Ilkiu-Borges (1996) already cited *B. capillare* as an indicator species for Fe, and our *B. capillare* specimens also hyperaccumulate iron (among other elements).

Even though bryophytes have no developed roots and do not absorb particles from the substrata (Fernández *et al.* 1999), some of them are capable of inhabiting mineral-rich polluted substrata such as Cu-polluted substrata of coal mines [*Scopelophila cataractae* (Mitten) Brotherus] or substrata enriched with heavy metals (e.g., serpentines). The water solution of the substratum can affect the level of metal uptake from it by bryophytes.

Hyperaccumulating heavy metals in mosses remain mostly extracellular, which is an advantage in comparison to vascular plants. Binding confirms to strict physico-chemical rules (Zechmeister *et al.* 2002). The CEC (cation exchange capacity) values cited by Brown (1984) refer mainly to unesterified polyuronic acid molecules and galacturonic or mannuronic acids in the cell walls. Total metal binding is determined by the number of available exchange sites and morphological structures of the bryophytes, which differ from species to species. Most species therefore have different uptake capacities (Zechmeister *et al.* 2002).

Younger parts of plants show higher amounts of monovalent cations and nutrient anions than older parts. Divalent cations, especially those of heavy metals, show

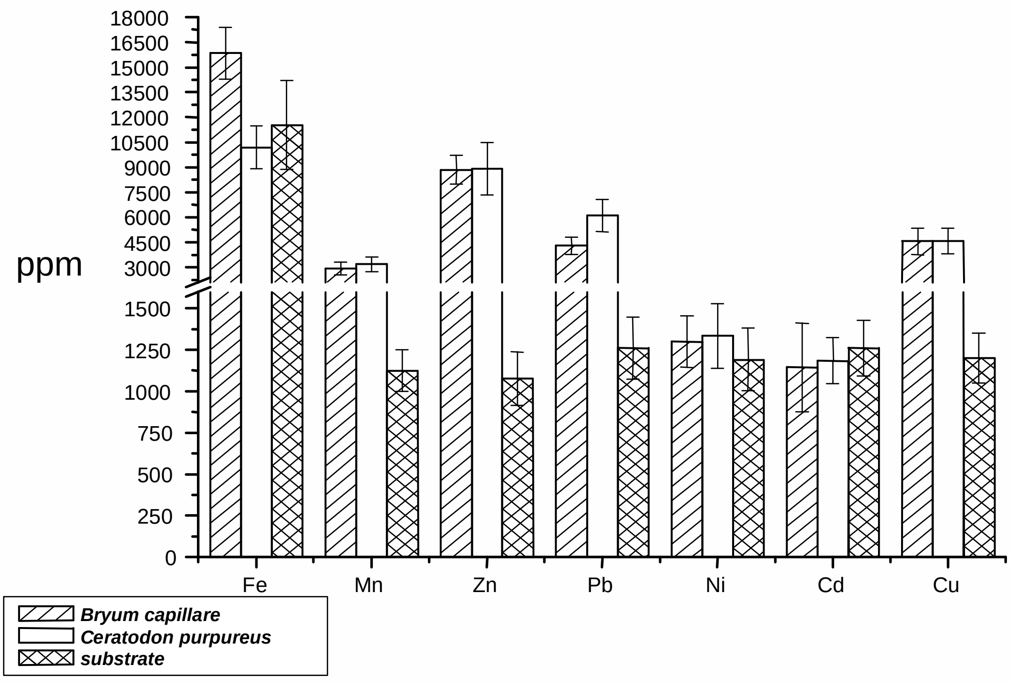

Fig. 8. Average metal concentrations in mosses and substrate (in ppm).

the reverse distribution. Dead tissues (not used in our study) retain polyvalent cations more effectively (Rühling and Tyler, 1970; Pakarinen and Rinne, 1979). Intracellular uptake of heavy metals, in contrast to extracellular uptake, is influenced by various aspects of plant metabolism, and metals mostly induce the production of thiol-containing peptides such as glutathiones, which can therefore be used as biomarkers for metal pollution, as in vascular plants. Young bryophyte shoots tend to have more effective barriers than older ones (Lüttge & Bauer, 1968)

Heavy metal-polluted soils usually lack an established vegetation cover due to toxic effects of pollutants or recent physical disturbance (Salt *et al.* 1995). Barren soils are more prone to erosion and leaching, which spread pollutants in the environment. A simple solution to the stabilization of these wastes is revegetation with metal-tolerant plant species. Mosses found to grow well in ash surfaces could be used as seed bed for some other plants in the process of revegetation. Sérgio (1987) observed that urban populations of *Tortula laevipila* Bridel. produced fewer sporophytes and more asexual gemmae than rural populations. Shaw (1994) and Shaw *et al*. (1991) stated that metal can decrease the sexual effort of mosses, certainly in *Ceratodon purpureus.* Indeed, both our plants were asexual, and the specimens of *Bryum capillare* bore many rhizoidal gemmae for vegetative reproduction. However, certain species (like *Funaria hygrometrica*) produce sporophytes abundantly under some types of highly polluted conditions (Gilbert, 1968).

Besides deposits from the atmosphere, these mosses also accumulate metals from the surface ash solution. Our three-year examination of metal content in the same subpopulations (60) shows that metal concentration decreases during the given period in young shoots of both moss species, as well in the ash substratum. This can be attributed to decreasing metal concentration in the substratum during the three years due to accumulation of metals by the mosses and rinsing of a certain percentage of metals to deeper levels of disposal site by water. However, the surface metal content is rinsed to a constant level much before settlements of the first spontaneous plants are established. The levels of metals in ash are periodically measured by the power plant's service department, and they correspond to our starting levels in ash in 1999. Also, only young shoots were used in analyzing to be sure that the accumulation of heavy metals was from just one year. The heavy metal concentration also decreased in the ash substratum during the year if moss patches grew on it. The high concentration of metals in these mosses is unquestionably due to the ash substratum solution, since atmospheric deposition is constant and standard in our measurements.

CONCLUSIONS

Bryum capillare and *Ceratodon purpureus* can be considered hyperaccumulator species for heavy metals, whose content in them comprises more than 0.1% dw in all cases and more than 1% for Fe, Zn, Pb, Cu. *Bryum capillare* has shown greater remedial potential, in view of higher hyperaccumulating values for Fe (up to 3.75% dw), Zn (up to 2.25% dw), Pb (up to 1.30% dw), and Cu (up to 2.00% dw) in comparison with *Ceratodon purpureus.* When these two species cover ash substrata, they bind surface ash and so prevent its being blown away to contaminate other surrounding surfaces. Protonema binding the surface of heavy metal-rich substrata stabilize them, and developing shoots, mats, and patches of bryophytes make seed beds for vascular plants, which develop spontaneously in these bare spaces. Because it is easier to cover bare spaces by spreading the propagules of bryophytes than by setting out seedlings of vascular plants, this can be a potential use of these two species in remediation of coal deposit sites. These species can be used for phytomining in view of the easy harvesting process.

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МАХОВИНЕ АКУМУЛИРАЈУ ТЕШКЕ МЕТАЛЕ СА ДЕПОНИЈА ПЕПЕЛА

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Биљке које могу да акумулирају и толеришу високе концентрације тешких метала (хгиперакумулатори) могу да се користе у фиторемедијацији (уклањању контаминаната из тла) или да се гаје на одређеним тлима да би се из њих издвајали контаминанти. Две врсте маховина, *Brym capillare* Hedw. и *Ceratodon purpureus* Hedw., су тестиране као потенцијални фиторемедијанти у условима *in vivo* на одлагалиштима пепела у Обреновцу (Србија). Током трогодишњег периода утврђиван је садржај тешких метала (гвожђе, манган, цинк, олово, никл, кадмијум и бакар) у маховинама и супстрату испод њих. Гвожђе и цинк су метали који се акумулирају у највећим концентрацијама у истраживаним врстамна маховина.