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## Heavy Metal Pollution in the Boatin Reserve (Bulgaria)

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**Abstract:** Background concentrations of the heavy metals Mn, Ni, Cd, Co, Zn, Cu, and Pb in the soils and plant monitors of the Boatin Reserve were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). Surface soil layers of the Boatin Reserve were polluted with Cu, as its concentration exceeded both the national legal standards and the content of Cu in non-polluted areas. Analysis of heavy metals accumulation in all studied plant monitors indicated that the content of Cu, Pb, and Zn exceeded their concentration in non-polluted areas. It may be concluded that the heavy metals Cu, Pb, and Zn are the main pollutants in the Boatin Reserve.

**Key Words:** Heavy metals, pollution, soils, plant monitors, Boatin reserve

### Introduction

Biological monitoring has been used for the control of anthropogenic pollution (Manning & Feder, 1980; Martin & Coughtrey, 1982). Biomonitoring has certain advantages in comparison to the direct measurement of industrial emission into the environment (Markert, 1996). Attention towards ecological monitoring of heavy metals has gradually increased, and investigations of their accumulation in soils and plants have been conducted (Dmuchovski & Bytnerowicz, 1995; Wenzel & Jokwer, 1999; Yilmaz & Zengin, 2004). Biomonitoring by means of mosses proved a useful tool for detecting pollution in natural, urban, and industrial areas (Vojtun, 1994; Bargagli et al., 2002; Aceto et al., 2003; Poikolainen et al. 2004; Culicov et al., 2005).

In Bulgaria, several studies of heavy metals content in soils, waters, and plant monitors have been carried out as part of the Program for Biological Monitoring (Vodenicharov et al., 1989; Yurukova et al., 1991; Yurukova & Nicolov, 1995). Recently, investigations of airborne pollution using mosses and lichens have also been conducted in Bulgaria (Yurukova et al., 1997; Yurukova & Ganeva, 1997; Yurukova & Damyanova, 1998; Yurukova, 2007).

The Boatin Reserve is a part of the national system for biotic control of environmental pollution. The purpose of

the present study was to determine the concentrations of the heavy metals Mn, Ni, Cd, Co, Zn, Cu, and Pb in surface soils and plant monitors of the Boatin Reserve.

### Materials and Methods

The Boatin Reserve was established in 1948. It is located on the northern slopes of the Central Balkan Range. The Boatin Reserve belongs to the Central Balkan National Park and occupies its westernmost part. The Reserve extends from 850 m to 2000 m above the sea level (Georgiev, 2004). Beech forests are most typical of the reserve and occupy 90% of its area. Pure spruce forests (10%) occur in the upper part of the reserve.

Soil and plant samples were collected from 4 sites within the Boatin Reserve (Table 1). The analysis of heavy metals in the soil followed Dimitrova & Yurukova (2005). A surface layer (0-10 cm) of soil samples (15 blocks per each sampling site) was cleaned from organic and mineral particles, grinded, passed through a 1-mm sieve, and dried at 85 °C for 48 h. About 1 g of soil sample was treated twice with a 10-ml mixture of perchloric and nitric acids (ratio 3:1) in a sand bath, and then with 20% HCl in a water bath. The soil sample was filtrated and the filtrate was diluted up to 50 ml with distilled water.

The content of heavy metals in plant monitors was

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Table 1. Sampling sites and plant monitor species.

Sampling Site	Altitude	Plant Monitor Species	
1	Gorski Dom	950 m	<i>Festuca nigrescens</i> Lam., <i>Agrostis capilaris</i> L., <i>Fagus sylvatica</i> L. (leaves and bark).
2	Kozarnika	1300 m	<i>F. nigrescens</i> , <i>A. capilaris</i> , <i>Arrhenatherum elatius</i> (L.), <i>P. Beauv. ex J. et C. Presl.</i> , <i>F. sylvatica</i> (leaves and bark), <i>Hypnum cupressiforme</i> Hedw.
3	Danchova Polyana	1350 m	<i>F. nigrescens</i> , <i>A. capilaris</i> , <i>F. sylvatica</i> (leaves and bark), <i>H. cupressiforme</i> .
4	Peak Baba	1800 m	<i>Juniperus sibirica</i> Burgsd. (leaves and branches).

determined according to Yurukova & Damyanova (1998). Plant samples consisted of the aboveground biomass of the examined monitor species (20-25 specimens per each species) collected at the peak of their development. About 2 g of plant material was dried at 85 °C for 48 h, then powdered and burnt to ashes in a muffle furnace for 12 h at 500 °C, followed by treatment with 20% HCl in a water bath. The sample was filtrated and the filtrate was made up to a volume of 50 ml with distilled water.

Each soil and plant sample was prepared in duplicate. The relative deviation between the duplicates was below 5%. Blanks were prepared in the same manner as the samples. The content of the heavy metals Mn, Ni, Cd, Co, Zn, Cu, and Pb was determined by inductively coupled plasma atom-emission spectrometry (ICP-AES) using a Perkin-Elmer instrument. Contents are expressed as mg kg<sup>-1</sup> dry weight.

Acidity of the soils (pH) was determined in a soil/water suspension (ratio: 1:2.5) 24 h after its preparation.

## Results

Acidity of the surface soil layers in the Boatin Reserve varied within the range of pH 4.9-5.1 (site 4 and 3, respectively). The concentrations of the heavy metals Mn, Ni, Cd, Co, Zn, Cu, and Pb are presented in Table 2. Mn content fluctuated within the range of 320-410 mg kg<sup>-1</sup>. Mean soil content of Mn for Bulgaria, averaged over 67 samples, is 880 mg kg<sup>-1</sup> (Mirchev, 1971), which is much higher than in the Boatin Reserve. Ni concentration ranged from 8.7 mg kg<sup>-1</sup> (site 3) to 10.6 mg kg<sup>-1</sup> (site 4), with a mean value of 8.6 mg kg<sup>-1</sup>. The maximum threshold concentration (MTC) for Ni, according to Bulgarian legal standards, is 35 mg kg<sup>-1</sup> at pH 5.0. The mean content of Cd in the soils of the Boatin Reserve was close to the MTC of 0.8 mg kg<sup>-1</sup> at pH 5.0. The content of Co ranged from 4.2 to 5.2 mg kg<sup>-1</sup>. The mean soil concentration of Co for Bulgaria, averaged over 34 samples, is 10 mg kg<sup>-1</sup> (Mirchev, 1971). The mean Co content in the Boatin Reserve was much less than the Bulgarian mean. Zn concentration ranged from 46.4 to 68.5 mg kg<sup>-1</sup>. The mean value for the Boatin Reserve was lower than the MTC of 50 mg kg<sup>-1</sup> at pH 5.0. Mean Cu

Table 2. Heavy metals content (mg kg<sup>-1</sup> dry weight) in the surface soil layers of the Boatin Reserve. Mean values are the average of 4 sampling sites.

Sampling sites	Heavy Metals Content						
	Mn	Ni	Cd	Co	Zn	Cu	Pb
1	320	9.2	0.6	4.2	47.2	35.8	39.3
2	372	10.3	0.8	5.1	59.9	45.4	31.8
3	410	8.7	0.9	4.7	46.4	59.9	32.3
4	388	10.6	1.1	5.2	68.5	56.5	40.7
Mean value ± SD	373 ± 30.8	9.64 ± 0.84	0.85 ± 0.18	4.8 ± 0.39	55.5 ± 9.1	49.4 ± 9.5	41.4 ± 5.1

content in the Boatin Reserve was higher than the MTC of 40 mg kg<sup>-1</sup> at pH 5.0. The soil concentration of Pb ranged from 31.8 to 40.7 mg kg<sup>-1</sup>. Mean Pb content in the Boatin Reserve was close to the MTC of 40 mg kg<sup>-1</sup> at pH 5.0. The descending order of heavy metals concentration in the Boatin Reserve (Mn > Zn > Cu > Pb > Ni > Co > Cd) was similar to the order in the nearby Steneto Reserve (Anchev et al., 1987); the only exception being Cu, which replaced Pb in the Boatin order.

The concentration of the heavy metals Mn, Ni, Cd, Co, Zn, Cu, and Pb in the plant monitor species are shown in Table 3. The content of Mn varied by a factor of 4 within the set of monitor species examined; from 106-432 mg kg<sup>-1</sup> in *H. cupressiforme* Hedw. and *A. capillaris* L., respectively. Ni content fluctuated within the range of 3.4-17.5 mg kg<sup>-1</sup>. Cd content varied in a narrower range, from 0.27 mg kg<sup>-1</sup> in *F. nigrescens* Lam. to 0.75 mg kg<sup>-1</sup> in *H. cupressiforme*. Excluding *Hypnum* species, mean content of Cd in all monitor species was 0.28 mg kg<sup>-1</sup>. This value did not exceed the norm of 0.3 mg kg<sup>-1</sup> for food and fodder set by Bulgarian legal standards. The content of Co ranged from 0.31 mg kg<sup>-1</sup> in *F. sylvatica* L. (bark) to 0.7 mg kg<sup>-1</sup> in *Arrhenatherum elatius* (L.) P. Beauv. ex J. et C. Presl. Zn content in the monitor species varied within the range of 17.1-89.5 mg kg<sup>-1</sup>, i.e. the maximum value exceeded the minimum by a factor of 5. Except for *Hypnum* species, mean Zn content (28.8 mg kg<sup>-1</sup>) was less than the legal norm of 50 mg kg<sup>-1</sup>. Average Cu content in all plant monitors, except *H. cupressiforme*, was 14.8 mg kg<sup>-1</sup>. This value was less than a third of the legal norm of 50 mg kg<sup>-1</sup>.

The maximum concentration of Pb exceeded the minimum by almost a factor of 7. Excluding *Hypnum* species, mean Pb content was 15.1 mg kg<sup>-1</sup>, 4 times higher than the legal norm of 4 mg kg<sup>-1</sup>.

## Discussion

The soil content of the heavy metals Mn, Ni, Cd, Co, Zn, Cu, and Pb determined by ICP-AES was studied at the Steneto Regional Background Station (RBS) (Anchev et al., 1987; Gerasimov et al., 1997). The concentrations of Mn, Ni, Cd, Co, Zn, and Pb in the Boatin Reserve were close to those in the soil samples collected from the Steneto Reserve. Cu content in Boatin was 3 times higher than in RBS Steneto. The investigations of soils at RBS Rozen (Gerasimov et al., 1997) revealed Mn, Ni, Cd, Co, and Pb content that is 2-5 times higher than observed in the present study. Cu content at RBS Rozen was nearly 50% less than in the Boatin Reserve. The descending order of the elements in the Boatin Reserve resembled that at RBS Rozen. The only exception was that Cu replaced Pb in the Boatin order. A large-scale study of heavy metals in the Central Balkan National Park (Delipavlov & Angelov, 2000) revealed that most samples had soil content of Mn, Zn, and Cd that was comparable to the values found in the present study. Cu and Pb soil concentrations in the westernmost part of the park were similar to the respective figures in the present study. It is noteworthy that the concentration of Cu in 2 heavily polluted sites exceeded the content of Cu in the Boatin Reserve by a factor of 2. These sites are on the southern

Table 3. Heavy metals content (mg kg<sup>-1</sup> dry weight) in plant monitors in the Boatin Reserve. Data on plant monitors *F. nigrescens*, *A. capillaris*, *F. sylvatica*, and *H. cupressiforme* are mean values averaged over the sampling sites.

Plant Monitors	Heavy Metals						
	Mn	Ni	Cd	Co	Zn	Cu	Pb
<i>F. nigrescens</i>	132.4 ± 26.6	5.6 ± 0.69	0.27 ± 0.03	0.35 ± 0.04	25.9 ± 0.99	14.2 ± 2.3	21.3 ± 3.2
<i>A. capillaris</i>	432.3 ± 45.1	6.8 ± 0.84	0.31 ± 0.03	0.34 ± 0.02	45.5 ± 7.4	32.3 ± 4.6	21.8 ± 5.1
<i>F. sylvatica</i> (leaves)	308.7 ± 37.6	7.2 ± 0.64	0.29 ± 0.05	0.32 ± 0.03	31.5 ± 7.1	18.3 ± 2.2	20.9 ± 3.8
<i>F. sylvatica</i> (bark)	345 ± 32.8	6.8 ± 0.57	0.32 ± 0.04	0.31 ± 0.05	18.5 ± 1.7	15.7 ± 2.4	19.4 ± 3.1
<i>A. elatius</i>	258.3	3.4	0.38	0.7	17.1	11.2	12.3
<i>J. sibirica</i> (leaves)	295.9	4.7	0.38	0.52	23.6	11.9	14.2
<i>J. sibirica</i> (branches)	330.4	5.8	0.42	0.6	25.3	11.2	16.5
<i>H. cupressiforme</i>	106.0 ± 19.5	17.5 ± 2.7	0.75 ± 0.11	0.65 ± 0.08	89.5 ± 13.2	95.1 ± 14.3	83.1 ± 11.7

slopes of the Balkan Range and are directly exposed to the gas emissions of a copper smelter near the town, Pirdop. Heavy metals content in the communities of *Pinus peuce* Griseb. in the Pirin Mountains was studied by Yurukova and Velchev (1987). Mean soil content of Cd and Ni were comparable to the respective values in Boatin. The concentration of Cu in the Boatin Reserve was 5 times higher than in the Pirin Mountains.

The concentration of the heavy metals Cd, Zn, Cu, and Pb in the plant monitors were determined at both RBS Steneto and Rozen (Anchev et al., 1987; Gerasimov et al., 1997). In most cases the Cd content was comparable to the values found in the present study. The content of Pb in Boatin was 2-7 times higher than the corresponding figures for RBS Steneto and Rozen. Cu content in the plant monitors of the Boatin Reserve was 2-3 times more than in RBS Steneto and Rozen. Excluding mosses, Zn content in Boatin was close to the respective values for RBS Steneto and Rozen. The species *J. sibirica* Burgsd. was used as a monitor in the study at RBS Steneto (Anchev et al., 1987). The concentration of the elements Mn, Ni, and Cd were close to the values found in the present study. The content of Cu in Boatin was 2-fold more than its content in RBS Steneto. The content of Pb in *J. sibirica* from the Boatin Reserve was 7 times more than the corresponding data for RBS Steneto. Different sets of mosses were used to estimate the background levels of some heavy metals in the Parangalitsa Biosphere Reserve (Ganeva, 1998) and Steneto (Anchev et al., 1987). The accumulation of Mn, Co, and Cd in the Parangalitsa Reserve was close to the data found in the present study. The content of Zn, Cu, and Pb in the moss monitor *H. cupressiforme* in the Boatin Reserve was 2-5 times greater than in both Parangalitsa and Steneto. It should be noted that both RBS Steneto and Rozen are situated in non-polluted areas, while the Boatin Reserve is close to an area of heavy industrial pollution. Similarly, biomonitoring surveys revealed higher concentrations of Cu, Pb, and Zn in areas of heavy anthropogenic impact (Tyler, 1984; Poikolainen et al., 2004, Orea et al., 2005).

It was found that surface soils in the Eastern Carpathians, Romania were mostly polluted by Pb (200-800 ppm), followed by Cu and Zn (Donisa et al., 2000). These figures were much higher than the respective values in the Boatin Reserve. Elevated mean Co, Cu, Pb, and Zn contents were found in the surface soils of the

area surrounding İzmit, Turkey (Yılmaz et al., 2005). The concentrations of Co, Pb, and Zn in the Boatin Reserve were lower (for Co as little as 25% of the İzmit concentration), whereas Cu content was higher than around İzmit. The accumulation of heavy metals in soils and plant monitors in the Boatin Reserve was higher than in the soils and the plant monitor *Taraxacum officinale* Webb in an ecologically clean region of Poland (Krolak, 2001). Analysis of the heavy metals Cd, Cu, Pb, and Zn in the plant monitor *Pinus nigra* Arnold revealed that the Thrace Region of Turkey is not affected by atmospheric air pollution (Coşkun, 2005). The content of these elements in plant monitors of the Boatin Reserve were higher, as compared to data of the above-cited study. Data gathered during moss surveys in Bulgaria, and cross-border data from Serbia and Greece were summarized by Yurukova (2007). The content of the heavy metals Cu, Pb and Zn in the moss species *H. cupressiforme* in the Boatin Reserve was higher than the average values for Bulgaria; however, it should be pointed out that the maximum concentration of heavy metals in mosses was found in the vicinity of local emission sources, e.g. Cu around a copper smelter (Yurukova & Ganeva, 1997). The Boatin Reserve is situated close to a copper smelter, which explains the comparatively high Cu accumulation in the moss monitor *H. cupressiforme*. Due to a copper smelter, the content of Cu in East Serbia was much higher as compared to the moss accumulation in Bulgaria. A study of Cu deposition in mosses of northern Greece revealed lower concentrations in comparison to data from Bulgaria (Yurukova, 2007).

Finally, it could be stated that the surface soil layers of the Boatin Reserve are polluted with Cu, as its concentration exceeded the national legal standards. Cu content in Boatin also exceeded the concentration of this element at RBS Steneto, Rozen, and the Pirin Mountains, which are situated in comparatively non-polluted areas. The main local source of Cu contamination is the nearby copper smelter on the southern side, opposite the Boatin Reserve. Air traffic in the area of the Boatin Reserve is a possible source of Pb pollution. An analysis of the heavy metals accumulation in plant monitors of Boatin indicated that the content of Cu, Pb, and Zn exceeded their concentration in non-polluted areas. It may be concluded that the heavy metals Cu, Pb, and Zn are the main pollutants in the Boatin Reserve.

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