

## ASSESSMENT OF POTENTIALLY TOXIC METALS CONCENTRATION IN KARST AREAS OF THE MEHEDINȚI PLATEAU GEOPARK (ROMANIA)

Andrei GIURGINCA<sup>1a</sup>, Cristian-Mihai MUNTEANU<sup>1b</sup>, Maria Luiza STANOMIR<sup>2a</sup>,  
Gheorghe NICULESCU<sup>3</sup> & Maria GIURGINCA<sup>2b</sup>

<sup>1</sup>“Emil Racoviță” Institute of Speleology, 13 Septembrie Road, No. 13, Sector 5, Bucharest 050711, Romania;  
<sup>a</sup>sankao2@yahoo.com; <sup>b</sup>criscarst@yahoo.com;

<sup>2</sup>University Politehnica of Bucharest, Polizu Street, No. 1, Sector 1, Bucharest 011061, Romania;

<sup>a</sup>stanomir\_luiza@yahoo.com; <sup>b</sup>m\_giurginca@yahoo.com;

<sup>3</sup>National Institute for Researches in Conservation and Restoration, Victoriei Road, No. 12, Sector 1, Bucharest 030026, Romania; niculescu.geo@gmail.com.

**Abstract:** The contamination with potentially toxic metals (PTM) represents an important threat to various ecosystem components, which are subjected to accumulation processes and along food chains it can also affect the human communities inhabiting the region. Our study was focused on a test area from the Mehedinți Plateau Geopark (Ponoarele) and also on a reference area (the Lupșa Valley, Gorj County), from which we collected samples of soil, leaf-litter and fauna (pertaining to two groups of invertebrates - Oniscidea and Diplopoda). On all samples, we performed AAS and XRF analyses, in order to assess the concentration of PTM. We found 13 PTM, but a significant level for the Ponoarele area was recorded only for copper. We determined also the food-biota accumulation factor (FBAF). A main challenge for future studies is to make the distinction between natural background level and the presence of a certain element as a result of the anthropogenic impact.

**Keywords:** Karst ecosystems, leaf-litter, Oniscidea, Diplopoda, PTM, AAS, XRF, FBAF, Mehedinți Plateau Geopark.

### 1. INTRODUCTION

Many papers are dealing with potentially toxic metals (PTM) contamination in Romania, but most of them are focused only on urban areas (e.g. Bucharest - Lăcătușu et al., 2004; Mihai et al., 2007; Giurginca et al., 2008; Lăcătușu et al., 2008; Râmnicu Vâlcea - Brănescu & Popescu, 2008; Brănescu et al., 2008; Rovinari - Lazăr et al., 2008; Baia Mare - Damian et al., 2008 and Zlatna - Damian et al., 2008), but no areas as the karst landscapes.

Two major categories of metal sources are documented, the natural background (air, water, bedrock, soil and biota) and the anthropogenic factor (raised levels of human-induced PTM).

Karst landscapes are more vulnerable to potentially toxic metals contamination, mainly due to the remarkable dynamics of processes running within these terrains (Vesper et al., 2000; Vlaicu et

al., 2007; Vlaicu et al., 2010). Moreover, the specificity of karst landscapes leads to a fast transfer of the contaminants, spread downstream on wide areas, including the non-karst ones. The three dimensional framework in which contamination extends has been presented by Doerflinger & Zwahlen, 1998, referring to groundwater vulnerability assessment by the EPIK method.

We focused our study to a representative test area from the southwestern part of Romania - Mehedinți Plateau Geopark, comprising an extended karst system (Zăton-Bulba). In order to identify reference elements, ecological investigations on the Lupșa Valley (located about 15 km northward, with a low level of anthropogenic influence) have been performed (Fig. 1).

We used two groups of invertebrates - the Oniscidea and the Diplopoda, for the assessment of the potentially toxic metals concentration, both being among the dominant groups of the soil

arthropod macrodecomposer community in many temperate habitats and key system regulators of decomposition and nutrient recycling ecosystem functions. Moreover, these two groups are potentially useful as bioindicators, quickly reacting to environmental impact (Paoletti & Hassall, 1999).



Figure 1. The location of the test area.

## 2. GEOLOGICAL AND GEOMORPHOLOGICAL SETTING

Although the limestones only limitedly outcrop in the Mehedinți Plateau, on 25 km<sup>2</sup> (Vlaicu et al., 2007), the functioning of the karst systems, and the diversity of the exokarst and endokarst features (more than 200 caves, with a cumulative development exceeding 45 km - Vlaicu et al., 2007) are ranking this area among the most significant karst terrains of Romania.

The Zăton-Bulba karst test area is located on the northern boundary of the Mehedinți Plateau, unit important due to the “limestone bar karst”, to the wide karren fields and also due to an interesting relief type - “cornete” (limestone hills), whose genesis is related to the limestone bars fragmentation and to a subsequent separation of the resulted subunits, by the interagency of the karst piracy depressions (Vlaicu et al., 2007).

The Zăton-Bulba karst system (Fig. 2) is developed in the region of the Ponoarele locality (Mehedinți County), marked by a series of well-known karst features: a natural bridge, crossed by the road Drobeta-Turnu Severin - Baia de Aramă - Herculan Spa, karren fields - like the one from the Peșterii Hill, sinkholes, springs and caves, such as the Zăton Cave (total development: 105 m), the Podul Natural Cave (734 m) and the Bulba Cave (5 km, extended on three levels).

In the region, there are two parallel limestone bars, developed on the Carpathian structures direction (NNE-SSW) and hydro- geologically connected.

The eastern one (Gărdăneasa-Băluța), is higher and authigenic, while the one we focused on, the western area (Baia de Aramă-Ponoarele) is located in a corridor (striking NE-SW, bounded by the Brebina Valley, in the north, and by the Zăton Lake alignment, in the south, 250-450 m a.s.l.), shows binary functioning and hydrographic network convergence in the limestone bar.

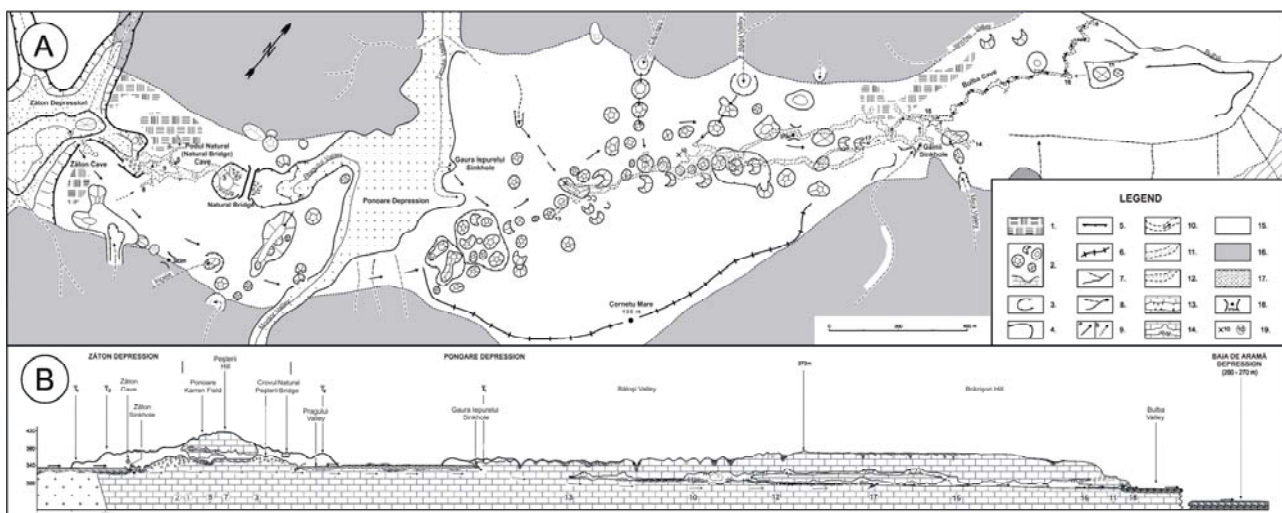


Figure 2. The Zăton-Bulba karst system (Mehedinți Plateau): 1. karren field; 2. sinkhole; 3. blind valley; 4. karst depression; 5. vertical cliff; 6. limestone ridge; 7. river; 8. swallow hole; 9. underground drainage; 10. active passageway; 11. temporary active passageway; 12. dry passageway; 13. speleothems; 14. collapsed rocks; 15. limestone; 16. non-karst rocks; 17. alluvial layer; 18. settlement; 19. mapping point (after Goran, 1978, modified, in Vlaicu et al., 2007).

Upstream, two closed depressions (Zăton and Bulba), flooded at high flow rates, can be noted. The inlet of the karst system is the Zăton Sinkhole, while the outlet is the Bulba Cave; the entire surface hydrographic network is caught in the subterranean realm (Vlaicu et al., 2007).

In the tectono-erosional window, the Mesozoic sedimentary outcrops, along with the crystalline basement of the Danubian Autochthonous (metamorphic rocks, intruded by the Tismana granites). The succession includes: non-karst detrital deposits (Lower Jurassic), limestones (Upper Jurassic-Lower Cretaceous), marly limestones, marls and wildflysch (Cenomanian-Middle Turonian and Upper Turonian-Senonian). The Zăton-Bulba karst system is developed within the Badenian-Aptian limestones (grey or white, massive or bedded Urgonian bioclastic limestones).

On the corridor margins, two synclinaloriums, comprising Paleozoic crystalline schists pertaining to the Getic Nappe and forming higher parallel ridges (600-700 m a.s.l.), can be identified.

The deposits of the Severin Unit also outcrop in the region: marly limestones, sandstones, conglomerates (Tithonian-Aptian), along with ophiolitic rocks, containing Cyprus type copper-pyrite ores. The pyrite, chalcopyrite, pyrotine, blend and galena occur as lenses, disseminations and small dykes.

A previous microtectonic study highlighted the preferential fissure trends for the Peșterii Hill, close to the Zăton-Bulba karst system inlet - N15°E,

N15°W, N30°W (tension fissures), N80°W (pseudocleavage of the limestone outcrop), W-E (shear fissures, parallel to the Izverna - Ponoarele - Baia de Aramă strike-slip system), and for the Bulba Valley, in the proximity of the outlet - N-S (parallel to the Motru Fault, with extensional regime), W-E (the same type as the above-mentioned) and N85°E (Vlaicu et al., 2007).

The reference area (Fig. 3), located in the north of the Mehedinți Mountains, in the Gorj County, is pertaining to the Lupșa Valley Basin (6 km long, striking E-W), since it is developed on a major, left side tributary of it, Ogașul Feței lui Iancu. After the confluence with the Motru Sec River, the water flows towards the Motru River.

The deposits of the Danubian Autochthonous outcrop in the area; on regional scale, the metamorphic basement consisting in quartzites, paragneisses and micaschists, or the Lower Jurassic detrital deposits, are mostly covered by limestones (Middle Jurassic-Neocomian and Barremian-Aptian).

Notwithstanding, the Lupșa Valley Basin is developed on non-karst rocks, overlaying the carbonate deposits - the Wildflysch Formation (Upper Turonian-Senonian): marl/clay shales, alternating with sandstones, in the lower part of the succession, followed by sandstones interlayered with conglomerates, in the upper one. The marl shales, striking N25°E and dipping 25°E, outcrop on the widest area of the basin (Botoșăneanu et al., 1967).

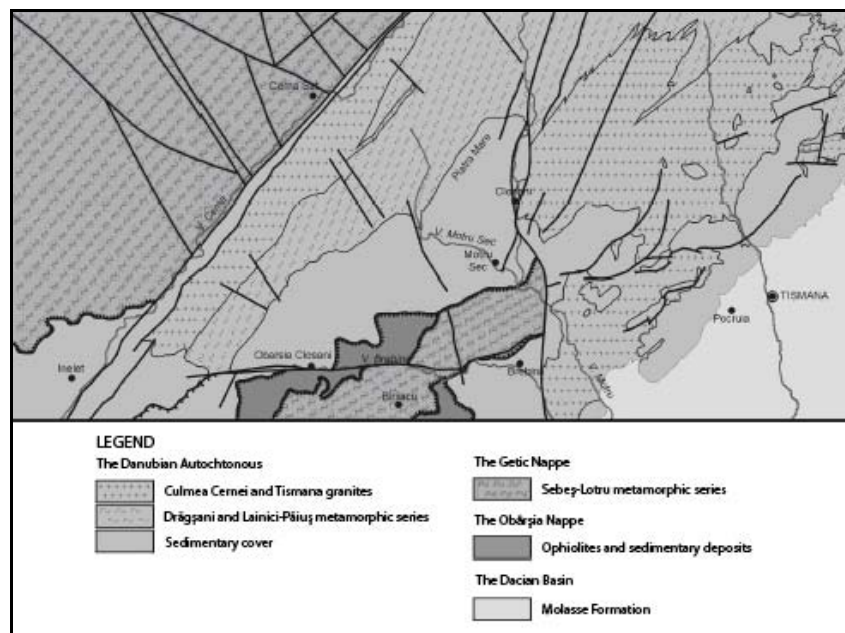


Figure 3. Lithostructural map of the region including the Lupșa Valley reference area (after Bercia et al., 1977 and Pop et al., 1975, modified, in Horoi, 2001).

The olistolithes of the Wildflysch Formation are just locally represented by Cenomanian-Middle Turonian marly limestones, marls and marl/clay shales, but widely by Barremian-Aptian limestones. The outcropping area of the limestone olistolithes includes the confluence between Ogaşul Feței lui Iancu and Lupşa Valley, but the tributary creek crosses non-karst deposits, and only downstream the carbonate rocks.

From a tectonic point of view, the main regional features are the Motru Fault, which brings into contact, along the N-S trend, the Tismana granites and the Barremian-Aptian limestones, and the Măgura Fault, striking NE-SW and establishing the contact between the carbonate rocks and the Wildflysch Formation (Diaconu, 1990). Regarding the morphology, it should be mentioned that 16

caves are related to the right slope of the Lupşa Valley, which presents vertical walls and erosion levels, only one pothole being identified on the left slope. The caves are located at 390-400 m a.s.l. (in the west) and at 364-373 m a.s.l. (in the east), the relative altitude ranging between 0 and 15 m, while the pothole is developed at 430 m a.s.l., 44 m relative altitude (Botoşăneanu et al., 1967).

### 3. MATERIALS AND METHODS

The biological samples have been collected by 21 pitfall traps (in groups of 3), placed in a beech forest (on Lupşa and Bulba valleys) and in a karren field (Ponoarele): 3 groups of pitfall traps on the Lupşa Valley and, respectively, at Ponoarele and one group of pitfall traps on the Bulba Valley.

Table 1. AAS results for the investigated area  
(L = Lupşa area; P = Ponoarele area; LL = leaf-litter; IS = invertebrate species).

Sample			Potentially toxic elements (µg/g)								
			Co	Mn	Zn	Cu	Hg	Fe	Cr	Pb	Cd
L	A <sub>1</sub>	LL	0.65	320	18.23	5.65	0.0002	830	3.85	13.41	2.82
		IS	1.19	230	180	170	0.0029	1690	4.27	1.07	1.20
	A <sub>2</sub>	LL	0.86	650	6.19	5.59	0.0002	560	3.40	16.82	1.69
		IS	1.23	100	180	180	0.0011	1800	4.82	0.58	2.38
	A <sub>3</sub>	LL	0.43	80	11.83	5.38	0.0002	210	2.04	23.89	1.78
		IS	1.04	920	150	130	0.0033	1890	6.54	0.91	0.31
P	A <sub>4</sub>	LL	0.40	650	13.48	3.62	0.0002	410	4.30	23.69	2.84
		IS	0.20	180	170	340	0.0099	270	0.91	1.67	0.54
	A <sub>5</sub>	LL	0.56	10	4.20	2.02	0.0008	190	3.14	26.06	2.27
		IS	0.33	20	130	120	0.0124	680	1.78	1.20	1.53
	A <sub>6</sub>	LL	0.19	30	7.44	3.83	0	160	1.93	15.91	2.88
		IS	0.10	130	180	120	0.0006	280	0.80	0.56	0.27
	A <sub>7</sub>	LL	0.51	10	9.04	4.30	0.0001	160	3.80	17.23	3.52
		IS	1.24	80	180	150	0.0005	1440	3.46	0.45	1.95

Table 2. XRF results for the investigated area (L = Lupşa area; P = Ponoarele area; LL = leaf-litter; IS = invertebrate species; n.a. = not available).

Sample			Potentially toxic elements (µg/g)							
			Mn	Fe	Cu	Zn	Rb	Sr	Zr	Pb
L	A <sub>1</sub>	LL	n.a.	12449	824	191	n.a.	80	102	n.a.
		IS	n.a.	428	103	87	n.a.	81	n.a.	n.a.
	A <sub>2</sub>	LL	7161	5470	791	144	35	98	62	101
		IS	n.a.	937	177	104	n.a.	95	n.a.	17
	A <sub>3</sub>	LL	4551	10896	1537	n.a.	n.a.	152	97	n.a.
		IS	135	n.a.	33	36	n.a.	132	N.A.	n.a.
P	A <sub>4</sub>	LL	2559	4625	679	168	33	93	62	n.a.
		IS	n.a.	114	63	63	n.a.	60	n.a.	n.a.
	A <sub>5</sub>	LL	n.a.	528	223	n.a.	22	41	69	48
		IS	n.a.	112	103	72	n.a.	47	n.a.	n.a.
	A <sub>6</sub>	LL	735	938	459	136	n.a.	47	75	80
		IS	n.a.	n.a.	71	96	n.a.	90	n.a.	n.a.
	A <sub>7</sub>	LL	n.a.	n.a.	225	n.a.	17	42	68	n.a.
		IS	103	509	144	116	n.a.	100	n.a.	n.a.

Two species of Oniscidea (*Armadillidium vulgare* - samples A<sub>1</sub> and A<sub>5</sub> - and *Trachelipus arcuatus* - samples A<sub>2</sub> and A<sub>7</sub>) and two species of Diplopoda (*Pachyiulus hungaricus* - samples A<sub>3</sub> and A<sub>4</sub> - and *Megaphyllum unilineatum* - sample A<sub>6</sub>) were used, the most common and abundant species of Oniscidea and Diplopoda in the investigated area.

The soil and vegetal samples were collected by average sampling from the same points where the pitfall traps were placed. The soil samples included districambosol from the Lupşa and Bulba valleys (4 samples) and erodosol from the Ponoarele karren field (3 samples). The vegetal samples consisted in leaf-litter from Lupşa and Bulba valleys (beech leaves - *Fagus sylvatica* L. - 4 samples) and grasses from the Ponoarele karren field (*Dichantium ischaemum* (L.) Roberty syn. *Andropogon ischaemum* L. - 3 samples).

All samples were subjected to AAS and XRF analyses. For the AAS, the sample preparation followed the procedure suggested by Drobne & Hopkin (1995): 1. drying at 60°C for 12 h; 2. wet grinding; 3. heat treatment with *aqua regia* - HNO<sub>3</sub> 65% (Merck) and HCl 37 % (Merck); 4. dilution with HNO<sub>3</sub> 1M; 5. filtering and filling up to 50 ml with bi-distilled water.

The concentration of Pb, Cd and Fe, Cu, Co, Cr, Mn, Zn was measured by an atomic absorption spectrometer AAS - Vario 6 (Analytik Jena). For Hg determination, a DMA 80 Hg analyzer (Milestone) was used.

In order to plot the calibration curves and for the optimization of the working conditions, Merck standard solutions (1000 mg/l) were used.

The XRF analyses were carried out by a portable X-ray spectrometer - Alpha Series (Innov-X Systems Inc.).

#### 4. RESULTS AND DISCUSSIONS

The following elements were determined by AAS analyses: Co, Mn, Zn, Cu, Hg, Fe, Cr, Pb and Cd (Tab. 1). The data show that: Co concentrations are less than 1 µg/g in the leaf-litter and about 1.2 µg/g in the invertebrate species; Fe and Pb concentrations are high in both leaf-litter and invertebrate species; the other metals are present in average quantities.

The results of XRF analyses are the following (Tab. 2): 13 metals were found in the samples - Mn, Fe, Cu, Zn, Rb, Sr, Zr, Pb, P, Ca, Ti, Ni and Ba; P, Ca, Ti, Ni and Ba are present only in traces, so they were not taken into consideration; Rb, Sr and Zr, found either in leaf-litter or in invertebrate species,

were presumably migrating from the soil. Rb and Zr were found only in the leaf-litter, while Sr was found both in the leaf-litter and in the investigated invertebrate species (Fig. 4 and 5). In order to compare the reference area (the Lupşa Valley) with the test area (Ponoarele), we determined the food-biota accumulation factor (FBAF = the element concentration in the invertebrate species / the same element concentration in the leaf-litter), using the PTM values resulted from AAS analyses (Tab. 3). The data show a higher concentration of potentially toxic metals in the Ponoarele area, while the lowest FBAF has been recorded for lead.

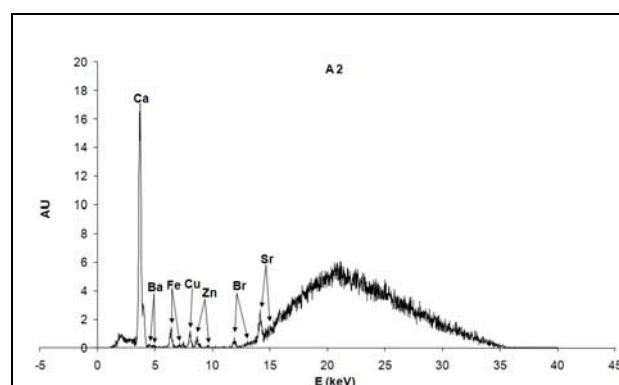


Figure 4. XRF spectra of the A<sub>2</sub> sample (*Trachelipus arcuatus*) from the Lupşa area showing the presence of Sr.

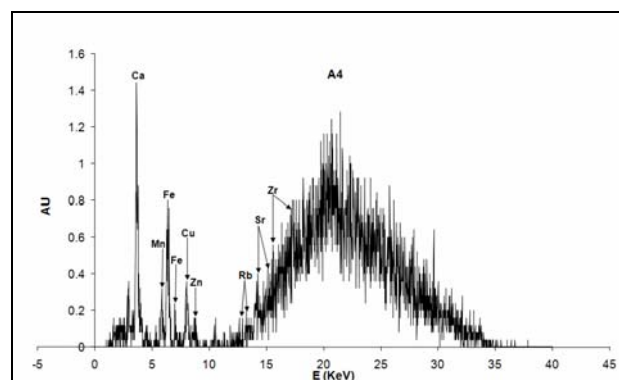


Figure 5. XRF spectra of the A<sub>4</sub> sample (*Dichantium ischaemum*) from the Ponoarele area, showing the presence of Rb and Zr.

All sensitive karst features of this area are placed under a constant anthropogenic pressure, due both to the road influence and to the residential impact (the dwellings of two villages with about 1000 inhabitants have no sewage or waste disposal facilities, the residues being directly discharged into the karst system; moreover, the manure is traditionally used as fertilizer for gardens and pastures).



Table 3. FBAF for Lupşa and Ponoarele areas.

Area	Sample	Potentially toxic elements								
		Cu	Zn	Hg	Co	Fe	Mn	Cr	Cd	Pb
Lupşa	A <sub>1</sub>	30.1	9.9	14.5	1.8	2.0	0.7	1.1	0.4	0.08
Ponoarele	A <sub>5</sub>	59.4	30.9	15.5	0.6	3.6	2.0	0.6	0.6	0.05
Lupşa	A <sub>2</sub>	32.2	29.1	5.5	1.4	3.2	0.2	1.4	1.4	0.03
Ponoarele	A <sub>7</sub>	34.9	19.9	5.0	2.4	9.0	8.0	0.9	0.6	0.02
Lupşa	A <sub>3</sub>	24.1	12.7	16.5	2.4	9.0	11.5	3.2	0.2	0.03
Ponoarele	A <sub>4</sub>	93.0	12.6	49.5	0.5	0.6	0.3	0.2	0.2	0.07

A vulnerability assessment study (Vlaicu et al., 2007) performed by the EPIK method (Doerfliger & Zwahlen, 1998) argued for a complex distribution pattern within the Ponoarele area, the most vulnerable terrains being assigned to the areas close to the Zăton Sinkhole and to the Găinii Sinkhole (Fig. 2) or to the diffuse infiltration areas, marked by the well-developed epikarst (karren fields) and by the thin soil layer; the rest of the karst area indicated a high or moderate vulnerability

The non-karst perimeters of the catchments, with lower slopes, were included in the category of the low vulnerable areas.

Copper is the only metal that suggests a higher pollution of the Ponoarele area, due to the natural background level (geological setting) and to the human impact (mining facilities). Related to the Severin Unit ophiolites, there are four ore fields in the Mehedinţi Plateau, the first two being important for our study: Cauna-Ponoarele, Gorunului Peak-Ocnelor Hill, Obârşia Cloşani-Podeni and Băroaia-Măgura. The Cauna-Ponoarele ore field (800 m long, 500 m wide) comprises a series of 13 pyrite-chalcopyrite lenses and disseminations, especially occurring in milonitized and chloritized basalts, as well as small dykes and interlayered bands in clays or basalts. The mineralization is thinning to zero towards south and west, but it intermittently continues 2 km more towards NE. The Gorunului Peak-Ocnelor Hill ore field shows nowadays the remnants of the copper mining field from Baia de Aramă, exploited since medieval or even earlier times, which generated over time slag dumps on the Bulba Valley. The compact, lens-shaped pyrite ore layer is confined between black shales (at the bottom) and intensely laminated basalts (on top). Due to the fact that before 1940 the mining was undertaken only for high copper content ores (over 2%), the dumps include sterile, but also ore with over 0.3% copper content as chemical analyses performed both on the primary mineralization and on the oxidized zone have shown (Ilie, 2008). In our opinion, this is the source for the copper found in the studied samples of leaf-litter and invertebrates.

The highest quantity of copper was found in

*Pachyiulus hungaricus*, followed by *Armadillidium vulgare* and *Trachelipus arcuatus*. A different case was recorded for Zn, its highest level being found in *Armadillidium vulgare*, followed by *Trachelipus arcuatus* and *Pachyiulus hungaricus*. The highest concentration of Hg was found in *Pachyiulus hungaricus*, followed by *Armadillidium vulgare* and *Trachelipus arcuatus*.

The correlation between the PTM and the studied invertebrates should be considered in the context of the invertebrates food preferences. Oniscidea and Diplopoda are mainly saprophytophagous primary decomposers, preferring to feed on dead vegetal material, already attacked and degraded by microbes (Eisenbeis & Wichard, 1987), as the decaying leaf-litter is easier to digest (Zimmer, 2002).

The amount of PTM (in our case Cu, Zn, Hg) assimilated from the leaf-litter by the primary decomposers depends as much on the form, as on the concentrations, of metals in their food (Hopkin et al. 1986). Thus, in aerielly contaminated sites, most of the metals detected in the leaf-litter are present as a blanket deposit of fine particles on leaf surfaces and, as such, are easily removed, as the food items transit the guts of the invertebrates. In contrast, metals in vegetation growing on contaminated soil (located on top of the ore fields discussed above) are much less available as they have been uptaken via the roots and are bound within the plant tissues (Hopkin et al., 1986). However, the availability of the PTM from the plant tissues might be enhanced by the decaying process and, as the Oniscidea and Diplopoda feed on decaying leaf-litter (macerated leaves, but also raw humus), the correlation between the PTM in the soil, leaf-litter and the studied invertebrates becomes clear.

Another factor, influencing the correlation of PTM in leaf-litter and in the studied invertebrates, is the specific food preference. For example, the concentrations of Cu, Zn and Hg in *Armadillidium vulgare* are higher in the Lupşa area than in the Ponoarele area. The explanation lies in the different vegetation of the two areas, but also in the food preferences of *A. vulgare*. The Lupşa Valley is

covered by a beech forest (*Fagus sylvatica*), while the vegetation of the Ponoarele karren field comprises mainly *Dichantium ischaemum* grasses. *A. vulgare* preferentially feeds on dicotyledonous plants (Souty-Grosset et al., 2005), *Fagus sylvatica* in this particular situation, rather than on monocotyledonous - the *Dichantium ischaemum* grasses, in our case. This preferential (and selective) consumption implies a higher uptake of beech leaf-litter, leading to a higher content of PTM (Tab. 1) in *A. vulgare* individuals collected from the Lupșa Valley, than in those sampled from the Ponoarele area.

## 5. CONCLUSIONS

A series of samples comprising leaf-litter and four species of invertebrates (two species of Oniscidea - *Armadillidium vulgare* and *Trachelipus arcuatus* - and two species of Diplopoda - *Pachyiulus hungaricus* and *Megaphyllum unilineatum*) from a representative test area of the Mehedinți Plateau Geopark were subjected to AAS and XRF analyses, showing the presence of 13 potentially toxic metals.

The computed FBAF has shown that copper was the most important metal in both investigated areas - the Lupșa Valley (the reference area) and Ponoarele (the test area), followed by Hg and Zn. The Cu content is the result of the natural background level (Cyprus type copper-pyrite ores) and of the human impact (copper mining facilities); the presence of Hg and Zn is presumably due to the human activities in this area.

Our data represent a first step in order to build a database concerning the impact of the potentially toxic metals on the karst areas.

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