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BIOACCUMULATION OF Cs, Ra, Pb AND U IN TISSUES OF THE TERRESTRIAL INVERTEBRATES (LUMBRICIDAE and JULIDAE) COLLECTED FROM LIQUIDATED URANIUM MINE

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SYNOPSIS

In this investigation, accumulation data of metals in terrestrial invertebrates were collected and compared. Based on total body concentrations, values were calculated for each metal (Cs, Ra, Pb and U) and each taxonomic group (Lumbricidae and Julidae). The analyses were performed by a standard method, including preparation, processing of collected material and dosimetric laboratory tests.

INTRODUCTION

The human activity may lead to a serious increase in the natural levels of heavy metals and radioactive substances in soil. Heavy metals pollution of ecosystems resulting from mine activity is an ecological factor in the living world, affecting individuals, populations, communities and ecosystems as a whole. Elevated concentrations of metals in soils can be detrimental to organisms and may have a lethal effect for some of them or lead to bioaccumulation in the tissues of the biological systems.

Ecological risk assessment of metal-polluted ecosystems is commonly based on total soil metal concentrations (Crommentuijn et al., 2000; Lock & Janssen, 2001). The total concentrations do not necessarily indicate bioaccumulation and toxicity of metals to biota. Often, soil specific parameters as pH and organic matter content can strongly determine the chemical availability of metals in soils (Sauve et al., 2000).

Uranium mining still is a cause of concern requiring assessment and the implementation of remedial actions. The bioaccumulation of radioactive substances and heavy metals can seriously affect human health, as well as other important

groups of terrestrial and aquatic organisms. Thus, there is a need to understand the interactions between uranium and living organisms in order to provide useful tools for the prediction of possible effects of environmental exposure.

The determination of the risks of such hazards is usually carried out through chemical and biological analyses of environmental samples, neglecting the assessment of biological effects (Antunes et al., 2008). Among soil organisms, earthworms (Lumbricidae), are considered to be particular interest, because they have a comparatively long lifetime in relation to other soil invertebrates and they can directly or indirectly modulating the transformation of organic and inorganic substances (Cooke et al., 1992). The earthworms (Lumbricidae) are located at the beginning of the consumer food chain and can transfer the contaminated food to their predators. (Kratz 1994; Alberti et al., 1996; Van Straalen et al., 2001). For many years they have been considered an interesting biological indicator of many metals in soils (Suthar et al., 2008). Soft bodied animals like earthworms are exposed to metals directly through their skins as well as via their diet (Eijsackers, 1998). As a consequence of metal deposition, soil organisms may fail to survive in contaminated environments or their functions in decomposition processes may be reduced. Several studies have reported that reduced species diversity, density and biomass in lumbricids due to increased metal concentrations in soil (Bengtsson & Tranvik 1989; Spurgeon & Hopkin 1999; Lukkari et al., 2004).

The main aim of this study was to investigate the heavy metal accumulation important for biological monitoring.

MATERIALS AND METHODS

Uranium mine Senokos is located at the watershed between the rivers Luda and Senokoska and at 1 km west-northwest of Senokos Village in Pirin Mountain. The mining in the region started in 1988 and continued until 1991 as an open mine. The mine was rehabilitated in the beginning of the 90's, but the lack of maintenance has led to intense surface erosion of the protective layer.

The changes and the bioaccumulation in tissues in terrestrial communities in the impacted area in the vicinity of uranium mine Senokos were assessed. All researching were done during April 2009 – June 2010.

The invertebrates (Lumbricidae and Julidae) were collected by digging (0-40 cm) from four different sample sites of the mine and one from the background, following by hand-sorting. Sampled invertebrates were taken to the laboratory and stored at cool temperature (15˚C) from one to five days. After this they were removed from the soil and transferred to clean jars filled with moistened filter paper, for one day or until they had emptied their gut contents. The collected invertebrates were identified in laboratory, and the identification of species was ensured by Narcis

Mesic (1991) and Milli Peet (2007). Analyses for metal concentrations in tissues were conducted in the ECOLAB Testing Laboratory at “DIAL” Ltd. Buhovo, Sofia town. The samples were dried at 105˚ C for two days or more, then ground to fine powder, and Gama spectrometric analyzed by BulgarianStateStandard IEC 61452.

RESULTS

The obtained data of our investigation shown a clearly affect in the studied families. The concentrations of the investigated heavy metals in soil, especially U238, were higher than the background (Bogoev et al., 2010). The soil in the vicinity of the mine is sandy loam with low contents of organic matter. The soil texture, organic matter and the concentration of studied elements in soil are given in Table 1.

Table 1: Soil texture, organic matter and concentration of studied elements in soil in the mine area.

Parameter	Unit	Mine
Soil texture	(%)	sand - 66.19±22 silt - 31.07±23 clay - 2.80±2.1
Organic matter	(%)	3.87±1.8
Element ($Bqkg^{-1}$)	**Background**	**Mine**
U238	38±4	265±25
Ra226	96±10	449±44
Pb210	76±7	408±37

Six species of earthworms belonging to six families were identified during the study *Eiseniella tetraedra tetraedra* (Savigny 1826), *Dendrobaena byblica* (Rosa 1893), *Aporrectodea rosea balcanica* (Savigny 1826), *Allolobophora tuleskovi* (Cern 1934), *Helodrilus balkanicu plavensis* (Karaman 1972), *Eisenie foetida* (Savigny 1826). The hightest percent of earthworms (Lumbricidae) were reported at 10.10.2009, perhaps as a result from higher soil moisure. With a highest abundance was spesies *E. tetraedra* and *D. byblica*. Only one species *D. byblica* was common for the mine area and the background. The distribution of *Lumbricidae* collected from the mine during April 2009-June 2010 is given in Table 2, and for the background is given in Table 2.1.

Two species of Myriapoda (*Pachyiulus cattarensis* (Latzel 1884) and *Megaphyllum hercules* (Verhoeff 1900) belonging to one family (Julidae) were found during the study. The total number of collected individuals was 273, 161 of them

were sexually mature. The sex structure of millipedes was dominated by females (94) over males (14) in the all phases of investigation. The distribution and the sex structure of Julidae collected from the mine during April 2009-June 2010 are given in Table 3.

Table 2: The distribution of *Lumbricidae* collected from liquidated uranium mine Senokos during April 2009-June 2010.

Date	Family	Genus	Species	Mature
23.03.2009	Lumbricidae	*Eiseniella*	*Eiseniella tetraedra tetraedra* (Savigny 1826)	2
10.10.2009				7
23.03.2009	Lumbricidae	*Dendrobaena*	*Dendrobaena byblica* (Rosa 1893)	3
16.05.2010				4
10.10.2009	Lumbricidae	*Aporrectodea*	*Aporrectodea rosea balcanica* (Savigny 1826)	3
10.10.2009	Lumbricidae	*Allolobophora*	*Allolobophora tuleskovi* (Cern., 1934)	3
16.05.2010	Lumbricidae	*Helodrilus*	*Helodrilus balkanicus plavensis* (Karaman 1972)	2
23.03.2009	Lumbricidae		immature	22
10.10.2009				40
16.05.2010				11

Table 2.1: The distribution of *Lumbricidae* collected from the background during April 2009-June 2010.

Date	Family	Genus	Species	Mature
16.05.2010	Lumbricidae	*Eisenia*	*Eisenia foetida* (Savigny 1826)	19
16.05.2010	Lumbricidae	*Dendrobaena*	*Dendrobaena byblica* (Rosa 1893)	2
16.05.2010	Lumbricidae		immature	8

The diversity of the studied invertebrates was low and unevenly distributed across the sites surveyed. According to some authors (Doblas-Miranda et al., 2009; Kicaj & Qirjo, 2010; Rybalov et al., 2000) invertebrate richness, abundance, biomass, species distribution and communities' composition depend on factors such as geographical region, climate, environment and soil depth. So one of the reasons for the low species diversity of the Lumbricidae and Julidae, in the area of the mine, probably due to the soil texture (sandy loam) and the low soil depth in some sites.

From an ecological point of view, the data obtained for the total bioaccumulation of these heavy metals in the tissues of the Lumbricidae and Julidae are very important because they show an average assessment of the organism's intoxication – a fact that could be used for prognoses in biological monitoring.

Table 3: The distribution and the sex structure of *Julidae* collected from liquidated uranium mine Senokos during April 2009-June 2010.

Date	Family	Species	Female	Male	Immature
21.05.2009	Julidae	*Pachyilus cattarensis* (Latzel 1884)	2	1	7
26.07.2009	(Myriapoda)		16	2	4
15.08.2009			38	3	19
10.10.2009			2	1	17
16.05.2010			4	1	8
21.05.2009	Julidae	*Megaphyllum hercules* (Verhoeff 1900)	5	1	4
26.07.2009	(Myriapoda)		4		2
15.08.2009			3	1	9
10.10.2009				1	9
16.05.2010			20	3	86

The bioaccumulation exposure toward to Cs, Ra, Pb and U and uptake of metals was evaluated by analyzing metal concentrations in invertebrates tissues and given in Table 4. The results showed elevated metal concentrations in the soil earthworms sampled from sites close to the Mine than the worms collected from the background. The highest accumulation ability, the earthworms (Lumbricidae) showed to U238 (2,1 ± 0,2 Bq/g). High concentration was registered for Ra226 too (0,051 Bq/g), five times more than the background (0,011 Bq/g). Only Cs137 was without any change in the mine area and at the background. Almost the same but with around ten times lower concentrations for the heavy metals, was observed for the Julidae family. This can be explained with the type of the used feed for millipedes and their vertical stratification in soil. *P. cattarensis* and *M. Hercules* are herbivores and scavengers, feeding primarily on decaying plant material and animal matter. According some authors the soil decomposers like the earthworms (Lumbricidae) accumulate heavy metals at a higher level than predators which have effective pollutant excretory mechanisms (Heikens et al., 2001). The epigeic species like *P.cattarensis* and *M. Hercules* prefers the humus layer, so they have weak contact with the less polluted mineral soil than the other species (Tischer, 2009).

The Lumbricidae and Julidae families have shown high bioaccumulation ability to studied heavy metals and they changed some of the indexes characterizing the structure and dynamic of their populations. The results clearly show that the investigated families can be successfully used for environmental monitoring of the liquidated uranium mines.

Table 4: In-tissue contents (Bq/g) of heavy metals in invertebrates (*Lumbricidae* and *Julidae*) collected from liquidated uranium mine Senokos during April 2009-June 2010.

Sample	Element	Unit	Results
Julidae	137 Cs		< 0,001
mine	210 Pb	Bq/g	< 0,008
	226 Ra		< 0,023
	238 U		0,21 ± 0,02
Lumbricidae	137 Cs		< 0,001
background	210 Pb	Bq/g	< 0,005
	226 Ra		< 0,011
	238 U		< 0,101
Lumbricidae	137 Cs		< 0,001
mine	210 Pb	Bq/g	< 0,023
	226 Ra		< 0,051
	238 U		2,1 ± 0,2

Table 4.1: (water content).

Sample	water content %
Julidae, mine	66,70%
Lumbricidae,background	75,20%
Lumbricidae, mine	71%

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