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## HEAVY METAL CONTENT IN SOIL AND THEIR BIOACCUMULATION IN EARTHWORMS (*Lumbricus terrestris* L.)

### SUMMARY

The aim of this study was to determine the concentrations of heavy metals (Cu, Fe, Zn, Pb, and Cd) in the soil and earthworm body (*Lumbricus terrestris*), as well to estimate Bioaccumulation Factor (BAF) in earthworm body. The soil and earthworm samples were taken three times, from March to June 2018, in six different locations in Kosovo (Mitrovicë, Kishnicë, Kastriot, Barilevë, Drenas and Lipjan) and were brought to the laboratory for heavy metal analysis. Concentrations of heavy metals in soil and earthworm samples were determined by an Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer brand model 1100 (Boston, MA, USA). Mean heavy metal concentration in soils were, 0.03-0.78mg/kg, 70.62-264.29mg/kg, 18.96-82.24mg/kg, 0.11-0.52 and 0.03-0.42mg/kg for Cu, Fe, Zn, Pb, and Cd respectively. The mean concentration range recorded in earthworms (*Lumbricus terrestris*) were 0.02-0.42mg/kg for Cu, 53.11-205.31mg/kg for Fe, 15.74-53.15mg/kg for Zn, 0.07-0.43mg/kg for Pb, and 0.01-0.37mg/kg for Cd.

Based on the results obtained it was shown that there are statistically significant differences of different levels of significance regarding the content of heavy metals according to locality (Factor A), substrate (Factor B), heavy metal (Factor C) and factor interactions (A\*B), (A\*C), (B\*C), and (A\*B\*C). The accumulation of heavy metals in earthworm samples maintains the same profile as mean heavy metal concentration of the soil Fe>Zn>Pb>Cu>Cd. Since some organisms like reptiles, birds and some other vertebrates feed on earthworms, transfer of these metals across the food chain is most likely, and therefore this research will be useful for risk assessment by relevant institutions responsible for the monitoring and surveying of environmental pollution and food security and safety in Kosovo.

**Keywords:** heavy metals, bioaccumulation factor, earthworms, ANOVA.

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## INTRODUCTION

One of the most important environmental problems in the world is the soils contamination by heavy metals in the industrial areas, and especially the contamination of the agricultural lands. Heavy metal can be defined as any metal with specific gravity higher than 4.00 g/cm<sup>3</sup> and is toxic and poisonous even at low concentration (Duruibe et al., 2007). Heavy metals are considered serious pollutants because they are toxic and non-degradable (Nwuche and Ugoji, 2008). When agricultural soils are polluted, these metals are taken up by plants and consequently accumulate in their tissues. Animals that graze on such contaminated plants and drink from polluted waters, as well as marine lives that breed in heavy metal polluted waters also accumulate such metals in their tissues. Humans are in turn exposed to heavy metals by consuming contaminated plants and animals, and this has been known to result in various biochemical disorders (Duruibe et al., 2007).

The loading of ecosystems with heavy metals can be due to excessive fertilizer and pesticide use, irrigation, atmospheric deposition, and pollution by waste materials (Aydinalp and Marinova, 2003).

Some authors reveals that lead (Pb) accumulates in the body organs (i.e., brain), which may lead to poisoning (plumbism) or even death. The gastrointestinal tract, kidneys, and central nervous system are also affected by the presence of lead, whereas regarding cadmium (Cd) the major threat to human health is chronic accumulation in the kidneys leading to kidney dysfunction (Wuana and Okieimen, 2011). According to the same authors copper in high doses can cause anaemia, liver and kidney damage, and stomach and intestinal irritation while zinc (Zn) is essential for human health and its shortages can cause birth defects. Most Zn is coming into the water resources during industrial activities, such as mining, coal, and waste combustion and steel processing. From water the Zn is entered in food chain through plants and finally arrives in the human body.

In recent years, earthworms have been widely used in the breakdown of a wide range of organic residues including sewage sludge, animal wastes, crop residues and industrial refuse in producing vermicomposts (Dominguez and Edwards, 1997; Kale, 1998).

Earthworms constitute an important group of soil organisms in terms of biomass, as food for many animals and in maintaining soil structure and fertility. Earthworms are located at the beginning of the consumer food chain and can transfer the contaminated food to their predators and ultimately to humans. According to some authors earthworms utilize a significant amount of soil organic matter for feeding, produce huge amounts of biogenic structures, and determine the activities of micro-organisms and other smaller invertebrates included in their 'functional domains' defined as the sum of biogenic structures that they have created in soil and the organisms that inhabit them (Lee, 1985). The use of earthworm as a bio-indicator for soil pollution was shown by Morgan and Morgan (1998). Stafford and Mc Grath (1986) also reported positive

correlation between earthworm and total soil Cu, Pb and Zn concentration from various metal contaminated sites.

Uba et al., (2009), carrying out a study on content of heavy metals in earthworms (*Lumbricus terrestris*) and associated soils in dump sites, confirms that earthworms accumulated some amount of heavy metals from dumpsite soils and levels of these metals accumulated in the earthworms tissues were less than 1 mg/kg for Cd, Cu, Pb and Mn while the ratio was higher than 1 mg/kg for Zn metal. The other authors, Bamgbose et al., (2000), in a study on the physico-chemical properties and heavy metal accumulation in contaminated and uncontaminated soil, shows that the concentration of heavy metals influences the pH and organic matter of the soil. Dumpsites usually contain various kinds and concentrations of heavy metals, which are dependent on the age, location and type of waste, (Ebong, et al., 2007).

In some regions and areas worldwide, especially around the mines where the industry is highly developed, soil pollution from heavy metal contamination has increased to the point that it endangers human life, and the reduction and eventual elimination of pollution in these areas is urgently more than necessary. A variety of physical, chemical and biological techniques have been proposed and implemented to achieve this aim. One of the methods is the usage of earthworms to clean up the soil from various pollutants, such as heavy metals, by the process of vermicomposting (Bianchin, 2009; Cheng-Kima et al., 2016). The term vermicomposting represents the process where earthworms ingest, grind and digest organic waste and finally convert it into a much finer, humified, microbially active material by the cooperative action of earthworms and microorganisms (Roshan Singh et al., 2014).

The indices of metal accumulation by organisms in their body tissue is known as bioaccumulation factor (BAF). It is the ratio of the level of metals in organisms to the soil substrate (Owagboriaye et al., 2015).

The aim of this work is to assess the level of some heavy metals in soil and earthworms as well to determine the bioaccumulation factor (BAF) of some heavy metals (Cu, Fe, Zn, Pb and Cd) in earthworm body with the total contents in the soils.

## MATERIAL AND METHODS

The present study was conducted in the year of 2018 by collection of heavy metal contaminated soil and earthworm samples from five different sites in the Republic of Kosovo: 1) Mitrovicë 2) Kishnicë 3) Kastriot 4) Barilevë, 5) Drenas and 6) Lipjan. For our purposes the agricultural land was used. The main heavy metal source contaminating the environment and agricultural soils in Kosovo is the industry, which is mostly located in the eastern part of the country (Zogaj et al., 2014). The following heavy industry sites are found in this area: the ore-metallurgic combine “Trepça” in Mitrovica, the Kosovo Energetic Corporation in Obiliq (Kastriot), “Ferronikeli” in Drenas, the Battery Factory Ni-Cd “IBG-Gjilan”, „Cementorja“ Hani i Elezit.

### **Soil sampling**

Soil samples were collected from agricultural land with a hand soil auger at least 10 meters away from the main stream and about 2 kg of soil was collected from top 5 cm layer at each site, after removal of surface vegetation and litter, in transparent polythene bags.

### **Earthworm sampling**

Regarding earthworms (*Lumbricus terrestris*) they were collected by digging into the soil from the same sites and then placed in sample bottles and labeled. All the bags (soil and earthworm samples) were marked with regard to the sites from which the samples were collected. Since freshly collected soil were moist the first step in preparation of soil sample was to dry with air or oven-dry as soon as possible to halt all biological transformation activities (Ndubuisi et al. 2017).

### **Preparation and preservation of soil samples**

The soil samples were spread out on a flat surface sheet of paper and left in the oven at the temperature of 105°C for three days to fully dry all moisture present. After this step, the sieving of soil samples was performed, in order to remove gravel, stones and plant debris. The materials that was able to pass through the 2mm mesh, as fine earth material, was selected and kept for analysis.

### **Preparation and preservation of earthworm samples**

The earthworm samples were placed in Petri dishes, and then refrigerated for 24 hours in order to purge the soil in the gut. After that they were removed, rinsed slightly with de-ionized water and then frozen pending analysis (Bamgbose et al., 2000).

### **Physicochemical properties of the soil samples**

The soil physicochemical properties were determined using the approved specific standard methods for each of them according to Miller et al., 2013. Soil acidity or pH value was measured in soil water solution 1:2 (Method S-2.40), organic matter with loss on ignition (Method S-9.20), nitrogen with Kjeldahl (Method S-8.10), phosphorus according to Olsen et al. (Method S-4.10), potassium, calcium and magnesium with Ammonium Acetat (Method S-5.10).

### **Heavy metal determination in soil and earthworm**

Samples in the amount of 3 g each (soil and earthworm), were treated with a 10 ml concentration HNO<sub>3</sub> and 10 ml concentration of H<sub>2</sub> SO<sub>4</sub> at a temperature of 400°C for over 1 hour (Latifi et al., 2017). Mineralized samples were mixed with distilled water and filtered with filter paper (Whatman No. 0.45 µm). The filtrate was set at 50 cm<sup>3</sup> in a volumetric balloon and leveled up to the mark with distilled water. Concentrations of heavy metals in soil and earthworm samples were determined by an Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer brand model 1100 (Boston, MA, USA).

### **Estimation of Bioaccumulation Factor**

Bioaccumulation factor (BAF) was calculated using the formula:  $BAF = C_{biota}/C_{substrate}$ , where  $C_{biota}$  and  $C_{substrate}$  are the total concentrations of heavy metals in earthworms and soil substrate respectively (Owagboriaye et al.,

2015). The higher the BAF value is means that the greater the amount of heavy metal is accumulated in the body of the earthworm and vice versa.

### Statistical Analysis

The data was statistically analyzed by analysis of variance (ANOVA) using computer software MSTAT-C from the University of Michigan, USA. Analysis of variance (ANOVA) and LSD -test were used to assess the differences in metal concentrations in soil and in earthworms as well, whereas the level of significance for the differences was set at  $p < 0.05$  and  $p < 0.01$  as well. Interaction factors were calculated from the table of ANOVA which was according to split-split plot experimental design. Factor A represents Locality (Mitrovicë, Kishnicë, Kastriot, Barilevë, Drenas and Lipjan), Factor B represents substrate (soil and earthworm), and Factor C represents heavy metal (Cu, Fe, Zn, Pb and Cd). Interaction between factors were A\*B (locality \* substrate), A\*C (substrate \* heavy metal), B\*C (substrate \* heavy metal) and A\*B\*C (locality \* substrate \* heavy metal).

## RESULTS AND DISCUSSION

Table 1 presents the results of the physicochemical properties of the soil samples from the localities where the experiment was conducted.

Table 1. Physicochemical properties of the soil samples

Locations	pH	Organic matter %	Nitrogen %	Nutrients mg/100g soil			
				P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	Ca	Mg
Mitrovicë	6.69	3.15	0.17	10.9	14.5	178	16.4
Kishnicë	6.85	2.73	0.13	12.1	15.7	189	15.9
Kastriot	6.43	2.08	0.10	9.7	15.3	154	15.2
Barilevë	7.12	2.95	0.14	12.3	13.6	194	15.5
Drenas	7.51	2.76	0.11	9.2	12.3	206	15.6
Lipjan	7.09	2.84	0.08	3.9	14.8	197	16.1
Average	6.95	2.75	0.12	9.7	14.4	186	15.8

Table 2. Heavy metal content in soil (mg/kg)

Locality	Heavy metal (mg/kg)				
	Cu	Fe	Zn	Pb	Cd
Mitrovicë	0.38	264.29	82.24	0.23	0.42
Kishnicë	0.78	174.15	76.18	0.52	0.25
Kastriot	0.11	114.69	33.62	0.39	0.13
Barilevë	0.03	70.62	18.96	0.11	0.04
Drenas	0.08	213.64	28.47	0.14	0.12
Lipjan	0.04	152.44	19.50	0.12	0.03
Average	0.24	164.97	43.16	0.25	0.17

As it can be seen the average of pH is 7.24 (neutral) and the organic matter 2.75%. With regard to the content of Nitrogen, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, Ca and Mg the values are 0.12 %, 9.7, 14.4, 186 and 15.8 mg/100 g soil respectively.

The results obtained from the heavy metal content in the soil have shown that the values obtained for all the analyzed metals are below the permissible limit according to the critical limits for EU countries and Kosovo legislation in force.

The results of these study regarding heavy metal concentrations in soil and earthworm samples from the six study locations in Republic of Kosovo is presented in tables 2 and 4.

In table 3 there are threshold reference values for some heavy metals in soil in EU countries and in Kosovo (Zogaj *et al.*, 2014).

Similar results for the level of heavy metals in the soils of Kosovo have also been reported by other authors and our data are consistent with those of these authors (Zogaj *et al.*, 2014).

Table 3. Heavy metal limits according to EU and National Legislation

Metals	Threshold		
	EU	Kosovo	Germany
Pb	300	50	100
Zn	300	300	200
Cu	140	100	60
Cd	1-3	2	1.5

The copper content in the soil samples ranged from 0.03 to 0.78 mg/kg (average 0.24 mg/kg). Copper is classified as micronutrient and earthworms did not accumulate this metal too much. The content of this metal in the earthworm's bodies ranged from 0.02-0.42 mg/kg (average 0.14 mg/kg) and was lower than that of the soil (Tab. 4).

Table 4. Heavy metal content in earthworms (mg/kg)

Locality	Heavy metal (mg/kg)				
	Cu	Fe	Zn	Pb	Cd
Mitrovicë	0.23	205.31	53.15	0.18	0.37
Kishnicë	0.42	162.41	46.92	0.43	0.16
Kastriot	0.07	92.10	29.10	0.34	0.09
Barilevë	0.02	53.11	15.74	0.07	0.02
Drenas	0.05	185.17	22.59	0.10	0.07
Lipjan	0.03	105.81	16.34	0.08	0.01
Average	0.14	133.99	30.64	0.20	0.12

Iron (Fe) in the soil samples was determined in the range of 70.62 to 264.29 mg/kg (average 164.97 mg/kg) and in the earthworm's bodies from 53.11 to 205.31 mg/kg with an average of 133.99 mg/kg and these differences are statistically highly significant, for the  $p > 0.01$  (Tab. 6). The zinc content in soil samples ranged from 18.96 to 82.24 mg/kg (average 43.16 mg/kg). Zinc, similar

to copper, is a microelement and its content in the earthworm's bodies was within limits 15.74-53.15 mg/kg (average 30.64 mg/kg). The range of lead content in soil was within 0.11 to 0.52 mg/kg with an average of 0.25 mg/kg (Tab. 2). The range of Pb content in the earthworm's bodies was 0.07-0.43 mg/kg with an average of 0.20 mg/kg (Tab. 4). Cadmium content in compost samples ranged from 0.03-0.42 mg/kg (average 0.17 mg/kg). The earthworms accumulated cadmium; the content of cadmium in the earthworm's bodies were lower (0.01-0.37 mg/kg) than in the soil.

These results has indicated that earthworm's bio-accumulate heavy metals in their tissue having the soil as its habitat, they feed on the debris of dead plants and animals which are components of the soil organic matter. The degree of bio-accumulation of these heavy metals by earthworms is dependent on the degree of accumulation in the soil. In our case the bioaccumulation of heavy metals in earthworm samples maintains the same profile as mean heavy metal concentration of the soil i.e. Fe>Zn>Pb>Cu>Cd.

By analyzing the obtained data regarding the bioaccumulation factor (BAF) of heavy metals in soil and earthworm's (Tab.5), it can be seen that the values are different for different heavy metals.

Table 5. Bioaccumulation factor of heavy metals in soil/earthworm

Locality	Heavy metal (mg/kg)				
	Cu	Fe	Zn	Pb	Cd
Mitrovicë	0.61	0.78	0.65	0.78	0.88
Kishnicë	0.54	0.93	0.62	0.83	0.64
Kastriot	0.64	0.80	0.87	0.87	0.69
Barilevë	0.67	0.75	0.83	0.64	0.50
Drenas	0.63	0.87	0.79	0.71	0.58
Lipjan	0.75	0.69	0.84	0.67	0.33
Average	0.64	0.80	0.77	0.75	0.60

The highest value of bioaccumulation factor, as average, was found to be for Fe (0.80 mg/kg) and the lowest one for Cd (0.60 mg/kg). Regarding other metals it was different and had these values of 0.77 mg/kg, 0.75 mg/kg and 0.65 mg/kg for Zn, Pb and Cu respectively. The bioaccumulation factor in all heavy metals was less than 1 mg/kg. This trend is similar to the result obtained by other authors (Agbaire & Emoyan, 2012, Uba et al., 2009). According to Ma et al., (1983), the amount of heavy metals accumulated within earthworm tissues is partly dependent on the absolute concentration of metal within a given soil and the physico-chemical interactions.

In recent years, many researchers (Marta Bożym, 2017, Shahmansouri et al., 2005, Gaganmeet and Hundal, 2015) have focused on heavy metals accumulation in earthworm's and their relationships with total and bioavailable fractions in soil, and reported that metals in earthworms were significantly related

to the concentration of heavy metals in a soil. Our results are in concordance with the results reported by these authors.

Based on the results of the analysis of variance (Tab. 6), it was shown that there are statistically significant differences of different levels of significance regarding the content of heavy metals according to locality (Factor A), substrate (Factor B), heavy metal (Factor C) and factor interactions (A\*B), (AxC), (BxC), and (AxBxC).

Table 6. Heavy metal concentrations in soil and earthworms (ANOVA)

Locality (A)	Substrate (B)	Heavy metal mg/kg (C)					Average (A*B)	Average (A)
		Cu	Fe	Zn	Pb	Cd		
Mitrovicë	Earthworm	0.23	205.31	53.00	0.18	0.37	51.82	60.57**
	Soil	0.38	264.29	81.24	0.23	0.42	69.31	
	Average (A*C)	0.31	234.80	67.12	0.21	0.40		
Kishnicë	Earthworm	0.42	162.41	46.92	0.43	0.16	42.07	46.23
	Soil	0.78	174.15	76.18	0.52	0.25	50.38	
	Average (A*C)	0.60	168.28	61.55	0.48	0.21		
Kastriot	Earthworm	0.07	92.10	29.10	0.34	0.09	24.34	26.97
	Soil	0.11	114.69	32.62	0.39	0.13	29.59	
	Average (A*C)	0.09	103.40	30.86	0.37	0.11		
Barilevë	Earthworm	0.02	53.11	15.74	0.07	0.02	13.79	15.87**
	Soil	0.03	70.62	18.96	0.11	0.04	17.95	
	Average (A*C)	0.03	61.87	17.35	0.09	0.03		
Drenas	Earthworm	0.05	185.17	22.59	0.10	0.07	41.60	45.05
	Soil	0.08	213.64	28.47	0.14	0.15	48.50	
	Average (A*C)	0.07	199.41	25.53	0.12	0.11		
Lipjan	Earthworm	0.03	105.81	16.34	0.08	0.01	24.45	29.44
	Soil	0.04	152.44	19.50	0.12	0.03	34.43	
	Average (A*C)	0.04	129.13	17.92	0.10	0.02	Average B	
Average (B*C)	Earthworm	0.14	133.98	30.62	0.20	0.12	B1 33.01**	
	Soil	0.24	164.97	42.83	0.25	0.17	B2 41.69**	
Average (C)		0.19Ns	149.48**	36.73	0.23Ns	0.15**	Average A*B*C	
FACTOR A B C AB AC BC ABC								
LSD	1 %	13.3438	5.9719	9.1075	16.7091	32.0536	14.3676	71.4261
	5 %	9.9084	4.5368	6.9189	12.0825	21.1587	10.4887	38.9108

Legend: Ns = No significant,  $p > 0.05$  = significant,  $p > 0.01$  = highly significant



As far as the localities (Factor A) are concerned, statistically significant differences have been observed, where the content of heavy metals as average during these surveys was higher in the locality of Mitrovicë (60.57 mg/kg), in relation to the Barilevë site where the content of heavy metals as average, regardless of the type of metal, was lowest (15.87 mg/kg). In this regard we can say that the locality Mitrovicë has been most polluted with heavy metals and that the observed differences are highly significant compared to the other localities. To our opinion this was expected given that in Mitrovica there is a metallurgical plant "Trepça" where zinc, iron and lead are produced and the pollution of soil, air and water is at a higher rate compared to other localities involved in these researches.

Statistically significant differences were also observed with respect to the substrate (Factor B). The content of heavy metals as average was higher in the analyzed soil samples (41.69 mg/kg), while the lowest in analyzed samples of earthworms (33.01 mg/kg) and the observed differences were very significant. Regarding the type of heavy metal (Factor C), regardless substrate (soil or earthworm's), statistically significant differences was also found (Average C). The highest content is found in Fe content (149.48 mg/kg) while the lowest in Cd (0.15 mg/kg) and the differences between them are highly significant. As a metal Fe is more widespread because Kosovo soils contain higher levels of Fe compared to other metals. In the locality of Drenas works Ferronickel combine where extraction of iron and nickel is done. This justifies the presence of Fe in larger quantities compared to the other metals. The average content of other heavy metals was 36.72 mg/kg, 0.23 mg/kg and 0.19 mg/kg for Zn, Pb and Cu respectively. Even among these elements, statistical differences of different levels of significance have been found.

With respect to factor interaction A\*B, A\*C, B\*C and A\*B\*C statistical significant differences of different levels of significances have been found as well (Tab. 6). Regarding interaction of the factors AxB (locality x substrate) there are many combinations, for instance if we compare heavy metal content in the soil at Drenas (48.50 mg/kg) with heavy metal content in Mitrovicë (69.31 mg/kg) these differences are statistically highly significant since the difference is higher than 16.7091 mg/kg which is at level of 1% of LSD (Tab. 6). In other case if we compare heavy metal concentration in soil in Lipjan (34.43 mg/kg) with that in the soil in Kastriot (29.59 mg/kg) we can see that there are no statistically significant differences since the difference is less than 12.0825 mg/kg (Tab. 6). The same comparison we can undertake at all interactions between factors we are interested to know and to confirm the level of significances.

## CONCLUSIONS

Heavy metal pollution of the soil and Ecosystem has become something of a global concern and this is as a result of its adverse effect on living organisms when they come in contact with these metals. They tend to be toxic and of a detrimental effect, this can be seen as a result of its ability to bio-accumulate in

the body of living organisms thus interfering with the food chain. In conclusion, this study confirms that earthworms accumulated some amount of heavy metals from soils in investigated localities. The study also showed that the levels of these metals accumulated in the earthworm tissue were less than 1 mg/kg. This study therefore confirms the potentials of earthworms to accumulate heavy metals from soils and so can be used as a bioindicator for pollution studies. Since some organisms like reptiles, birds and some other vertebrates feed on earthworms, transfer of these metals across the food chain is most likely, and therefore this research will be useful for risk assessment by relevant institutions responsible for the monitoring and surveying of environmental pollution and food security and safety in Kosovo.

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