TRACE METALS IN A HOST – PARASITE SYSTEM (*RATTUS* SPP. – *HYMENOLEPIS* SPP.) IN THE AREA OF A COAL MINE COMPLEX MARITZA IZTOK IN BULGARIA

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ABSTRACT

Trace metals (**Al, Fe, Mn, Zn, Cu, Pb, Ni**) accumulation and their dynamics in the system *soil-rodents-cestodes* from natural site located around complex *Maritza Iztok* were tracked. Accumulation factors (ratio of metals contents in rats to these in soil) and as well bioaccumulation factors in the *host – parasite system* (ratio of content of metals in cestodes to this in the rats) were calculated. Metal conten was determined in the soil, the liver of rats (infected and non-infected) and in the tapeworms. The accumulation factor was the highest for **Zn** followed of **Cu** and **Pb**. Cestodes *Hymenolepis spp.* have higher bioaccumulation capacity for toxic metals, than their infected host, especially for **Ni**. Data demonstrated an increase in pollution due to a coal industry in a region of *Maritsa Iztok* coal complex.

Key words: rats, helminth, contamination, trace metals.

Introduction

Coal is used extensively in electricity generation in different countries. The main components of coal are carbon, sulfur, oxygen, hydrogen and heavy metals.

Mining operations negatively impact the surrounding ecosystems through drainage of acid mine waters, deposit of mine waste rocks or mine dust (Bian et al. 2010; Liu et al., 2019). Biota are exposed to a mixture of several chemical pollutants (Bian et al. 2010). In general, non-essential elements are available to biota over a long time and are usually strongly accumulated in tissues of animals. When non-degradable pollutants are released into the environment, they can be taken up and transferred through food chains and may accumulate in animals. Free-living small mammals (mainly rodents) are sensitive and relevant zoomonitors of heavy metals in the polluted areas (Milton & Jonson, 1999; Topashka- Ancheva et al.1999; Petkovsek et al., 2014). Small mammals are used for determination of contaminants level. Higher concentrations of Cd, Pb, Zn and Cu are detected in the soft and hard tissues of small rodents inhabiting areas with a coal industry (Milton et al. 2003; Martiniakova et al., 2011; Sanches-Chardi et al., 2007).

Mammals are frequently infected with endohelminths. Several helminths are able to accumulate considerable concentrations of heavy metals (Sures et al., 1999). Parasites are considered as bio-indicators capable and indicating possible environmental burden, due to their higher potential to accumulate heavy metals. Several studies have reported researches on the use of parasites as indicators of environment quality. Rattus spp. are one of the most common harmful rodents. They are typical omnivorous mammals. Rat helminth fauna includes a variety of species, as cestodes *Hymenolepis* spp. .Rattus spp.- cestodes *Hymenolepis* spp. system has been recently used as a bioindicator of heavy metal pollution in the terrestrial environment (Kovacheva et al. 2020).

The aim of this work was to study trace metals accumulation and their dynamics in a system *soil-rats-endohelminths* from a natural site located in coal complex *Maritza Iztok*, Bulgaria.

Coal complex Maritza Iztok is the largest coal industrial complex in South-Eastern Europe with 3 coal mines and 3 thermal electric power plants (TEPP). Among them TEPP "Maritza iztok" 2 is the biggest one.

Materials and Methods

The study was performed in the region of the TEPP "Maritza iztok" 2, near the coal mine Trayanovo 1, east of Radnevo city, Bulgaria. Samples were collected in May 2018.

Soil samples

The soil samples were collected from the TEPP "Maritza iztok" 2. Each sample consists of 5 individual sub-samples (approximately 200 g each), obtained at a depth of 0.10–0.20 m from a square with dimensions 0.5×0.5 m and mixed. The soil type was cinnamon forest soils. Each soil sample was air-dried at room temperature, hand-ground and sieved through a 2 mm mesh sieve. The total Al, Fe, Mn, Ni, Cu, Zn and Pb were determined by ICP-OES (PRODIGY 7, Teledyne Leeman Labs, USA), following their extraction in *aqua regia.* (Totev et al., 1991; Essington, 2004).

Animal samples

Rats (*Rattus* spp.) 37 in total were captured by snap traps in the zone studied. Only adult rats were used in the study, on the basis of their body weight. Their age was determined according to criteria of molar root development and growth (Gustavsson et al, 1982). The rats are in permanent contact with soils and are territorially conservative. In the study only male rats were used.

The identification of helminths in the rodents was according (Genov, 1984). The dominant species of helminths were *Hymenolepis spp.* According to our data, wild small rodents rarely remain uninfected (Metcheva et al. 2001).

After trapping dead rats were dissected for removal of their liver and digestive tract. The digestive tract was investigated according to standard helminthological procedures. Rodents infected with trematodes and acanthocephalans and those with mixed infections were excluded from the study. Only rats infected with *Hymenolepis spp.* and non-infected ones were used in the study. Livers and tapeworms were taken from each animal, dried and the tissues were digested by HNO_3. The content of trace metals Al, Fe, Mn, Cu, Zn, Ni and Pb was determined using ICP-OES. The accumulation factor was calculated as a ratio of metals contents in liver rats to the content of the same metals in soils. The Bioaccumulation factor (BAF) was calculated as a ratio of metals concentrations in parasites to the metal concentrations in liver rats.The study was conducted in compliance with the requirements of the European Convention for the protection of Vertebrate animals used for experimental and other specific purposes and current Bulgarian laws and regulations. All procedures for animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences (Permit number: 11 30 127).

Results

All soils examined were found to have low concentrations of trace metals, with their contents of primary mineral composition of the soil, while iron and to a lesser extent manganese oxides form the secondary mineral composition. Among the rest of the microelements studied, the content of Zn was the highest, followed by that of Cu, Pb and Ni (Table 1).

Table 1: Trace metal contents in the studied soil, rats and their endohelminths

ME	MAC mg/kg	SOIL mg/kg	NON-INFECTED RATS mg/kg	INFECTED RATS mg/kg	ENDOHELMINTHS mg/kg
Al	-	31606	16.30±4.10	17.07±4.71	8.55±0.89
Fe	-	23969	263.83±25.75	270.74±16.83	271.81±19.84
Mn	-	863	3.04±0.77	4.21±0.96	8.05±0.67
Ni	110	29.6	0.46±0.09	0.55±0.07	1.33±0.05
Cu	220	36.8	10.01±1.25	10.63±2.70	11.53±0.98
Zn	450	83.5	71.73±22.59	87.10±2.63	63.33±12.76
Pb	150	25.9	5.11±10.45	4.99±0.79	6.71±1.09

Note: MAC – maximum allowed concentrations for permanent grass areas, pH_{H2O} > 7.4, BG regulation no. 3/2008

Small mammals play an important role in food chain by acting at different trophic levels. It is assumed that the metal concentrations in small rodents are reflected not only by trophic level but also by exposure time and metal concentrations in the exposure areas. In the case of *host-parasite* systems that live around the zone studied we have determined the trace metal contents (Al, Fe, Mn, Ni, Cu, Zn, Pb) in the liver of uninfected and parasite-infected with *Hymenolepis spp.* rats, as well as in the parasite *Hymenolepis spp.* itself (Table 1).

The concentration of elements studied in the livers of both types of rats are arranged as follows: Fe>Zn>Al>Cu>Pb>Mn>Ni. The results showed that the concentrations of all trace metals studied are below the critical levels, which were consider non-dangerous for mammals (Milton & Jonson, 1999) nevertheless the rats were infected or not.

The content of trace metals in the parasites followed a different order – after Fe and Zn are Cu, Mn, Al, Pb и Ni. This sequence of metals in the studied host-parasite system corresponded to the relative concentrations in ошгеи host/rodents/- parasite /cestodes/ system in habiting non-loaded or extremely loaded environments (Sures et al., 2002). Although the concentration of Fe in the liver tissue of rats is higher, it is at physiological levels..

The trace metals mobility from soils to rats we have determined by the calculation of accumulation factor (FA), which is the ratio of trace metal concentration in the rats to those in the soils (Fig. 1). The accumulation factor of metals in infected rats is higher than in non infected being the highest for Zn followed of Cu and Pb. It was corresponded to the sequence of the corresponded metals in the soils and we can observe their influence on the metals contents in the rats. Cu and Zn are essentials for the living organisms and are kept at relatively constant concentrations through the homeostatic mechanisms of the animals. The metals Al, Fe, Mn and Ni did not accumulate in the liver of the rats.

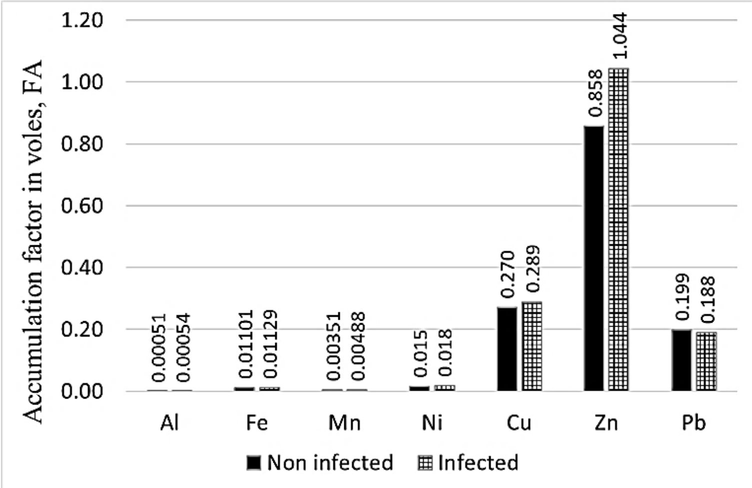

Figure 1: Accumulation factor (F) in rats

The ability of the parasites to accumulate metals is affected by numerous factors including the nature of metal, itself (Yossifova & Dimitrova, 1999). The bioaccumulation factors of trace metals for *Hymenolepis spp.* against tissues from infected rats are presented in Fig 2.

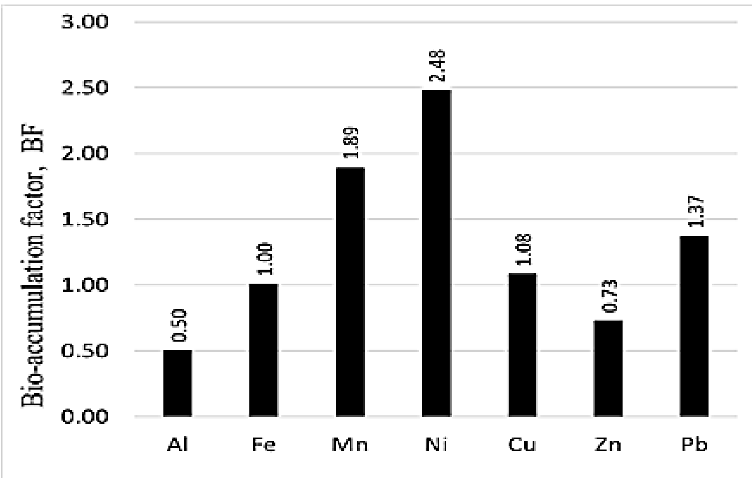

Figure 2: Bioaccumulation factors of trace metals in the parasite *Hymenolepis* spp. against tissues from infected rats/liver

Discussion

Pollution assessment in the region of complex *Maritza iztok*, Radnevo city with developed coal and coal processing industry, was done on the bases of data for trace metals accumulation and their dynamics in *soils – host – parasite* system *Rattus spp*.- *Hymenolepis* spp.The accumulation factors for metals in the rats to those soil was the highest for zinc. The AF for Pb and Cu were lower than 1. The rest AF were very lower due to the low metals levels in the soil.

The parasites had higher bioaccumulation capacity in comparison with the infected rats in regard to Fe, Mn, Ni, Cu and Pb. The biggest difference was observed in Ni content, where the bioaccumulation capacity was 10 times higher in the helminths than that found in the liver. Al and Zn accumulation in the parasite was lower than that in their host.

The BAF /parasites to rats/ of Al and Zn were below than 1. It is assumed that the metal concentrations in small rodents are reflected not only by trophic level but also by exposure time and metal concentrations in the exposure zone (Weissmannova & Pavlovsky, 2017). Species- specific metal concentrations were expected to be related to the metal levels in the soil of a trapping location (Sures et al, 1999).

We can consider that endoparasites are important element in the food chain. Our metal food chain model shows the direct/ indirect responses of parasites to anthropogenic changes. There is a significant relation between the levels of toxic metals in soil and a host-parasite system *(rats – cestodes*). It demonstrates an increase in pollution due to a coal industry in a region of Maritza Iztok. The dust emitted contains Zn, Cu, Pb, Cd, Mn and this contamination may increase the content of these elements in the tissues of rodents inhabiting polluted areas. It was observed that the host-parasite system *Rattus spp.- Hymenolepis spp.* living around the studied zone had the highest accumulation coefficient of Zn, followed by Cu and Pb.

Conclussion

The metal concentrations in the studied rodent-endohelminth system reflected metal concentrations in the exposure areas. Although the concentration of the determined metals in the rats were lower than the levels causing toxicological effects, they may predict changes at the level of higher links of the trophic chain. The system *Rattus* spp. – *Hymenolepis* spp. as a food sub-web model could be useful in the understanding the direct/indirect responses of parasites to anthropogenic changes. The rat-endohelminth scheme studied could be proposed for monitoring pollution in the contaminated ecosystems.

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