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Heavy Metals in a Host-Parasite System from a Cooper Mining Region in Bulgaria

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A host-parasite system *Rattus norvegicus/ Hymenolepis spp.* has been used as a bioindicator of heavy metals in the copper mining region of Chelopech, Bulgaria. The region has been polluted from the mining industry for copper and gold. Studies were done for contents of heavy metals zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb), nickel (Ni), aluminium (Al), iron (Fe), manganese (Mn) in tissues (liver and kidney) of rats (infected with tapeworms and non-infected) as well as in strobila of the parasites in comparison aspect using ICP-OES. Bioaccumulation factor was determined as a ratio between the content of metal in cestodes to a content of the same metal in rat tissue (liver or kidney). The high BF-s for Pb and Cu indicated that the amount of metals in the environment may result in significant uptake by the tapeworms and their host.

Key words: Hymenolepis spp., rat, heavy metals

Introduction

The intake and bioaccumulation of trace metals by mammals is known to occur [14]. Rodents and voles are mammals suitable for a bioindication of metals in the environment. These small mammals are widely used for determination of the level of contaminants based on mainly the detoxifying organs (liver and kidneys) due to their high capability for accumulating heavy metals [20]. There is a close interaction between the effects of pollutants and parasites on terrestrial animals [18]. Small mammals most frequently are infected with endohelminths. Both hosts and parasites are being exposed simultaneously to the concentrations of pollutants that are available in the environment. In most cases the intestinal parasites accumulated higher concentrations of heavy metals than their hosts, which could, in turn, even be beneficial for their host [18, 23]. Parasites are accepted as an integral part of the environment, with natural and anthropogenic variables influencing their various life stages [12,13].

Rattus norvegicus is widespread in rural and urban habitats, commonly found living near sources of food and water and is a good bioindicator for heavy metal pollution [9]. A tapeworm *Hymenolepis spp.* is one of the endohelminths with increasing abundance in the wild rats [10, 19, 21]. A host-parasite system *H. diminuta/R. norvegicus* has been used as a bioindicator of heavy metal pollution in the terrestrial environment [2, 4, 18, 20].

The aim of the study was to determine the main heavy metals content in the host-parasite system /*Rattus norvegicus – Hymenolepis spp.*/ collected in the copper mining region Chelopech, Bulgaria.

Material and Methods

Experimental material was obtained from a total of 96 rats in an area localized in the South West of Chelopech mining, near the village of Chavdar. The lands of the region have been polluted from the mining industry for copper and gold. The analyses of complex soil problems allowed us to point out that the main problem is related to acidity and the lack of nutrient elements. Gold mining has greatly increased copper concentration [3]. Low pH and high copper contamination reflect on the biota [6, 15]. The concentration of heavy metals in the region exceeded the accepted maximal permissible levels in respect to Cd (0.6mg/L), Cu (118,5 mg/L), Zn (98,1 mg/L) and Pb (39,5 mg/L) in randomly spread soil sample [6, 15].

Rattus norvegicus is a very spread rodents in this area. The wild rats were captured during 1 year from May to September 2018. They were captured by snap traps. Only adult rats were used in the study > 2,5 months old, according to body weight (border value 200 g). Their age was determined according to criteria of molar root development and growth [8].

The identification of endohelminths was according [7]. The dominant species of helminths were tapeworms *Hymenolepis* spp. Wild small rodents rarely remain uninfected (**Table 1**). A high prevalence of infection with intestinal helminths may be due to high reproductive potential, moved more often and faster than uninfected rats [15].

Table 1. Number of rats (parasitized and non-parasitized captured around Chelopech (Bulgaria)

Un-infected	Infected
19	52

After trapping dead rats were dissected for removal of the liver, kidney and digestive tract. The digestive tract was investigated according to standard helminthological procedures. Rats infected with trematodes and acanthocephalans and those with mixed infections were excluded from the study. Samples of the target organs were deep-frozen until posterior processing for chemical analysis. Livers, kidneys and tapeworms were taken from each rat, dried and the tissues were digested by dry ashing procedure according [11]. The concentration of samples in the homogenates was determined using ICP-OES. Studies were done for heavy metals Zn, Cu, Cd, Pb, Ni, Al, Fe, Mn in tissues (liver and kidney) of rats (infected and non-infected) as well as in strobila of tapeworms in comparison aspect.

The study was conducted in a 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: 96, 22.05.2014). Bioaccumulation factor (BF) was determined as a ratio between the content of metal in cestodes to a content of the same metal in rat tissue (liver or kidney) [19]. The statistical analysis was carried out on the Prism 6 programme. The distribution of data was determined – a Gaussian one (normal for all values). The determination of the distribution was performed using the test of Kolmogorov-Smirnov and D`Agostino-Pearson. In the Grubb test application no extreme value have been found (they are strongly differing from the mean one, usually neglible). Variation analysis was used for determining the mean values, the standard deviation (SD) and the significance criterion (P). The comparison of the mean values of parameters was carried out using the one-way analysis of variance, Dunnett's Multiple Comparison Test. The results from these comparisons were also statistically significant: * (P≤0.05), ** (P≤0.001), *** (P≤0.0001).

Results and Discussion

Data of the study give the possibility to compare the concentration of metals in tissues from non-infected and infected rats as well as to compare concentrations of metals in *Hymenolepis spp.* and host tissues of the infected rats. (**Fig. 1a, b, c**).

The levels of metals in the rat tissues and cestode tissues were in the next descending order:

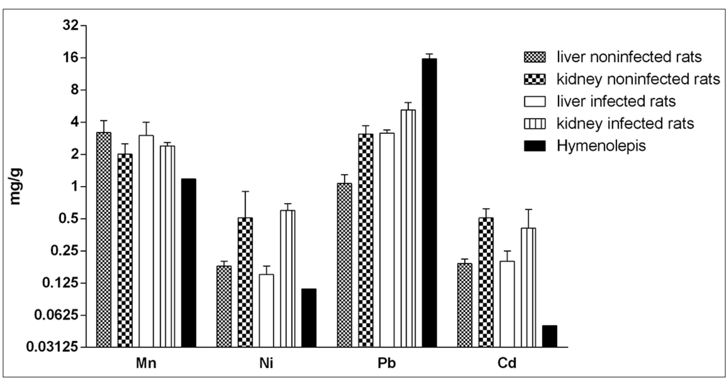

Fig. 1A

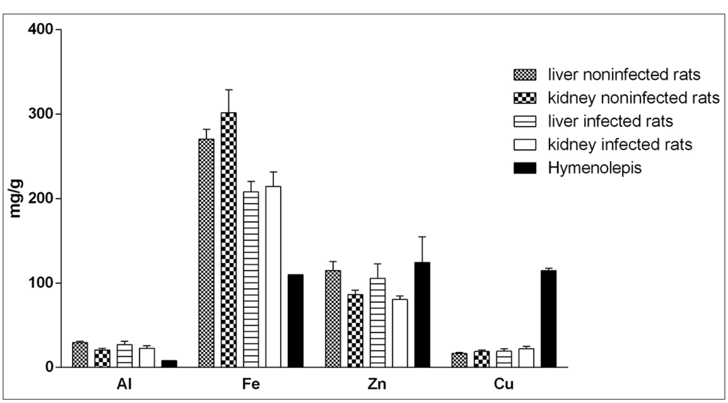

Fig. 1B

Fig. 1C

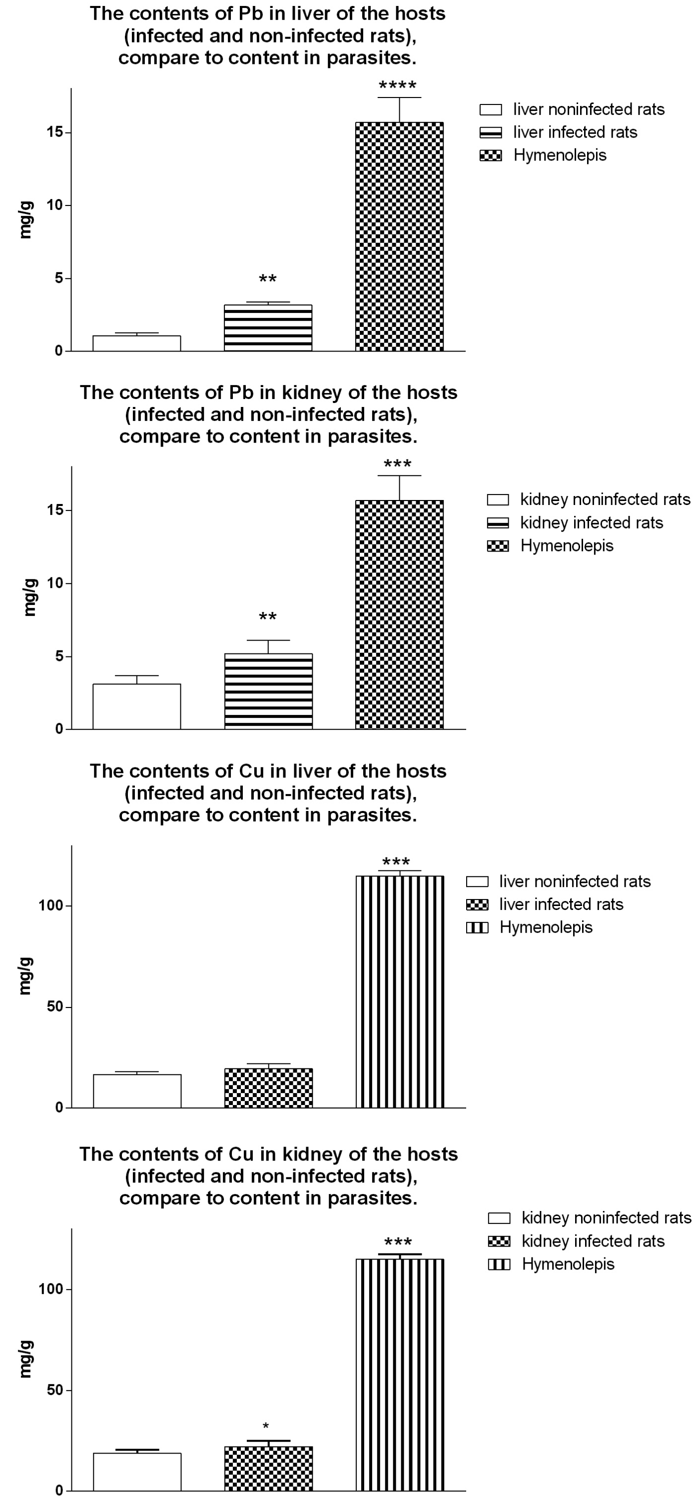

Fig.1. The concentration of metals in tissues from non-infected and infected rats compare to concentrations of metals in *Hymenolepis spp.* and host tissues of the infected rats.

Liver from infected rats: Fe> Zn> Al>Cu> Pb>Mn> Ni> Cd
Liver from un-infected rats: Fe> Zn> Al>Cu> Pb> Mn > Ni> Cd
Kidney from infected rats: Fe> Zn > Cu> Al> Mn> Pb> Ni> Cd
Kidney from un-infected rats: Fe> Zn > Cu> Al> Pb> Mn> Ni> Cd
Hymenolepis spp.: Zn> Fe>Cu > Al> Pb > Mn> Ni> Cd

Concentration of metals in descending order did not differ between these in the livers of infected and non-infected rats. The contents of Fe, Cu, Cd and Pb were higher in the kidneys than the livers of the hosts (infected and non-infected rats). Contents of Al, Cu and Fe were reduced in the livers of infected rats compared with non-infected individual. Concentration of Fe was reduced in infected rats compared with that in non-infected rats. The descending order of metals in the tapeworm was presented in different way to these in the hosts. The levels of Cu in the parasite were about 7 times higher than that in the infected rats. Zn content in the parasite was insignificantly higher compared to Zn level in the host. High ability of cestodes to accumulate heavy metals was present by BF of ratio of metal content in cestodes to that in host tissues. BF for liver and kidney was above 5 for Cu and Pb, and for Zn was above 1. Cu, Zn and Pb were bioaccumulated more in cestodes than in the host tissues. Concentration of Zn is an essential nutrient required for growth and maintenance of many biological systems in a host and a cestode [12, 13]. *Hymenolepis* strobila tissues showed significantly higher Pb and Cu content than the host tissues. It is in a good accordance with studies of [19] which indicated that tapeworms are able to accumulate heavy metals much more rapidly than the host soft tissues.

The system of barriers maintains body homeostasis in animals. One of these is the gastrointestinal barrier, which effectively protects from deleterious effect various factors (biological, chemical etc.) by means of their selective transport. Because the small intestine is simultaneously the predilection habitat of Hymenolepidae and the main site for heavy metal absorption after oral intake, tapeworms may interact with metals in the rat intestine and their absorption into the host body. This interaction may affect the course of heavy metals in the intestine. Helminth paratization may disrupt internal homeostasis, modulates immunological regulation of the host. Cestoda with lacking digestive tract concentrate metals to a higher degree than the host tissues [22].

The studies showed elevated tapeworm BFs in animals living in in polluted areas [5]. Our studies support the hypothesis that cestodes with a relatively large absorbance surface have reached high BFs. It may be due to their lower metabolic activities or due to a fact that the endohelminths lacking digestive tracts (Cestoda and Acanthocephala) and using their teguments for absorbing substances from the host digestive tract [10]. Tapeworms take up certain biogenic elements as Fe, Zn, Cu from the chyme into their tissues, and this uptake could free up transport routes for Pb absorption because these elements use the same pathways. Parasites may also cause an increase in host heavy metals by disrupting their risk elements regulation system. The content of Pb was higher in the kidneys than in the livers in the infected and non-infected hosts. Median values of Pb content differed insignificantly among parasitized and non-parasitized animals. Our results have confirmed those of [5] done only at low Pb exposure. Generally, helminths interfere with the host absorption, metabolism, uptake and regulation of the mineral elements [19]. Helminths may demonstrate higher sensitivity to the toxic effect of Pb.

Endohelminths and their hosts compete for biogenic elements Zn, Fe, Cu. The digestive tracts are absent in the cestodes, therefore they are able to concentrate metals to a higher degree than the host tissues. Worms are not able to synthesize their own cholesterol and fatty acids, so they take bile from a host intestine which in turn reduces their ability to absorb metals [18]. Cestodes use the bile salts for their egg formation and hatching. This could possibly explain the high concentration of metals in the cestodes.

Our data do not fully support the prior hypotheses of [10] which state that intestinal helminths are able to prevent their host from absorbing ingested heavy metals and their accumulation in the tissues of the host. The high Pb and Cu concentration in the tapeworms were not associated with simultaneously significant decrease of these elements in host tissues [5, 19]. Thus tapeworms are not able to actively eliminate pollutants from host tissues. Lead content represents the most important risk, which is probably linked to the nature of the ore exploited, to the low mobility of Pb in the environment and the acidification of the soil in the studied region. The concentration of heavy metals in the region exceeded the accepted maximal permissible levels in respect to Cd (0.6mg/L), Cu (118,5 mg/L), Zn (98,1 mg/L) and Pb (39,5 mg/L) in randomly spread soil sample [17], [16], [6]. Mammals and birds are 100-1000 times more resistant to Cu than other animals. Despite the fact that almost heavy metal values may not indicate a severe risk of toxic effects on wildlife but after a long period of time could exert an impact on individuals, communities and ecosystems.

Conclusions

Concentrations of heavy metals were determined in sub-web a host-parasite system *Rattus norvegicus – Hymenoleps spp*. in copper polluted region. A high ratio C parasite/C host was indicative of acute pollutant exposure or long/chronic exposure of the pollutants correlated with high concentration in both the host and parasite. Parasite infections have been attributed to man-made impact and environmental changes in terrestrial habitat. The position of parasite as a trophic consumer can infer details about the chemical state of the environment as a consequence of food web biomagnifications. The host-helminth system wild rat/ *H. diminuta* reflects the content of heavy metals in the terrestrial environment and could be used as a bioindicator for heavy metal pollution. The high BFs for Pb and Cu indicated that the amount of metals in the environment may result in significant uptake by the tapeworms (**Fig. 2.**). Despite the fact that almost of heavy metal values may not indicate a severe risk of contamination in the environment.

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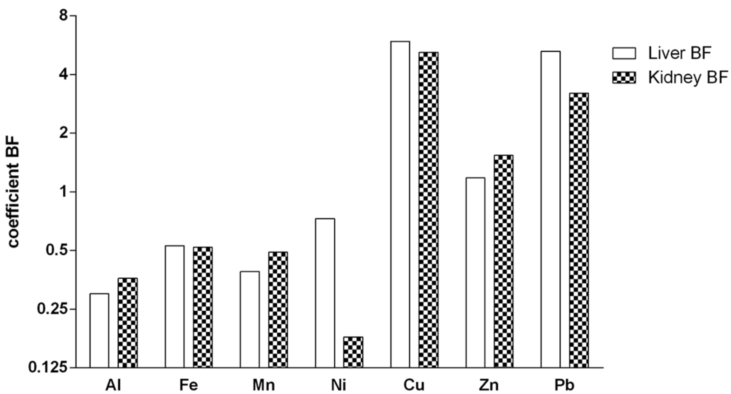

Fig. 2. BF of ratio of metal content in cestodes to that in host tissues.

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