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BIOACCUMULATION OF METALS IN TISSUES AND HELMINTHS OF HARES IN KARDZHALI REGION

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ABSTRACT

This study was aimed to evaluate content of heavy metals in Lepus europeas Pallas 1778 and its helminth *Trichuris sylvilagi* (Nematoda) in Kardzhali region, Bulgaria. The contents of Cd, Pb, Zn, Mo, Mn, and Co were determined in liver, kidney, muscle and helminths. A metal bioaccumulation factors were calculated. Significant differences in the concentrations were found between parasites and host tissues. The content of Zn, Pb and Cd was higher in the nematodes than in the hare tissues. The studied model could be a promising bio-indication system to evaluate the ecological state in terrestrial habitats.

Key words: *Trichuris sylvilagi*, hare, heavy metals.

Introduction

Heavy metal pollution is one of the most serious environment problems. Recent studies show that a brown hare is a good metal pollution bioindicator in terrestrial environment (Baikov et al., 1994; Wajdzik et al., 2017). The majority of heavy metals enters into the body of living organisms via oral intake and is absorbed in the intestinal tract (Takac et al., 2009; Arnaudova and Sapundzhiev, 2012), where many of them are absorbed by the helminths. Studies in recent decades indicate that several parasitic helminths are able to bioaccumulate higher heavy metal concentrations than the tissues of their hosts, which make them good bioindicators of metal pollution (Sures et al., 2004; Jankovska et al., 2008). The information on the combined effects of metal pollution and parasitism on terrestrial animals are scare. It became clear the acanthocephalans and cestodes parasitizing small mammals can bioconcentrate several toxic metals to higher concentrations than the tissues of their definitive hosts (Sures et al., 2004). The possibility of nematodes to accumulate heavy metals is not studied enough.

Our study was to assess the contents of heavy metals in the brown hare and its helminth a whipworm *Trichuris sylvilagi* from polluted Kardjali region, Bulgaria. The contents of Cd, Pb, Zn, Mo, Mn, and Co were established in liver, kidney, muscle and helminthes. Their bioaccumulation coefficient was calculated as well.

Material and methods

During the hunting season hares were sampled in 2017 year in a region of town Kardjali. Hares were obtained in cooperation with a local hunting organization. Their age was above 1 year. The animals were dissected to collect liver, kidney, muscle (m. semimembranosus of the hind leg) and digestive tract. The digestive tract was analyzed for nematodes. Parasitological negative hares and hares with cestoda or mixed infections were excluded from this study. Liver, kidney, muscle and helminth specimens were deep-frozen until posterior processing for analysis. Heavy metals contents

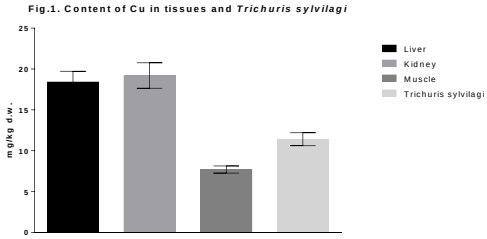
(Cd, Pb, Zn, Mo, Mn, and Co) were determined in the samples using flame atomic absorption spectrometry. Statistical analyses were performed according to Statview 4.5 software package. The bioaccumulation factor (BF) was determined according Sures et al., 1998 as the ratio of the element concentration in the parasites to that in different host tissues (BF=C parasite/C host tissue).

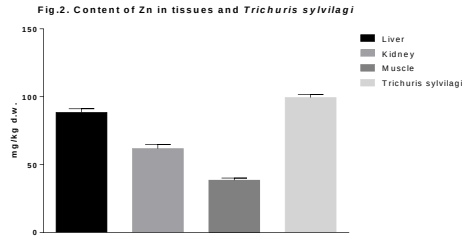
Results

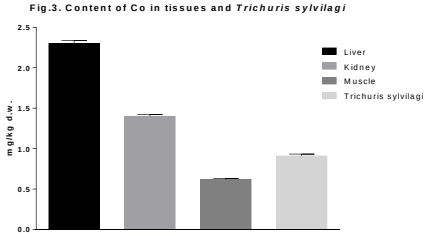
Eleven hares were infected with *Trichuris sylvilagi* with high intensity – above 120 specimens per a hare.

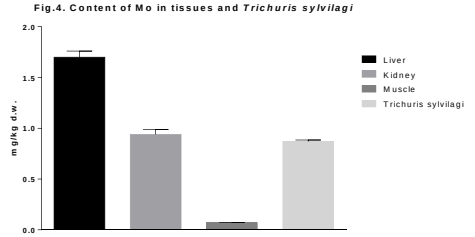
Elemental concentrations of hosts and parasites is presented in Fig. 1. Copper level was the lowest in muscle and highest in the liver and kidney. Cu content in the helminths was similar to that in the muscle. Zn content was significantly higher in the nematode and in the liver than in muscle and kidney (Fig. 2). Content of cobalt, cadmium, lead, molybdenum and manganese is presented in Fig. 3, 4, 5, 6. Co concentration was the highest in the liver of hares and the lowest in the muscle and nematode. Mn and Mo levels were significantly lower in the helminths than the host tissues – liver and kidney. The level of Cd was higher in the helminth compared to that in the kidney ($p<0.05$) and in the muscle ($P< 0.001$). Pb concentration was higher in the nematode than in the host tissues. Toxic metals Cd and Pb were higher than in the liver and muscle of the host.

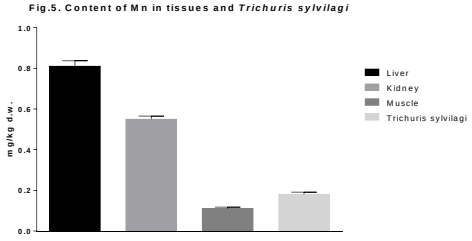
Metal BF was calculated to characterize the bioconcentration capacity of the helminths. The BF are presented in Fig. 8–13. The BF of the all elements was higher in the muscle as well. The highest BF was obtained for Pb and Cd in the muscle. (BF=C parasite/C muscle).











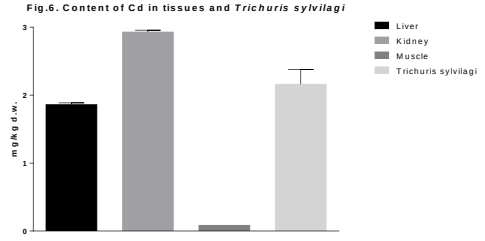


Fig.7. Content of Pb in tissues and *Trichuris sylvilagi*

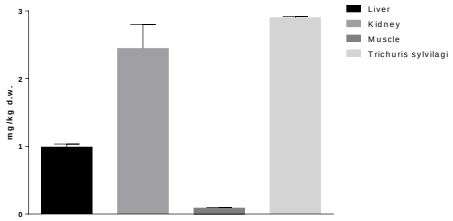


Fig.8. Bioaccumulation coefficient for *T.sylvilagi* of Cu

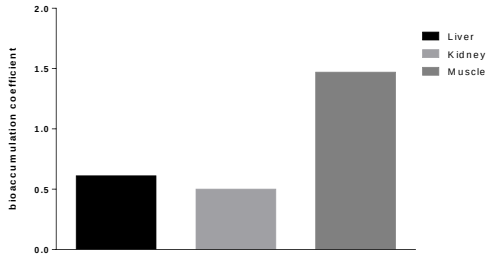


Fig.9. Bioaccumulation coefficient for *T.sylvilagi* of Zn

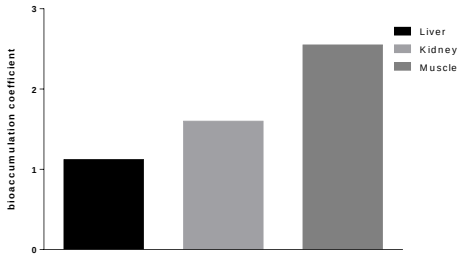


Fig.10. Bioaccumulation coefficient for *T.sylvilagi* of Co

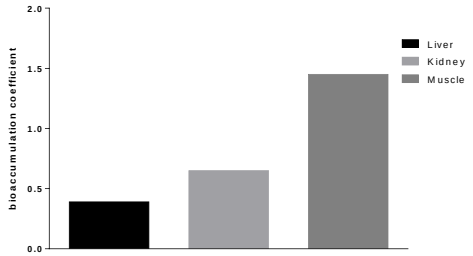


Fig.11. Bioaccumulation coefficient for *T.sylvilagi* of Mo

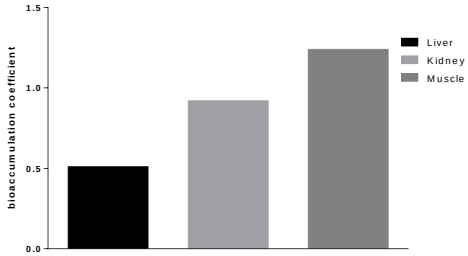


Fig.12. Bioaccumulation coefficient for *T.sylvilagi* of Cd

Fig.13. Bioaccumulation coefficient for *T.sylvilagi* of Pb

Discussion

Variations in the metal concentrations among tissues of the infected hares as well as in the nematode indicate differences in the degree of accumulation of them. Zn and Cu are involved in the series of metabolic processes and were therefore found in very large amounts in all three tissues. Toxic metals Cd and Pb show a different pattern with low values in muscle, and high levels of kidney and liver. Our findings confirm the data that the kidney and the liver are organs in which metals are present in the highest concentrations for terrestrial mammals.

The impact of the helminth burden of the bioaccumulation of heavy metals in the hares was well exhibited in our study. Comparison of heavy metal concentration in the organs of hares infected with nematodes showed similar accumulation capacity

The differences among most concentrations of heavy metals accumulated in the muscle, kidneys, liver of hares and those in parasites were statistically significant. The highest BFs of Cd and Pb were stated for muscle. In parasites mean Pb and Cd concentrations were higher than those detected in muscle, liver and kidney, respectively. The toxicity of heavy metals known to be wholly harmful like Cd and Pb rises with their concentrations in an accessible form especially by organisms in food chains. The concentrations of heavy metals in water and soil have increased due to increased human activities. The target tissues of heavy metals in hares are those metabolically more active, accumulating high levels of them, such as liver and kidneys. The liver serves as a storage site for heavy metals and it plays an important role in detoxification and elimination of many pollutants from the body. It has been found that the kidneys was the target organ receiving the largest quantities of heavy metals in hares. It is characterized as a metabolically active and eliminative organ that performs many vital functions including the removal of heavy metals from the body. High levels of some metals found in it in this study might also suggest this function. Present findings are in agreement with those of (Jankovska et al., 2008). According to some eco-toxicological reports the muscle represents one of the least metal-containing tissues (Baikov et al, 1994).

Higher accumulation of Pb and Cd was observed in the nematodes compared to the host tissues. The accumulation of the individual metals in the studied system was in different extent. Their levels have reflected the environmental contamination with metal toxicants. The hares used in our study were captured in industrial emission affects area of the town Kardzhali. One of the most important sources of contamination with heavy metals was metallurgic plant in this region. Increased Zn, Cd and Pb levels in water and soils were observed by Staykova and Naydenova (2008). According to the Commission Regulation No 1881/ 2006 the maximum level in meat of bovine animals is 0.050 microgram/g for Cd and 0.1 microgram/g for Pb. These concentration limits have been exceeded in almost all endoparasites samples.

A few articles were have been published on how mammalian nematodes accumulate heavy metals. The intestinal nematode *Ascaris suum* had the concentration of Pb and Cd significantly low than in their host tissues (Sures et al., 1998). Barus et al., (1997) reported that the concentration of Pb and Cd was significantly higher in specimens of Philometra ovata (Nematoda, Philometroidea) compared to the host muscle tissues.

Our results and of the above authors showed that some endohelminths can selectively accumulate certain metals, and others – cannot, unlike their hosts. It is possible that host organism performs a barrier function to the access of some elements to the parasites when there was increased concentration of them in the environment. The other possibility is that the helminths are adapted to the significant deviations of metal levels in the host – animals. The different accumulation capacity of diverse species parasites is difficult to explain. The influence of several factors and even their interaction (helminth microhabitat, location in the intestine, developmental stages, size, characteristics of tegument) should be taken into consideration. It is important to use organisms that are known to be infected, especially in studies examining the question of whether exposure to certain chemicals affects the physiological homeostasis of a test organism. Most free living animals used for biomarker studies are believed to be infected with a variety of parasites. Therefore, results from pollution test can be falsified if infected test organisms are involved (Sures et al., 1997).

Conclusion

The studied model including hare – *Trichuris sylvilagi*, could be a promising indicator to evaluate several toxic metals in the terrestrial habitats. Due to high abundance of industrial heavy metals in exposed area of Kardzhali hares migrating in affected territories are suitable bioindicator of anthropogenic environmental pollution. Survival of the hares and their nematode allows identify local environmental problems.

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