SHORT COMMUNICATION

Veterinary Research Forum. 2022; 13 (4) 611 - 614
doi: 10.30466/vrf.2020.135702.3043
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Evaluation of bioaccumulation of some heavy metals in liver flukes (*Fasciola hepatica* and *Dicrocoelium dendriticum*) and liver samples of sheep

Saeed Khatemeh[1], Abbas Imani Baran[2]*

[1] MSc Graduate, Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran; [2] Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article Info

Article history:

Received: 31 August 2020
Accepted: 08 November 2020
Available online: 15 December 2022

Keywords:

Bioaccumulation
Dicrocoelium dendriticum
Fasciola hepatica
Heavy metals
Sheep

Abstract

Different living organisms are used as applicable bioindicators to determine heavy metal pollutions. Recent studies have shown that helminths parasites can be used as efficient environmental sentinels. This study aimed to evaluate *Fasciola hepatica* and *Dicrocoelium dendriticum* as bioaccumulators of lead (Pb), chromium (Cr), cadmium (Cd), copper (Cu). For this work. A total of 50 samples (*F. hepatica*, *D. dendriticum*, and livers from the infected and uninfected sheep, each of 10 samples) were collected from sheep slaughtered in Tabriz abattoir. One gram of each sample was incinerated and analyzed by Flame Atomic Absorption Spectrometry. The analysis of samples showed that Pb, Cr and Cu values in *F. hepatica* were higher than those in *D. dendriticum*, but only the differences of Pb and Cu were significant. The values of heavy metals in *F. hepatica* were significantly higher than those in the infected livers (except for Cd), while in *D. dendriticum,* Cr and Cd were only higher. Based on metal levels in livers, it was found that bioconcentration factors (BCFs) of Cr, Pb and Cu for *F. hepatica* were much more than one, and BCFs of these three metals between two flukes were statistically significant. This study indicated that *F. hepatica* had a higher bioindicator potential than *D. dendriticum* to evaluate environmental pollutants by some metals.

Introduction

Heavy metals are of the natural composition of the earth's crust. Although they are in various concentrations, most of environmental contaminations result from uncontrolled and unconventional anthropogenic activities. Heavy metals are now considered as “detrimental contaminants of the environment” due to their toxicity, high biological capacity, and stability.[1,2] Humans and animals exposure to heavy metals leads to a variety of biological and health complications. Some heavy metals act as cofactors in many enzymatic reactions and are essential to maintain various functions when they are in very low levels, while exceeding certain threshold concentrations can be associated with serious threats to humans and animals, such as delayed growth, cardiovascular disorders, damage to nervous system, immune system, kidney, and finally death.[3-5]

Biomonitoring is currently a significant assignment to distinguish potential dangers of heavy metals. Different living organisms are used as bioindicators or efficient environmental sentinels mainly in aquatic ecosystems and partially terrestrial habitats.[6,7]

Several researchers have found that helminthic parasites are good biological indicators to monitor heavy metal pollutions.[3,7-15] Because these worms have higher potential to absorb heavy metals from their environment than their hosts, therefore helminthic parasites can be considered as promising applicable bioindicators to detect environmental pollutions.[2,7] Among helminthic parasites, commonly acanthocephalans, nematodes and cestodes of fish, and partially mammalian cestodes have been investigated, and the studies have resulted that cestodes and acanthocephalans, in particular, have an enormous heavy metal accumulation capacity.[14] However, the findings are preliminary and need to be confirmed by

***Correspondence:**

Abbas Imani Baran. DVM, PhD
Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
E-mail: a.imani@tabrizu.ac.ir

further studies using distinct species of helminthic parasites, particularly trematodes, under equal conditions. Despite the importance of environmental pollutions caused by heavy metals and consequently the pathological effects of theses pollutants on humans and animals, few studies have been conducted on the terrestrial mammalian helminthic parasites as the potential bioindicators to identify heavy metals,[12] and these studies are limited to cestoda-mammalian models.[7] This study aimed to evaluate and compare Pb, Cr, Cd and Cu in the liver trematodes (*F. hepatica* and *D. dendriticum*) and livers of slaughtered sheep in Tabriz abattoirs, East Azerbaijan province. The liver trematodes potential as bioindicators of heavy metals was also determined.

Materials and Methods

Sampling. From December 2019 to February 2020, adult helminths (*F. hepatica* and *D. dendriticum*) were collected separately from infected livers of 10 sheep slaughtered at Tabriz abattoir, East Azerbaijan province, Iran. Simultaneously, 30 samples were taken from the livers of the infected (n = 10) and uninfected (n = 10) sheep. The collected samples were transferred to the Faculty of Veterinary Medicine, University of Tabriz. The samples were washed separately in phosphate-buffered saline (pH 7.30) and stored at – 26.00 ˚C.[15]

Sample preparation. The frozen samples were transferred to the Analytical Chemistry Laboratory of the Faculty of Chemistry, University of Tabriz, Tabriz, Iran. After thawing, one gram of each sample was weighed and dried (subjected to ethanol 70.00 ˚C for 5 min, ethanol 90.00 ˚C for 10 min, finally acetone for 1 hr).[13] To incinerate, dried samples were transferred to the crucibles (crucibles were already floated once in diluted HNO_3 (Merck, Darmstadt, Germany), washed with ddH_2O (produced by Faculty of Chemistry, University of Tabriz, Tabriz, Iran), and then thoroughly dried). The crucibles were then transferred to a muffle furnace to be incinerated samples at 600 ˚C for 4 hr. The contents of the crucibles were transferred to falcon tubes and dissolved in 5.00 mL of 60.00% HNO_3; the tube volume was finally brought to 25.00 mL using ddH_2O.

Determination of heavy metals. The samples were analyzed using flame (air-acetylene) atomic absorption spectrometry (novAA 800; Analytik Jena AG, Jena, Germany). Firstly, the spectrometer was pre-calibrated by certified standard of each metal. After homogenizing prepared solutions, the samples were injected to flame atomic absorption spectrometry and the absorbed value of each metal was recorded. The procedure was repeated three times to accurately measure the concentration of heavy metals in all samples. The selected operational indicators of spectrometry to determine the considered metals were as follows; Pb: Wave Length (WL) = 283.30 nm, Current (C) = 0.50 mA, Gap Width (GW) = 3.00 nm; Cr: WL = 357.90 nm, C = 0.50 mA, GW = 5.00 nm; Cd: WL = 228.80 nm, C = 0.50 mA, GW = 3.00 nm; Cu: WL = 324.80 nm, C = 0.50 mA, GW = 3.00 nm.[15]

Evaluation of bioconcentration. The bio-concentration factor in parasites was calculated by the following formula:[13]

$$\textit{Bio-concentration factor} = \frac{\textit{Concentration of the heavy metal in the parasite tissue}}{\textit{Concentration of the heavy metal in the host liver tissue}}$$

Statistical analyses. Metal concentrations were expressed in micrograms per gram (μg g^{-1}) wet weight as mean ± SEM. Before analyzing, the normal distribution of data was tested by Kolmogorov-Smirnov and Shapiro-Wilk tests. Data analysis was done by non-parametric tests. Mann-Whitney test was used to evaluate the significant differences of the heavy metals concentrations between parasites and livers. Kruskal-Wallis equality ranked test was used to evaluate the differences between BCFs of metals. Data analysis was done using SPSS Software (version 21.0; IBM Corp., Armonk, USA). The $p < 0.05$ was considered as significant.

Results

The comparison of the heavy metal values in flukes indicated that Pb, Cr, and Cu in *F. hepatica* were higher than *D. dendriticum*, but only the differences of Pb and Cu were significant. The values of metals in *F. hepatica* were higher than those of in the *F. hepatica*-infected livers, except for Cd. While in *D. dendriticum,* Cr and Cd were only higher than those in the *D. dendriticum*-infected livers. Significantly, Cd values in the infected livers were higher than those in the uninfected ones, while, Cr values were higher in the *F. hepatica*-infected livers and lower in the *D. dendriticum*-infected livers (Table 1).

Table 1. The comparison of heavy metals (μg g^{-1}) in *Fasciola hepatica*, *Dicrocoelium dendriticum*, and the infected and uninfected livers.

Metals	*Fasciola hepatica*	*Dicrocoelium dendriticum*	*p*-value[a]	*F. hepatica* infected livers	*P*-value[b]	*D. dendriticum* infected livers	*p*-value[c]	Uninfected livers	*p*-value[d]	*p*-value[e]
Pb	0.43 ± 0.04	0.14 ± 0.01	0.000	0.10 ± 0.01	0.000	0.19 ± 0.05	0.705	0.12 ± 0.00	0.059	0.290
Cr	0.62 ± 0.08	0.59 ± 0.03	0.597	0.11 ± 0.04	0.000	0.24 ± 0.01	0.000	0.40 ± 0.04	0.001	0.002
Cd	0.08 ± 0.01	0.09 ± 0.00	0.257	0.11 ± 0.00	0.003	0.08 ± 0.00	0.031	0.07 ± 0.01	0.000	0.000
Cu	5.97 ± 0.61	0.87 ± 0.11	0.000	3.63 ± 1.02	0.049	4.09 ± 0.68	0.000	2.92 ± 0.34	0.762	0.151

[a] *p*-value for *F. hepatica* + *D. dendriticum*; [b] *p*-value for *F. hepatica* + infected livers; [c] *p*-value for *D. dendriticum* + infected livers; [d] *p*-value for *F. hepatica*-infected livers + uninfected livers; [e] *p*-value for *D. dendriticum*-infected livers + uninfected livers.

The Cr BCF for *D. dendriticum* was higher than 1. Regarding *F. hepatica*, the BCFs of Cr, Pb and Cu were much more than one. The differences of BCFs between two trematodes were statistically significant (Table 2).

Table 2. The bioconcentration factors of *Fasciola hepatica* and *Dicrocoelium dendriticum.*

Metals	*Fasciola hepatica*	*Dicrocoelium dendriticum*	*p*-value
Pb	5.06 ± 1.04	0.94 ± 0.12	0.000
Cr	17.27 ± 6.35	2.58 ± 0.20	0.01
Cd	0.76 ± 0.08	0.93 ± 0.03	0.049
Cu	3.70 ± 1.03	0.28 ± 0.07	0.001

Discussion

Our results showed that Pb concentration in *F. hepatica* was significantly higher than livers. This result was consistent with Acosta *et al*., Sures *et al*. and Lotfy *et al*.[3,12,13] The Pb value of 6.89 μg g^{-1} in *F. hepatica* was reported by Sures *et al*.[12] Lotfy *et al*. reported the Pb values of 20.21 and 23.97 μg g^{-1} in *F. hepatica* and *F. gigantica*, respectively; while it was reported 6.74 μg g^{-1} in the infected livers of buffaloes.[13] Also, Acosta *et al*. reported the Pb value of 20.8 μg g^{-1} in *F. gigantica* (4.7 μg g^{-1} in the infected livers of buffaloes).[3] There were reliable evidences regarding excretion of Pb from the liver into the intestine of mammals and since *F. hepatica* lives in the bile ducts, this metal could be absorbed across the tegument of the flukes in a lipophilic form after combining with bile acids. Formation of the bile acid-Pb complex may indicate high bioconcentration of Pb in the flukes.[12] Also, migrating immature forms of *Fasciola* spp. and *D. dendriticum* in the liver and utilizing the liver parenchyma as the main site of infection, impairs hepatic xenobiotic-metabolizing activity. As a result, liver damage may lead to the accumulation of heavy metals in the host liver.[13,16]

In this work, Cu showed maximum level in *F. hepatica*, and also in the infected and uninfected livers. Lotfy *et al*. reported the Cu concentrations of 19.32 and 24.91 μg g^{-1} in *F. hepatica* and *F. gigantica*, and of 13.67 and 15.22 μg g^{-1} in the livers, respectively.[13] Acosta *et al*. also reported the Cu concentrations of 72.23 μg g^{-1} in *F. gigantica*, and of 9.12 μg g^{-1} in the livers.[3] The high Cu concentration in *F. hepatica* and in the infected and uninfected livers can be partially related to the ceruloplasmin acute phase protein.[17] The Cd value in the infected livers was higher than those in *F. hepatica* and *D. dendriticum*. While, the Cr values were only significantly higher in *F. hepatica* and in the *D. dendriticum*-infected livers. Similar to our results, Sures *et al*. reported that Cd concentration in the infected livers of cattle was significantly higher than *F. hepatica*.[12]

Conversely, Acosta *et al*. reported a higher Cd value in *F. gigantica* than that in the infected livers of buffaloes.[3] However, Lofty *et al*. reported that the Cd value in the liver flukes and in the infected livers of buffaloes didn't show any significant difference.[13]

Compared to the previously reported results, in our work the heavy metal levels were lower in parasites and infected livers. Several studies demonstrated that the difference in the accumulation and distribution of heavy metals in different tissues depends on the distinct physiological characteristics of tissues, duration of exposure, metals concentrations, and physiological conditions of the environmental factors in the host.[7,16] However, Lotfy *et al*. attributed the difference between the metals concentrations (including Cd) to the different sample sizes in various studies.[13] As our results showed, the concentrations of heavy metals were, totally, higher in *F. hepatica* than *D. dendriticum*. That may be due to the large size and highly anatomical difference of *F. hepatica* digestive tract compared to *D. dendriticum*. *D. dendriticum* caecum is very simple and without any branches, whereas *F. hepatica* possesses highly branched caecum, therefore, the area of the branched intestinal wall and enclosed contents in *F. hepatica* is much larger than that of *D. dendriticum*.[18] Sures *et al.* indicated the highest Cd amount was found in *Ascaris suum* intestine. Compared to the parasite intestine, the Cd amounts in the reproductive system, perienteric liquid and body wall were very low. Thus, it appears that the relatively high Cd amount of the whole worms merely reflects the elevated concentration within the intestinal wall and its enclosed contents.[12]

The statistical analysis of the heavy metals BCFs in the infected livers with two flukes showed that the highest BCFs of Cr, Pb and Cu were linked to *F. hepatica*. Although Cd BCF was found to be highest for *D. dendriticum*, it was either insignificant or lower than 1. Therefore, *D. dendriticum* cannot be used as bioindicator for Cd. Generally, our results indicated that the highest BCFs was mostly related to *F. hepatica*. Sures *et al*. reported BCF ranges of Pb and Cd in the cattle livers infected with *F. hepatica* from 4 to 80 and from 0.10 to 3.00, respectively.[12] Lotfy *et al*. recorded the highest BCF of Pb, Cu and Cr in *F. hepatica* and the highest BCF of Pb and Cu in *F. gigantica*.[13] Acosta *et al*. also recorded the highest BCF of Cu, Pb, and Cd in *F. gigantica*.[3]

These results indicate that trematodes are able to accumulate heavy metals at concentrations much higher than those in their host. BCFs of heavy metals indicated that *F. hepatica* had higher bioindicator potential to detect environmental pollutants than *D. dendriticum*. Nevertheless, further studies are needed to confirm our findings. To our knowledge, this study is the first work to evaluate some of the most important environmental pollutants using two major trematodes in Iran.

Acknowledgments

The authors would like to acknowledge the support of Mr. Kanaani for setting up atomic absorption spectrometer and sample analysis. Dr. Gholamreza Hamidian helped us in statistical analysis. The personnel of the industrial abattoir of Tabriz were helpful in the collection of samples.

Conflict of interest

There was no conflict of interest.

References

1. Hamzeh MA, Bumari M, Rezaei H, et al. Environmental geochemistry of heavy metals in coastal sediments of Gawatr bay, southeast extreme of Iran. J Oceanogr 2012; 2(8): 11-20.
2. Najm M, Fakhar M. Helminthic parasites as heavy metal bioindicators in aquatic ecosystem. Med Lab J 2015; 9(4): 26-32.
3. Acosta AGD, Camara CNM, Ongsiako JRMJ, et al. Bioaccumulation of cadmium, copper, lead, and zinc in water buffaloes (*Bubalus bubalis*) infected with liver flukes (*Fasciola gigantica*). Orient J Chem 2017; 33(4): 1684-1688.
4. Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. Curr Med Chem 2005; 12: 1161-1208.
5. Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants. Afr J Food Sci 2010; 4(5): 200-222.
6. Rosenberg DM, Resh VH. Freshwater biomonitoring and benthic macroinvertebrates. 1st ed. New York, USA: Chapman and Hall 1993; 198-259.
7. Abed Alrikaby NJ, Maktoof AA, Hafedh AA. Bioaccumulation of some heavy elements in different tissue of *Cotugnia polycantha* and two parasites (*Raillietina tetragona* and *Streptopellia senegalensis*) infected with birds. Eurasia J Biosci 2020; 14(1): 395-399.
8. Sures B. Environmental parasitology: relevancy of parasites in monitoring environmental pollution. Trends Parasitol 2004; 20(4): 170-177.
9. Vidal-Martinez VM, Pech D, Sures B, et al. Can parasites really reveal environmental impact? Trends Parasitol 2010; 26(1): 44-51.
10. Sures B, Siddall R, Taraschewski H. Parasites as accumulation indicators of heavy metal pollution. Parasitol Today 1999; 15(1): 16-21.
11. Sures B, Taraschewski H. Cadmium concentrations in two adult acanthocephalans *Pomphorhynchus leavis* and *Acanthocephalus lucii*, as compared with their fish hosts and cadmium and lead levels in larvae of *A. lucii* as compared with their crustacean host. Parasitol Res 1995; 81(6): 494-497.
12. Sures B, Jürges G, Taraschewski H. Relative concentrations of heavy metals in the parasites *Ascaris suum* (Nematoda) and *Fasciola hepatica* (Digenea) and their respective porcine and bovine definitive hosts. Int J Parasitol 1998; 28(8): 1173-1178.
13. Lotfy WM, Ezz AM, Hassan AAM. Bioaccumulation of some heavy metals in the liver flukes *Fasciola hepatica* and *F. gigantica*. Iran J Parasitol 2013; 8(4): 552-558.
14. Sures B. Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective. Parasitology 2003; 126 Suppl: S53-S60.
15. Jankovská I, Vadlejch J, Száková J, et al. Experimental studies on the lead accumulation in the cestode *Moniezia expansa* (Cestoda: Anoplocephalidae) and its final host (*Ovis aries*). Ecotoxicology 2010; 19(5): 928-932.
16. Leyendecker M, Korsten P, Reinehr R, et al. Ceruloplasmin expression in rat liver cells is attenuated by insulin: Role of FoxO transcription factors. Horm Metab Res 2011; 43(4): 268-274.
17. Mersch J, Pihan JC. Simultaneous assessment of environmental impact on condition and trace metal availability in zebra mussels *Dreissena polymorpha* transplanted into the Wiltz River, Luxembourg. Comparison with the aquatic moss. Arch Environ Contam Toxicol 1993; 25: 353-364.
18. Eslami A. Veterinary helminthology: Trematoda. Vol. 1. 2nd ed. Tehran, Iran: Tehran University Press 1998; 54-195.