Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(17):90-92
(http://www.derpharmachemica.com/archive.html)

Bioaccumulation of Copper and Lead in Water Buffaloes (*Bubalus bubalis*) Infected with Liver Flukes

Joko Miguel A Aguila[1], Gian Franco B Saldonido[1], Mary Jane C Flores[1], Jose Isagani B Janairo[1], Jose Santos R Carandang VI[1], Marlon B Ocampo[2], Derick Erl P Sumalapao[1,3,4*]

[1]*Department of Biology, College of Science, De La Salle University, Manila, Philippines*
[2]*Philippine Carabao Center Reproductive Biotechnology Unit, Science City of Muñoz, Nueva Ecija, Philippines*
[3]*School of Multidisciplinary Studies, De La Salle-College of Saint Benilde, Manila, Philippines*
[4]*Department of Medical Microbiology, College of Public Health, University of the Philippines Manila, Manila, Philippines*

ABSTRACT

Heavy metals such as lead and copper when present in large concentrations in the environment can have detrimental effects on living organisms. This study identified the presence of lead and copper in liver flukes, Fasciola hepatica and Fasciola gigantica and in liver tissues of water buffaloes (Bubalus bubalis) as potential heavy metal bioaccumulators using atomic absorption spectroscopy. Both the uninfected and infected liver tissues and the liver flukes were found positive for lead and copper. Lead concentration levels of 5.162 µg/g in uninfected liver tissue sample, 5.422 µg/g in infected liver tissue, 9.692 µg/g in F. hepatica, and 7.754 µg/g in F. gigantica were detected, while copper concentration levels of 6.742 µg/g in uninfected liver tissue, 2.278 µg/g in infected liver tissue, 24.75 µg/g in F. hepatica, and 32.98 µg/g in F. gigantica were observed. Liver flukes showed a greater concentration of both copper and lead than the liver tissues suggesting that these parasites can serve as bioaccumulators of heavy metals and bioindicators of environmental pollution.

Keywords*:* Heavy metals, Bioaccumulators, Bioindicators, Liver flukes, Water buffaloes

INTRODUCTION

One of the significant problems society faces nowadays is the heavy metal contamination of the environment. Power plants, chemical shops, and auto shops contribute to the wide spread of heavy metals in the environment as a direct result of technological advancement [1]. Common examples of these heavy metal pollutants include Copper (Cu) and Lead (Pb). Mining sites and metallurgical industries were found to be the primary culprits in the spread of copper in the environment [2]. Copper is a vital dietary nutrient. However, high dosages of copper can result to serious health complications with detrimental effects. Meanwhile, lead is naturally occurring, it is a toxic metal with no known beneficial effect on living organisms [3]. Excessive accumulation of lead in the body results to a decline in the mental, cognitive, and physical state of an individual [4]. An *in vitro* study revealed that liver flukes subjected to lethal concentration of lead exhibited manifestations of physiological stress such as curling, surface alterations, and body folding [5].

Human exposure to heavy metals poses huge health risk. Heavy metal accumulation through ingestion is a common route in which humans are exposed to this class of pollutants. Humans as consumers depend on animals for meat products and their daily consumption of food. Meat contains proteins, fats, and other essential elements that are found to be an essential part of the human diet as well as good source of nutrients [6]. Ingestion of food produced from animals exhibiting heavy metal accumulation can cause many health complications when trace elements of these heavy metals accumulate in large concentrations within the organs of living organisms. Thus, monitoring and quantifying the concentration of the heavy metals in the environment, especially in organisms represent an important component of environmental assessment and protection. As such, the use of living organisms as bioindicators in determining environmental pollutants plays a major role in the field of environmental parasitology. Organisms such as *Acanthocephalus lucii* [7-9], *Ascaris suum*, the trematodes *Fasciola hepatica* and *Fasciola gigantica* [10], ectoparasites and plants have been used as pollutant bioindicators and bioaccumulators. These plants and parasites as environmental indicators can detect elements describing the environmental condition of a specific place within a given period. Pollution and contamination of natural resources can be determined by parasites inhabiting the organs of some grazing animals [2]. Parasites, specifically *Fasciola* can be a potential bioaccumulators and bioindicators for heavy metals.

Flukes under the genus *Fasciola* accumulated lead, copper, cadmium, zinc, and chromium [11]. In this study, it determined the presence of lead and copper in the liver flukes and liver tissues from water buffaloes (carabao) sourced from Central Luzon, Philippines. Carabaos are important agricultural animals in the Philippines for their utility in farming as well as for their meat and milk. Thus, knowledge about the lead and copper concentration on the liver tissues of these fluke infected carabaos helps assess the local environment where these animals are raised. Moreover, exploring the use of liver flukes as bioindicators for heavy metals within the context of a Philippine environment can also help assess the health and conditions of the carabaos.

MATERIALS AND METHODS

Collection of samples

For the extraction of liver flukes, two available liver samples collected from the death of a stray water buffalo from Muñoz, Nueva Ecija and from an uninfected water buffalo from San Jose City slaughterhouse were utilized. Species identification was done based solely on morphological characteristics [12]. The collected liver samples were then brought to the Zoology and Parasitology Laboratory, Science and Technology Research Center, De La Salle University for extraction procedure and preparation for atomic absorption spectroscopy.

Extraction and preparation of samples for acid digestion

The flukes were extracted from the biliary ducts, separated based on species [12] and oven dried at 70°C for two days. Corresponding liver samples were likewise oven dried. The dry weight of the samples was noted and approximately 5 g of each of the samples were obtained. However, only 3.6 g of *F. hepatica* were obtained from the infected liver sample.

Acid digestion

The samples were chopped into tiny pieces using plastic knives. The liver and fluke samples were then separately digested in a mixture of 6 ml of 30% hydrogen peroxide and 12 ml of 60% nitric acid. The samples were heated at a low temperature inside the fume hood until the samples are completely digested and a white smoke appeared [13].

Analysis of samples for heavy metal sample preparation

The digested samples of liver tissue and liver flukes were filtered twice and the filtrates obtained were discarded until the solution became clear. Using volumetric flasks, the filtered samples were diluted up to the 100 ml mark with distilled water [13].

Preparation of standards

The initial stock solution concentration for Pb and Cu were adjusted from 100 ppm to 10 ppm. From the 10 ppm working solution, the standards of 0.1250, 0.2500, 0.5000 and 1.000 ppm of Cu and Pb were prepared [14].

Spectrophotometric analysis

The absorbance of the standards of Cu and Pb were measured using the Shimadzu-6700 spectrophotometer with corresponding hollow cathode lamps. The spectrophotometer generated a standard curve for the absorbance and concentration of the standard samples for each metal of interest. The absorbance of the acid digested samples, namely: uninfected liver tissue, infected liver tissue, and the liver flukes were measured for Cu and Pb [14].

RESULTS AND DISCUSSIONS

Two species of liver flukes, namely *F. hepatica* and *F. gigantica* were identified from infected liver of water buffaloes. Upon extraction of the liver flukes, they were immediately identified and grouped per species. It is interesting to note the coexistence of *F. gigantica* and *F. hepatica* from the infected liver, whereas only *F. gigantica* was identified in the liver sample of water buffalo from Nueva Ecija [15]. Twenty liver flukes from each species and their corresponding liver tissues were utilized for heavy metal analysis.

Results of spectrophotometry revealed that uninfected and infected liver samples, *F. hepatica*, and *F. gigantica*, were positive for both lead and copper accumulation (Figure 1). Uninfected liver samples had an average lead and copper concentrations of 5.162 and 6.742 µg/g, respectively. Infected liver sample had an average lead concentration of 5.422 µg/g and copper of 2.278. *F. hepatica* had an average of 9.692 µg/g lead and 24.75 µg/g copper while *F. gigantica* has an average of 7.754 and 32.98 µg/g for lead and copper concentrations, respectively. The highest and lowest lead concentrations were obtained from *F. hepatica* and uninfected liver sample, respectively. Regarding copper concentrations, the highest and the lowest values were identified from *F. gigantica* and infected liver, respectively. Regardless of the liver fluke species, higher concentrations of copper and lead were identifies suggesting the potential of the parasites as heavy metal bioindicators. As such, findings of this study provide baseline information on heavy metal accumulation in carabaos in the Philippines. Due to scarcity of information regarding heavy metals, only heavy metal content of carabao milk sourced from the same location was studied [16]. Considering the significance of carabaos to Philippine society, studies monitoring concentrations of these heavy metals in the animal are of paramount importance.

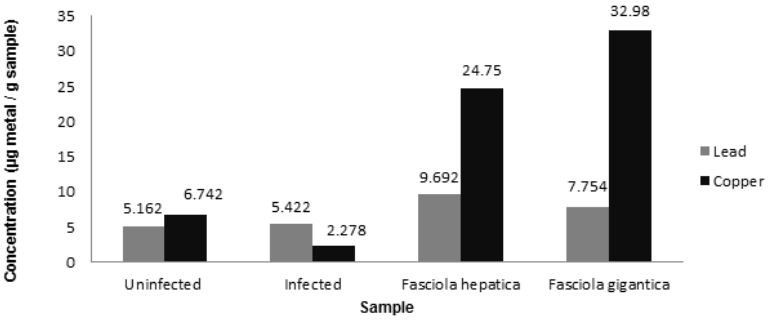

Figure 1: Lead and copper concentration levels (µg/g) in uninfected and infected liver tissues, *Fasciola hepatica* and *Fasciola gigantic*

CONCLUSION

Lead and copper concentrations in liver and flukes from carabaos sourced from Central Luzon, Philippines were successfully measured. Flukes accumulate and exhibit greater concentrations of these heavy metals than the liver tissue samples where they were extracted. *F. hepatica* and *F. gigantica* show their potentials as bioaccumulators by exhibiting the greatest concentration for both lead and copper when compared to liver tissue samples. It is recommended that other heavy metals be tested and other parasites be studied as potential bioaccumulators indicating the condition of the ecosystem. Moreover, comparisons can be made on soil and plant samples where the water buffaloes graze be done for this heavy metal contamination.

ACKNOWLEDGEMENT

The authors would like to thank De La Salle University and Philippine Carabao Center for supplying materials used in the study and allowing the use of their facilities.

REFERENCES

[1] B. Wei, L. Yang, *Microjournal.*, **2009**, 94(99), 107.
[2] B. Ashish, K. Neeti, K. Himanshu, *Research Journal of Recent Sciences.*, **2013**, 2277, 2502.
[3] M. Nkansah, J. Ansah, *International Journal of Scientific and Research Publications.*, **2014**, 4(8), 2250.
[4] A. Alberto, G. Segua, B. Baui, J. Prudente, *Environ. Sci. Pollut. Res. Int.*, **2007**, 14(2), 498.
[5] A. Chang, M. Flores, *Asian Pac. J. Trop. Biomed.*, **2015**, 5(6), 493.
[6] J. Akan, F. Abdu, O. Irahman, C. Sodipo, *Research Journal of Applied Sciences, Engineering and Technology*., **2010**, 2(8), 743.
[7] B. Sures, *Parasitol Res*., **1995**, 81(6), 494.
[8] B. Sures, *Parassitologia.*, **1997**, 39(3), 213.
[9] I. Jankovska, D. Miholova, M. Petrtyl, S. Romocusky, L. Kalous, J. Vadlejch, *Bull. Environ. Contam. Toxicol*., **2011**, 86(3), 342.
[10] B. Sures, G. Jurges, H. Taraschewski, *Int. J. Parasitol*., **1998**, 28(8), 1173.
[11] W. Lotfy, A. Ezz, A. Hassan, *Iran. J. Parasitol.*, **2013**, 8(4), 552.
[12] M. Rokni, H. Mirhendi, A. Minzani, M. Mohebali, M. Sharbatkhori, E. Kia, H. Abdoli, S. Izadi, *Experimen. Parasitol.*, **2009**, 124, 209.
[13] J. Furukawa, *University of Tsukuba*., **2013**.
[14] P. Srikanth, S. Somasekhar, G. Kanthi, K. Raghu Babu, *International Journal of Environment, Ecology, Family and Urban Studies*, **2013**, 3(1), 127.
[15] A. Chang, M. Flores, *Manila Journal of Science.*, **2016**, 9, 1.
[16] R.A. Magbitang, B. Rodriguez, *Science Diliman.*, **2012**, 24, 1.