Pollution 2022, 8(4): 1164-1171
DOI: 10.22059/POLL.2022.340517.1399

RESEARCH PAPER

Levels of Heavy Metals in the Water, Sediments, and Tissues of Golden Apple Snail (*Pomacea canaliculata*) from Lake Dakong Napo, Esperanza, Agusan del Sur, Philippines

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Received: 14.02.2022, Revised: 20.05.2022, Accepted: 20.07.2022

Abstract
This study was conducted in order to assess the level of heavy metals in the water, soil, and tissues of *Pomacea canaliculata* from Lake Dakong Napo, Esperanza, Philippines as well as identify the histopathological alterations in the gonads and muscles of the snail. Heavy metals were detected using Atomic Absorption Spectrometry and slides for histological studies were prepared using histological routine procedure. Results revealed that in sediments Cr (174.67±62 mg/L) and Ni (269.33±17.56 mg/L) were above from the recommended safe limits of the international standards, US EPA (≤25) and FAO (≤50) while concentrations of Pb (16.35± 0.58 mg/L), Cd (5±1.42 mg/L), and Cr (1±0 mg/L) in water were above from the recommended safe limits of the national standards, DAO (≤0.05; ≤0.01; ≤0.05). These heavy metals were below the permissible limits in the gonads and muscles of *P. canaliculata* however, alterations in the tissues of the snail are evident suggesting that these heavy metals and other environmental stressors are negatively affecting the organisms inhabiting the lake.

Keywords: Bioaccumulation, golden apple snail, histological alterations

INTRODUCTION

Aquatic ecosystems are exposed to excessive input of pollutants and contaminants from various sources like domestic and industrial sewage, agricultural processes, heavy metals and others (Santos *et al.,* 2014). Metals are naturally occurring in the environment and its concentrations varies across geographic regions (Alhashemi *et al.,* 2011). Heavy metals have no known biological importance, and its contamination in aquatic ecosystem has been recognized as a severe and prolonged pollution problem in most developing countries, and the world over. Toxic heavy metal contamination mostly occurred in aquaculture farms and frequently occurs in groundwater, rivers, estuaries, wetland and coastal areas (Kaoud & El-Dahshan, 2010).

Accumulation of heavy metals in aquatic food chain can cause health risk in aquatic organisms, including human population. The prolonged exposure to water pollutants even in very low concentrations have been reported to induce morphological, histological and biochemical alterations in the tissues of aquatic organisms and have been associated with decreased fertility and other reproductive abnormalities and altered immune function in vertebrates and non-vertebrates (Kaoud & El-Dahshan, 2010; Falusi & Olanipekun, 2007).

In the Philippines, studies regarding assessment and determination of heavy metal contamination focuses only in huge aquatic environment and commonly caught fish population

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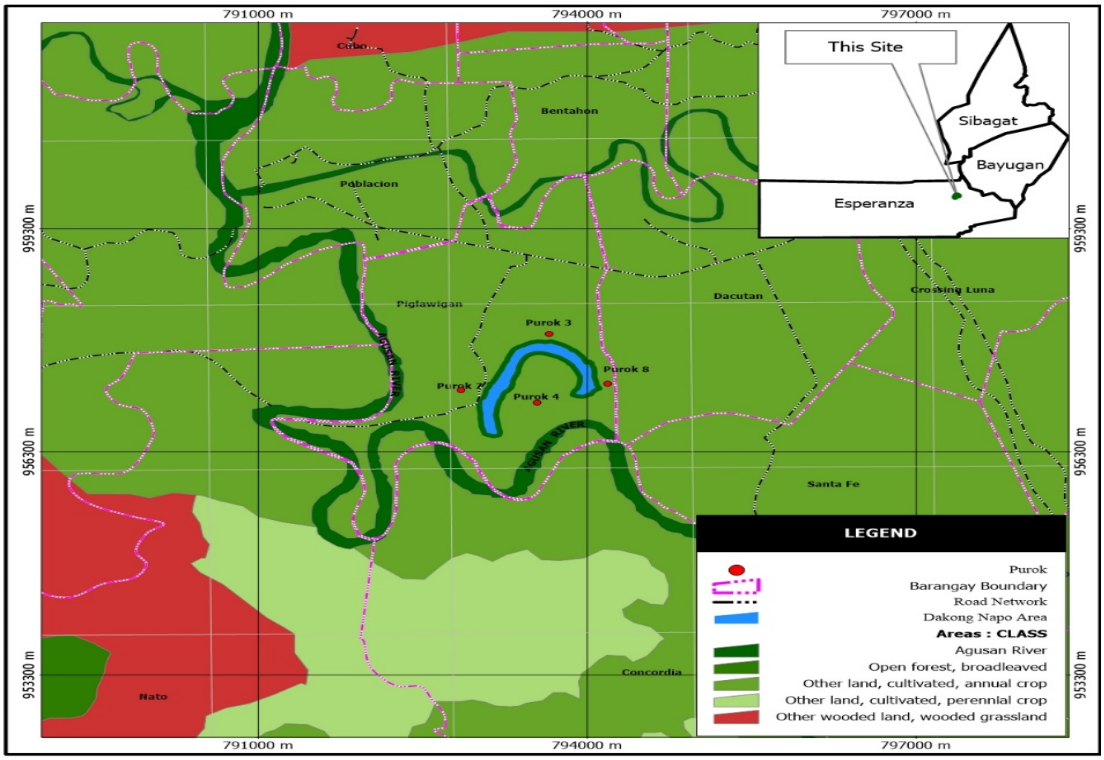

Fig. 1. Map showing the location of Lake Dakong Napo in Esperanza, Agusan del Sur, Philippines. Map credited to MENRO Esperanza.

in the county such as in Manila Bay (Sia Su *et al.*, 2009; Sia Su *et al.*, 2013), and Agusan River in Mindanao (Capangpangan *et al.*, 2016, Velasco *et al.*, 2016) with small lakes and other aquatic organisms remained understudied.

Lake Dakong Napo is very significant in the community of Esperanza where it is situated, for it serves as habitat for endemic and indigenous flora and fauna and it also provides water for irrigation to farmlands surrounding the lake. Aside from ichthyofauna and other aquatic organisms, Golden Apple Snail (*Pomacea canaliculata*) is another inhabitant of the said lake. Though, considered as invasive and pest in the country, this snail is one of the important sources of protein in the area and is part of the food chain. Currently, studies concerning the status of the lake and its inhabitants is very scarce, thus, this study was conducted in order to provide baseline information concerning the levels of heavy metal contaminations in the water, sediments and tissues of golden apple snail. The results of the study will be an important source of information for the management of Lake Dakong Napo, including the community which utilizes the said lake.

MATERIALS AND METHODS

Lake Dakong Napo covers an area of 35.00 hectares and is situated in Puroks 3, 4, 7 and 8 of Barangay Piglawigan, Esperanza, Agusan del Sur, Philippines (Figure 1). The lake is surrounded with residential area, rice and cornfields and an important source of water for irrigation. Recently, Lake Dakong Napo is one of the upcoming projects of the Municipality of Esperanza, being proposed to be a protected area because of its unique biological features.

Water samples were collected prior to the collection of the sediments. 1.5 liters of plastic bottle was filled with water. This was done in triplicate, and each bottle were properly labelled. The samples of sediments were acquired from the same points were water samples are collected.

One kilogram of sediments was placed in properly labeled polyethylene bag. Water and sediment samples were transported to the Regional Soils Laboratory, Department of Agriculture Taguibo, Butuan City, Philippines for heavy metal analysis using Microwave Plasma – Atomic Emission Spectrometer Method with a detection limit of 0.1 mg/L. On the other hand, Golden apple snail were collected in the same site were sediment and water samples were collected. In the laboratory, a total of 20 individuals were randomly selected from the pool of samples. Individuals were then washed with distilled water in order to remove the dirt from the shell of the snail. In order to expose the internal parts of the snail, shell was removed by gently beating it using a hammer.

Using a scalpel, 0.5g of gonads and muscles were dissected per individual, and were separately placed in a vial with proper label. 10% formalin was used as a buffer solution, these samples were then subjected for histological slide preparation. Another 0.5g of gonads and muscles were dissected from the same individuals and were placed in a separate vial with proper label which were subjected for heavy metal analysis. All samples subjected for both analysis were done in triplicate. The samples for histological analysis were immediately transported in Hi-Precision diagnostics laboratory in while samples subjected for heavy metal analysis were transported to Davao Analytical Laboratory.

Gonads and muscles of golden apple snail were ash and digested following the Standard Method of Analysis for soil, fertilizer, plant tissue, and water and the Official Methods of Analysis of AOAC International, 17th edition (2000). All samples were read in duplicate using Atomic Absorption Spectrophotometry. For histological slide preparations, after fixation of the gonad and muscle tissues in 10% formalin for 24 hours, tissues were slowly dehydrated using three grades or percentages of alcohol (70%, 80% and 90%). Xylene was applied to remove the alcohol from the tissue and paraffin was used for embedding the tissue. Using microtome, tissues were cut into thin sections and were placed in glass slide and were rehydrated using absolute alcohol. Tissues were stained using hematoxylin and eosin. Slides were viewed using a compound microscope with 1000x magnification.

RESULTS AND DISCUSSION

This study was the first to document the level of selected heavy metals in the water, sediments, including in the gonads and muscles of *P. canaliculata* in Lake Dakong Napo, Esperanza, Agusan del Sur, Philippines. The heavy metal concentration in the sediments collected at the lake was in the order of Ni>Cr>Pb>Cd (Table 1). Among the four metals analysed, Nickel had the highest mean concentration (269.33±17.56 mg/L) which exceeds the recommended safe limits of FAO (1999) which is ≤50 mg/L. Concentration of Chromium (174.67±62.0), on the other hand also exceed the recommended safe limits of US EPA (2002) which is ≤50 mg/L. Moreover, Lead (56±41.58) and Cadmium (1±0) concentrations are below the recommended safe limits of FAO

Table 1. Mean concentration of heavy metals in the sediments collected from Lake Dakong Napo, Esperanza, Agusan del Sur.

Heavy Metals	Results for Sediments	Recommended Safe Limits	Agency
Pb (mg/L)	56±41.58	≤100	FAO (1999)
Cd (mg/L)	1±0	≤3	FAO (1999)
Cr (mg/L)	174.67±62.0	≤25	US EPA (2002)
Ni (mg/L)	269.33±17.56	≤50	FAO (1999)

USEPA-United States Environment Protection Agency; FAO- Food and Agriculture Organization

Table 2. Mean concentration of heavy metals in the water samples collected from Lake Dakong Napo, Esperanza, Agusan del Sur.

Parameters	Results for Water	Recommended Safe Limits (DAO, 2016)
Pb (mg/L)	16.35±0.58	≤0.05
Cd (mg/L)	5±1.41	≤0.01
Cr (mg/L)	1±0	≤0.05
Ni (mg/L)	BDL	≤0.05

Department of Environment and Natural Resources (DENR Administrative Order); BDL- Below Detection Limit

(1999) which are ≤100 and ≤3 respectively.

The concentration of Ni and Cr in the sediments exceeded the recommended safe limits set by international agencies. Concentrations of Pb and Cd were also detected in the sediments of the lake. This indicates that the sediments in the area retained pollutants. The high level of Ni and Cr and other metals in Lake Dakong Napo may be attributed to the various by-products such as industrial and domestic sewages, animal/poultry wastes product, land-based fertilizers and pesticides. As the waste is disposed to the lake, it flows along the water as it will not degrade in general (Gonzales & Cossa, 2008) and will accumulate in the soil of the lake. In this way, heavy metals accumulate through time in the sediments. This might be the reason of the concentrations of heavy metals in the sediments of the lake, given that the lake is surrounded with agricultural area such as corn and rice fields, in addition households are also present around the lake wherein wastes from these households might directly discharge to the lake. With this, regulation of this human activities around the lake should be included in crafting legislations, as these heavy metals may directly affect the biological state of aquatic organisms in the lake.

The level of heavy metals in the water collected from Lake Dakong Napo was in the order of Pb>Cd>Cr>Ni. Among the four heavy metals analysed in the water samples, only the concentration of Ni has below the recommended safe limits of the Department of Environment and Natural Resources Administrative Order (DAO, 2016) for fresh surface water which is ≤0.05. Concentrations of Pb (16.35±0.58 mg/L), Cd (5±1.41 mg/L) and Cr (1±0 mg/L) are all above the maximum limits recommended by the same agency which are ≤0.05 for Pb, ≤0.01 for Cd and ≤0.05 for Cr respectively.

In nature, occurrence of metals may occur by geographical phenomena like volcanic eruptions, weathering of rocks, leaching into rivers, lakes and oceans due to action of water (Bagul *et al.,* 2015). However, ignition of these metals in aquatic ecosystem were caused by human activities. Wogu and Okaka (2011) reported that aquatic ecosystem which receives the industrial, agricultural, and urban sewage has concentrations of heavy metals above standard limits.

In addition, the presence of the heavy metals in the water samples of the lake particularly Lead maybe attributed to the by-product of human activity which includes combustion of petroleum fuels from automobile exhaust (Juberg, 2000), household plumbing (Edward *et al.*, 2013) and use of metal containing pesticides and agricultural run-off (Castro-Gonzales & Mendez-Armenta, 2008). High level of other heavy metals in the surface water of the lakes could also be attributed to local point sources and by the direct deposition of heavy metals from air pollution to the water during precipitation (Skejelkvale, 2001). This might be the reasons of the high level of some heavy metals in the surface water of the lake. Aside from agricultural areas, the lake is also situated close to a highway in which vehicles are passing to the different barangays of the municipality.

The high level of some heavy metals in the sediments and water samples collected from

Table 3. Concentration of heavy metals in the gonads and muscles of *P. canaliculata* collected from Lake Dakong Napo, Esperanza, Agusan del Sur

Parameters	Gonads	Muscles	Recommended tolerable limits for Molluscs	Agency
Pb (mg/L)	ND	ND	≤1.5	FAO/WHO (1984)
Cd (mg/L)	ND	ND	≤0.05	FAO/WHO (1984)
Mn (mg/L)	8.49±1.08	18.14±2.56	≤0.05	US EPA (2002)
Ni (mg/L)	28.3+6.76	ND	≤80	FAO/WHO (1984)

US EPA- United States Environment Protection Agency; ND- Not Detected

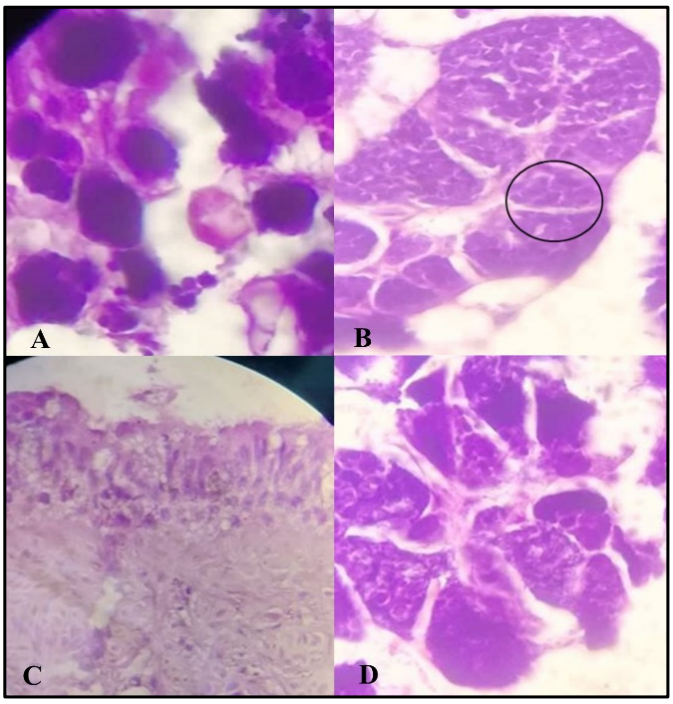

Fig. 2. A) Normal gonads; B) spaces between the gonad tissues; C) hyaline degeneration; D) separation of gonads.

Lake Dakong Napo have deleterious effect and potential risk of bioaccumulation to humans and other aquatic organisms in the area. Heavy metals such as Lead, Nickel and Cadmium are very harmful even in low concentrations. Long term exposure to these heavy metals can cause behaviour and learning problems, memory and concentration problems, hearing problems and slowed growth, it also cause damages in kidney, liver, circulatory and nerve tissues (Sankhla *et al*., 2016).

Manganese were detected both in the gonads and muscles of *P. canaliculata* (8.49±1.08 mg/L and 18.14±2.56 mg/L), whereas Nickel was detected only in the gonads of the snail (28.3+6.76 mg/L), while Lead and Cadmium were not detected in both samples (Table 3). Manganese is an essential element for all species however, based on the results, the concentration in both gonads and muscles is very high and above the safe limits set by the US EPA (2002). Thus, can cause harm to the aquatic organisms and its consumers, more likely humans.

Histological evaluation revealed no gross lesion on muscle tissues examined however, displayed hyaline degeneration of and separation of gonads, disintegration of muscle fibers and exhibited shrinkage away from the surrounding perimysium (Figures 2 and 3). This result

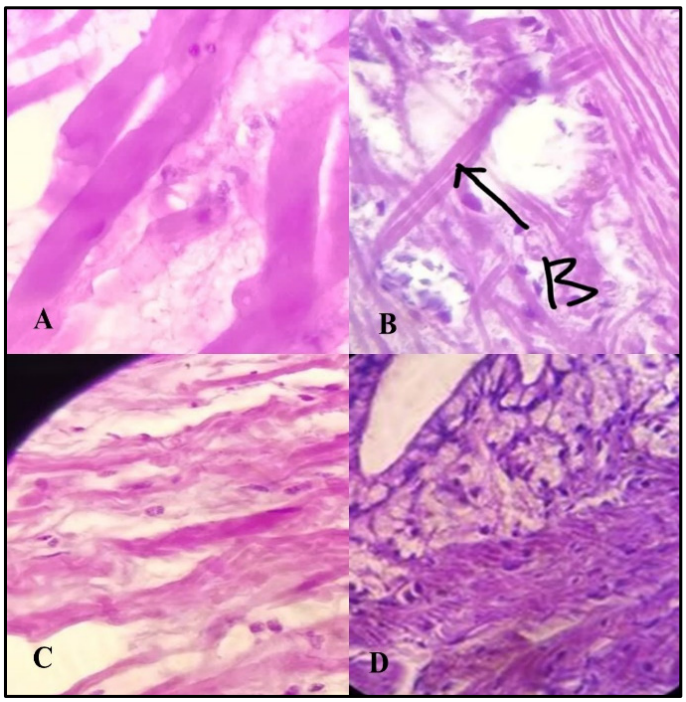

Fig. 3. A) Normal muscle bundle; (B Spaces between muscle bundles; C) Shrinkage away from the surrounding perimysium; D) proliferation of epithelium.

concurs with the study of Ebrahimi and Taherianfard (2011) that though muscles accumulate heavy metals, no pathological changes in the muscle tissues can be observed. Gbem *et al.* (2001) noted in their findings that the liver had the most altered tissues and histopathological changes compared to muscles given that the liver plays an immense role in accumulation and detoxification. On the other hand, the most notable changes in the muscle tissues of aquatic organisms that accumulate heavy metals such include disintegration and splitting of muscle fibers and degeneration of muscle bundles (Kaoud & El-Dahshan, 2010; Patnaik *et al.,* 2011; Sia Su *et al.,* 2013). These histological alterations might be due to the accumulation of heavy metals in the samples being examined. Heavy metals are toxic even at low concentrations and has no known function in biochemical processes (Burden *et al*., 1998), and were found to inhibit the impulse conductivity by inhibiting the activities of monoamine oxidase and acetylcholine esterase to cause pathological changes in tissue and organs (Rubio *et al*., 1991).

CONCLUSION AND RECOMMENDATIONS

This study provides baseline information concerning the levels of heavy metals in the sediments, water, and tissues of *P. canaliculata* in one of the important water resources in Esperanza, Agusan del Sur, Philippines. In sediments, only Cr (174.67±62 mg/L) and Ni (269.33±17.56 mg/L) were above from the recommended safe limits of the international standards while in water, the concentrations of Pb (16.35± 0.58 mg/L), Cd (5±1.42 mg/L), and Cr (1±0 mg/L) were above from the recommended safe limits of the national standards. These heavy metals were below the permissible limits in the gonads and muscles of *P. canaliculata* except for Mn. Alterations in the tissues of the snail are evident suggesting that these heavy metals and other environmental stressors are negatively affecting the organisms inhabiting the lake. Further studies should be done using different organisms such as fish and determination of other heavy metals such as mercury, arsenic and others should also be included in future studies.

ACKNOWLEDGMENT

The authors are thankful to the Municipal Environmental and Natural Resources Office of Esperanza, Agusan del Sur, Philippines for the support they extended to the authors during the conduct of the study.

GRANT SUPPORT DETAILS

The research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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