Journal of Environmental Science and Management 25-2: 20- 27 (December 2022) ISSN 0119-1144

JESAM

Lead Uptake of the Asian Clam (*Corbicula fluminea*), Nile Tilapia (*Oreochromis niloticus*) and its Parasite (*Acanthogyrus* sp.) in Yambo Lake, Laguna, Philippines

ABSTRACT

The contamination of heavy metals in the aquatic environment poses a threat due to its persistence and potential accumulation in aquatic life forms. In order to detect such pollution, the use of organisms as bioindicators was explored. This study investigated the use of fish acanthocephalan parasites as bioindicator of lead (Pb) in a semi-pristine Yambo Lake in San Pablo, Laguna, Philippines. Lake water samples, wild Nile tilapias (Oreochromis niloticus), its acanthocephalan parasites (Acanthogyrus sp.), and Asian clams (Corbicula fluminea) were collected and subjected to Pb concentration detection through heavy metal analysis. Higher accumulated mean concentration levels of Pb is recorded in Acanthogyrus sp. (10.13 mg kg^{-1}*), followed by the fish host tissues: liver (6.19 mg kg*$^{-1}$*), intestine (2.80 mg kg*$^{-1}$*), and muscle (0.75 mg kg*$^{-1}$*). An established bioindicator, C. fluminea, only accumulated an average of 0.16 mg kg*$^{-1}$* Pb in its soft tissues. The bioaccumulation capacity of Acanthogyrus sp. to the fish host and water samples were 35 times higher than the liver, 190 times the intestine and 211 times than the muscle. Furthermore, the accumulated Pb in the parasite was 3,015 times higher than C. fluminea in lake water. Among all samples analyzed, Acanthogyrus sp. showed the highest accumulation capacity. These findings provide useful information to the bio indicator potential of this parasite, and that local environment authorities can utilize its sensitivity to detect and monitor traces of heavy metals even in less polluted aquatic environments.*

Keywords: *Acanthogyrus sp., bioindicator, Yambo Lake, Lead (Pb)*

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INTRODUCTION

The Seven Freshwater Lakes situated in the city of San Pablo are famous spots for aquaculture production and livelihood in Laguna, Philippines. Out of the seven lakes, Yambo Lake (14.121°N 121.365°E) is well-known for its less-polluted, oligotrophic quality, and its industrial, aquaculture, and ecotourism potentials. However, along with the increasing developments are the threatening impacts of anthropogenic pollution which may alter the quality of the lake (*LLDA 2005*). One of the most alarming problems is the rise in heavy metal pollution particularly lead (Pb) (*Singh et al. 2014*). Lead is found naturally occurring in the environment, and is also an anthropogenic pollutant which can be derived from sources such as road run-off that include vehicle tires, brake linings and fuel (*Cheng et al. 2015, Akan et al. 2012, Corradi and Mutti 2011*). Heavy metals cannot be broken down and can stay in the environment for longer periods, which result in accumulation in the sediments of estuaries, lakes and sea. (*Xu et al. 2017; Dong et al. 2014*). This accumulation in aquatic ecosystems may affect organisms inhabiting in them because it can interfere with the growth, consumption and reproduction of the animals (*Akankali and Elenwo 2015*). Anticipating the possible health hazards that the local aquatic biota and the immediate community may acquire, environmental biomonitoring efforts should be considered to preserve and protect the lake from harboring elevated levels of metal pollution.

At present, one of the most effective ways to detect heavy metal contamination in the aquatic ecosystem is through the use of pollution bioindicators such as sentinels. Sentinels are organisms that accumulate and concentrate pollutants from their environment. Their tissues provide a time-integrated indicator estimate of the environmentally available concentration of pollutants (*Najm and Fakhar 2015; Basu et al. 2007*). Previous studies suggest the use of *Corbicula fluminea*, also known as the Asian clam as a heavy metal bioindicator (*Dabney et al. 2018, Arini et al. 2014*). This organism meets the criteria of a good bioindicator such as their long lifespan, (average of 1 to 5 years) (*McMahon 2002*), ability to bioaccumulate pollutants (*Huang et al. 2007, Dos Santos and Martinez 2014*), limited movement which contributes to site-specific bioaccumulation, its abundance in the ecosystem (*Sousa et al. 2008*), and adequate tissue mass for analysis (*Sures 2005; Rosa et al. 2014*).

However, recent studies show a developing interest in the association of pollution and parasitism in the aquatic ecosystem (*Nachev and Sures 2016a*). Various studies in temperate countries (demonstrated and proved the parasite's sentinel features, showing fish acanthocephalans as one of the promising group of pollution indicators in a highly contaminated environmental setting *Kennedy 2006, Jankovska et al. 2011, Marijic et al. 2013; Brazova et al. 2012*). In a tropical setting, some members of the Phylum Acanthocephala, particularly *Acanthogyrus* sp. are now being studied for their efficiency in detecting contamination of heavy metal in the aquatic environment (*Paller et al. 2016; Al-Hasawi 2019*). *Acanthogyrus* sp. commonly parasitize the intestinal part of fishes, and can accumulate heavy metals in its body as it responds to changes in the environment (*Sures 2003*).

Most bioaccumulation studies of freshwater mollusks and acanthocephalan parasites have been conducted in temperate and tropical countries, under the conditions of extreme aquatic pollution. To fully highlight the potential of acanthocephalan parasites as sentinels of heavy metal pollution, it is important to test the capacity and sensitivity of this organism in a seemingly pristine environment with very low levels of Pb concentration detected in ambient waters. This study aimed to determine the potential use of acanthocephalan parasites in naturally-infected freshwater fishes as a lead (Pb) indicator in a semi-pristine, oligotrophic setting of Yambo Lake, Laguna, Philippines.

MATERIALS AND METHODS

Sample Collection

Collection of samples were done during the wet season. One hundred seventy Nile Tilapia (*O. niloticus*) samples were collected using trap nets by fishermen, and 100 Asian clams (*C. fluminea*) were obtained in the substratum using hand method (*Gabal 2007*). Samples were placed in separate ziplock bags and were transported to the laboratory for processing. Lake water samples were collected in triplicates using 1L sterile high density polyethylene (HDPE) bottles and transported to the laboratory using a cooling container. In order to obtain adult *Acanthogyrus* sp., *O. niloticus* collected from the lake were dissected. Adult forms of this parasite possess retractable proboscis armed with hooks that attaches to the intestine of its host (*Bayoumy 2006*).

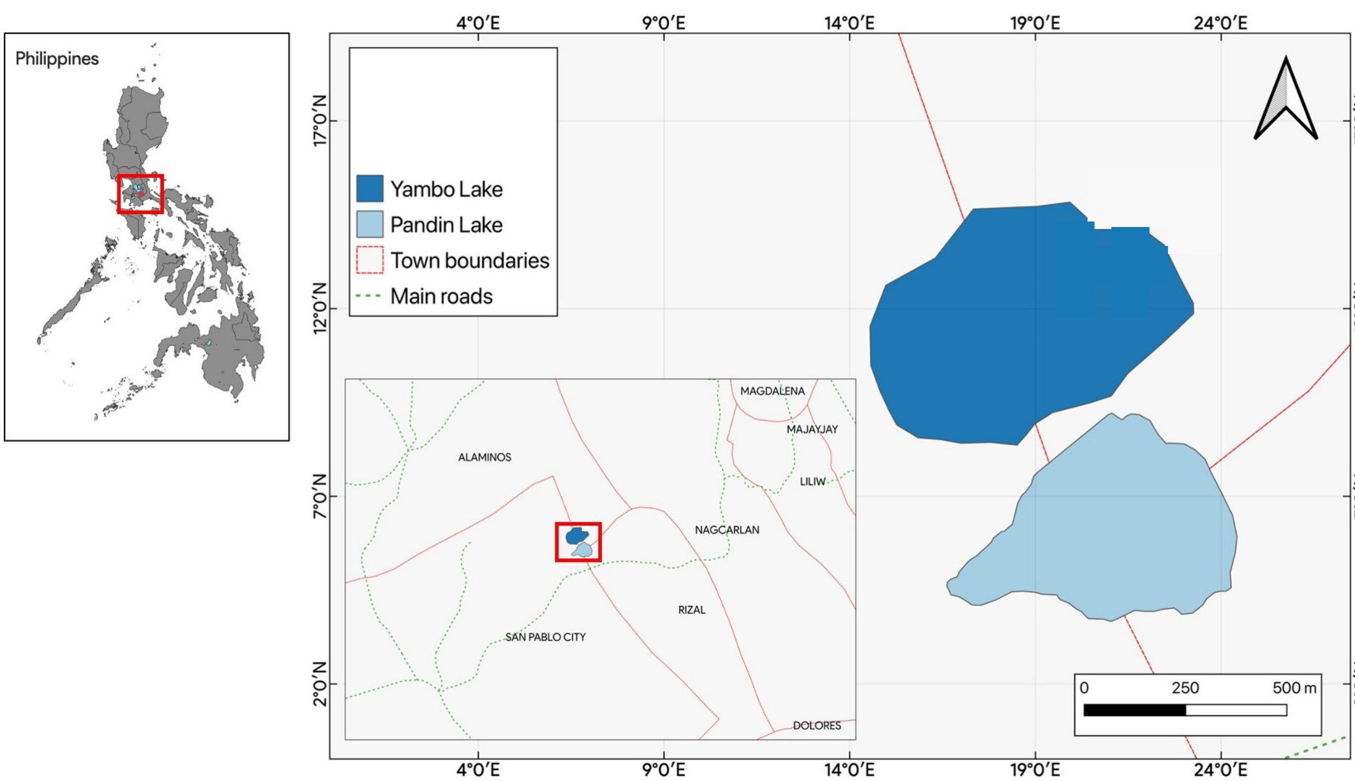

Figure 1. Map showing the location of Yambo Lake, one of the seven crater Lakes of San Pablo Laguna, Philippines.

Sample Preparation for Lead (Pb) Analysis

Collected water samples were acidified using concentrated nitric acid (HNO_3) and refrigerated at 4°C prior to heavy metal analysis. For fish and clam samples, the weight (g) and the total length (cm) were recorded. Fish samples were anaesthetized using clove oil (*Thielen et al. 2004*) and then were dissected. The fish intestines were placed in a petri dish and teased using forceps in order to check for the presence of the Acanthocephalan parasites. The acanthocephalans were immersed in cold distilled water for approximately 2 hr in order to evert the proboscis and were examined under the microscope for parasite identification. Ten fish with relatively heavy infections were selected and subjected for lead (Pb) concentration analysis. *Acanthogyrus* sp. collected with varying intensity between 81 and 130 parasites per fish were grouped and identified as one sample. Clam samples were washed thoroughly with distilled water to remove dirt (*Sow et al. 2019*). The soft tissues of the clam samples, the intestine, muscle and liver of the infected fish were collected using forceps and scissors. Samples were heated gently using a plate until surface water subsided. Afterwards, samples were placed in a sterilized plastic container and stored at -20°C prior to its treatment.

Lead (Pb) Analysis of Collected Samples

Pb detection in samples was performed at the Analytical Services Laboratory of the Institute of Chemistry, University of the Philippines Los Baños. Prepared lake water samples were subjected to Pb analysis using Atomic Absorption Spectrometry (AAS Varian 55B). The samples of parasite, fish, and clams were thawed, weighed and homogenized. Digestion of the samples followed using concentrated 5mL H_2SO_4 and 10 mL HNO_3, and were subjected to heating at 60°C and increased gradually to 120°C until most of the nitrous fumes were dissolved. Dropwise of HNO_3 were added to the samples until clear, and the volume was adjusted to 25 mL and 10 mL for tissue samples and parasites, respectively. Lead was analyzed using the external standard from 1,000 ppm Pb stock solution (traceable to the National Institute of Standards and Technology), following the working standards of 0.02-10 ppm prepared by serial dilution. The concentration was analyzed at 217 nm with flow rate at 5 mL min^{-1} and air-acetylene as fuel. In Pb detection, samples were prepared in triplicates with mean concentration determined as mg kg^{-1} wet weight. The limits of detection (LOD) for Pb analysis in both tissue samples and parasiteswere determined as three times the standard deviation of the measurement of 10 blanks (*Paller et al. 2016*).

Data and Statistical Analysis

The lead concentration ratio between the parasite and tissues of the fish hosts were calculated as follows:

$$C_{[parasite]}/C_{[host\ tissue]} \quad (1)$$

while the bioconcentration factors (BAF) of the parasite against the ambient lake water were computed as:

$$C_{[parasite]}/C_{[water]} \quad (2)$$

wherein C is the concentration of lead (*Thielen et al. 2001*). To test the Pb concentration differences between the tissues of the host, acanthocephalans and clam samples, data were subjected to Kruskal-Wallis test at 95% level of significance. All graphs and statistical computations were constructed and performed using Statistical Package for Social Sciences (SPSS Statistics) 2.0 software (IBM Corporation USA).

RESULTS AND DISCUSSIONS

Lead (Pb) Concentration in Ambient Waters and Collected samples

The mean weight and length of *O. niloticus* fish samples collected from Yambo Lake were 198.90 ± 58.39 g and 19.47 ± 5.06 cm. The acanthocephalan *Acanthogyrus* sp. were collected in 143 out of 179 (79.9%) fish, with a mean intensity of 66 worms per host. The intensity of infection ranged from very low (one worm per fish) to high (130 worms per fish). The parasite pooled weight per sample ranged between 3.11 and 25.35 mg (wet weight). Water samples collected from three different points of the lake concentrated in the north east showed that lead (Pb) is present in relatively low average concentration of 0.02 mg L^{-1} (**Table 1**) and is within the acceptable Pb limit (0.05 mg L^{-1}) for freshwater quality under Class C (*DENR 2016*).

In aquatic environments, heavy metals are produced from natural and anthropogenic sources (*Ghasemi and Moazed 2014*). Detected Pb concentrations in Yambo Lake could be attributed to its volcanic origin. In water bodies with volcanic origin such as lakes, enrichment of heavy metals occurs by 100 to 10,000 fold compared to concentration in the ocean water (*White et al. 1971*). Recently, the wastes coming from the upland residential areas and the increase in tourism could also contribute to Pb pollution in the previously pristine lake. Majority of the toxic contaminants in fresh water come from runoffs in urban areas, and the frequently detected contaminant due to runoff included the heavy metal Pb ranges from 6 to 460 µg L^{-1} (*Cole et al. 1984*). Also, the products of transportation wear-offs, the existing upland agricultural plantation, which employs the use of fertilizers, and nearby industrial operations may carry the metal effluents towards the lake basin during precipitation. Once they reach the lake, the heavy metals accumulate and concentrate in the sediments (*Duncan et al. 2018*). The suspended sediments then adsorb pollutants, causing the pollutant concentration in the water column to lower (*Yi et al. 2011*). During the rainy season, the disturbance created in the water surface produces upwelling of the sediments settled in the bottom, thereby releasing Pb to the water column (*Beiras et al. 2003*). The dissolved oxygen during the wet season increases in the lake because the rain interacts with oxygen in the air as it falls, thus, increasing the respiration activity of the aquatic organisms. Hence, aquatic organisms such as fishes are susceptible to Pb accumulation since lead is taken in easily at the event of increased respiration activity (*Mziray and Kimirei 2016*).

The heavy metal analysis revealed the presence of Pb in the fish host tissues namely: liver, intestine, muscle, in the fish parasite, *Acanthogyrus* sp. and in the Asian clam, *C. fluminea* (**Figure 2**). Pb analysis revealed that the host tissues and *C. fluminea* accumulated lower Pb concentrations than *Acanthogyrus* sp., and that Pb concentration was significantly higher in parasites than in fish tissues and clams ($X^2 = 10.12$, $p < 0.05$). The fish parasite *Acanthogyrus* sp. showed the highest level of Pb with a mean concentration equal to 10.13 mg kg^{-1}, followed by the liver (6.19 mg kg^{-1}), intestine (2.80 mg kg^{-1}), muscle (0.75 mg kg^{-1}), and *C. fluminea* (0.16 mg kg^{-1}) (**Table 1**).

Bioaccumulation capacity between *Acanthogyrus* sp, *C. fluminea* and *O. niloticus* tissues

The ratio of Pb bioaccumulation capacity between *Acanthogyrus* sp. and the fish host tissues is 190 times compared to the intestine, 35 times than the liver and 211 times than the muscle (**Table 2**). Also, the bioaccumulation factor of Pb in the parasite, *Acanthogyrus* sp. was found to be higher (3,015), than *C. fluminea* (29) collected from the same site.

The sedimentary filter feeder, *C. fluminea* has high ability for bioaccumulation of chemical substances in ambient waters (*Fournier et al. 2005*). However, the higher bioaccumulation capacity of *Acanthogyrus* sp. in this study compared to the rest of the samples can be attributed to the absence of its digestive tract, the manner of absorption of all nutrients through their body wall (*Wolff 2013*); and their ability to obtain bile-bound lead from the lumen of the intestine which reduces its entero-hepatic cycling (*Nachev and Sures 2016b*).

The high Pb bioconcentration capacity of the fish host

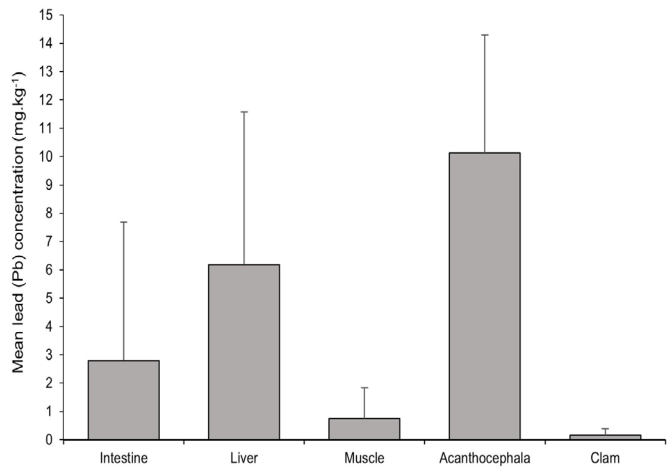

Figure 2. Mean Lead (Pb) concentrations (mg kg^{-1} wet wt.) in infected *Oreochromis niloticus* tissues (intestine, liver and muscle), their acanthocephalan parasite, *Acanthogyrus* sp. and the Asian clam, *Corbicula fluminea* collected from Yambo Lake, San Pablo Laguna, Philippines (Bars denote +SD).

Table 1. Mean Lead (Pb) concentrations (mg kg^{-1} wet wt.) in the tissues of infected *Oreochromis niloticus*, *Acanthogyrus* sp., lake water samples, and in *Corbicula fluminea**.

Sample		Mean ± SE (mg kg^{-1} wet wt.)	Minimum	Maximum
Infected fish	Liver	6.19 ± 3.28^{ab}	<0.075	29.32
(*Oreochromis niloticus*)	Intestine	2.80 ± 2.80^{ab}	<0.075	15.24
	Muscle	0.75 ± 0.34^{ab}	<0.075	2.57
Parasite	*Acanthogyrus* sp.	10.13 ± 8.27^{a}	<0.075	82.85
Water		0.02** ± 0.00	0.02	0.02
Clam	*C. fluminea*	0.16 ± 0.07^{ab}	<0.075	0.16

Fish tissues, parasite, and water samples were analyzed in three replicates.
** Concentration unit for water presented as mg L^{-1}
ab denote significant differences among samples ($p < 0.05$)

Table 2. Lead (Pb) concentration ratio between *Acanthogyrus* sp. and *Oreochromis niloticus* tissues and the bioaccumulation factor of *Acanthogyrus* sp. and *Corbicula fluminea*. (C-concentration).

	Oreochromis niloticus		
Ratio	Intestine	Liver	Mucle
	190	35	211
Bioaccumulation factor	*Acanthogyrus* sp.	*Corbicula fluminea*	
	3,015	25	

liver tissues in *O. niloticus* is expected since this organ performs filtration functions including accumulation of Pb (*Rajeshkumar and Li 2018*). The rapid rise of metal levels in the liver takes place during exposure, and remains high for a long time of depuration, when other organs have already been cleared (*Jezierska and Witeska 2006*). The accumulation in both liver and intestine were also expected since these organs are involved in heavy metal detoxification by the entero-hepatic pathway (*Nachev and Sures 2016a*). On the other hand, metals that bind in bile complexes that are excreted in the small intestine are acquired by acanthocephalans; making it unavailable for intestinal reabsorption (*Nachev et al. 2013a*). As a result, fish muscles only absorb low metal concentrations (*Jezierska and Witeska 2006*).

A number of field studies showed similar findings with this study on the accumulation capacity of acanthocephalan parasites, fishes and that of the bivalve mollusks as routinely used sentinel. Studies reported *Pomphoryncus laevis* to accumulate higher concentrations of different heavy metals than its fish host (*Vidal-Martinez 2010; Sures 2003*). This is also in agreement with the results of the efficiency of *Acanthocephalus lucii* in accumulating heavy metals (Pb and Cd) against the tissues of its host, *P. fluviatilis* (*Sures et al. 2009*). *A. lucii* accumulated 30-38 times (6.4-8.7 mg kg^{-1}) more Pb than the intestinal wall of its fish host, and 120-230 times more than the tissues of the zebra mussel, *Dreissena polymorpha*. Also, existing studies on the heavy metal accumulation of acanthocephalans reveals its efficiency in a polluted freshwater setting. Acanthocephalans were highly prevalent in infected *O. niloticus* from the metal contaminated Burullus Lake in Egypt, yet non-infected fish in this study possess significantly higher metal levels including Pb. This suggests that parasites absorb high metal levels (*Radwan et al. 2022*). Similar findings were observed by *Taraschewski* (*2000*) where 27,000-fold higher than water metal exposure concentration has been accumulated by isolated acanthocephalan. *Paller et al.* (*2016*) also reported that Pb levels in *Acanthogyrus* sp. were 988 times higher relative to the ambient waters of the highly productive Sampaloc Lake in Laguna, Philippines. On the contrary, this study revealed the efficiency of *Acanthogyrus* sp. in accumulating Pb in a less-polluted aquatic environment. Ambient waters of Yambo Lake showed relatively low levels of Pb compared to the Pb absorbed by acanthocephalan parasite suggesting the parasite's sensitivity to the heavy metal may it be in a high or less contamination freshwater setting. This study highlights the efficiency of *Acanthogyrus* sp. as a good bioindicator of metal pollution (*Sures et al. 1997, Dos Santos and Martinez 2014*).

CONCLUSIONS AND RECOMMENDATIONS

The detection of Pb in a semi-pristine setting using *C. fluminea*, the fish acanthocephalan parasite, *Acanthogyrus* sp. and *O. niloticus* tissues was conducted. In this study, Yambo Lake showed relatively low levels of Pb concentrations but this metal can be accumulated by fish through respiration, and by clams through filter-feeding. The results of this study reveal that even in a less-polluted aquatic environment, a totally different ecological setting, Acanthocephalan parasites are better bio indicators of pollution and environmental degradation compared to their fish hosts and bivalves. Parasitism in this setting is advantageous for the fish hosts, since parasites can act as a metal sink, accumulating more Pb concentrations than the fish host tissues. The results of this study provide evidentiary support to *Acanthogyrus* sp. as a potential sentinel for detecting heavy metal contamination in the environment, and that environmental managers may consider this aspect of parasitology in monitoring environmental health.

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