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Comparative sensitivity of *Caridina nilotica***,** *Haplochromis nubilus***,** *Bulinus africanus* **and** *Bulinus forskalii* **from Lake Victoria, Tanzania to mercury chloride**

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Lake Victoria has been under increasing threat of mercury pollution derived from artisanal gold mining, where mercury is amalgamated with gold. Despite the fact that mercury and its derivatives can adversely affect the aquatic biota in Lake Victoria, comparative studies to determine the susceptibility of native biota to mercury have seldom been done. This study was done to compare the susceptibility of shrimps (*Caridina nilotica*), fish Astatotilapia nubia (Boulenger, 1906) or *Haplochromis nubilus*, and two species of fresh water snails (*Bulinus africanus* and *Bulinus forskalii*) to mercury chloride (HgCl₂) in water. The results indicated that *C. nilotica* is the most susceptible to mercury-induced toxicity among the tested organisms with 96 h LC50 of 8μg*/*l. The second most susceptible species was *B. forskalii* which exhibited LC50 of 98 and 68μg*/*l after 72 h and 96 h of exposure respectively. *Hypochromis nubilus* was the least sensitive species to mercury toxicity of the four tested organisms with 96 h LC50 of 162μg*/*l. Findings from the present study suggest that *C. nilotica* is sensitive to mercury chloride and can be used to assess Hg environmental risk in Lake Victoria.

Keywords: *Caridina nilotica*; *Haplochromis nubilus*; *Bulinus africanus*; *Bulinus forskalii*; mercury; Lake Victoria

1. Introduction

In the last decade there have been considerable amount of research about Lake Victoria contamination with mercury (Hg) [1–6]. Many of the Hg studies in Lake Victoria have been triggered by concerns about the gold (Au) ore processing practices in artisanal gold mines that use mercury amalgamation for extraction of gold. Studies conducted along the major amalgamation areas around Lake Victoria, identified high mercury concentrations in sediment and water. Other possible major sources of mercury to Lake Victoria basin though less studied include biomass burnings, atmospheric deposition and soil erosion. In the aquatic environment inorganic mercury is methylated by micro organisms into its organic forms that are capable of biomagnification along the food chain [6].

Most of the research work on mercury toxicity in Lake Victoria basin has been focused mainly on Hg bioaccumulation in fish and its eventual possible effects to human health [5,7,8]. Such

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information, though valuable, is less relevant to safegurd the important biodiversity in Lake Victoria ecosystem. On the other hand, the regulation of substances discharged to aquatic systems relies on data derived from ecotoxicity tests [9,10]. However, most of the available ecotoxicological data were generated in developed western world, thus are based on temperate and coldwater species which are found in Europe and North America [11]. There is less available ecotoxicological data for species from tropical aquatic ecosystems [12] and Lake Victoria in particular. Because of this paucity of data, water quality criteria (WQC) in Tanzania like in most African countries are based on extrapolations of data from developed countries. This extrapolation approach is based on the assumption that local species respond in the same way like temperate species. This assumption tends to ignore the fact that biodiversity in the tropic ecosystems is substantially higher than in temperate waters, thus, the number of species affected by pollutants are always greater and that there may be variation in sensitivities to pollutants. Recently, Kwok and his team [13] demonstrated that the sensitivities of tropical species are different from their counterparts in the temperate zone for some pollutants.

The present study was therefore, undertaken to compare the susceptibility of fresh water shrimps (*Caridina nilotica)*, fish Astatotilapia nubia (Boulenger, 1906) or *Haplochromis nubilus*, two species of fresh water snails (*Bulinus africanus* and *Bulinus forskalii*) which are native organisms in Lake Victoria. The tested species carry significant importance in Lake Victoria ecosystem. *C. nilotica* is the only shrimp species known in Lake Victoria and is an important prey species for Nile perch (*Lates nilotica*) [14]. *H. nubilus* is one species of the haplochromine cichlids which was once considered extinct, and only recently there have been signs of its resurgence in some parts of Lake Victoria [15]. *B. africanus* and *B. forskalii* are resident species of snails which plays an important role in Lake Victoria food chain and have been shown to accumulate pollutants including mercury [7]. All the tested species were acclimatised in the laboratory for two weeks before the start of the experiments.

2. Materials and methods

2.1. *Toxicity test with shrimps*

Ninety six hours semi static acute assays with shrimps (*Caridina nilotic*a (Roux) (Crustacea) were conducted according to standardised protocol. Shrimps (average fresh weight 0.83 g) were collected by using vertical plankton net in pelagic waters in Lake Victoria. Tests were conducted in 3000 ml glass aquaria containing synthetic water made in our laboratory which had moderate hardness (44 mg/l as CaCO₃), pH of 7.5 ± 1 , dissolved oxygen of $4-5$ mg/l and maintained at 25 ± 1◦C under a 12:12 h light*/*dark cycle. Each tested mercury concentration was replicated six times, such that a total of thirty shrimps were exposed (5 per aquaria) to each concentration of mercury chloride (Merck-Germany) (0, 2.5, 4.5, 10, 18, 32, 60 and 108μg*/*l). Testing solutions were renewed every 24 h, and shrimps lethality was recorded and LC50 values calculated, at 24, 48, 72 and 96 h of exposure.

2.2. *Toxicity test with snails*

Ninety six hours semi static acute tests with snails *B. africanus* and *B. forskalii* (Mollusca; Gastropoda) were carried out in 1200 ml glass containers using synthetic water (pH 7*.*0 ± 0*.*2, water hardness of 44 mg/l as CaCO₃, dissolved oxygen of 4–5 mg/l). Snails of average body weight of 0.217 g for *B. africanus* and 0.093 g for *B. forskalii* respectively were collected from areas of Lake Victoria in Sengerema district, which is situated far from gold mining areas. Each tested concentration of mercury $(0, 20, 36, 64, 116, 210, 376 \mu g/l)$ was replicated six times,

i.e. 30 snails were used per tested mercury concentration. The choice of mercury concentration to be used was based on our earlier range finding test. Room temperature ($25 \pm 1°C$) and 12:12 light*/*dark cycle were kept constant. Testing solutions were renewed every 24 h and the survival of snails was evaluated at 24, 48 and 96 h of exposure and respective LC50 values were determined.

2.3. *Toxicity test with* **Hypochromis nubilus**

Ninety six hours semi static acute assays with haplochromines fish were conducted according to standardised protocol. Juvenile fish (average weight 2.1 g) were collected by using plankton net in pelagic waters in Lake Victoria. Tests were conducted in 5000 ml glass aquaria containing synthetic water with moderate hardness of 44 mg/l as CaCO₃), pH of 7.5 \pm 1, dissolved oxygen of 4–5 mg/l and maintained at 25 ± 1 [°]C under a 12:12 h light/dark cycle. A total of thirty (30) fish were exposed (5 per aquaria) to each concentration $(0, 25, 45, 81, 146, 263, 472, 485, 472)$ as mercury chloride. Testing solutions were renewed every 24 h, and fish lethality was recorded and LC50 values calculated, at 24, 48, 72 and 96 h of exposure.

2.4. *Mercury analysis*

Samples of testing water were collected for mercury analysis at the end of each experiment. For. Analysis 10 ml of filtered sample (using $0.45 \,\mu\text{m}$ filters) was mixed with 2 ml of 1:1 H₂SO₄ to 2% potassium permanganate solution. The mixture was allowed to stand for 15 min; subsequently 0.5 ml of 5% potassium sulphate was added. The mixture was heated in a water-bath at 95° C for 1 h. After cooling, the 3% hydroxylamine solution was added drop wise, until the permanganate colour discharged completely. Five ml of the digested sample was acidified, using 5 ml 6M HCl. Samples were then analysed for total mercury by Atomic Absorption Spectrophotometer with cold vapour generation technique (ICP Ultima 2, Horiba JobinYvon, France). Acid washed glassware, analytical grade reagents and double distilled and deionised water were used in the analysis. In order to check purity of the chemicals used, one blank was run every 10 samples. Additionally, to ensure the validity of the calibration of the instrument throughout the analysis, the replicates of Hg standard solutions was run every 10 samples. There was no evidence of contamination in the blanks and the variability in Hg standards measurement was low $(\pm 0.05 \,\mu g/l)$. Analytical quality control was ensured through the analysis of replicates of Hg standard solutions. The limit of detection was 0.02μg*/*l.

2.5. *Statistical analysis*

The LC50 (lethality) values and their 95% confidence were determined by probit analysis (Finney, 1971) using a computer software (STATISTICA version 6).

3. Results

As shown in Tables 1, 2, 3 and 4, the *C. nilotica* LC50s (28, 22, 20, and 8 for 24, 48, 72 and 96 h respectively) proved to be, the most susceptible to mercury induced toxicity, among the four organisms tested. The second most susceptible species was *B. forskalii* which exhibited LC50s of 98 and 68μg*/*l after 72 h and 96 h of exposure, respectively. The *B. africanus* snails (72 and 96 h LC50s of 116 and 76μg Hg*/*l) were less susceptible to mercury toxicity in water compared to *B. forskalii* snails. On the other hand, *Hypochromis nubilus fish* was the least sensitive species to mercury toxicity of the four tested organisms with LC50s of 350, 251, 200 and 162 for 24, 48, 72 and 96 h, respectively.

Measured exposure concentration of Hg $(\mu g/l)$	24h	48h	72 h	96 h	
	Survivorship (%)				
${<}0.02$	100	100	100	93	
0.9	100	100	100	90	
2.6	97	100	87	80	
12	93	90	87	33	
21	90	83	80	17	
24	57	27	3.3	0	
42.1	13	θ	0	Ω	
110	0	0	0	Ω	
LC50 (95% confidence interval)	$28(18-41)$	$22(11-37)$	$20(9-34)$	$8(4-24)$	

Table 1. Toxicity test with *Caridina nilotica*.

Table 2. Toxicity test with *Hypochlomis nubilus*.

Measured exposure concentration of Hg $(\mu g/l)$	24h	48h	72h	96 h		
	Survivorship (%)					
${<}0.02$	100	100	100	97		
21	100	100	100	100		
37	100	100	100	100		
72	100	97	97	90		
149	100	87	64	60		
223	80	63	43	27		
363	52	10				
792	Ω	0	0			
LC50 (95% confidence interval)	350 (282-432)	251 (218-278)	200 (155-289)	$162(86 - 221)$		

Table 3. Toxicity test with *Bulinus forskalii*.

Measured exposure concentration of Hg $(\mu g/l)$	24h	48h	72h	96 h		
	Survivorship (%)					
${<}0.02$	100	100	97	97		
21	100	100	100	90		
24	100	100	93	83		
42	100	80	73	60		
110	83	67	43	13		
176	33	10	10	3		
209	0	0	0	0		
LC50 (95% confidence interval)	$161.5(98-214)$	148 (103-204)	$98(72 - 102)$	$68(38-105)$		

Table 4. Toxicity test with *Bulinus africanus*.

4. Discussion

Compared to studies done previously to other types of shrimps, *C. nilotica* appear to be more sensitive to mercury. For example, in an aqueous experiment on white shrimp (*Penaeus setiferus*) with mercury chloride the calculated 96 h LC₅₀ was $17 \mu g/l$ [16], while the 96 h LC₅₀ for adult brown shrimps (*Crangon crangon*) ranged between 100–300μg*/*l [17]. Nevertheless, brown shrimps which are marine organisms can not be exactly compared with *C. nilotica*, a fresh water shrimp, because fresh water invertebrates tend to be more susceptible to mercury than their counterparts in the marine environment [17].

There is no available data on the sensitivity of *Hypochlomis nubilus* to mercury, but it has been documented that the intestinal wall in fish present an effective barrier to mercury chloride permeability [17]. It is probably due to this reason that *H. nubilus* exhibited the least sensitivity to mercury in the present study. Though, sensitivity of different fish species to mercury varies widely, compared to data reported in the literature it appears that *H. nubilus* is more sensitive to mercury. Studies with *Tilapia mossambica, Heteropneustes fossilis, Channa marulius* and Rainbow trout resulted into 96 h LC50 of 350, 350, 131 and $280 \mu g/l$, respectively [17] which are higher than 162μg*/*l calculated in the present study. But, testing of *Sarotherodon mossambicus* and *Roccuss saxatilis* with mercury chloride resulted into 96 h LC50 of 75 and $90 \mu g/l$, respectively [17]. These levels are lower than ones calculated for *H. nubilus* in this study.

There have been some studies that have examined the toxicity of Hg to snails of fresh water. Data compiled by the World Health Organisation (1989) reported 96 h LC50 values of 80μg*/*l and 135μg*/*l for *Amnicola* sp. and *Lymnaea luteola* snails, respectively [17]. In another experiment it was reported that *Pila globosa* snails resulted into a 72-h LC50 of 296μg*/*l of mercury. Calculated 96-h LC50 in the present study for *B. forskalii* (68μg*/*l) and *B. africanus* (76μg*/*l) are lower than ones reported in the literature for *Amnicola* sp. and *L. luteola*. Similarly, the 72-h LC50 calculated in the present study for *B. forskalii* (96μg*/*l) and *B. africanus* (116μg*/*l) are much lower than 296μg*/*l reported for *P. globosa*. Piyatiratitivorakul and his team [18] estimated the LC50 of the golden apple snail, *Pomacea* sp. to be $1570 \mu g/l$, which is above the one calculated in this study.

Toxicity of mercury like other metals to aquatic species depends, on one side, on the organism sensitivity and on the other side, on the concentration of mercury and its bioavailability. In natural settings, mercury bioavailability varies depending on a variety of factors such as adsorption to particles, complexation by organic matter (e.g. humic and fulmic acids), presence of other cations [19] and pH [20]. Based on these factors, Hg bioavailability in a certain natural water body may be different from that obtained in a standard laboratory bioassay, and thus laboratory data should be used with caution by risk assessors. The physical-chemical characteristics (pH, dissolved oxygen, temperature and water hardness) of the testing media in the present study were near or equal to those measured at the areas where test animals were collected in Lake Victoria. These parameters were 7.2 to 7.4, 3.6 to 5.8 mg/l, 23 to 26.5 °C and 44 to 58 mg/l (as $CaCO₃$) for pH, dissolved oxygen, temperature and water hardness, respectively. Results from the present studies indicate that *C. nilotica* a tyisid shrimp was the most susceptible organisms of the four tested species. The snails (*B. forskalii* and *B. africanus)* were the second most sensitive tested organisms, and *H. nubilis* fish were the least sensitive. The results from this study are very useful as they have indicated that the tested organisms which were collected from Lake Victoria exhibited higher sensitivity to mercury chloride than similar organisms described in the literature. However, the high concentrations of Hg at which mortalities were observed in the present study may be ecologically less relevant as measured mercury concentrations in Lake Victoria water is lower [6]. Additionally, these results suggest that *C. nilotica*, which is the only shrimp reported occurring in Lake Victoria may be appropriate for assessing Hg environmental risk in Lake Victoria. However, more tests should be done to assess the sensitivity of more living organisms in Lake Victoria to

different pollutants and develop the multi-species toxicity tests so as to develop realistic pollutants water quality criteria instead of relying on data generated using temperate organisms.

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