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Levels of Mercury Species in Nile Perch (*Lates Niloticus*) from Lake Victoria, Tanzania

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Abstract**—** *Mercury speciation in fish tissues is a very important information for assessing the potential toxicological effects of mercury species in fish and subsequently humans. Apparently, there is scarce information and comparative studies on speciation between total-Hg and MeHg in Nile perch obtained from Lake Victoria. In this study the levels of total-Hg and MeHg in twenty-seven (27) samples of Nile perch (Lates niloticus) from three regions around Lake Victoria, namely Mwanza, Geita, and Mara were analysed. Both total-Hg and MeHg were qualitatively and quantitatively analyzed and determined using Tricell DMA-80 direct mercury analyzer. The mean concentration of total-Hg in Nile perch obtained at Mwanza, Geita and Mara region were 1.6954, 0.0452 and 0.0472 ng/kg respectively and the mean concentration of MeHg in Nile perch obtained at Mwanza, Geita and Mara region were 0.0425, 0.0380 and 0.0404 ng/kg respectively. Nile perch obtained in Mwanza region showed the highest concentration for both total-Hg and MeHg, Nile perch obtained in Geita region showed the lowest concentrations for both total-Hg and MeHg. The percentage of MeHg in total-Hg in Nile perch obtained at Mwanza town, Mswahili, Kikongo, Bukome, Senga, Bukondo, Mwigobero, Mugango, and Suguti were 95.44, 01.60, 01.71, 78.32, 86.23, 87.61, 88.33, 80.30 and 87.93 respectively. This results indicate that the specific chemical forms of an element must be taken into account in its individual forms, also human activities and environmental factors cause diversity of chemical concentrations from village to village or region to region. Further studies about speciation on mercury and other heavy metals should be conducted in water, sediment and biota.*

Keywords**—** *Total-Hg, MeHg, Nile perch, Lake Victoria, DMA-80.*

I. INTRODUCTION

Lake Victoria is the second largest lake in the world covering about 68,800 km^2, and is about 400 km from North to South and about 240 km East to West. Lake Victoria accounts for 51%, 43% and 6% of the area covered in the three countries of Tanzania, Uganda and Kenya (Okungu et al. 2005). Lake Victoria has many species of fish, but the most common species of fish are Nile tilapia (Oreochromis niloticus), native daga (Rastrineobola argentea) and Nile perch (Lates niloticus) (Tumwebaze 2018). Increased human activities such as agriculture, industrialization and urbanization have continuously caused adverse effects on the lake causing changes in the lake's ecosystem (Okungu et al. 2005).

Due to concerns about possible presence of contaminants caused by gold ore processing, biomass burning and soil erosion on the southern parts of the lake, research interests have been drawn to analyze concentrations of total-Hg (Campbell et al. 2003). Sources of mercury are burning of coal, mining activities and extraction of metals, volcanoes, intercontinental transport, evaporation of mercury from soil and water surface, degradation of minerals and burning of forest (Travnikov 2005). Mercury exists in different forms of species such as elemental mercury, inorganic mercury and organic mercury that is classified as alkylmercury and allylmercury (Broussard et al. 2002). Recent studies have shown that the HgcAB gene cluster in some microorganisms is responsible for the conversion of divalent mercury (HgII) to MeHg by methylation (Bravo and Kozio 2020). Through the proposed mechanism of mercury methylation (scheme 3), gene cluster HgcAB present in microorganism contribute the formation of $CH3Hg^+$ through the following steps: (i) corrinoid cofactor reduced to form Co^I, (ii) methylation of Co^I to form CH_3-Co^{III} species, (iii) methyl shifting to form CH_3Hg^+ (Cooper et al. 2020).

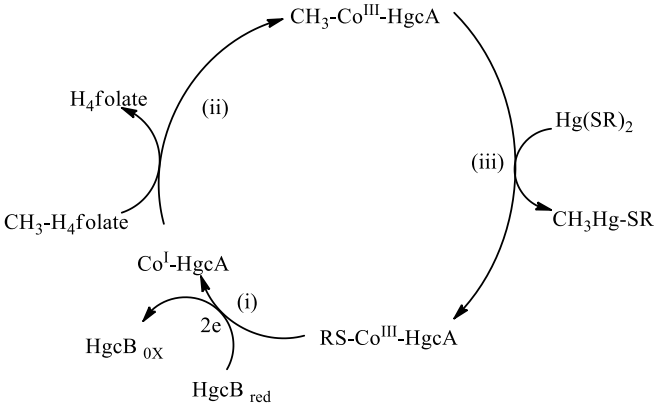

Figure 1: Proposed mechanism of Mercury methylation (Cooper et al. 2020).

The study on animals showed that inorganic mercuric forms have high affinity for metallothionein in renal cells, and can cause problems in the central nervous system (CNS) and damage to unborn children (Broussard et al. 2002). Exposure to MeHg causes central nervous system problem and for pregnant women it causes abnormalities and cerebral palsy even if the mother cannot show any symptoms. MeHg affects infants more than adults (Tsuchiya 1984). The toxicity of mercury varies through its forms, exposure amount, inflow path and individual susceptibility. Fish and fish products are a major source of MeHg for human through diet (UNEP 1990).

A number of researches have been done to analyze total concentration of mercury in Lake Victoria (Campbell et al. 2003, Ramlal et al. 2003, Machiwa 2004) and showed that water, sediments and organisms in Lake Victoria contain mercury (Ramlal et al. 2003). Studies on toxic dynamic and kinetic of both inorganic mercury and MeHg show that the two species are different and MeHg is very toxic compare to inorganic mercury due to its higher rate of absorption and

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accumulation in fatty tissues (Bravo and Cosio 2020). Lavoie et al. (2018) reported that in 175 countries where studies were conducted showed that 38% of populations were exposed to doses of MeHg above thresholds limits permitted by regulatory authorities (Lavoie et al. 2018). The average amount of MeHg that may causes health effects to human are 300 µg/day or 4.3 µg Hg/day/kg body weight (Jones 1999). In Tanzania there is no information on concentrations of MeHg especially for Lake Victoria. Most of the data and information available are of total-Hg concentration in water, sediments, fish, biota and humans (Campbell et al. 2003). This is why this study intends to investigate and avail data on chemical speciation of mercury, i.e., MeHg and total-Hg concentrations in Nile Perch from Lake Victoria in Mara, Mwanza and Geita regions in Tanzania.

II. Materials and Methods

2.1 Materials

2.1.1 Sample collection

Fish samples of this study were collected from three regions of Tanzania i.e. Mara, Mwanza and Geita. Samples were collected from different villages in three regions. In Mara region the Nile perch samples were collected at Mwigobero, Mugango and Suguti, in Mwanza regions the samples were collected at Mwanza town (Mwaloni market), Mswahili and Kikongo, while in Geita region the samples were collected at Bukome, Senga and Bukondo.

Nine samples were collected from each of the three regions making a total of twenty-seven (27) samples. Samples were collected directly from fishermen of Lake Victoria where weight and length of samples were recorded. The size of fish sampled ranged from 50 - 61 cm length and the weights ranged from 1.34 up to 1.96 kg. Each sample was kept in separate labeled polyethylene plastic bags, and then placed in ice cold containers before being transported to the Chief Chemist Laboratory Agency (CGCLA) Zanzibar for further analysis.

2.2 Methods

The DMA-80 was used for both qualitative and quantitative determination of total-Hg and MeHg. Double liquid extraction technique was used to extract MeHg then the extract obtained was analyzed using DMA-80. For determination of total-Hg, the sample was ground using a blender and weighed before analyzing using DMA-80.

2.2.1 Preparation of stock solutions

1% of L-cysteine was prepared by mixing 12.5 g of sodium acetate (CH_3COONa) anhydrous, 0.8 g of sodium sulfate ($NaSO_4$) in 100 mL of distilled water and 1 g of **L**-cysteine hydrochloride monohydrate. 10 mg/L of mercury was prepared through the dilution of 1000 mg/L standard solution of mercury with 1% potassium dichromate and hydrochloric acid (HCl) 1:1 (v/v). From 10 mg/L of mercury, 500 µg/L of mercury was prepared by dilution (Valsecchi 2021). Both solutions were stored in the freezer at -20 ºC

2.2.2 Total-Hg analysis

The procedures were performed with minor modifications as described according to US EPA (2007) and May (2005). The Nile perch fillet sample was cut, ground by using a blender, measured its weighs and put in polyethylene plastic container with a plastic lid and labelled, and stored in a freezer at -20 ºC.

0.1000 g up to 0.2300 g was measured by using analytical balance and transferred into a small boat container of Direct Mercury Analyzer (DMA-80). Then boat container was arranged in a DMA tray and the sample was analyzed. Each sample took 5 – 6 min for completing the analysis (US EPA 2007 and May 2005).

2.2.3 MeHg analysis

All materials used for MeHg analysis were soaked in clean dilute nitric acid HNO_3 10 % (v/v) bath for 24 h and rinsed with distilled water and put in an oven for drying. With some modifications, 10 mL of hydrobromic acid was poured into a container and about 1.0 – 1.5 g of Nile perch fillet sample was added in the same container and shaken before adding 20 mL of toluene. Then 20 mL of toluene were poured and the solution was then vortexed for 2 min followed with centrifugation at 5000 rpm for 10 min. About 15 mL of toluene were recovered and added into a second container. 15 mL of toluene was poured to the remaining sample of the first container, then the solution vortexed for 2 min followed with centrifugation at 5000 rpm for 10 min. Again about 15 mL of toluene were recovered and added into a second container followed with addition of 6 mL L-cysteine solution. Then, the solution in the second flask was vortexed for 2 minutes and then centrifuged at 5000 rpm for 10 minutes. After that the toluene phase was recovered and considered as a waste. The supernatant (aliquot of aqueous phase) obtained in a centrifugation was taken for DMA – 80 analyses (Calderón 2013)

0.1989 up to 0.3720 g of the supernatant (aliquot of aqueous phase) obtained after sample preparation for MeHg was added in a boat container of DMA, then the boat container was inserted in DMA tray, final the DMA-80 analyzed the sample for qualitative and quantitative determination of MeHg, each sample took 5 – 6 min to complete analysis (Calderón 2013). The concentration of MeHg was converted from aqueous extract to solid (fillet) sample by using the following formula: $MeHg = \frac{6 \times C}{w} \times f$ Note: MeHg is the concentration of MeHg in the sample, C is the concentration of MeHg in the extract, w is the weight of the sample, 6 mL is % L-cysteine solution and f is the ratio between the molecular weigth of MeHg and mercury (1.075) which used to convert the value of mercury to MeHg (Valsecchi 2021).

2.2.4 Statistical analysis

Microsoft excel was used to calculate descriptive statistics (mean and standard deviation) and Origin 8.5 (Data Analysis and Graphing Software) was used to plot the column and scatter central graphs.

III. Result and Discussion

3.1 Introduction

This chapter reports and discusses results obtained on analysis of levels of total-Hg and MeHg from twenty seven (27) Nile perch that make a total of fifty four (54) of Nile perch fillet samples from Lake Victoria. The analysis of total-Hg concentration was performed according to US EPA (2007) and May (2005) at the CoAF laboratory of the University of Dar es

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Salaam. Analysis of MeHg concentration was performed at CoAF Laboratory of the University of Dar es Salaam according to Valsecchi (2021) and Calderon (2013).

3.2 The concentrations of mercury species in Mwanza region

In Mwanza region the samples were collected in three places which are Mwanza town, Mswahili and Kikongo. Kikongo samples village exhibited the maximum average concentration of total-Hg of 2.5850 ng/kg compared to Mwanza town and Mswahili where the average concentrations of total-Hg were 0.0461 and 2.4550 ng/kg. Kikongo showed high average concentration of MeHg that was 0.0441 ng/kg compared to Mwanza town and Mswahili which showed 0.0440 and 0.0393 ng/kg average concentrations of MeHg respectively

Levels of mercury species were determined in three locations: Mwanza town, Mswahili and Kikongo villages. The % concentration of MeHg from total-Hg in Mwanza town, Mswahili and Kikongo were 95.44, 1.60 and 1.71 respectively.

3.3 The concentrations of mercury species in Geita region

In Geita region the samples were collected from three villages which are Bukome, Senga and Bukondo. Bukondo village samples showed the highest average concentration of total-Hg that was 0.0460 ng/kg compared with Bukome and Senga samples where the average concentrations of total-Hg were 0.0452 and 0.0443 ng/kg. Bukondo samples village showed the highest concentration of MeHg which was 0.0403 ng/kg compared with Bukome and Senga samples which showed 0.0354 and 0.0382 ng/kg concentrations of MeHg respectively.

Speciation of mercury species was done in three villages of Geita region i.e. Bukome, Senga and Bukondo. The % concentration of MeHg from total-Hg in Bukome, Senga and Bukondo samples were 78.32, 86.23 and 87.61 respectively.

3.4 The concentration of mercury species at Mara region

In Mara region the samples were collected from three villages which were Mwigobero, Mugango and Suguti. Mwigobero samples showed the highest average concentration of total-Hg that was 0.0480 ng/kg compared to Mugango and Suguti where the average concentrations of total-Hg were 0.0472 and 0.0464 ng/kg respectively.

Speciation of total-Hg and MeHg was done in three villages of Mara region i.e. Mwigobero, Mugango and Suguti. The % concentration of MeHg from total-Hg at Mwigobero, Mugango and Suguti were 88.33, 80.30 and 87.93 respectively.

TABLE 1: Total-Hg and MeHg concentrations in Nile perch fillet

Region	Village	Average concentration of total-Hg ± SD (ng/kg)	Average concentration of MeHg ± SD (ng/kg)	$\frac{MeHg}{THg} x\ 100$ (%)
Mwanza	Mwanza town	0.0461 ± 0.0030	0.0440 ± 0.0019	95.44
	Mswahili	2.4550 ± 0.0849	0.0393 ± 0.0020	01.60
	Kikongo	2.5850 ± 0.1223	0.0441 ± 0.0026	01.71
Geita	Bukome	0.0452 ± 0.0015	0.0354 ± 0.0027	78.32
	Senga	0.0443 ± 0.0020	0.0382 ± 0.0012	86.23
	Bukondo	0.0460 ± 0.0020	0.0403 ± 0.0021	87.61
Mara	Mwigobero	0.0480 ± 0.0028	0.0424 ± 0.0022	88.33
	Mugango	0.0472 ± 0.0040	0.0379 ± 0.0015	80.30
	Suguti	0.0464 ± 0.0019	0.0408 ± 0.0031	87.93

The mean concentration of total-Hg in Nile perch fillet obtained in Mwanza, Geita and Mara region were 1.6954, 0.0452 and 0.0472 ng/kg respectively and the mean concentration of MeHg in Nile perch fillet obtained in Mwanza, Geita and Mara region were 0.0425, 0.0380 and 0.0404 ng/kg respectively. Nile perch fillet obtained in Mwanza region had the highest mercury species for both total-Hg concentration and MeHg concentration followed by Nile perch fillet of Mara region and the last was Nile perch fillet of Geita region. The main sources of activities that cause contaminations of Lake Victoria in Mwanza region could be industrial activities such as mining activities and discharging of sewage wastes (Makundi 2001), these factors could be the reasons of highest concentration of total-Hg and MeHg in Nile perch obtained in Mwanza region compared with Nile perch of Mara and Geita region. The report of NEMC of 22nd March, 2022 reported that the sources of human activities that caused contaminations of Lake Victoria in Mara region were mining, expansion of agricultural land, settlement, deforestation, excessive livestock keeping and use of fertilizers (Minister of State 2022). The Geita region was blessed with industries such as mining, livestock and agriculture (United Republic of Tanzania 2019). While mining activities is among of the source of contamination in Lake Victoria (Minister of State 2022). NEMC is among of the institutions in Tanzania that contribute to prevent, treat, control and manage the ecosystem of Lake Victoria through its laws and regulations to make sure the quality of the Lake remains in its good standard. Through NEMC investigation report of March 2022 showed that NEMC conducted field survey at the Mara River wetland areas along Lake Victoria to test for contaminations, microbes and other physical parameter such as pH in the polluted water and sediments (Minister of State 2022).

The speciation results from this study are comparable with the results of the mercury speciation from a study done by Maggi et al. (2009) which was to determine levels of total-Hg and MeHg using direct mercury analyzer (DMA) in the muscles of Antarctic fishes (*T. pennelli, G. acuticeps, T. bernacchii, C. mawsoni and C. hamatus*) from sea water. According to Maggi et al. (2009), fish samples collected from coastal area at Terra Nova Bay in Italy and their results were categorized into three parameters, the first parameter was percentage concentration of MeHg from total-Hg, the second parameter was levels of total-Hg concentration and the third parameter was levels MeHg concentrations (Maggi et al. 2009). There were slight differences of the total-Hg and MeHg concentrations between the study of Maggi et al. (2009) and the present study. Results of Maggi et al. (2009) are shown in the table below;

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TABLE 2: Total-Hg, MeHg, and percentage of MeHg/T-Hg in muscles of Antarctic fishes [46]

Antarctic fishes	total-Hg (ng/kg)	MeHg (ng/kg)	MeHg/T-Hg (%)
T. bernacchii	0.0926 ± 0.0008	0.0623 ± 0.0041	67.3
T. pennelli	0.2992 ± 0.0036	0.1356 ± 0.0053	45.3
G. acuticeps	0.3799 ± 0.0023	0.2301 ± 0.0018	60.6
G. acuticeps	0.3959 ± 0.0045	0.2785 ± 0.0028	70.3
C. hamatus	0.3786 ± 0.0062	0.3070 ± 0.0021	81.9
C. mawsoni	0.9073 ± 0.0047	0.6702 ± 0.0009	73.9

Machiwa (2004) reported 0.0614 ng/kg concentration of total-Hg from Nile perch in two regions of Mara and Mwanza and the Range total length was 9.8 – 83 cm (Machiwa 2004) these results are comparable with the results of this study whereby the mean concentrations of total-Hg from Nile perch in two regions of Mara and Mwanza was 0.8713 ng/kg. Both levels of total-Hg concentrations in Nile perch from Lake Victoria from this study and the study done by Machiwa (2004) were below the permissible levels of FAO/WHO. There were slight differences of total-Hg concentrations in Nile perch between the study done by Machiwa (2004) and the present study, it concluded that variation of time is among the factor causes changing of total-Hg concentrations.

Ramlal et al. (2003) reported 0.090 to 0.250 ng/kg wet weight concentration of total-Hg in Nile perch found in the northern part of Lake Victoria (Ramlal et al. 2003) while in this study the concentration of total-Hg in Nile perch found in southern part (Mwanza, Geita and Mara) was ranging from 0.0443 to 2.5850 ng/kg wet weight.

Carbonell et al. (2009) performed analysis of total-Hg and MeHg in muscle of seawater fish (sardine, anchovy and tuna fish). Their results are shown on the following table;

TABLE 3: total-Hg and MeHg concentrations in muscles of sardine, anchovy and tuna fish (Carbonell et al. 2009)

Fish name	Mean of total-Hg ± SD (mg/kg) wet wt	Mean of MeHg ± SD (mg/kg) wet wt	MeHg %
Sardine	0.026 ± 0.004	0.018 ± 0.001	72.66
Anchovy	0.038 ± 0.004	0.031 ± 0.002	80.39
Tuna fish	0.244 ± 0.011	0.262 ± 0.005	106.28

The results of Carbonell et al. (2009) from the above table exhibited higher levels for both total-Hg and MeHg compare with the results of total-Hg and MeHg from this study. Although there are other factors that cause differences of mercury species levels, also fish sample from different fish species, water sources and analysis of the same instrument (DMA-80) can possess different levels of mercury species.

The deviation of results from this study and other studies could be attributed to different factors that are natural and anthropogenic such as time, length and weight of the fish, air intercontinental, mining activities, nature of the soil and plants, industrial and domestic waste, climate condition, physical characteristic of water, contaminations of metals in biota, volcanoes and air pollution.

Variation of length and weight against concentration of total-Hg and MeHg

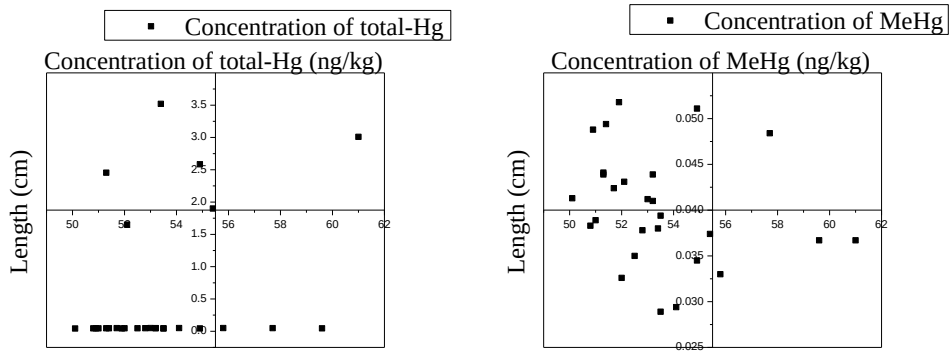

Figure 2: Variation of length against concentrations of total-Hg and MeHg

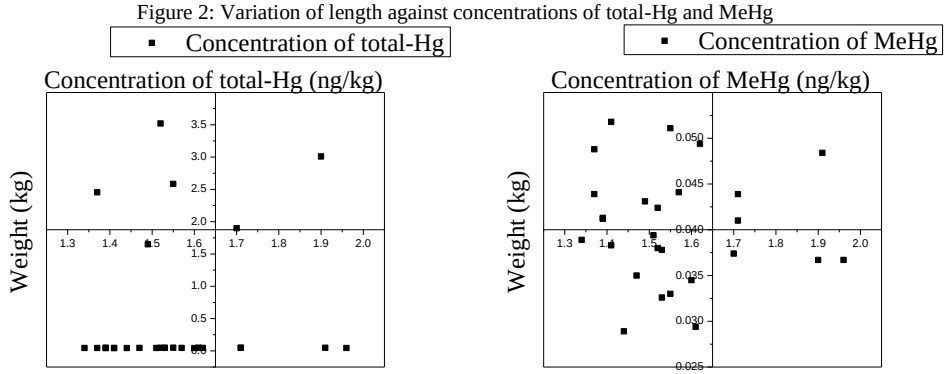

Figure 3: Variation of weight against concentrations of total-Hg and MeHg

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The variations of length for fish samples taken for analysis of this study were not much higher, therefore the concentrations of total-Hg and MeHg in the fish samples were depending on its specific place of sampling and not on its length. Also, the variations of weight for fish samples from this study were not much difference, therefore the concentrations of total-Hg and MeHg in the fish samples were depending on its specific location of sampling and not on its weight. The variations of total-Hg concentrations and length of Nile perch from this study are compared with the results of variations between total-Hg concentrations and length of Nile perch from the study of Machiwa (2004). According to Machiwa (2004) there was low correlation coefficient (0.591) between total-Hg concentrations and length of Nile perch (Machiwa 2004). This indication shows how total-Hg concentrations of Nile perch were not much depend on their length.

IV. Conclusion and Recommendations

4.1 Conclusion

The aim of this study was to investigate the speciation between total-Hg and MeHg around regions of Lake Victoria and the results were obtained from Mwanza, Geita and Mara region. Mwanza town, Mswahili, Kikongo, Bukome, Senga, Bukondo, Mwigobero, Mugango and Suguti were the villages where the samples were collected.

In this study a simple and cost effective method to determine MeHg was used. Basically, this method combines the extraction followed by clean-up with cysteine acetate solution and MeHg quantification by a direct mercury analyzer (DMA). The most relevant advantage of this method is the short processing time to extract and analyze a considerable number of samples. In addition, precise and rapid determination of both chemical forms (THg and MeHg) in fish tissues can be performed using the same analytical system thus reducing the variability in reported results.

Based on the results of this study it is concluded that the total-Hg concentrations were higher than MeHg concentrations for all locations where samples were collected. The source of high concentration can be attributed by high population, extensive land use, industrial and domestic waste water disposal. Kikongo in Mwanza was observed to have highest both total-Hg and MeHg concentration.

The results have contributed much in area of chemical speciation of mercury by showing how chemical speciation distributes individual chemical species from a total element. The trend of total-Hg concentration in Nile perch was not increasing since 2004 from the study done by Machiwa (2004) up to 2022 from the results of this study.

4.2 Recommendations

Since this study was done on mercury speciation in Nile Perch, it is recommended to widen the it to cover not only other species such as Nile tilapia (*Oreochromis niloticus*) and sardine (*Rastrineobola argentea*) but also investigate the speciation to include other elements diversify it water, sediment and biota to make a good correlation.

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