TRACE METALS IN WATER AND FISH (Unga species, Pungu maclareni, catfish Clarias maclareni) FROM LAKE BAROMBI MBO, CAMEROON.

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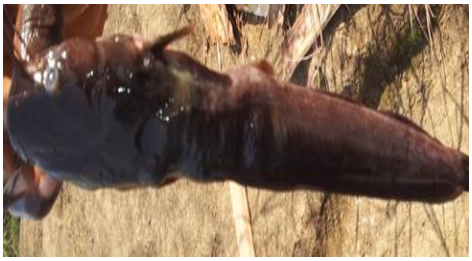

A THESIS

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ABSTRACT

Lake Barombi Mbo is an isolated oligotrophic lake situated in the volcanic range of West Cameroon and home to several endangered endemic cichlids. A fieldwork was carried out at the lake where water and fish samples were collected as part of an investigation. The aim of this study was to investigate (i) whether studied trace metals were present at levels exceeding ambient water criteria, (ii) link uptake of trace metals in gills and liver of fish to water chemistry, (iii) accumulation of mercury in muscles and biomagnification along the food chain. ICP-MS and ICP-OES analysis for concentration of trace metals in water samples from the lake showed that, the total concentrations of investigated trace metals were below U.S. Environmental Protection Agency (EPA) criteria limits, Canadian Council of Ministers of the Environment (CCME), South Africa Water Quality Guidelines and World Health Organization (WHO) guidelines for protecting aquatic life. Linking uptake and water chemistry, bioconcentration factor (BCF) analyses showed accumulation of trace metals in both gills and liver of fish. With minor differences in accumulation sequence, all fish species accumulated Al, Mn, and Sr in highest concentration in their gills with Cu, Cd, Co, Cr, Pb, and U highest in liver. The highest mean concentration of metal accumulated was observed for Cu (1153 μg/g dw) in liver of U. species. *P. maclareni* accumulated Al, Cr, Co, Sr, and Pb in highest concentrations. While U. species had Mn and Cu in highest concentration, Cd was present in highest concentration in *C. maclareni*. The high accumulation of Al, Mn and Sr on gills of the three fish species, indicates that they are bioavailable and probably high levels in Lake Barombi Mbo. Total mercury concentrations (mg/kg ww) were low with mean values of 0.0093 in U. species, 0.0274 in *P. maclareni* and 0.0266 in *C. maclareni* compared to 0.2 mg/kg WHO recommended guideline for Total Dietary Intake (TDI) to protect vulnerable groups (pregnant women and children) from mercury toxicity. Stable isotope analysis of carbon $\delta^{13}C$ used as index for carbon source and flow, and nitrogen $\delta^{15}N$ as index for trophic position within the aquatic food chain were determined. Hg concentrations in muscle of fish coupled mean $\delta^{13}C$ (‰) and nitrogen $\delta^{15}N$ (‰), showed that U. species had the lowest Hg concentrations, $\delta^{13}C$ (- 32.9 ‰) and $\delta^{15}N$ (6.6 ‰), and so occupied the lowest position of the food chain. *C. maclareni* and *P. maclareni* both had the highest Hg levels, but *C. maclareni* had the highest $\delta^{15}N$ (9.9 ‰) and occupied the highest trophic level. Log THg vs. $\delta^{15}N$ among all species sampled showed a significant positive relationship indicative of mercury biomagnification along the food web of Lake Barombi Mbo. Results of trace metal levels in water and fish tissues suggest that trace metals do not pose a serious threat to the aquatic biota of Lake Barombi Mbo.

DEDICATION

To mum and dad for every sacrifice in instilling the quest of knowledge in me and taking me this far in life to learn all I have had the opportunity of learning.

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CHAPTER ONE

INTRODUCTION

Trace metals occur naturally in the environment and are usually present in low concentrations in freshwater (Ward, 2000; van Loon & Duffy, 2011). Though naturally present at low concentrations, anthropogenic inputs continuously increase their concentrations above natural geological and biological alterations (Ward, 2000). The non-biodegradable nature and toxicity of trace metals is a global issue (Ikem et al., 2003; Malik et al., 2010; Olowu et al., 2010). While some trace metals (e.g. copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn)) are essential at levels of safe exposure, others (e.g. aluminium (Al), cadmium (Cd), mercury (Hg), lead (Pb)) exert toxic effects even at low concentrations. The bioavailability and toxicity of trace metals in aquatic ecosystems is controlled by their speciation which depends on pH, solubility, temperature, nature of other species present and other factors of solution chemistry (Franklin et al., 2000; Ward, 2000; Teien et al., 2004**)**. However, the distribution of the different physicochemical forms in an aquatic ecosystem vary in terms of size and charged fractions between high molecular mass (HMM) (particles and colloids; > 10kDa) to low molecular mass (LMM) species usually the bioavailable fractions (Masresha et al., 2011). Their uptake and toxicity generally correlates to their free metal ion than their total concentration (Campbell, 1995). The accumulation of trace metals in aquatic ecosystems may result in adverse effects on both biota and humans through consumption due to bioaccumulation and bio-magnification over time (Malik et al., 2010**)**. This may result either in death, reduced growth, or in impaired reproduction and lower species diversity (Praveena et al., 2007). Fish occupy highest trophic level in many aquatic food chains and are constantly exposed to pollutants (Agah et al., 2009**)**. Fish therefore serve as excellent biomarkers of trace metals in aquatic ecosystems (Nsikak et al., 2007) and provide long-term measure of pollutant bioavailability (Nehring et al., 1979), accumulating trace metals in different organs to concentrations many times higher than the low levels in water (Namminga and Whilm, 1976; Noor El Deen et al., 2010). The gills are the dominant physiological organ directly in contact with water. They accumulate bioavailable trace metals and their measurements can reflect their speciation and concentration in water (Rosseland et al., 1992). The presence of trace metals in the liver reflects storage from water (Romeo et al. 1999) and potential assimilation through food. Lake Barombi Mbo in Cameroon is the largest Crater Lake within the South West eco-region and home to several endemic cichlids and some incipient fish species. Serving as local fisheries for nearby inhabitants of Barombi Mbo village and

drinking water source for the city of Kumba, Reid (1995) identifies water pollution as a potential threat to its water quality. Based on literature survey, to the best of my knowledge except for in-depth study on the feeding biology of its fishes, no work has been carried out on the environmental quality and biota of the lake.

The objectives of this study were to:

- establish the concentrations of trace metals in water, and verify if concentration of analyzed trace metals exceeded permissible ambient water quality criteria that may cause negative effects on the aquatic organisms;
- obtain information about trace metal bioavailability and accumulation in fish (gills, liver and muscle) of three different fish species representing different trophic levels and evaluate the main uptake pathways and risk assessment of accumulation of trace metals.

The predictions were that:

- high concentration of trace metals in fish is due to exposure of bioavailable trace metals;
- increased concentration of trace metals with trophic level, illustrates biomagnification effects.

CHAPTER TWO

BACKGROUND AND LITERATURE REVIEW

2.1. Lake Barombi Mbo

Barombi Mbo is an oligotrophic lake situated in the volcanic range of west Cameroon, about 35 miles north-north-east of Mount Cameroon at 9˚22′E and 4˚38′N (Fig. 1). The lake is clear and lies in a small forested crater. Roughly circular with a diameter of about 2.5 km, it has a maximum depth of 111m and an altitude of 301m a.s.l. with one major outflow through the Kake Gorge at the South-eastern corner and several small inflows, some of them seasonal. A dam has been constructed at its outlet, raising its level, and surrounding the lake's crater rim is felled forest, much of which is used for agriculture. Isolated from the nearby river system, the small size of the lake renders it extremely vulnerable to even minor disturbances. There are growing concerns on the ecological status of the lake due to partial deforestation of the interior crater rim for agricultural land use, water extraction, pollution associated to urbanization and introduction of exotic fish species.

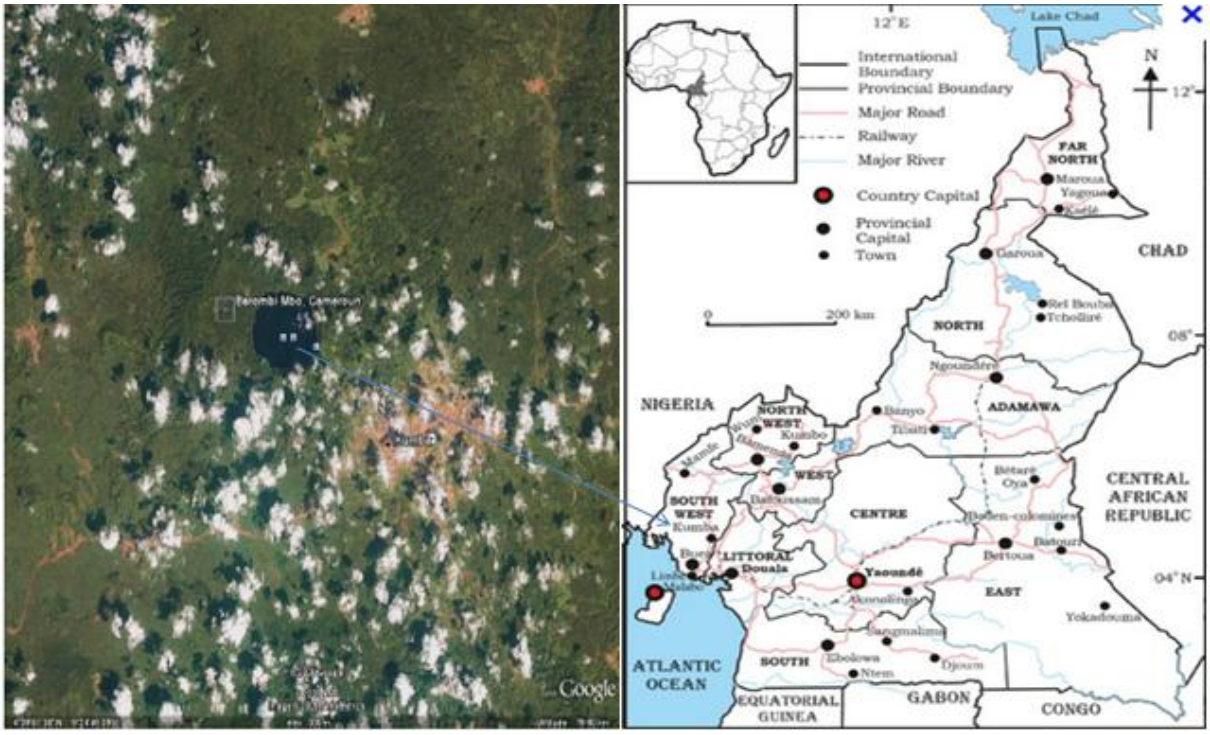


Figure 1. Map of Lake Barombi Mbo, Cameroon (Source: google.com)

The main anthropogenic activity around the forested Crater Lake Barombi Mbo is agriculture. This mostly entails the farming of cocoa and the subsequent use of pesticides for spraying cocoa plants. Felt trees from the crater rim were also observed in the water probably due to wind or human impact.

Lake Barombi Mbo with a high index of endemicity per area is home to 12 endemic cichlids (Trewavas et al., 1972). Of the 12 endemic species, all endemics are tilapiine cichlid fishes except for the catfish, *Clarias maclareni.* Four of the five tilapiine genera are endemic: *Konia* except for the clariid catfish *C. maclareni*. Four of the five tilapiine genera are endemic: *Konia* (two species), *stomatepia* (three species), *Pungu* (one species), and *Myaka* (one species). Ecological surveys conducted by Trewavas et al. (1972) based on the examination of stomach contents of the fishes revealed that Unga species and *Myaka myaka* feed on phytoplankton and chaoborus larvae. *C. maclareni* preyed on *M. myaka* and *Konia dikume*. *Stomatepia mariae* was also observed to be piscivorous. With the established food chain, bioaccumulation and biomagnification of trace metals can be investigated.

2.2. Trace metals in water

Trace metals are pollutants which though naturally occurring enters aquatic ecosystems from a variety of anthropogenic sources which increase their concentrations at levels exceeding their natural background levels. Extensive studies have been carried out for the following metals either on an individual or group basis, Cd, Hg, Pb, Al, Cu, Zn, Fe, Mn, Cr, Ni and Co (Förstner & Whittmann, 1979, Handy, 1992). However, these metals are classified on the basis of their essentiality (Fe, Zn, Cu, Co, and Mn) and non-essentiality (Hg, Cd, and Pb). Although Cu, Fe, and Zn are known required elements for metabolic activities (Frieden, 1972), at high concentrations they may be toxic. The non essential trace metals such as Hg, Cd, and Pb are potentially toxic to biota even at levels of low exposure (Förstner & Whittmann, 1979). However, once in aquatic ecosystems like freshwaters, many factors affect the dynamics and extent of trace metal bioaccumulation in fish (Campbell, 1995). These include, the characteristics of the trace metal in view of its solubility and physicochemical form (speciation) controlled by pH, temperature, dissolved organic carbon (DOC), dissolved oxygen (DO), alkalinity, hardness, total dissolved solids (TDS), and electrical conductivity (EC) which characterize the water quality (Franklin et al., 2000; Ward, 2000; Teien et al., 2004). These physicochemical characteristics are also linked to biological characteristics of the exposed organism (Luoma, 1983). These include behaviour, modes and feeding frequency with the specific type of food being ingested, age and size of the organism (Campbell, 1985; Luoma, 1983). The parameters for metal speciation differ between metals and the effects exerted by bioavailable metals also differ between species of organisms. Whereas microbial activity, and dissolved organic carbon (DOC) influence the speciation, bioavailability and toxicity of mercury, pH, reduced DOC, and temperature enhance aluminium (Al) speciation, bioavailability, and toxicity. The extent of metal

toxicity on fish also depends on the sensitivity and life history stage of the fish species, as smolt are more sensitive to Al toxicity than parr (Kroglund et al., 2008).

Several studies suggest waterborne and dietary uptake of trace metals as the main sources of metal uptake in fish (Spry et al., 1988; Spry & Wiener, 1991; Rosseland et al., 1992; Romeo et al., 1999). The possible routes of waterborne uptake and accumulation are by direct uptake through the gills which is the primary physiological organ in contact with water and concentration in gills are correlated with waterborne exposure (Rosseland et al., 1992). Dietary uptake of trace metals is reflected by accumulation in the liver either through absorption from water and/or ingestion from food (Romeo et al., 1999). The bioavailable and absorbed metals are redistributed from active uptake sites through the blood and accumulate at other target organs distant from the point of entry (Handy, 1992), resulting in systemic effects (Fig. 2**)**. Metals differ in their ability to accumulate at specific uptake sites, as such, though specific metals may target specific tissues such as bone, spleen, kidney, muscle and intestines, and the pattern of distribution in tissues may reflect the route of metal uptake in fish. Tissue localization studies have shown that fish liver tissue generally accumulates highest concentrations of trace metals (Bendell-Young et al., 1986; Ewers & Schlipkoter, 1991). This is also due to the fact that the liver is the major producer of the metal binding protein metallothionein (Kalay & Canli, 2000). Also, the liver acts as the organ for storage and detoxification of contaminants. The accumulation of trace metals in fish can be quantified either on a whole body or on an organ specific basis as bioconcentration factor. The bioconcentration factor (BCF) represents the extent or ratio to which the concentration of a specific metal in the tissue of an aquatic organism (e.g., fish) exceeds levels in the surrounding environment (water) in which it is exposed (Wood, 2012). Once present in the aquatic environment and accumulated in fish tissues, trace metals can exert toxic effects. The total concentrations in water provide no information concerning the fate of trace metals in terms of their interaction with various matrices of the aquatic environment, their ability to cross biological membranes, or their resultant toxicity (Christie, 2000). Hence, the bioavailability and potential of trace metals to exert toxic effects is generally correlated to the concentrations of the free metal ions (Campbell, 1995). The bioavailable free ion concentrations of trace metals are compared with ambient water quality criteria (AWQC) based on water hardness as safety limits from acute and chronic levels for aquatic life protection. The higher the water hardness, the larger the criteria limit and AWQC varies between jurisdictions due to differences in geology and sensitivity of aquatic organisms within a given aquatic ecosystem.

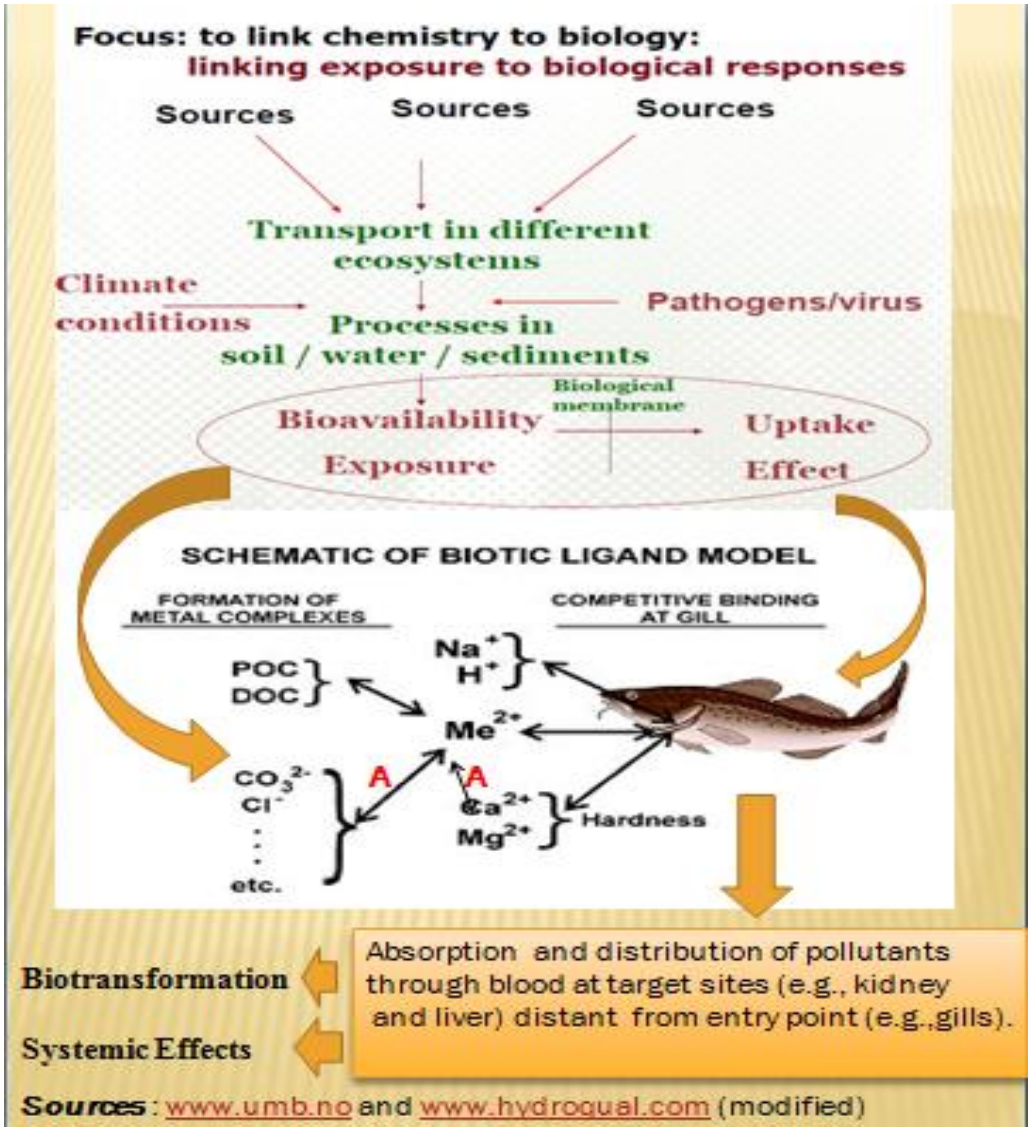


Figure 2. Linking metal bioavailability and accumulation in fish tissues (A = antagonistic interaction between trace metal ions and major cations and anions). Sources: www.umb.no & www.hydroqual.com.

Water hardness plays an important role in altering trace metal uptake at competitive binding sites on the calcified tissues of fish. Whilst toxicity is related to the specific trace metal and its concentration, some trace metals are more toxic than others even at low concentrations and consequently have varying AWQCs. However, high concentrations of some trace metals (e.g., Cu) in the liver do not necessarily result in toxic effects. This is as a result of homeostatic control through which their levels in liver can be regulated by metabolic processes. In addition, metals such as Cu which are in continuous interaction with the gills of fish can be valuable indicators of acute lethal exposure (van Hoof & van San, 1981) due to accumulation, but uptake through food might be different. On the contrary, the very toxic metals (e.g., Hg, Cd, and Pb) are poorly regulated and their increased half

lives in tissues may result in adverse acute and chronic effects. This is due to their slow excretion and poor homeostatic control which increases their resident time in fish tissues. Gill and liver of fish sampled for trace metal concentrations should reflect the trace metal concentrations from water and food, respectively. As such, to determine the extent of contaminants (e.g., trace metals), the natural levels or background concentrations need to be established (Velz, 1984). There is lack of comparable data on national guidelines for Cameroon on trace metals in fish tissues. Results of trace metals in sampled tissues are therefore compared with background levels reported from other countries with respect to similarities in geographical settings and fish species studied. It is rare to find one contaminant at a time released into the aquatic ecosystem (Kumar & Singh, 2010). When toxicants such as trace metals enter the environment, they become subject to various interactions with naturally occurring constituents and other toxicants present (Anderson & D'Apollonia, 1978). Most of the trace metals interact with each other and are influenced by other ions (Kumar & Singh, 2010). Thus, the effect of a toxicant on an organism it encounters can be modified. For example Zn, Cd, Co, Pb, and Sr mimic calcium on the calcified tissues of fish by competing for active binding sites on calcium rich bone and filaments on the gills arch (Bury et al., 2003). Trace elements entertain synergistic or antagonistic interactions whenever in a mixture (Sprague, 1985) and as a result of their bio-accumulative and non-biodegradable properties, they constitute an important category of aquatic pollutants.

2.3. Selected trace elements

The trace metals, Al, Cd, Cr, Pb, Hg, Cu and Mn were chosen for study because they are among the most studied and also reported to be present in freshwater ecosystems. Based on this, I characterized them in view of their essentiality (Cu, Mn) and non essentiality (Al, Cd, Cr, Pb, Hg) in fish. Some of these metals (Cd, Pb, Hg) are reported to be particularly toxic at low concentrations. So, because I want to investigate if they were at levels exceeding background concentration, I felt there was a need to investigate them over other possible trace metals.

2.3.1. Aluminium (Al)

Al occurs naturally in the environment as aluminosilicates, oxides, and hydroxides, combined with other elements and complexed with organic matter (Landler, 1988). However, soil minerals are the primary source of aluminium to aqueous and biological environments (Driscoll & Schecher, 1990) as acid rain dramatically influences the leashing of Al from soils into aquatic ecosystems (Ward, 2000). Once in aquatic systems, Al can be present in different physicochemical forms, varying from particles, colloids, simple monomeric ions to polymers (Salbu & Oughton, 1995). Waterborne Al

toxicity is controlled by factors of solution chemistry such pH, temperature, hardness and complexation (Teien et al., 2004). According to Wilson (2012), the dietary uptake of Al and associated toxicity is very negligible and its bioaccumulation through the aquatic food chain does not occur. Though, dietary uptake to internal organs such as muscle, liver, kidney, and gills may occur (Handy, 1994). Al predominates as inorganic monomeric species [$Al(H_2O)_6^{3+}$, Al^{3+}, $Al(OH)_2^{+}$] within pH 4.0-5.0, $Al(OH)_3$ at intermediate pH values and $Al(OH)_4^{-}$ under alkaline conditions in aquatic ecosystems (Teien et al., 2004; Wilson, 2012). Of all Al species formed under varying acidity, the inorganic monomeric species having a positive charge (e.g., Al^{3+}) are the most toxic (Schlindler, 1988; Gensemer & Playle, 1999). According to Rosseland et al. (1990) and Teien et al. (2006), Al^{3+} is readily bioavailable and very gill-reactive. So, the physiological effects of gill Al vary with acidity of the aquatic environment. Below pH 5.0, Al $(OH)_3$ precipitates due to reduced solubility of Al and bioaccumulates on fish gills (Rosseland & Staurnes, 1994). Between pH 5.0-6.0, short lived Al^{3+} cellular internalization through Al polymerization is said to occur (Exley et al., 1991) with toxicity decreasing at low temperatures (Poléo et al., 1991). The resulting effects of gill Al are respiratory dysfunction due to impediment of the interlamellar space with excessive mucus production, decreased membrane fluidity; disruptive ion regulation associated with increasing loss of plasma and haemolymph ions, cell necrosis and increased mortality (Rosseland et al., 1990; Exley et al., 1991; Peuranen et al., 2003; Moiseenko & Sharowa, 2006). However, the above mentioned effects of Al toxicity are also dependent on fish species, development stages or size (Baker & Schofield, 1982). In a study exposing parr and smolts of Atlantic salmon (*Salmo salar* L.) in the same experimental tanks, Monette & McCormick (2007) observed that parr was more Al tolerant as it accumulated six fold more Al on its gills than smolt. The concentration of Al in ambient water which is highly dependent on speciation is a prerequisite to possible toxic effects on fish when background levels are exceeded. The ambient water quality guideline for dissolved Al in freshwater is 750 μg/L and 87 μg/L for acute and chronic effects, respectively (EPA, 1998). However, toxicity is dependent upon speciation and protecting factors. For Atlantic salmon (*Salmo salar* L.) smolt exposed for a prolonged period of <10 days, a concentration of <20 μg/L labile Al results in a gill Al concentration of up to 300 μg/g Al dry weight associated with hypo-osmoregulatory disturbance. But, >40 μg/L labile Al results in a gill Al concentration >450 μg/g Al dry weight with high mortality (Kroglund et al., 2008).

2.3.2. Cadmium (Cd)

Cd occurs ubiquitously in the environment at generally low concentrations with no functional role in biological systems (Almeida, 2001; Lydersen et al., 2002; McGeer et al., 2012). With its potential for long-range transport, anthropogenic sources have increased Cd levels in the environment beyond natural inputs (Okada et al., 1997; Lydersen et al., 2002). Waterborne Cd can result from weathering of rocks and also leashing from soils. Once in aquatic ecosystems, Cd predominates as Cd^{2+} across pH 4.5 to 7.0 and at higher alkalinity $CdCO_3$ complex becomes predominant (Lydersen et al., 2002). However, the concentration and subsequent bioavailability of Cd in aquatic ecosystems depends on numerous factors. These include its interaction with other constituents such as particulate matter which result in adsorption or desorption to sediments altering the concentration of cadmium in the water column and bioavailability to the biota therein (Thornton, 1995; Lawrence et al., 1996; Skeaff et al., 2002; Lydersen et al., 2002)**.** Although, various modes of Cd uptake exist in aquatic organisms, Cd^{2+} is readily absorbed directly from water by organisms (AMAP, 1998). McGeer et al. (2012) confirm that Cd toxicity to aquatic species depends on its speciation which is proportional to the predominant bioavailable Cd^{2+} concentration. In fish, during short exposures with high Cd concentrations, the gills are thought to be the primary site of damage and accumulation (Lydersen et al., 2002)**.** Following chronic exposure, the kidney is the main target organ for Cd with the liver storing considerable amounts (Kumar & Singh, 2010). Wren & Stephenson (1991) suggest that though Cd readily bioaccumulates and bioconcentrates in aquatic organisms, it does not biomagnify in aquatic food chains. The bioavailability and toxicity of Cd^{2+} can be altered by inorganic cations such as Na^{+}, Mg^{2+} and especially Ca^{2+} through competitive interactions for active binding sites on the chloride cells of the gills and organic cadmium complexes which are comparatively non bioavailable (Lydersen et al., 2002; McGeer et al., 2012). Cd exerts a variety of acute and chronic effects on fish. Among these is accumulation on gills which disrupts ion homeostasis as Ca uptake from water is inhibited causing hypocalcemia. Pratap & Bonga (1993), observed changes in gill ultrastructure and degeneration of pavement and chloride cells of the freshwater cichlid *Oreochromis mossambicus* exposed to waterborne and dietary Cd. With varying Cd toxicity in fish, salmonides are thought to be the most sensitive taxonomic group (Lydersen et al., 2002)**.** The most susceptible life stages are the embryo and early larva, while eggs are the least susceptive (Lydersen et al., 2002)**.** Several studies document the chronic effects of Cd as potentially affecting ion regulation, growth, reproduction, immunological and histopathological parameters, behaviour, development and endocrine functions (Pratap et al., 1989; McGeer et al., 2000a; Thophon et al., 2003). Worthy of note, is the fact that Cd accumulation causes oxidative stress resulting from the production of

Reactive Oxygen Species (ROS). Pickering & Gast (1972), reported survival over growth as a more sensitive endpoint for Cd effects on fathead minnows. Exposed rainbow trout (*Oncorhynchus mykiss*) to concentrations up to 5.5 μg/L Cd showed no effects on growth or survival over 65 weeks, but reproductive development was delayed at an even lower exposure (1.8 μg/L Cd). Though Cd induces metallothionein in the gills, it is readily detoxified in the liver (Olsson & Hogstrand, 1987). The ambient water quality criteria set for Cd is 0.6 μg/L for acute levels and 0.11 μg/L for chronic levels based on a water hardness of 30 mg/L (EPA, 2001).

2.3.3. Chromium (Cr)

Cr though naturally occurring enters various environmental components (air, water, soil) from a wide variety of natural and anthropogenic sources which have further increased its environmental concentration above permissible limits (Lydersen et al., 2012; Reid, 2012). The element exist in several oxidation states ranging from -2 to +6, with the insoluble trivalent (Cr^{3+}, Cr $(OH)^{2+}$, $Cr(OH)_2^{+}$) and highly soluble hexavalent (CrO_4^{2-}, H CrO_4^{-} , $Cr_2O_7^{2-}$) species being the most stable forms in aquatic ecosystems (Lydersen et al., 2012; Reid, 2012**).** Although Cr^{3+} serves as cofactor for insulin action in glucose metabolism (Vincent et al., 1995) and maintains efficient lipid and protein metabolism in mammals, it has no known biological function in aquatic organisms (Lydersen et al., 2002). Both Cr^{3+} and Cr^{6+} can exist in water with little organic matter (Towill et al., 1978), but Cr^{6+} predominates in oxic conditions as the dissolved stable species (Reid, 2012) being toxic to organisms due to its strong oxidative ability (Langard & Nordseth, 1979; Eisler, 1986). Chromium toxicity to aquatic biota depends on both biotic (species, age and developmental stage) and abiotic (temperature, oxidation state and concentration of Cr, pH, alkalinity, salinity and water hardness) factors (Eisler, 1986). Chromium exposure to fish can initiate a variety of acute and chronic effects from physiology, histopathology, biochemical as well as enzymatic and genetic parameters. Chromium accumulation seems to be highest in the gill, liver and intestine (Kuhnert & Kuhnert, 1976; Van der Putte et al., 1981), with the gill representing the primary site of uptake (Van der Putte et al., 1981a)**.** Hexavalent Cr has been reported to inhibit Na/K-ATPase in gill, liver and intestine of rainbow trout exposed at different pH (Van der Putte et al., 1982) and that of coastal teleost at different concentrations (5, 10, 15 mg/L) (Thaker et al., 1996). As a result, acute hexavalent Cr exposure leads to loss of osmoregulatory and respiratory abilities in fish. The chronic effects of Cr toxicity which include changes in histology, reduced survival and growth, production of ROS, and impaired immune function are all well documented. Mishra & Mohanty (2009) reported changes in gill, liver, and kidney histology, plasma cortisol, and growth after exposing spotted snakehead (*Channa*

punctata) for 1 and 2 months durations to 2 or 4 mg/L Cr^{6+} as potassium dichromate ($K_2Cr_2O_7$) at pH 7.3. *Tilapia sparrmanii* exposed to potassium dichromate (0.098 mg/L) at different pH levels showed an increase in the clotting time and in a different study haemoglobin concentration significantly decreased at high pH and slightly increased at pH 5.0 affecting hematological indices (Gey van Pittius et al., 1992). Farag et al. (2006) observed reduced growth and increased mortality of Chinook salmon parr exposed to 120 µg/L and 266 µg/L respectively. Krumschnabel & Nawaz (2004) reported increased ROS production and reduced cell viability in isolated hepatocytes of goldfish (*Carassius auratus*) exposed to 13 mg/L Cr^{6+}. Ambient water quality criteria for Cr based on Cr^{6+} is defined as < 0.29 µg/L as 24h average for acute levels and not to exceed 21µg/L at any time for chronic levels (EPA, 1980).

2.3.4. Lead (Pb)

Pb is a non-essential metal which reaches the aquatic environment through natural mineralization, mining, industrial effluents and in highway runoff (Ward, 2000). Natural waters contain approximately 0.1to 1 µg/L Pb (Ward, 2000). The speciation of Pb in freshwaters is influenced by pH, alkalinity, hardness, particle size of inorganic colloids, the concentration and quality of natural organic matter (Benes et al., 1985; Driscoll et al., 1988; Eisler, 1988a; Mager, 2012). Lead toxicity to aquatic environments is related to its dissolved fraction with its organic forms being generally more toxic than the inorganic forms (Lydersen et al., 2002). Sediments act as sink for Pb, and evidence suggests biomethylation takes place within the sediment-water interface producing very toxic Pb species $(CH_3)_3Pb^+$ and $(CH_3)_4Pb$ through alkylation (Ward, 2000) with $(CH_3)_4Pb$ being the most toxic to juvenile rainbow trout (Chau et al., 1980; Wong et al., 1981). However, Pb does not biomagnify along the food web (Settle & Patterson, 1980; Demayo et al., 1982) due to its low trophic bioavailabilty and sequential biopurification by Ca^{2+} (Mager, 2012). Lead exposure to fish causes both acute (mucus production and ionoregulatory) and chronic (hematological, neurological, growth and development) effects. Elevated Pb concentrations are normally found in blood, bone, gill, liver and kidney and adversely affect survival, growth, reproduction, development and metabolism of most species, with the younger, immature organisms being most susceptible to its toxicity (Lydersen et al., 2002). The morphological effects of lead on fish are most prominent on the gills associated with increased mucus production, disruptive Ca^{2+} homeostasis resulting in respiratory asphyxiation and disruption of ionoregulatory homeostasis (Mager, 2012). Some studies report detrimental effects of acute Pb exposure on ion homeostasis in rainbow trout (Rogers et al., 2003, 2005; Rogers & Wood, 2004). Holcombe et al. (1976) having exposed three generations of brook trout (*Salvelinus*

fontinalis) to mean total lead concentration, observed that, gill, liver, and kidney tissues of first- and second generations accumulated the greatest amount of lead with reduced growth in third generation. Lead exposure to fish affects heme biosynthesis (hematological dysfunction) causing measurable inhibition of aminolevulinic acid (ALAD) activity (Manly, 2000) making ALAD a common biomarker of Pb exposure in fish (Mager, 2012). The ambient water quality criteria for dissolved Pb based on a water hardness of 20 mg/L is 10.8 μg/L and 0.4 μg/L for acute and chronic levels respectively (EPA, 1985).

2.3.5. Mercury (Hg)

Hg occurs naturally in the environment but enters the environment in different forms from a combination of natural and anthropogenic sources (National Research Council, 2000), with human related emissions having increased relative to natural loads (Fitzgerald et al, 1998; Chan et al, 2003). Atmospheric transport and deposition at normal temperature is mercury's pathway to many of the world's aquatic ecosystems (Limbong et al., 2003). Elevated levels of Hg in aquatic ecosystems remote from industrial sources have been largely attributed to long-range atmospheric transport and deposition of anthropogenic Hg (Fitzgerald et al, 1998). Once Hg enters aquatic ecosystems through surface runoff or atmospheric transport and deposition (Rytuba, 2003), its transformation to the most toxic and bioavailable form, methylmercury (CH_3Hg^+) (Westcott and Kalff 1996) is influenced by both microbial methylation and photon influx (Black et al., 2011). The solubility of CH_3Hg^+ is enhanced when complexed with organic (e.g. DOC) and inorganic (e.g. SO_4^{2-}, Cl^-) ligands (Kidd & Batchelar, 2012) and the presence of organic matter is positively correlated with the presence of Hg (Babiarz et al., 2001; Black et al., 2011). Apart from the presence of DOC, pH and redox of the water strongly influence the speciation of both Hg^{2+} and CH_3Hg^+ (Kidd & Batchelar, 2012). Unlike other forms of Hg which tend to accumulate in aquatic biota, methyl mercury (CH_3Hg^+) is the lone species that biomagnifies up aquatic food chains (Chan et al., 2003) and typically constitutes more than 90% of the total mercury (THg) found in fish (Ikingura & Akagi , 2003; Wiener et al, 2003). The principal route of Hg uptake in fish is the gut though the gills present a larger surface area (Hall et al., 1997). The muscles bear the majority of the total Hg body burden redistributed from other tissues (Amlund et al., 2007). The biomagnification of Hg across the food chain is a potential threat to aquatic biota (particularly in piscivores), wildlife and humans (Campbell et al., 2003d; Kidd et al., 2003). Stable isotopes of carbon in fish muscle serve as index for carbon source in the aquatic environment and stable isotopes of nitrogen indicate the level of the food chain of the individual specimen due to biomagnification rate of Hg across an aquatic food chain (Cabana & Rasmussen,

1994). Waterborne or dietary exposure of fish to Hg^{2+} and CH_3Hg^+ lead to both chronic and acute toxicity affecting growth, development and reproduction. CH_3Hg^+ exposure of Atlantic salmon (*Salmo salar* L.) parr through dietary supplements resulted in pathological damage, altered feeding behaviour and oxidative stress through production of ROS, but no mortality or reduced growth was observed (Berntssen et al., 2003). Examining the effect of dietary CH_3Hg^+ on reproduction of fathead minnows (*Pimephales promelas),* Hammerschmidt et al. (2002) observed that though CH_3Hg^+ delayed spawning in fish at phase 1 (juvenile fish until sexual maturity) and phase 2 (at sexual maturity male and female allowed to reproduce), it also decreased reproduction of adult fathead minnows at dietary concentrations. The toxicity of CH_3Hg^+ is also dependent on life stage and species of fish and larger fish tend to be less sensitive than smaller fish of the same species (Kidd & Batchelar, 2012). The ambient water quality criteria for total Hg for the protection of aquatic life is 1.4 µg/L and 0.77 µg/L representing acute and chronic levels respectively (EPA, 2009). However, because Hg biomagnifies and assimilates in fish muscle, fish being an important food source for humans, guidelines are also set for Hg in edible fish muscle. The recommended Total Dietary Intake (TDI) is 0.3 mg/kg set to protect groups vulnerable to Hg toxicity (FAO/WHO, 2003).

2.3.6. Copper (Cu)

Cu is a naturally occurring metal essential within permissible limits for all organisms as a component in many metalloenzymes and proteins (Lydersen et al., 2002). Cu is found in natural waters usually at concentrations of < 5µg/L, where mining activities, industrial processes and its use as an algicide and molluscicide increase its concentration to harmful levels in fish (Alabaster & Lloyd, 1980). Shaw and Brown (1974) suggest the toxicity of Cu in aquatic ecosystems is related to the total concentrations of soluble Cu^{2+} and $CuCO_3$. Though, the toxic forms are mainly associated to Cu^{2+} either as free Cu^{2+} or as ionic hydroxide complexes ($Cu(OH)^+$, $Cu_2(OH)_2{}^{2+}$) (Lydersen et al., 2002) the most important form is Cu^{2+} (Howarth & Sprague, 1978). Water hardness, temperatures, low dissolved oxygen and reduced chelation of Cu on to inorganic and organic substances are the parameters that enhance its toxicity to fish in aquatic ecosystems (Spear & Pierce, 1979; Alabaster & Lloyd, 1980; Erickson et al., 1996). Several studies document the effects of Cu toxicity on fish parameters. In an experiment exposing *Poecilia reticulata* to a mixture of Ni, Cu and Zn, Khunyakari et al. (2001) observed increased mucus secretion over gills, excessive excretion, anorexia and increased fin movement. Also, skin and gill mucus production coupled with heavily bleeding gills were observed in carp *Heteropneustes fossils* (Svobodova et al., 1994). Ionoregulatory disturbance was observed in rainbow trout (Sayer et al., 1989; Wilson & Taylor, 1993), and inhibition of whole

body sodium influx in tilapia *Oreochromis mossambicus* (Pelgrom et al., 1995) exposed to Cu. However, copper toxicity differs with fish species, life stages and size. Cu retarded the growth and development of early life stages of brown trout (*Salmo trutta* L., Sayer et al., 1991) and reduced growth of Asian catfish (*Saccobranchus fossils*) (Khangarot & Tripathi, 1991). Primary life stages of brook trout and Chinook salmon were more Cu sensitive than other life stages (Chapman, 1978). Exposure to Cu also impaired swimming performance of brown trout as a behavioural effect (Beaumont et al., 1995). The ambient water quality criteria for dissolved Cu based on a water hardness < 60 mg/L is 1.6 μg/L and 0.53 μg/L for acute and chronic levels respectively (South Africa Water Quality Guidelines, 1996).

2.3.7. Manganese (Mn)

Mn is ubiquitous in the environment and essential for both plant and animal life forms in very small concentrations (Hem, 1985; Nealson et al., 1988), with elevated concentrations being toxic to fish (Heal, 2001). The natural sources of Mn in aquatic ecosystems include soils, sediments, igneous and metamorphic rocks (Hem, 1985), with negligible direct atmospheric deposition (Eisenreich, 1980). Mn is most common in nature with an oxidation state of +2, +3, and +4, though Mn^{2+} and Mn^{4+} are the main existing forms in aquatic ecosystems (Hem, 1985; Nealson et al., 1988; Lydersen et al., 2002). The solution chemistry of Mn (Fig. 3) is greatly influenced by pH and redox potential (E_h), as Mn^{2+} predominates as the soluble and bioavailable species at low E_h and pH over Mn^{4+} mostly present as insoluble oxides and oxyhydroxides, with abiotic or microbial transformation of both species (Nealson et al., 1985; Heal, 2001). However, complexation of Mn^{2+} with organic matter is presumed to be weak (Davison et al., 1988; L'Her Roux et al., 1988) as bacteria are believed to utilize Mn-oxides for respiration of organic matter under anoxic conditions (Gounot, 1994). Manganese is soluble, bioavailable and toxic as Mn^{2+} to fish. Nyberg et al. (1995**)** assumed brown trout mortality was significantly correlated to the concentration and rate of accumulation of Mn^{2+} on gills. Examining the mechanism of Mn toxicity to the South African banded tilapia (*Tilapia sparrmanii*) exposed to 4.43 mg/L Mn at pH 7.4 and 5, Wepener et al. (1992) observed significant changes in hematological indices and no mortalities. Decreases in white blood cells, red blood cells, hemoglobin, hematocrit and mean cell volume were attributed to internal hemorrhaging possibly as a result of necrosis of the intestinal mucosa and kidneys.

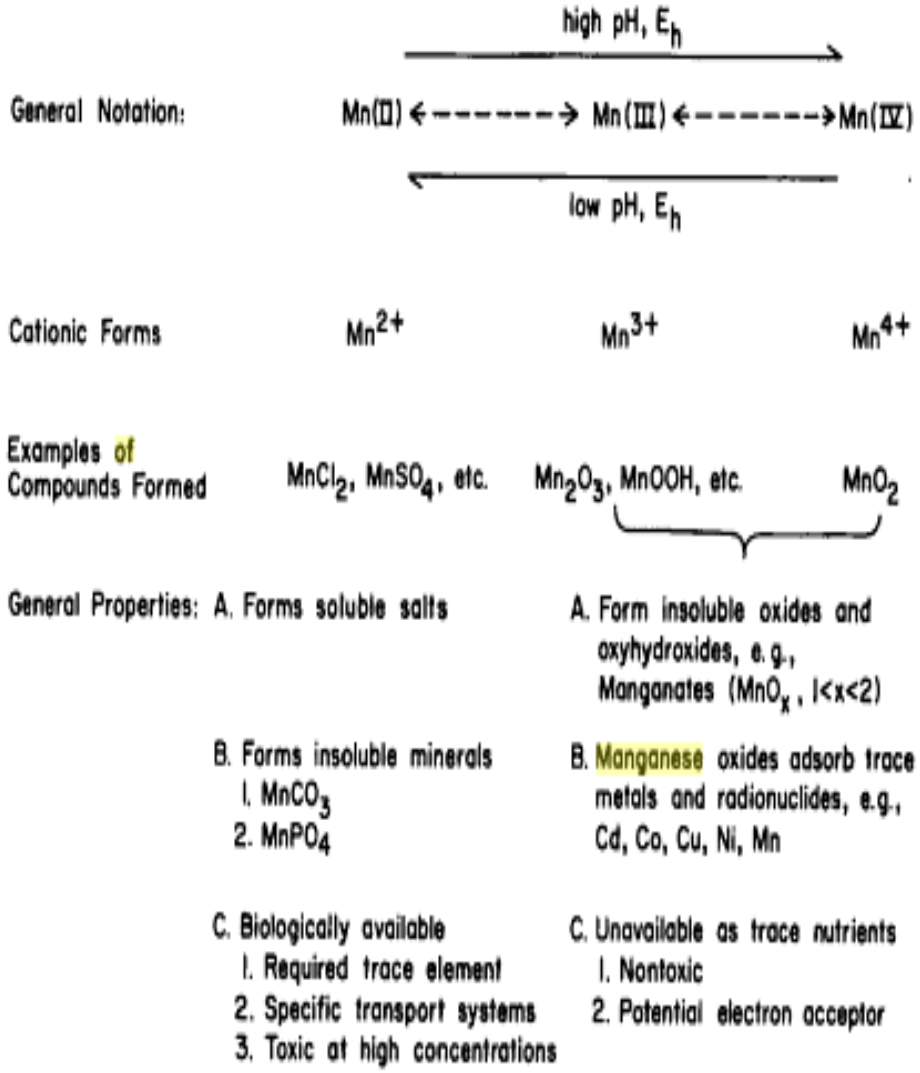


Figure 3. Microbial oxidation of manganese. (Source: Nealson et al., 1988)

The essentiality of Mn cannot be overlooked amidst its toxicity. Manganese deficient diet (4.4mg Mn/kg diet) fed on by rainbow trout for 60 weeks resulted in lens cataracts, short body dwarfism with no effect on growth (Yamamoto et al., 1983). Low dietary Mn effects on plasma ion levels, hepatic minerals and hepatic enzyme activity in trout with no effect on growth are reported (Knox et al., 1981). In natural waters, the toxicity of Mn^{2+} can be affected by water hardness. The toxicity of Mn^{2+} was observed to decrease with increasing water hardness (30-450 mg $CaCO_3$/L) in the early life stage of brown trout during 62 days test (Stubblefield et al., 1997). Lewis (1976), observed significant mortality in rainbow trout eggs exposed for 29 days to 1mg MnSO4/L in soft water. The ambient water quality criteria for dissolved Mn based on a water hardness of 20 mg/L is 120 µg/L for chronic levels (EPA, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study area

Lake Barombi Mbo situated in the volcanic range of west Cameroon, about 35 miles north-north-east of Mount Cameroon was the site of investigation. I carried out the fieldwork from November 2011 to January 2012. This period of the year was the dry season and the weather was hot with temperatures around 30 °C. During the study no inflows were observed but rather the only outflow to Kake Gorge persisted (Fig. 4).

Figure 4. Major outflow to Kake Gorge showing water clarity of Lake Barombi Mbo.

3.2. Water sampling

3.2.1. Collection of water samples

Since, most trace metals to be measured in aquatic systems are often present at very low or ultra trace levels (µg/L or ng/L) sample contamination and analyte losses are potential problems. Water samples were collected from four different locations to obtain both representative and reproducible samples. The first water sampling was carried out in the morning of 29/12/2011. Triplicate 1.5 L water samples from four different locations were collected and their coordinates obtained using a GPS Silva Multi Navigator (Table 1). Low density plastic bottles were rinsed thrice with ambient water prior to sampling.

Table 1. GPS coordinates for lake water samples

Sample N^{o}	GPS coordinates	GPS Elevation (m)	Barometric reading(mmHg)	Altitude (m) a.s.l.
1	4˚08'59.8N 9˚17'05.8E	408	731	239±5.0
2	4˚08'59.8N 9˚17'05.8E	408	730	249±5.0
3	4˚08'59.8N 9˚17'05.8E	408	730	255±5.0
4	4˚39'08.3N 9˚24'32.7E	332	729	260±5.0

GPS: SILVA Multi Navigator.

One representative 1.5 L composite for each location was then obtained. Thereafter:

- 0.5 L from each composite sample was transferred into HDPE bottles, capped, marked, and stored cold in a cooler for transport and subsequent storage.
- 50 ml of raw water samples from each composite were collected and kept cold prior to total measurements of trace metals.
- 50 ml were used to obtain pH and temperature values
- 50 ml triplicates for total organic carbon (TOC) and anion measurements
- 0.9 L was used for fractionation purposes.

3.2.2. Determination of General Water Quality

WTW multi 340i with SenTix pH electrode was used to obtain pH measurements at site. The pH meter was calibrated in field before usage. Buffer 4 was used as first standard for calibrating the pH probe after being cleaned with distilled water. Obtained readings of pH and temperature with respect to the standard buffer were recorded. Once the probe was thoroughly rinsed with distilled water, it

was inserted into the second standard, buffer 7 and obtained readings also recorded. Then the slope mV/pH was obtained before taking off the probe. The reason for this was to ensure that the probe was calibrated and readings obtained were acceptable. Thereafter, the probe was rinsed again and inserted into the raw water sample collected from the lake. 10 ml of buffers 4 and 7 were separately transferred into two 50ml tubes to avoid contamination of the bulk volume while calibrating the WTW multi 340i for pH. HANNA Instruments HI9811 pH-EC-TDS was used to obtain electrical conductivity (EC) and total dissolved solid (TDS) values. A waterproof thermometer Extech Instruments 39240 was used to obtain temperature readings. Prior to obtaining pH and temperature readings from each sample, the pH probe and thermometer were cleaned with distilled water from an LDPE wash bottle. The entire sampling within the lake took approximately two (2) hours from the hours of ten to about midday. Ten readings of EC and TDS were obtained from three different locations of the lake (Appendix 1). This was aimed at verifying for any differences between sampling locations. In order to verify for consistency in obtained readings, triplicate readings of pH, temperature, TDS and EC were obtained for the littoral and in lake regions of the lake (Appendix 1 & 2). Based on the samples collected, other water quality parameters; TOC, anions (Cl^-, SO_4^{2-}, NO^{3-}**),** major cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) and Si were determined after storage and transportation to Norway for analysis at the Norwegian University of Life Sciences (UMB), Department of Plant and Environmental Sciences (IPM). The 50 ml samples were acidified with 1 ml ultrapure HNO_3 (i.e. 2vol % HNO_3) before analyses for major cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) and Si using ICP-OES (Perkin Elmer, Optima 5300 DV). The non acidified 50 ml samples were used for analyses of major anions (Cl^-, SO_4^{2-}, NO_3^-**)** and TOC using ion-chromatography and a carbon analyzer, respectively.

3.2.3. Fractionation of water

To obtain information of trace metal speciation, size and charge fractionation was performed. Filtration and fractionation were performed immediately at the site to reduce the storage time of the composite water samples and also minimize subsequent changes in the water. In the absence of a peristaltic pump, filtration was performed under gravity (Fig. 5). A 0.45µm membrane filter paper was inserted into a filter paper holder and tightly locked. Thereafter, a substantial amount of the first water composite was transferred into a plastic bottle and fitted to the filter paper holder by means of a plastic tube. The water was then gently passed through the filter paper by pressing the bottle to exclude any air trapped within the filter paper holder. Once this was achieved, the bottle was exchanged with another which had no bottom and constant level of water to enhance free and constant flow. Because the process was manual and time consuming to get a substantial volume of the 0.45µm membrane filtrate, the filter paper was changed several times to avoid clogging and

reduce the residence time of the water through the filter paper. With a substantial volume of the 0.45µm filtrate enough to ensure continuous flow, charge fractionation was started. Portions of the filtrate were poured into a clamped plastic vessel with holes at the walls. The containing vessel was fitted to a clamped tube containing 15 ml Chelex100 resin by means of a junction tap (T_1) to control flow. At the end of the tube containing the resin was another tap (T_2). With the set-up complete, both taps were opened for fractionation of the filtrate through the Chelex 100 cation exchange resin. The first chelex filtrate was collected in a 50 ml tube.

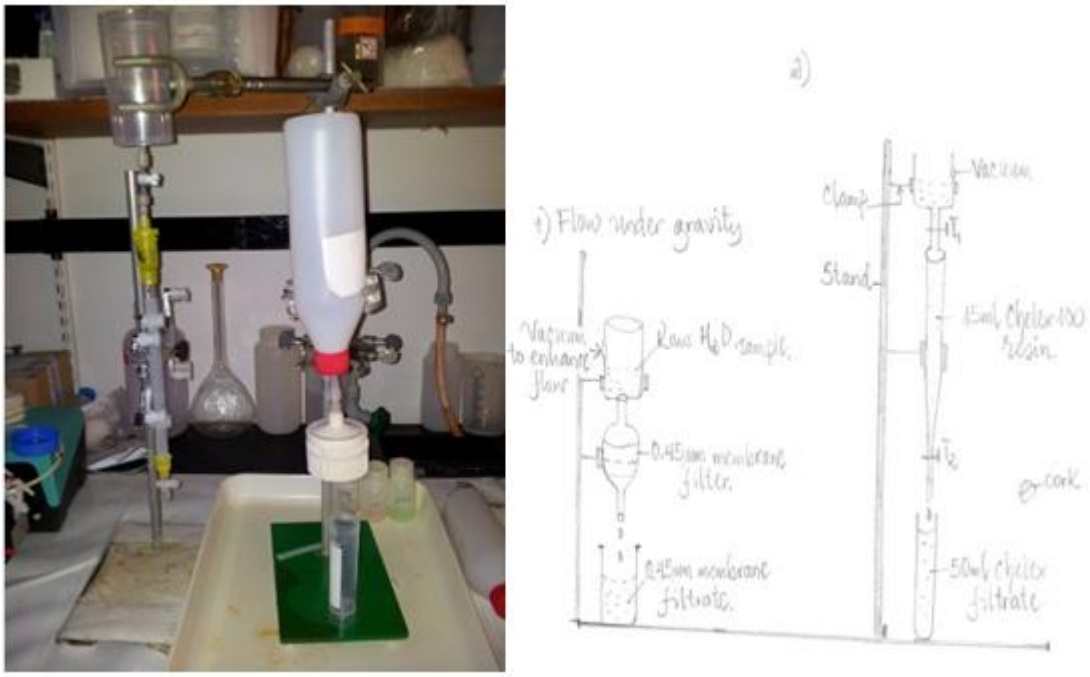


Figure 5. Set up for in-situ filtration and fractionation of raw water samples.

The first 50 ml of eluate obtained through the Chelex 100 resin was used for conditioning purpose and to ascertain flow and calculate pH again, before a second 50 ml of the eluate was collected. The flow rate was 9 ml/15 ml resin/min. The same procedure was performed for all four composite water samples. Triplicate 50 ml of the chelex eluate were obtained for each of the four composites. They were then kept into a cooler, transported and then stored cold devoid of light. The same fractionation procedure was performed for all four composite water samples. Then, 50 ml triplicates of the Chelex 100 eluate were obtained for each of them and marked with their sample numbers. They were then kept in a cooler containing ice blocks, transported and later stored in a fridge devoid of light. The filtration and charge fractionation of all four 0.9 L raw water composites took at most four (4) hours and their temporal storage time to final storage location was about two (2) hours. From then, all samples were stored cold in the fridge at approximately 4 ˚C till their final transportation to Norway

for further analysis at the Norwegian University of Life Sciences (UMB), Department of Plant and Environmental Sciences (IPM).

3.2.4. Trace metals in water

Triplicate 50 ml water samples representative of each fractionation series (unfiltered, filtered and eluate from Chelex 100) were acidified with 1 ml ultrapure HNO_3 before determination using ICP-OES and ICP-MS (Perkin-Elmer Sciex ELAN 6000).

Thus, the following information was obtained:

- Concentration of total concentration of trace metals, based on unfiltered sample.
- Concentration of trace metals not retained in the Chelex 100 resin, analyses of trace metal in the eluate from the Chelex 100.
- Concentration of trace metals retained in the Chelex 100 resin, obtained by differences between trace metals in filtered samples and filtered before eluted from Chelex 100.

The differences between unfiltered sample and the eluate sample could be due to exclusion by the filter or retained by the Chelex 100. Unfortunately samples for only filtered water was not collected during the field work, thus, the concentration of trace metals retained in the Chelex 100 could not be calculated. Total concentration of trace metals was then used to characterize the water quality.

3.3. Fish species

3.3.1. Fish species of study

Fish was collected to obtain information of bioconcentration of trace metals and to obtain information of biomagnification, e.g., changes in the food chain. Different fish species were chosen to represent distinct trophic levels with the lake's aquatic food chain (Fig. 6). U. species represent the juveniles of *S. caroli and S. linellii* which are the most consumed fishes of the lake. U. species (phytoplankton feeder) was most abundant and most consumed of all three species chosen. Reasons for high consumption were its fleshy nature and sweet taste. *P. maclareni* (zooplankton feeder) was chosen over *Konia dikume* because it is said to be threatened and it is on the IUCN red list for critically endangered species (Reid, 1990). It was also observed to be very rare during the study. *P. maclareni* is abundant near shore lines (Fig. 7) and it is a sponge feeder. The catfish *C. maclareni* lives in deep and shallow areas of the lake. *C. maclareni* is a gill and lung breathing piscivorous fish and the top predator in the lake aquatic food chain.

Figure 6. Selected fish species of Lake Barombi Mbo, Cameroon. A) U. species, B) *P. maclareni*, C) *C. maclareni*.

P. maclareni is abundant near shore lines (Fig. 7) and has an average length of 10 cm. It is also a sponge feeder and most importantly it is on the IUCN red list for critically endangered species. The catfish *C. maclareni* is the only endemic piscivore of the lake and lives in deep and shallow areas of the lake.

3.3.2. Fish sampling

On a typical sampling day, pre-ordered fish samples were collected in the morning from local fishermen (Fig. 4). The fish supplied were always carefully selected to obtain fresh ones and most especially to have sampled organs in the best conditions possible. Except for *C. maclareni*, which was most often caught by nets set overnight, *P. maclareni* and U. species were both caught the same morning prior to sampling. Fish were then kept in zipped-plastic bags, stored in a cooler and transported for close to ninety (90) minutes to the laboratory for sampling. The total lengths and weights of the fish were obtained on different sampling days by using a measuring tape and electronic balance (Adventurer Ohaus AR3130) respectively and values recorded (Appendix 4). The secondary gill arch, liver, kidney and muscle (Hg, isotope analysis) were sampled following the procedures in the EMERGE Protocol (Rosseland et al., 2001) using slicers and scalpels. The

equipments were cleaned with distilled water and the slices changed frequently to avoid any risk of sample contamination. Liver and muscle were wrapped in Al-foil and the gills were kept in plastic vials, marked, sealed in plastic zip-bags and kept frozen devoid of light, until subsequent analysis after transportation to the Norwegian University of Life Sciences (UMB), Department of Plant and Environmental Sciences (IPM). In total, 20 U. species, 19 *P. maclareni* and 15 *C. maclareni* was sampled. Fishing was done on a daily basis only by indigenous fishermen of Barombi Mbo village with the use of gill nets. Only canoes were used for transportation and fishing.

Figure 7. Setting gill nets near shore line for harvesting of *P. maclareni*.

3.3.3. Isotope analysis of ^{15}N (‰) and ^{14}N (‰), and ^{13}C (‰) and ^{12}C (‰) in muscles

Analyses of stable isotopes were determined to obtain information on the trophic level of each fish. Isotope analyses were based on one separate sample tissue of the muscle wrapped in aluminium (Al) foil that had been stored frozen. The muscle tissues were homogenates. Milli-Q water was added to

them and homogenized by aid of an electric stirrer. The procedure was step-wise accompanied by sequential rinsing after every muscle extract sample to avoid contamination and interference in results obtained thereafter. The homogenates were transferred to labelled plastic vessels and sealed with perforated parafilm followed by freeze drying (Fig. 8). After freeze drying, the samples were weighed and prepared for Isotope-ratio mass spectrometry (IRMS) following standard procedures at the Isotope Laboratory of the Environmental Chemistry Section of the Department of Plant and Environmental Sciences (IPM), Norwegian University of Life Sciences (UMB), Norway (see Desta et al., 2007). Control sample results are given in stable isotope ratio of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$).

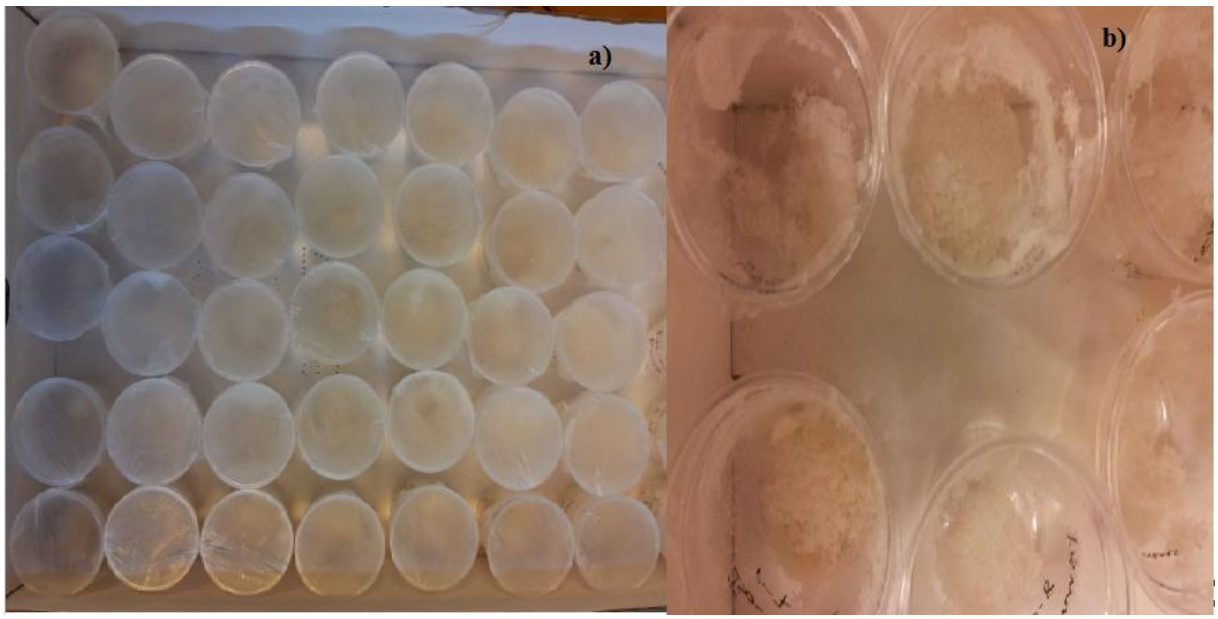


Figure 8. Muscle tissues prepared for stable isotope measurements. **a**) muscle homogenates prior to freeze drying, **b**) muscle tissues after freeze drying.

3.3.4. Determination of Trace Metals in Gills and Liver

At the laboratory (Fig. 9), gills and whole liver samples were freeze-dried, transferred to Teflon tubes and their dry weights determined in grams using an AG204 Delta Range Toledo electronic balance. To all dried samples (weighed ≤ 0.1 g) was added 1ml HNO_3 and 50 µL Internal Standard (IS) as standard procedure prior to digestion using ultraclave. Three blanks as well as DOLT-4 (piked dogfish) certified reference material from the National Research Council of Canada, Ottawa, were used as control and for traceability to control sample values and the accuracy of the methods respectively. The material of DOLT-4 added to the Teflon tubes was about 0.5 g, so 250 µL Internal Standard (IS) and 5 ml HNO_3 were added to it. After digestion, all samples and blanks were diluted with Milli-Q (MQ) water to 10ml and DOLT-4 to 50 ml respectively after transfer from Teflon tubes

(Fig. 10). Thus, all samples have the same concentration of HNO_3 (5 volume %) and the same concentration of IS. Trace metal concentration in digested gill and liver tissues were measured using ICP-MS. Analyzed and presented trace metals are selected based on the results from ICP-MS. Iron was not analyzed and Cobalt (Co), Strontium (Sr), and Uranium (U) were included in the list in addition to selected metals.

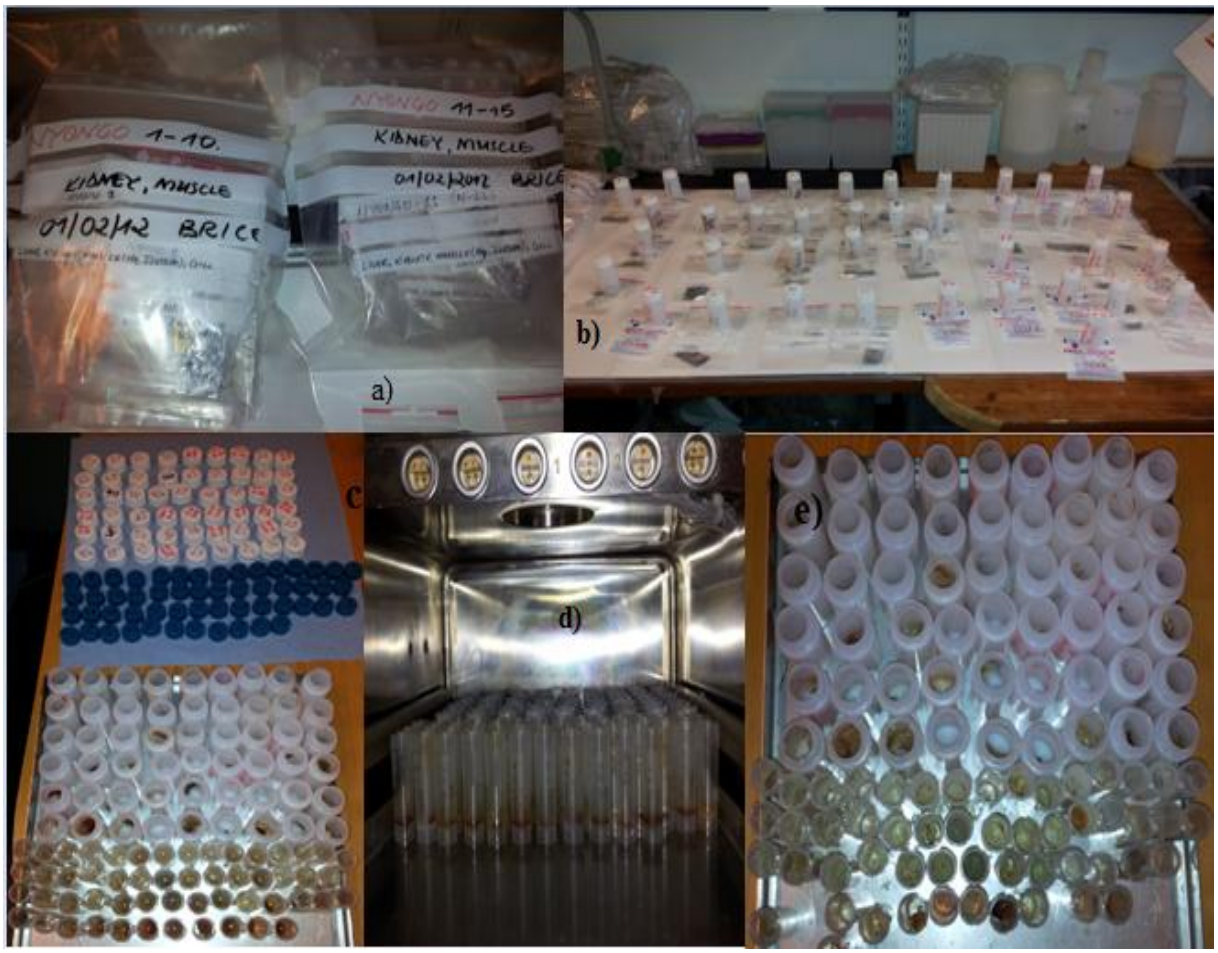


Figure 9. Gill and liver sample handling at IPM laboratory. **a**) sealed samples, **b**) sampled tissues sorted with respect to corresponding fish species and codes, **c**) samples prior to dry freezing, **d**) samples in freeze drier, **e**) freeze-dried samples.

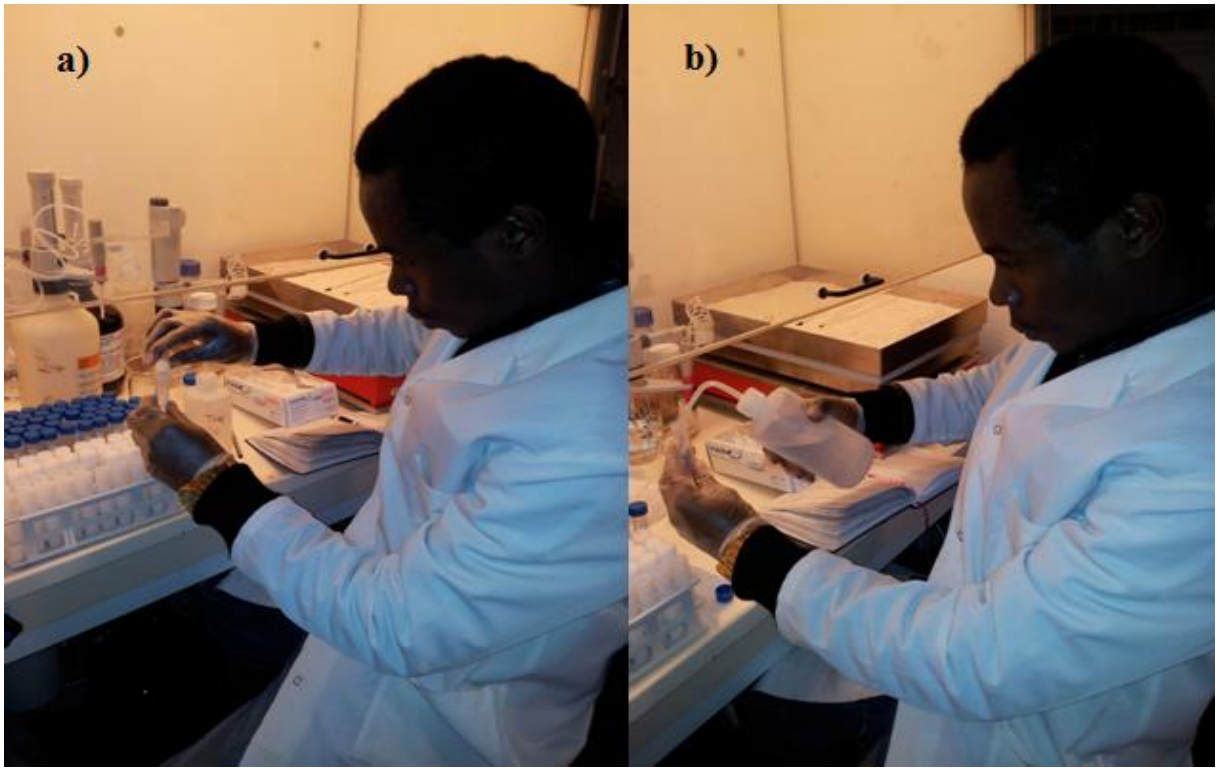


Figure 10. Standard dilution of digest. **a**) transfer of digested liver, gill, blanks and DOLT-4 from Teflon tubes to dilution tubes, **b**) dilution of digested samples with distilled water.

3.3.5. Mercury Analysis in Muscles

Mercury analyses were based on one separate sample tissue of the muscle wrapped in aluminium foil that had been stored frozen. Mercury (Hg) analysis in muscles of sampled fish species was performed following standard procedures in the Environmental Chemistry Section of the Department of Plant and Environmental Sciences (IPM), Norwegian University of Life Sciences (UMB), Norway. Blanks and DORM-2 (piked dogfish) certified reference material from the National Research Council of Canada, Ottawa, were used as control and accuracy of the method respectively.

3.4. Statistical analysis

Information on the concentration of trace metals in water and tissue samples were reported as mean (± SD) using MS-Excel. Trace metals in gills, liver and accumulation of total mercury concentration (THg) in muscles of fish species was tested for correlation with length, weight, $\delta^{13}C$ and $\delta^{15}N$ using Linear regression analysis described by r^2 and p-values ($p < 0.05$). Analysis of variance (ANOVA) was used to examine differences in mean values of THg, $\delta^{13}C$ and $\delta^{15}N$ among all species with significance at $p < 0.05$. All statistical procedures were performed using MINITAB 16 release.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Lake water

4.1.1. General water quality of Lake Barombi Mbo.

The main physicochemical properties of Lake Barombi Mbo water pertaining to general water quality including pH, electrical conductivity (EC), major cations (Na^+, K^+, Mg^{2+}, Ca^{2+}), Si, major anions (Cl^-, SO_4^{2-}, NO^{3-})**,** total organic carbon (TOC) and total dissolved solid (TDS) are given in Table 3. The pH range was in the range 7.04-7.62. The mean temperature of the lake water during the investigation was 30 °C. As EC and TDS are useful measures of ionic strength, low EC and TDS represent low ionic strength. From the investigation, low ionic strength, low hardness and especially low TOC content asserted by the clear nature of the lake characterized the lake water. Of note, is the fact that measured parameters between sites (Appendix 1) and also collected water samples did not show any differences for pH, EC, TDS and temperature (Table 2). This implied no changes for these parameters due to storage.

Table 2. Mean values for Lake Barombi Mbo water parameters in field.

Lake water	n	Temperature (°C)	pH	EC (mS/m)	TDS (mg/L)
In-situ	12	30.2	7.4	4.0	19.7
Bottled samples	3	30.0	7.3	4.0	20.0

n=sample size (based on triplicate measurements).

Hardness = $[Ca^{2+}]$ + $[Mg^{2+}]$, quantified as $CaCO_3$ equivalent in mg/L (Wood et al., 2012).

The hardness of the water was 6.6 mg/L $CaCO_3$ as $[Ca^{2+}]$ = 3.6 mg/L and $[Mg^{2+}]$ = 3.0 mg/L.

Table 3. Mean (±SD) values for general water quality parameters of Lake Barombi Mbo, Cameroon.

Parameters	Present Study
Field	
pH	7.4±0.2
Temp. (°C)	30.2±0.5
EC (mS/m)	4.0±0.2
TDS (mg/L)	19.7±1.7
Laboratory	
Ca^{2+} (mg/L)	3.6±0
Mg^{2+} (mg/L)	3±0
K^{+} (mg/L)	6.9±1
Na^{+} (mg/L)	2.8±0.2
Si (mg/L)	6.3±0
Cl^{-} (mg/L)	0.8±0
NO_3^{-} (mg/L)	<0.006
SO_4^{2-} (mg/L)	0.12±0

4.1.2. Trace metals in water

Table 4 show the concentration of analyzed trace metals in water samples from Lake Barombi Mbo. The sequence of trace metal concentrations in unfiltered water samples was Sr > Al > Mn > Cu > Cr > Pb > Co > Cd > U. The results of total concentration of trace metals in unfiltered water samples indicated that the concentrations of Sr, Al and Mn were higher than the other metals (Table 4 and Fig. 11). The mean total concentrations of trace metals in the water samples from Lake Barombi Mbo were below ambient water quality criteria (AWQC) and low compared to CCME and EPA limits for protection of aquatic life. Also, the total concentrations of the trace metals were lower than dissolved analyte concentrations with respect to EPA 2004 criteria limits. At the time of sampling, the use of pesticides for spraying of cocoa farms around the rim of the lake was the only observed anthropogenic activity. The lake being far removed and isolated from large urban settlement makes it presumably less susceptible to direct contaminant inputs from sewage and industrial effluents. This probably explains the very low levels of trace metals in the lake water. These results are in agreement with many of the African lake waters which have low concentrations of trace metals (Biney et al., 1994).

Table 4. Mean (±SD) concentration of trace metals (µg/L) in different size fractions of water samples from Lake Barombi Mbo (LBM), Cameroon compared with water quality criteria. Acute and chronic values are based on a hardness of 20 mg/L unless stated.

Metal	Unfiltered (Total)	*Chelex eluate (Lab)	Acute (µg/L)	Chronic (µg/L)	Guideline
Al	**5.8±0.9**	3.3±0.2	750	87	EPA (1998)
Cr	**0.3±0.1**	0.2±0		21	EPA (1980)
Mn	**2.5±0.4**	0.3±0.1		120	EPA (2004)
Co	**0.03±0.01**	0±0		8	WHO (2006)
Ni	**0.7±0.4**	0.3±0	120	13	EPA (2005)
Cu	**1±0.6**	1.1±0.1	1.6	0.53	[a] SA (1996)
Zn	**3.2±2**	0.6±0.1	30.6	30.2	EPA (1987)
Sr	**43±5**	0.04±0.02	40000	21000	[b] EPA (2009)
Cd	**0.004±0.004**	0±0	0.6	0.11	EPA (2001)
Pb	**0.048±0.049**	0±0	10.8	0.4	EPA (1985)
U	**<0.005**	<0.005	33	15	CCME (2011)
Fe	**28.9±4.1**	13.2±1.5		300	[**] CCREM (1987)

* sample filtered using 0.45 µm filter prior to ion exchange.

[a] SA (1996): South Africa Department of Water Affairs and Forestry (criteria value based on Hardness < 60 mg $CaCO_3$ /L); [b] EPA (2009): US Environmental Protection Agency, Ohio; [**] CCREM & CCME: Canadian Council of Ministers of the Environment based on total measured Fe concentration; WHO: World Health Organization.

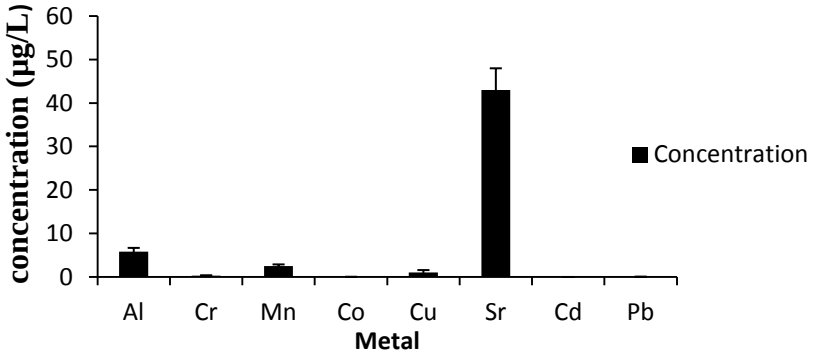
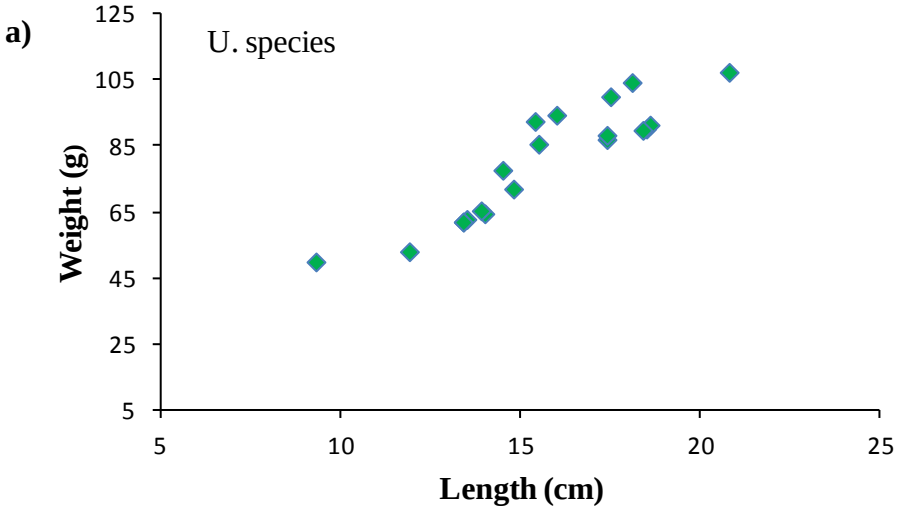


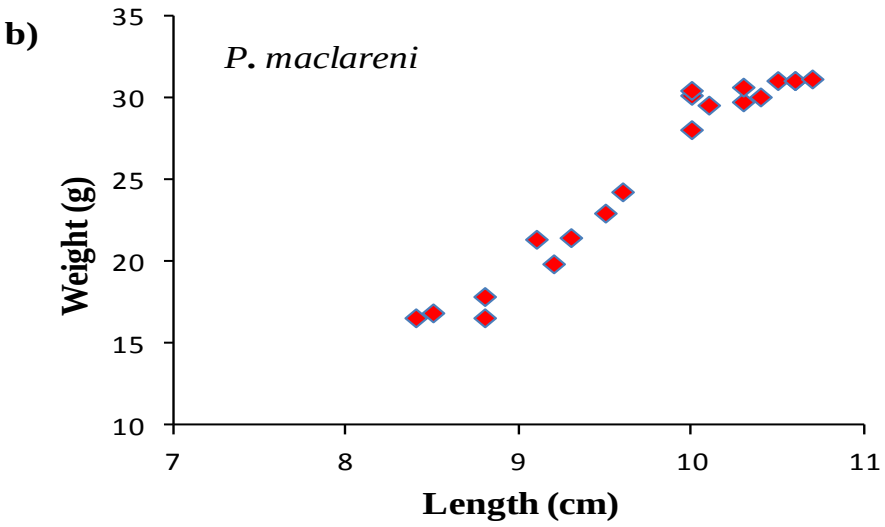
Figure 11. Concentration of trace metals in the water of Lake Barombi Mbo, Cameroon.

4.2. Fish

4.2.1. Characteristics of fish species collected

Based on literature survey, U. species and *P. maclareni* are tilapiine cichlids and *C. maclareni* is the only endemic catfish of Lake Barombi Mbo. All three fish species showed significant differences in their sizes (Table 5, Fig. 12, and see Appendix 4). The mean lengths and weights were 15.6 cm and 49.9 g in U. species, 9.7 cm and 16.6 g in *P. maclareni*, and 26.9 cm and 178.6 g in *C. maclareni* (Table 5).





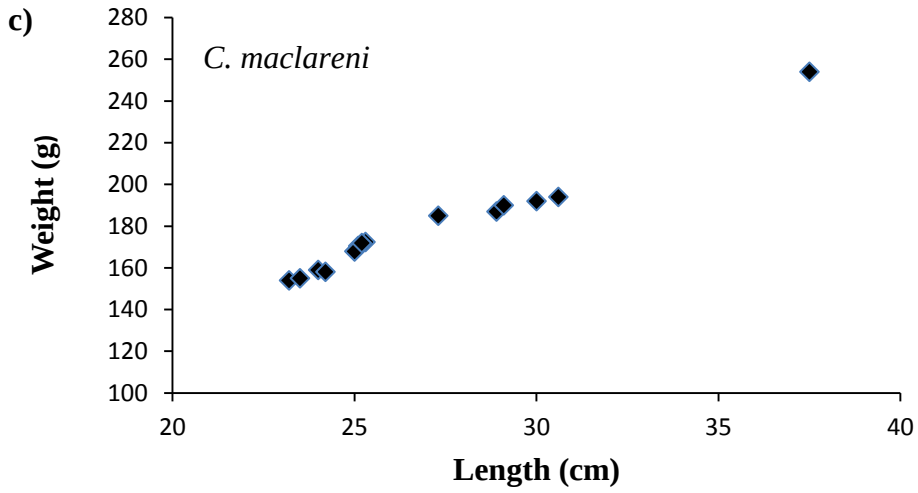


Figure 12. Length and weight of individual fish species in Lake Barombi Mbo, Cameroon. **a)** U. species, **b)** *P. maclareni*, **c)** *C. maclareni*.

Results of IRMS analysis showed that the mean $\delta^{13}C$ (‰) values was -32.9‰ in *U. species*, -30.2‰ in *P. maclareni* and -31.5‰ in *C. maclareni*. The sequence for decreasing stable carbon isotope ratio was *P. maclareni* > *C. maclareni* > U. species. So, *P. maclareni* had the highest $\delta^{13}C$ of all three fish species in the aquatic food chain (p = 0.000). However, $\delta^{13}C$ (‰) values showed no significant relationship with fish size in both U. species and *P. maclareni* (Fig. 13a & 13b). U. species being phytoplankton feeders have lower $\delta^{13}C$ compared to *P. maclareni* being zooplankton feeders. Conversely, there was a significant relationship between $\delta^{13}C$ and fish size in *C. maclareni*, with total length (p=0.016, r^2=0.371) being relatively more significant over total weight (p=0.021, r^2=0.348). This probably relates to changes in carbon source with increasing fish size depending on the fish species. Trophic index analysis for $\delta^{15}N$ showed that the mean values for individual species were 6.6 ‰ in U. species, 8.4 ‰ in *P. maclareni* and 9.9 ‰ in *C. maclareni* (Table 5). *C. maclareni* had the highest $\delta^{15}N$ value (9.9 ‰) and thus occupied the highest trophic level (Fig. 14). With the lowest $\delta^{15}N$ value, U. species occupied the lowest level of the food chain of the three fish species analyzed. There was no significant relationship between $\delta^{15}N$ and fish size for all species (p>>0.05) as shown in Table 8 and Figures 13. All pair wise comparison for mean values of $\delta^{15}N$ (n = 54, df = 2, F = 54.29, p = 0.000; ANOVA) and $\delta^{13}C$ (n = 54, df = 2, F = 10.09, p = 0.000; ANOVA) among levels of species were significantly different (Appendix 11). Pearson's correlation coefficient showed that there was a more significant correlation between $\delta^{15}N$ and species level (r^2 = 0.680) over $\delta^{13}C$ and species level (r^2 = 0.284). Apparently, observed variations in $\delta^{15}N$ (‰), and $\delta^{13}C$ (‰) with fish

size seemed to be more explanatory in *C. maclareni* than in U. species and *P. maclareni* (Table 6). Thus, *C. maclareni* represents a higher trophic level than U. species and *P. maclareni*, and based on $\delta^{13}C$, the carbon source for U. species is -32.9‰ and -30.2‰ for *P. maclareni* (Table 5). Significant differences in $\delta^{13}C$ and $\delta^{15}N$ between all fish species reveal diverse food prey items and thus different trophic positions of individual fish in the same size group.

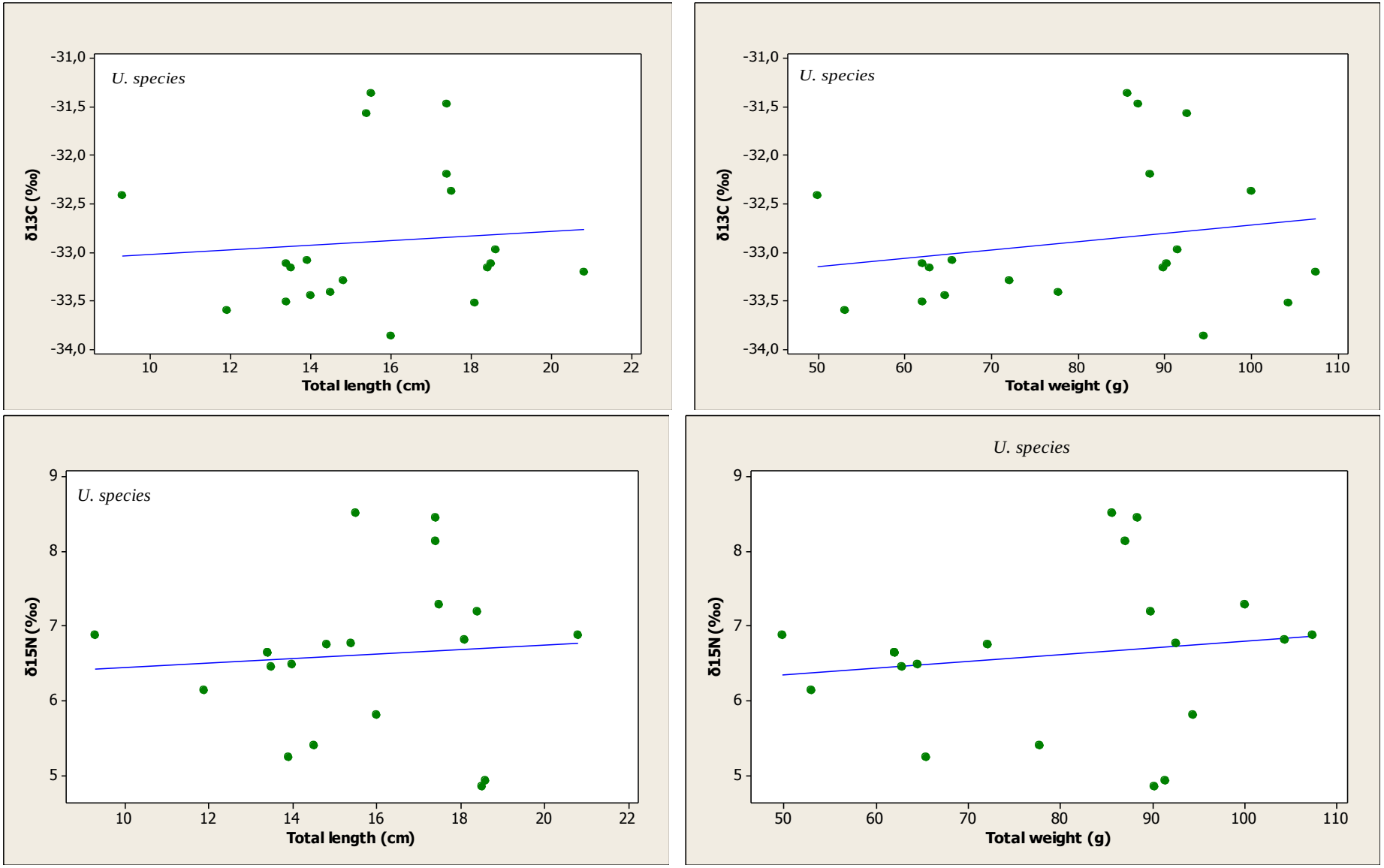


Figure13a. Relationship between stable isotopes of carbon and nitrogen with fish size in U. species from Lake Barombi Mbo, Cameroon.

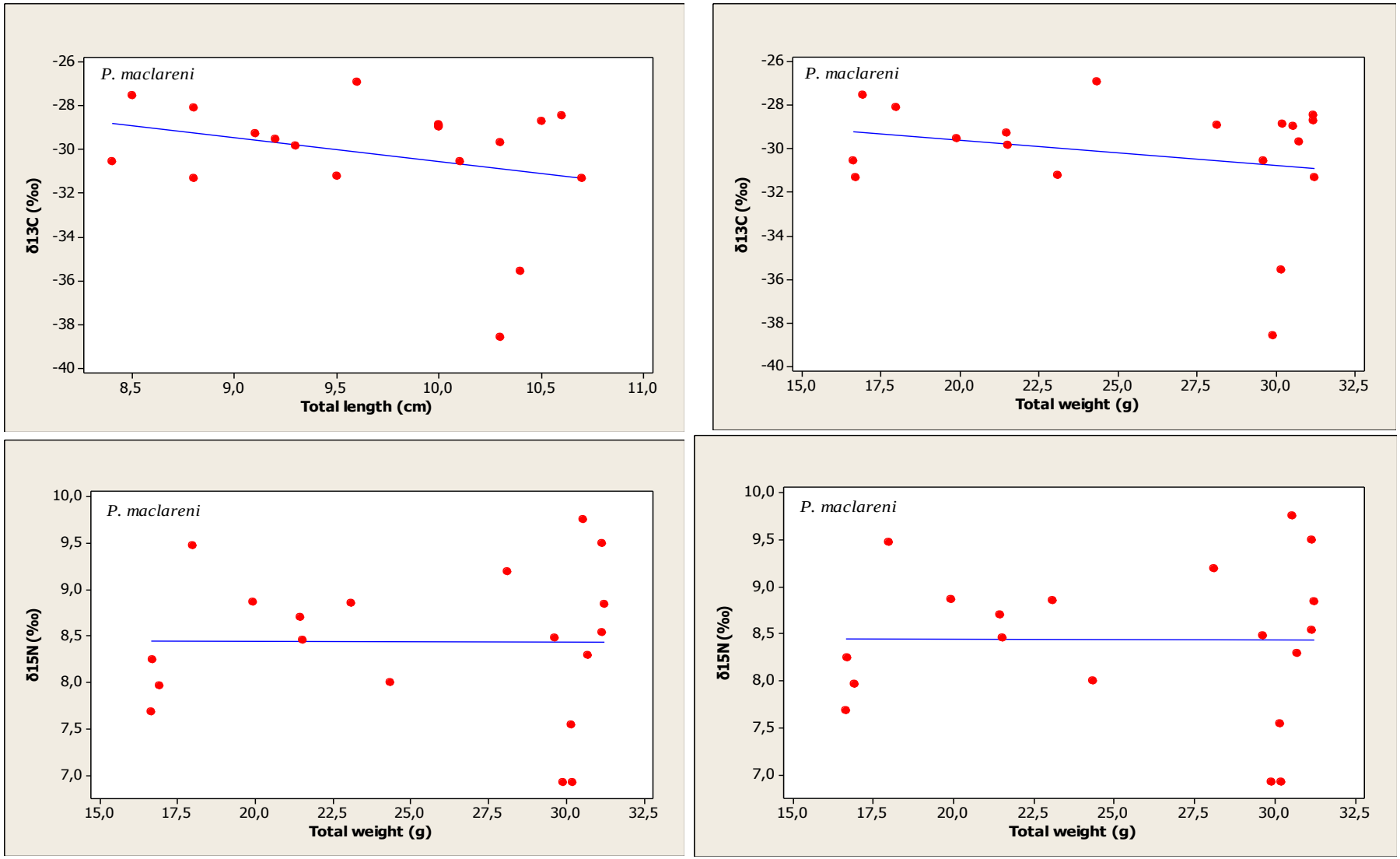


Figure 13b. Relationship between stable isotopes of carbon and nitrogen with fish size in *P. maclareni* from Lake Barombi Mbo, Cameroon.

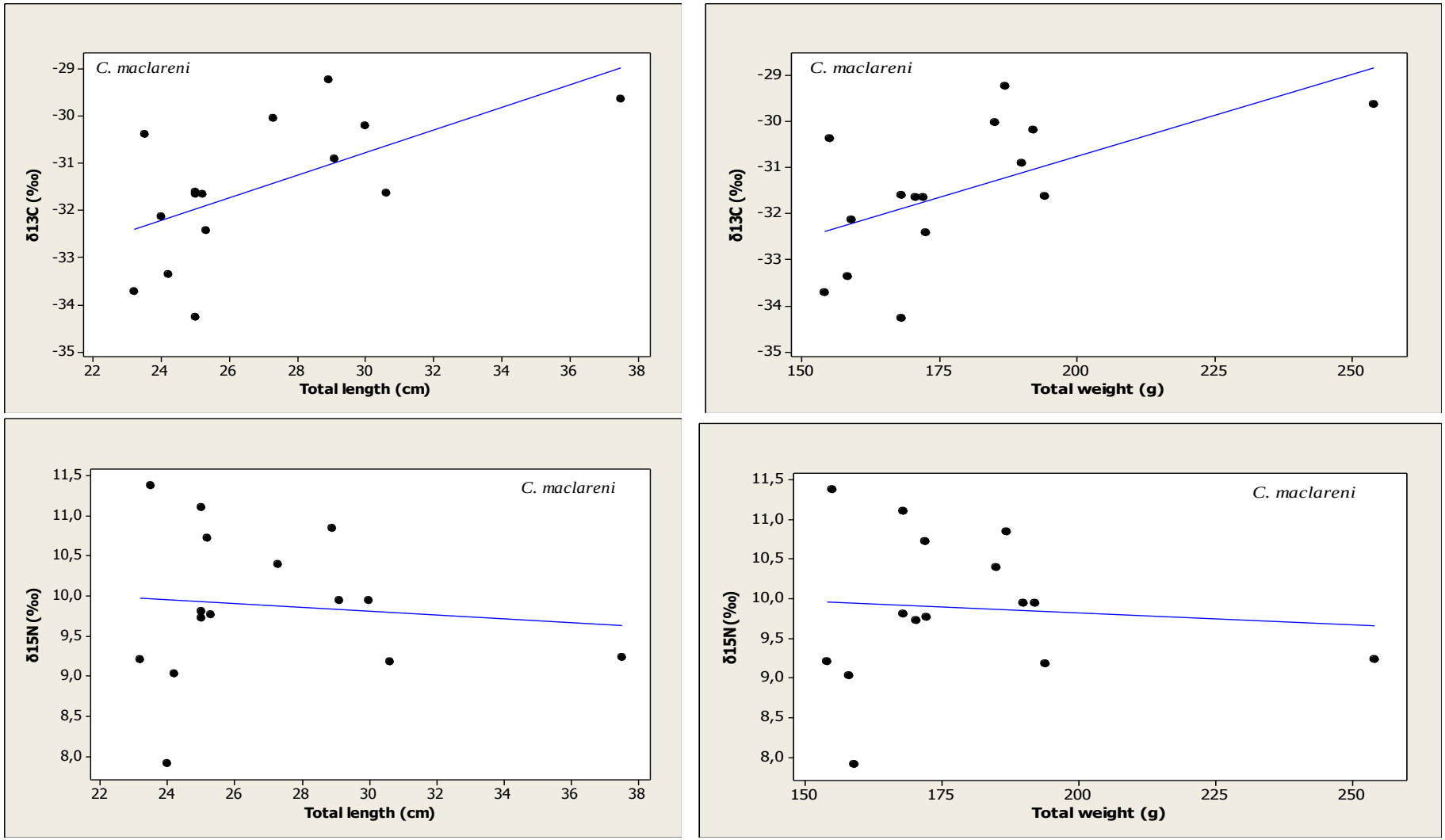


Figure 13c. Relationship between stable isotopes of carbon and nitrogen with fish size in *C. maclareni* from Lake Barombi Mbo, Cameroon.

Table 5. Mean (±SD) of total length (cm), total weight (g), $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of fish species from Lake Barombi Mbo, Cameroon. n = sample size.

Species	n	Length (cm)	Weight (g)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
U. species	20	15.6±2.7 (9.3, 20.8)	80.0±17.3 (49.9, 107.4)	-32.9±0.7 (-33.9, -31.4)	6.6±1.0 (4.8, 8.5)
P. maclareni	19	9.7±0.7 (8.4, 10.7)	25.3±5.7 (16.6, 31.2)	-30.2±2.8 (-38.6, -26.9)	8.4±0.8 (6.9, 9.8)
C. maclareni	15	26.9±3.8 (23.2, 37.5)	178.6±24.9 (154, 254)	-31.5±1.5 (-34.3, -29.2)	9.9±0.9 (7.9, 11.4)

Table 6.

Regression of $\delta^{15}N$ and $\delta^{13}C$ against total length (L_T) and total weight (W_T) for sampled fish species from Lake Barombi Mbo, Cameroon**.** For each regression, the sample size (n), intercept, slope, r^2, and p-values were given (bold numbers indicate significant regression).

Species	Regression	n	Intercept	Slope	r^2	p-value
U. species	$\delta^{15}N$ vs L_T	20	6.16	0.0293	0.006	0.746
	$\delta^{15}N$ vs W_T	20	5.89	0.0090	0.002	0.528
	$\delta^{13}C$ vs L_T	20	-33.3	0.0235	0.008	0.715
	$\delta^{13}C$ vs W_T	20	-33.6	0.0085	0.039	0.404
P. maclareni	$\delta^{15}N$ vs L_T	19	8.16	0.0280	0.001	0.916
	$\delta^{15}N$ vs W_T	19	8.45	-0.0007	0.000	0.983
	$\delta^{13}C$ vs L_T	19	-19.5	-1.1000	0.085	0.226
	$\delta^{13}C$ vs W_T	19	-27.2	-0.1160	0.058	0.320
C. maclareni	$\delta^{15}N$ vs L_T	15	10.5	-0.0231	0.009	0.733
	$\delta^{15}N$ vs W_T	15	10.4	-0.0029	0.006	0.779
	$\delta^{13}C$ vs L_T	15	-38.0	0.2400	0.371	**0.016**
	$\delta^{13}C$ vs. W_T	15	-37.9	0.0355	0.348	**0.021**

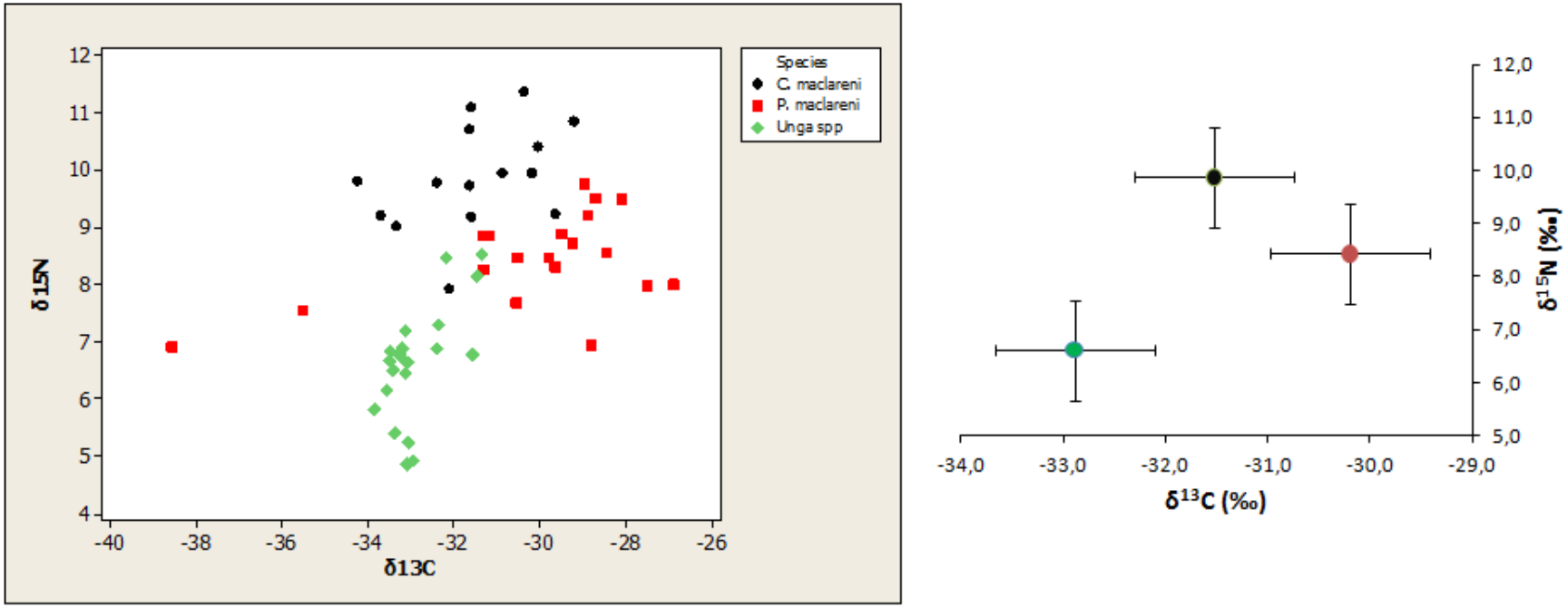


Figure 14. The relationship between $\delta^{15}N$ and $\delta^{13}C$ values of fish species sampled in Lake Barombi Mbo, Cameroon. Ranges of error bars indicate standard deviations from the mean, with vertical bars for $\delta^{15}N$ and horizontal bars $\delta^{13}C$ values (symbols: **green dots** = U. species, **red dots** = *P. maclareni*, **black dots** = *C. maclareni*).

4.3. Trace metals in fish tissues

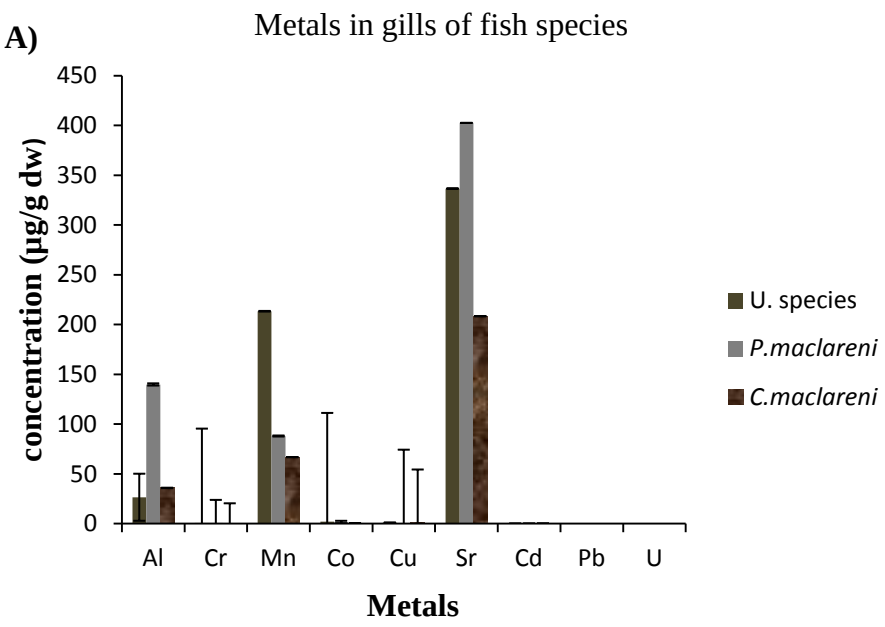
The results presented in Table 7 showed that trace metals in water were also present in fish tissues. Trace metal concentrations in fish tissues decreased generally in the sequence; Sr > Mn > Al > Co > Cu > Cr > Pb > U > Cd in gills - with minor changes between species and in the sequence Cu > Mn > Co > Sr > Cd > Cr > Pb > U in liver with minor changes between species. The differences in metal accumulation sequence revealed that tissues of fish species accumulated metals in varying amounts. All three fish species showed highest concentration of Al, Mn and Sr in their gills whereas Cr, Co, Cu, Cd, and U had higher concentrations in the liver. This suggests that gills were the major site for Al, Mn and Sr accumulation for the three fish species. There was no significant difference in Pb concentration for both tissues in all species ($p > 0.05$). Whilst the levels of metals in gills represent uptake of bioavailable forms from water (Rosseland et al., 1992), levels in liver represent storage of metals in the water (Romeo et al., 1999), hence, uptake through water and/or food by absorption or ingestion. The bioconcentration factor (BCF) between water and fish tissues, were greater than or equal to 1, except for Cu in *P. maclareni* (gills/water) and Sr in *C. maclareni* (liver/water) as presented in Table 8. Higher metal concentrations in fish tissues compared to water are indicative of bioaccumulation. BCF were highest for Al, Mn and Sr in gills of all species, whereas Co, Cu and Cd had the highest values in liver for all species. Cu had a very high BCF of 1153 in liver of tilapia U. species compared to *P. maclareni* and *C. maclareni*. Consistent with this study, Abdel-Baki et al. (2011) reported highest concentrations of 11533 ppb (11533 μg/g) and transfer factor of 263.9 for Cu in liver of tilapia species (liver/water) demonstrating trace metal bioaccumulation from water. In this study, BCF values suggest that Cr, Co, Cu, Cd, and U were stored and detoxified in the liver. BCF for Pb between water and tissues of the fish species did not show any clear difference especially for U. species and *C. maclareni*. However, Pb predominantly concentrates within calcified hard tissues (e.g., skeleton and scales) and it mimics Ca^{2+} uptake on the apical surface of the gill epithelium (Rogers et al., 2003). Pb also concentrates to a large extent within blood, gill, and kidney in fish (Mager, 2012). The concentration of Cu in liver of U. species was high, which may reflect feeding on Cu-containing algae. The variations in metal concentrations with fish size (length and weight) by linear regression, showed no significant relationship in *P. maclareni*. U. species showed a significant

Table 7. Trace metal concentrations (mean ± SD, µg/g d.w) in gills and liver of three selected fish species from Lake Barombi Mbo, Cameroon. n= sample size.

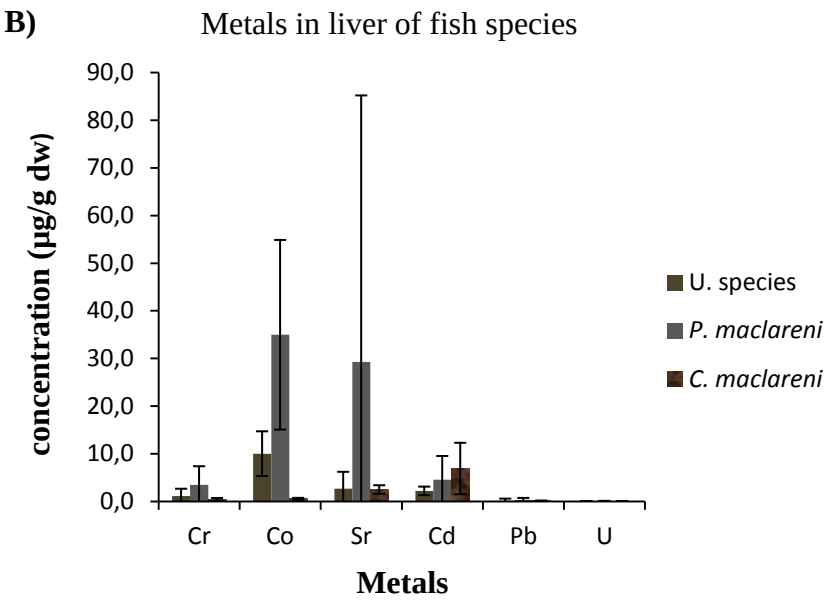
Species	Tissue	N	Al	Cr	Mn	Co	Cu	Sr	Cd	Pb	U
U. species	Gills	20	26.5±23.6	0.91±2.75	213.3±94.5	2.1±1.0	0.99±0.33	336.5±109.1	0.01±0.004	0.15±0.065	0.021±0.008
	Liver	17	-	1.15±1.52	75.5±102.4	10.0±4.7	1153±588	2.71±3.45	2.2±0.91	0.15±0.43	0.024±0.017
P. maclareni	Gills	19	140±199	0.90±1.11	88±23	1.7±0.5	0.19±1.25	402.5±74.1	0.024±0.011	0.45±0.47	0.036±0.013
	Liver	16	-	3.48±3.88	20.9±21.3	35.0±19.9	14±8.1	29.3±55.9	4.53±4.96	0.33±0.43	0.046±0.064
C. maclareni	Gills	15	36.0±36.6	0.26±0.18	66.8±20.2	0.25±0.094	0.99±0.37	208.3±53.4	0.073±0.040	0.12±0.27	0.001±0.0008
	Liver	11	-	0.41±0.30	11.9±4.7	0.54±0.24	46.3±32.3	2.5±0.9	6.9±5.4	0.111±0.068	0.002±0.004
CRM	certified										
DOLT-4	determined		-	**1.4**	-	**0.25**	**31.2±1.1**	**5.5**	**24.3±0.8**	**0.16±0.04**	-

DOLT is a reference material that was used to check if the ICP-MS instrument measured the right value that is certified for DOLT-4. See information values (Appendix 9).

A)


Metals in gills of fish species
concentration (µg/g dw)
450
400
350
300
250
200
150
100
50
0
Al
Cr
Mn
Co
Cu
Sr
Cd
Pb
U
Metals
U. species
P.maclareni
C.maclareni

B)


Metals in liver of fish species
concentration (µg/g dw)
90,0
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
Cr
Co
Sr
Cd
Pb
U
Metals
U. species
P. maclareni
C. maclareni

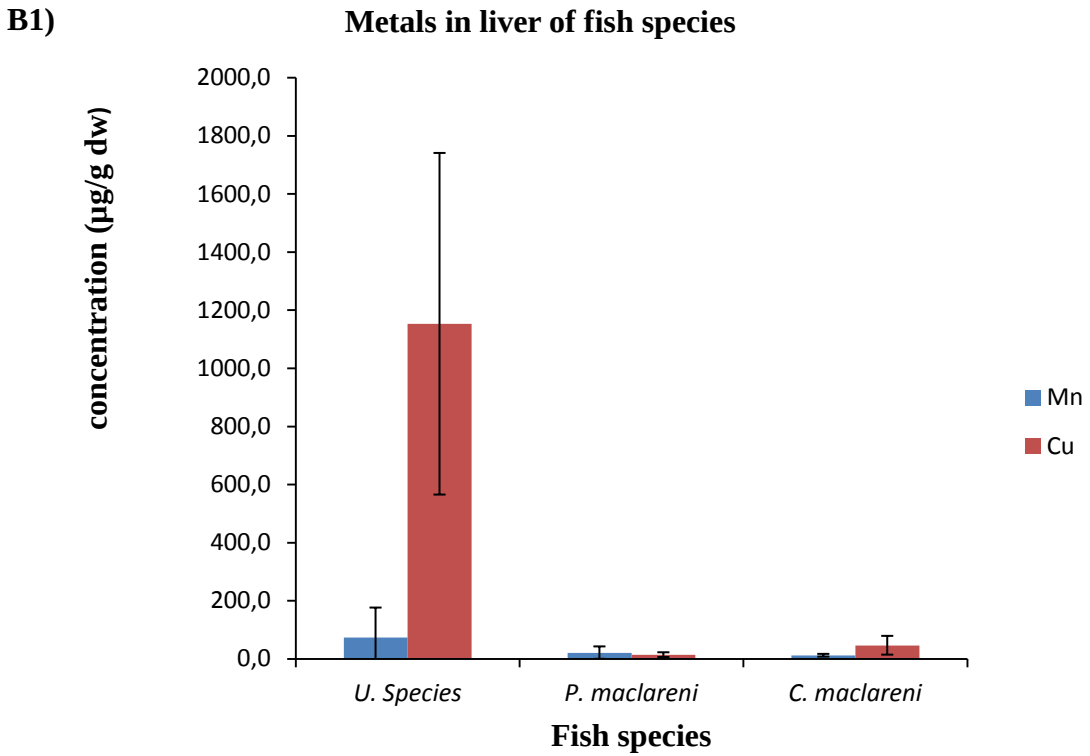


Figure 15. Comparing concentrations of trace metals in tissues (gills and liver) of three selected fish species from Lake Barombi Mbo, Cameroon. A) gills , B) liver, and B1) liver.

relationship between gill- metal concentration and fish size for Al, Cr and Co ($p < 0.05$, Appendix 12). But *C. macalreni* also showed a significant relationship (Appendix 12) between gill- metal concentration and fish size for Co ($p = 0.001$) and Sr ($p = 0.000$). Like several metals (e.g., Cd, Zn, and Pb), Co and Sr appear to specifically target Ca^{2+} channels on the gills through ionic mimicry (Bury et al., 2003**)** by competitive interaction with calcium at active binding sites**.** The gill arch consists of both filament and some calcified bone, and the size of the gill is correlated with the size of the fish. Thus, it is expected that with Sr mimicking Ca, the concentration of both Ca and Sr should increase with increasing fish size. There was also a significant relationship in *C. maclareni* between concentration of Co and weight in liver ($p = 0.023$, Appendix 12). Adeyeye et al. (1996), reported differences in tissue-metal concentrations being species dependent. But, results in this study seem to indicate such differences are influenced by both, the type of metal present and the species of fish exposed to the metal. Türkem et al. (2005) found that concentration of metals was significantly affected by the sampling site and fish species in three commercially valuable fish species. In contrast, Evans et al. (1993) reported that the concentrations of trace metals (Cd, Cu, Mn, Pb) in livers of Atlantic croaker (*Micropogonias undulatus*) increased with fish length. They suggested that the pattern of increase was expected for non essential metals such as Ag, Cd, Hg, and Pb given their poor homeostatic control. Nevertheless, the concentrations of trace metals in this study showed no significant relationship with fish length in liver.

Table 8. Bioconcentration factor (BCF) of trace metals from water into gills and liver of selected fish species from Lake Barombi Mbo. Concentration in tissue in µg/g tissue d.w.

Species	Parameter	Al	Cr	Mn	Co	Cu	Sr	Cd	Pb	U
U. species	Gills/Water	4.6	3.0	85.3	70.7	1	7.8	2.5	3.1	-
	Liver/Water	-	4	29.4	333	1153	0.06	550	3.1	-
P. maclareni	Gills/Water	24.1	3	35.2	56.7	0.2	9.4	5	9.2	-
	Liver/Water	-	11.7	8.4	1167	14	0.7	1125	6.3	-
C. maclareni	Gills/Water	6.2	1	26.7	8.3	1	4.8	17.5	2.5	-
	Liver/Water	-	1.3	4.8	16.7	46.3	0.06	1725	2.3	-

Concentration of Uranium in water was <0.005, this explains the absence of BCF values for Uranium in tissues. There are no BCF values for Al in liver/water because liver samples were stored in Al foil.

Bioconcentration Factor (BCF) = $[M]_{tissue}$ / $[M]_{water}$

Where, $[M]_{tissue}$ is the trace metal concentration in gills and/or liver

$[M]_{water}$ is the trace metal concentration in water.

However, some differences were observed in the concentration of trace metals between tissues sampled. *P. maclareni* had Al and Sr in gill with Cr, Co, Pb, and U in liver all in highest concentrations. On the other hand, U. species had Mn and Cu in highest concentration in their liver, while *C. maclareni* showed highest Cd concentration in liver. BCF analyses also illustrate the same pattern of metal accumulation differences between fish species. Differences in trace metal accumulation and their relationship to fish species could be further explained based on $\delta^{13}C$ and $\delta^{15}N$ values elucidating food sources and trophic levels of the fishes (Fig. 14). The significant variations in $\delta^{13}C$ and $\delta^{15}N$ between fish species indicate different carbon sources and trophic levels within the lake ecosystem. *P. maclareni* having the highest $\delta^{13}C$ (feeding closer to the littoral area) and the highest concentration of Cr, Co, Pb and U in liver, possibly linking the carbon source to the accumulation of metals. Though some trace metals perform essential biological functions by either acting as enzyme cofactors, enhancing glucose metabolism and heme synthesis, very high intakes may pose adverse effects in fish. Copper was found in highest concentration (1153µg/g dry weight) in liver of U. species (Table 7 & Fig. 15 B1)**.** At levels exceeding safe exposure, copper adversely affects fish by changing hematological parameter, disrupting migration and osmoregulation, impairing respiration, survival, growth and reproduction of fish (Hodson et al., 1979; Lwanga et al., 2003). However, such high metal concentrations for essential metals do not necessarily suggest potential adverse effects. Because tissue concentrations of essential elements are internally controlled by homeostasis (Liebscher & Smith, 1968), enzyme systems may maintain their optimal levels (Giesy & Wiener, 1977) and the better regulated the shorter their half lives in tissues (Heath, 1987). Toxic effects occur when homeostatic control is inefficient and detoxification mechanisms are no longer able to offset uptake or storage cells die and the "stored" metal episodic increase blood concentration to cause cardiac arrest among other effects (Heath, 1987).

The concentrations of some selected trace metals (Table 9) reported for brown trout gill-metal accumulation (Rosseland et al., 2007), are compared with fish gill-metal accumulation in this study. This is based on the similarity in water chemistry parameter and also the time of sampling. The pH of 7.4 (Table 3) for Lake Barombi Mbo water samples is presumed neutral or non-acidified as the neutral waters of Lochnagar. Although Lake Barombi Mbo is in the tropics and Lochnagar in the arctic, sampling was carried out during hot and dry periods. The highest mean gill-Al concentration (140 µg/g dw) was in *P. maclareni* and was greater than the maximum gill-Al concentration (108 µg/g dw) for Lochnagar trout. Aluminium is known to be highly gill reactive, impairing physiological functions by disrupting ion regulation, affecting growth and survival in fish (Rosseland et al., 1992; Kroglund et al., 2008). Most importantly, acute toxicity of Al is associated to ion

regulation disturbances at low pH, and chronic toxicity with respiratory disturbances at high pH (Kroglund et al., 2008; Rosseland et al., 2007). With Al concentration of 108 μg/g dw on trout gill, ion regulation was not severed (Rosseland et al., 2007) and at pH > 5.8 with < 300 μg/g dw Al on smolt gill, osmoregulatory capacity was disturbed (Kroglund et al., 2008). Thus, tilapia *P. maclareni* being more tolerant than trout and smolt might exhibit acute ion regulatory disturbances at much higher gill-Al concentrations than 140 μg/g dw. The concentrations of Cr, Cd and Pb were below concentrations reported for trout gill-metal accumulation. Based on fish species tolerance toward tissue metal accumulation it may be possible to set background levels. The effects trace metals relative to background levels in trout can be extrapolated at higher concentrations for more tolerant fish species in this study.

Table 9. Comparing selected gill-metal background concentrations (μg/g dw) in brown trout from Lochnagar with fish species sampled from Lake Barombi Mbo, Cameroon.

Fish species	Al	Cr	Mn	Cu	Cd	Pb	Reference
Brown trout	**108±24**	**3±0.5**	**21±4**	**2±0.1**	**10.5±5**	**19.5±3.5**	Rosseland et al., 2007
U. species	26.5±23.6	0.91±2.75	213±94.5	2.1±1.0	0.01±0.004	0.15±0.065	This study
P. maclareni	140±199	0.90±1.11	88±23	1.7±0.5	0.024±0.011	0.45±0.47	This study
C. maclareni	36±36.6	0.26±0.18	66.8±20.2	0.25±0.09	0.073±0.040	0.12±0.27	This study

Values in Rosseland et al., 2007 have been transformed by a factor of 5 from μg/g ww to μg/g dw for consistency and easy comparison. i.e., 1 μg/g ww = 5 μg/g dw.

The potential for trace metals to persist in the environment, bioaccumulate, and exhibit acute or toxic effects in aquatic biota is of serious concern particularly the non essential metals which are capable of causing deleterious effects even at levels of low exposure especially Cd and Pb. Both metals are reported to adversely affect ion-regulation, survival, growth, reproduction, histopathology and metabolism of most fish species (Lydersen et al., 2002; McGeer et al., 2000a). They are also readily accumulated in the kidney and detoxified by binding to metallothioneins in the liver of fish.

4.4. Mercury (Hg) concentration in fish muscle

The mean concentration of Hg in U. species was 0.0093±0.0010 mg/kg ww, while that in *P. maclareni* and *C. maclareni* were 0.0274±0.0082 mg/kg ww and 0.0266±0.0136 mg/kg ww respectively (Table 10). The total mercury concentration ranged from 0.008 mg/kg wet weight minimum concentration in U. species to 0.062 mg/kg wet weight maximum concentration in *C.*

maclareni. The mean Hg concentration was highest in *C. maclareni* and *P. maclareni* and lowest in U. species (Table 10). Pair wise comparisons (ANOVA) among levels of species for mean concentration of Hg showed no significant difference between *P. maclareni* and *C. maclareni*, though both were significantly different from U. species ($p = 0.000$). Thus, U. species had lower Hg concentration in their muscle tissue than the other fish species sampled.

The low concentrations can be explained based on water chemistry parameters recorded. Low pH and high DOC together enhance mercury uptake by fish and subsequently concentration of Hg (Tadiso et al., 2011; Watras et al., 1998). According to Xun et al. (1987), increasing acidity increases microbial methylation of Hg^{2+}. Spry & Wiener (1991) also acknowledge that in lakes, low pH, low alkalinity and high organic carbon concentrations are conditions which enhance Hg methylation and subsequently increase CH_3Hg^+ burden in fish. In this study a mean neutral pH of 7.4 and total organic carbon (TOC) of 1.56 mg/l (Table 3) is presumed not to greatly influence methylation of Hg and Hg uptake. According to Black et al. (2011), high organic content of freshwater reduce photon influx over microbial influence in the transformation of Hg^{2+} to CH_3Hg^+ and clearer water bodies are more susceptible to photon influx and less microbial influence. With respect to this study, water clarity, neutral pH and low organic matter could possibly explain the low Hg concentration in muscle tissues of all fish species sampled. Ramlal et al. (1993) suggest that low Hg concentration in fish is caused by low sediment fluxes of Hg and low net methylation rates in the cold and clear lakes. Similar to this study, low Hg concentration in muscle tissue of fish are reported by Voegborlo & Akagi (2007).Worth noting, is that the levels of Hg in fish from studied areas of tropical Africa are substantially lower than those recorded in freshwater fish from comparable regions globally (Black et al., 2011).

Table 10. Mean (±SD) of total mercury concentration (mg/kg, w.w) in fish species from Lake Barombi Mbo, Cameroon. n = sample size.

Species	N	Tot-Hg	Range
U. species	20	0.0093±0.001	(0.008, 0.011)
P. maclareni	19	0.0274±0.0082	(0.018, 0.049)
C. maclareni	15	0.0266±0.0136	(0.008, 0.062)

4.5. Relationship between Hg and fish size

The relationship between Hg concentration in muscle tissue and fish size (total length, T_L and total weight, T_W) was investigated by separately regressing, Log Hg against T_L and T_W. There was a

negative significant relationship in U. species for both length ($p=0.010 < 0.05$) and weight ($p=0.032$) but the variation in total Hg concentration explained by both length ($r^2=0.316$) and weight ($r^2=0.230$) was not high (Table 11; Fig. 16a & 16b). Thus, the Hg concentration reflects the phenomenon of Tilapia species which feed on zooplankton as small, and then turn to plant diet as they grow larger (Desta, 2007). In *C. maclareni* the relationship was positive and highly significant for both length ($p=0.000$) and weight ($p=0.000$) with Hg concentration respectively, and the variation in Hg explained by both length ($r^2=0.675$) and weight ($r^2=0.642$) was high. This means that total Hg concentration in muscle tissue increases with increased feeding and increased growth (and thus age) in *C. maclareni*. Conversely, there was no significant relationship between fish size and Hg concentration in *P. maclareni* (Table 11; Fig. 16a & 16b). The variation in total Hg concentration explained by both length ($r^2=0.069$) and weight ($r^2=0.067$) in *P. maclareni* was very low. The relationships between mercury concentrations against total length and total weight were positive, and strongly significant in *C. maclareni* ($p=0.000$), negative and significant in U. species, but not significant in *P. maclareni*.

Table 11. Regression of log-transformed total mercury concentration (log [THg] in mg/kg ww) against total length (L_T), total weight (W_T), $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) for sampled fish species from Lake Barombi Mbo, Cameroon. For each regression, the sample size (n), intercept, slope, r^2, and p-values are

Species	Regression	n	Intercept	Slope	r^2	p-value
U. species	log [THg] vs. L_T	20	-1.89	-0.0095	0.316	**0.010**
	log [THg] vs. W_T	20	-1.93	-0.0013	0.230	**0.032**
	log [THg] vs. $\delta^{15}N$	20	-2.03	-0.0007	0.000	0.949
	log [THg] vs. $\delta^{13}C$	20	-1.96	0.0024	0.001	0.872
P. maclareni	log [THg] vs. L_T	19	-2.01	0.0445	0.069	0.278
	log [THg] vs. W_T	19	-1.72	0.0056	0.067	0.286
	log [THg] vs. $\delta^{15}N$	19	-0.836	-0.0882	0.324	**0.011**
	log [THg] vs. $\delta^{13}C$	19	-2.43	-0.0280	0.390	**0.004**
C. maclareni	log [THg] vs. L_T	15	-2.91	0.0476	0.675	**0.000**
	log [THg] vs. W_T	15	-2.89	0.0071	0.642	**0.000**
	log [THg] vs. $\delta^{15}N$	15	-1.96	0.0335	0.002	0.621
	log [THg] vs. $\delta^{13}C$	15	2.18	0.1210	0.673	**0.000**

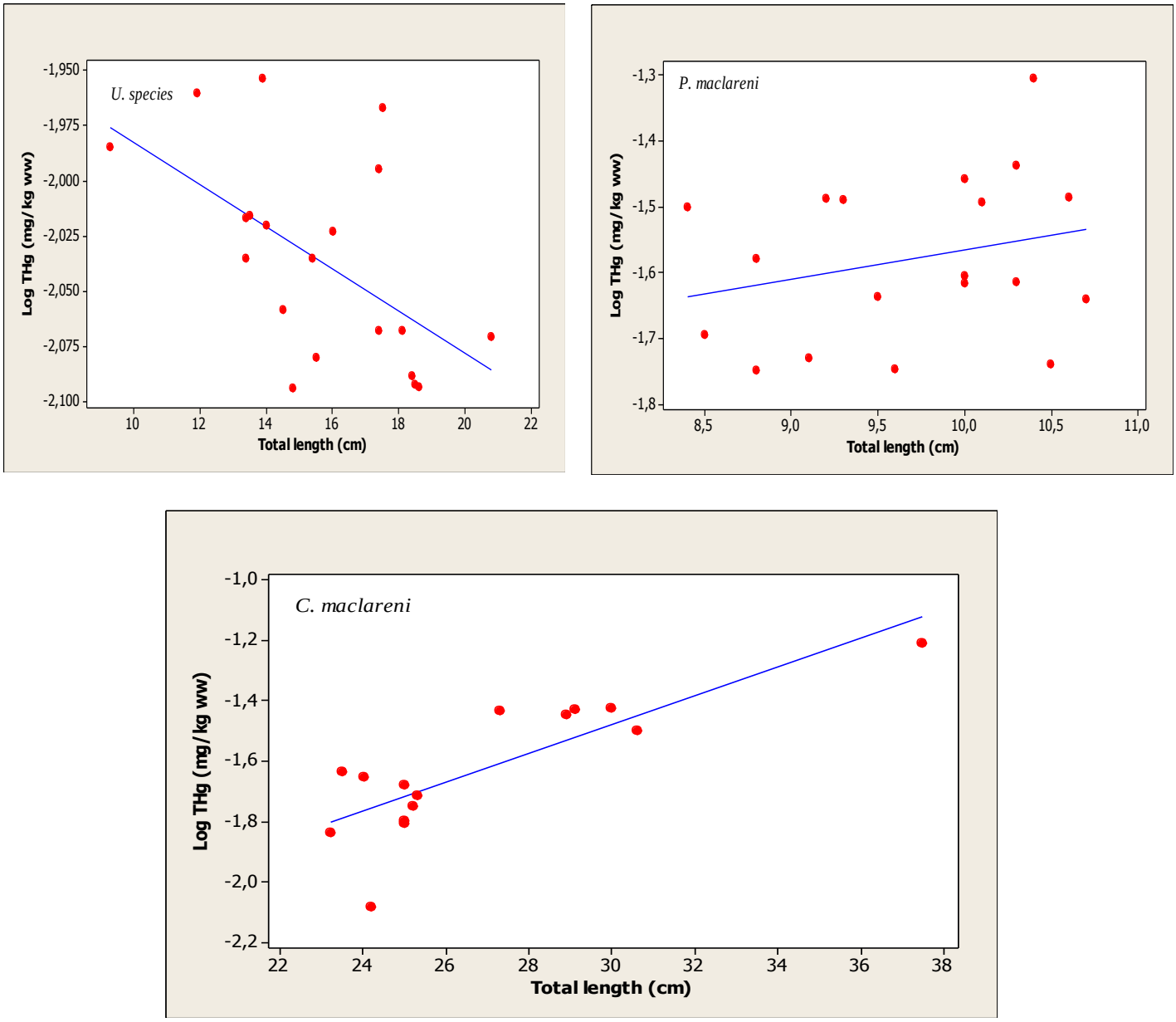


Figure 16a. Log (THg) (mg/kg, ww) vs. total length (cm) in U. species, *P. maclareni*, and *C. maclareni* from Lake Barombi Mbo, Cameroon.

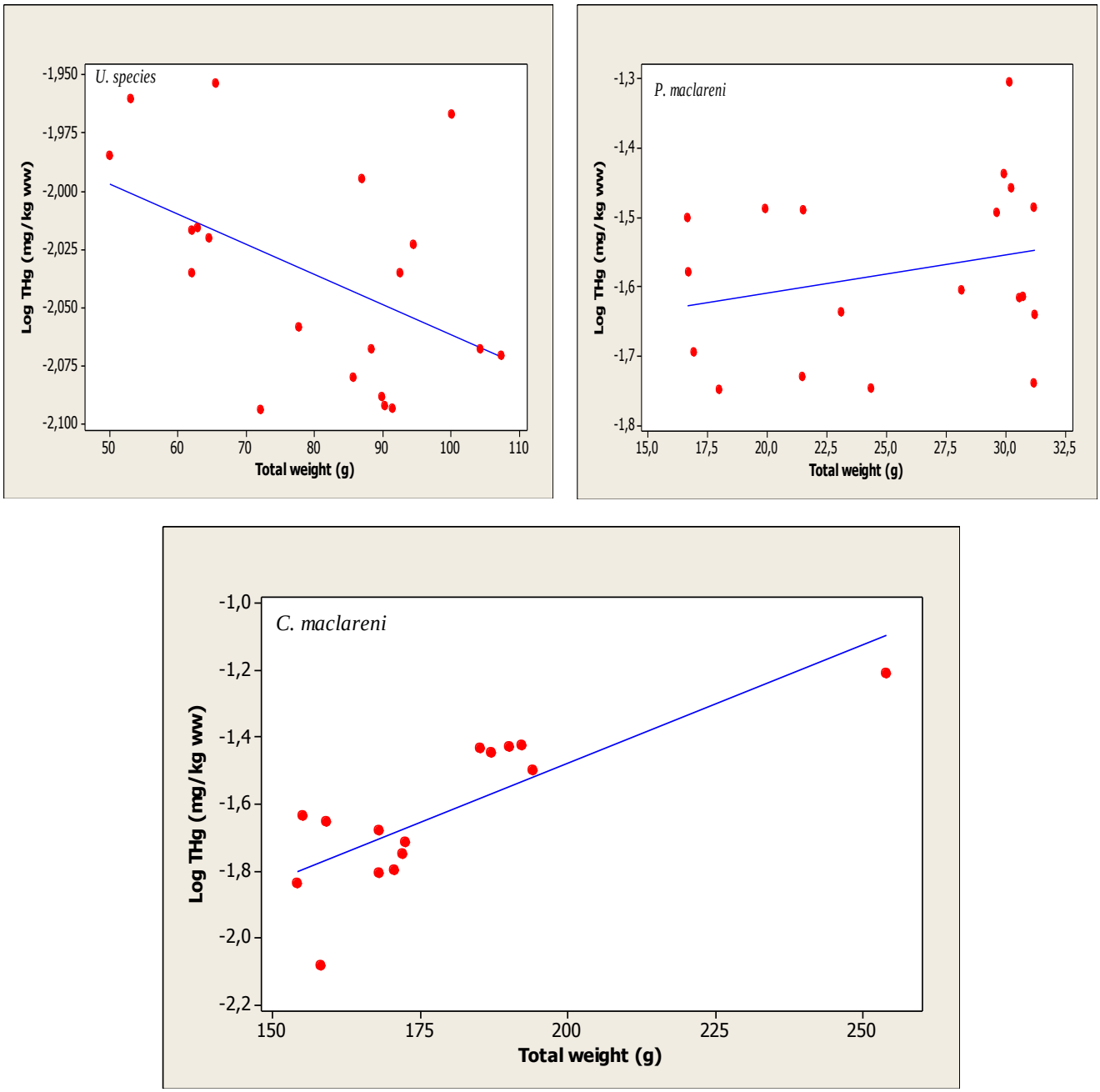


Figure 16b. Log [THg] (mg/kg, ww) vs. total weight (g) in U. species, *P. maclareni*, and *C. maclareni* from Lake Barombi Mbo, Cameroon.

4.6. Relationship between mercury (Hg) concentration and carbon source

Results from Table 5 & 10, showed that *P. maclareni* had the highest mean Hg concentration (0.0274 mg/kg ww) and highest $\delta^{13}C$ (- 30.2 ‰). *C. maclareni* had mean Hg concentration of 0.0266 mg/kg ww and mean $\delta^{13}C$ of -31.5 ‰, meanwhile U. species had mean values of 0.0093 mg/kg ww and -32.9 ‰, for mean Hg concentration and $\delta^{13}C$, respectively. The sequence for Hg concentration

and carbon source revealed that U. species had the lowest Hg concentration and $\delta^{13}C$ values. As a result, the carbon source influenced the Hg concentration in the U. species. Though, U. species showed no significant relationship between Hg concentration and $\delta^{13}C$, *P. maclareni* and *C. maclareni* both showed a significant relationship between Hg concentration and $\delta^{13}C$ (Table 11). While the relationship between Hg and $\delta^{13}C$ was positively correlated in U. species and *C. maclareni*, the relationship was negatively correlated in *P. maclareni*. Mean $\delta^{13}C$ values increased in the order U. species < *C. maclareni* < *P. maclareni*. Thus, U. species had the lowest stable isotope values of all three fish species sampled (Table 5). This suggests that U. species feed distant from the littoral region or within the pelagic region of the lake.

4.7. Relationship between mercury (Hg) concentration and $\delta^{15}N$

With no significant difference in Hg concentration between *P. maclareni* (0.0274 mg/kg ww) and *C. maclareni* (0.0266 mg/kg ww), *C. maclareni* had the highest mean $\delta^{15}N$ (9.9 ‰) . In contrast, U. species with lowest Hg concentration (0.0093 mg/kg ww) had the lowest $\delta^{15}N$ (6.6 ‰) and occupied the lowest trophic position of the aquatic food chain established by the three fish species (Table 10 & Fig. 14). The relationship between Hg concentration and $\delta^{15}N$ was significant ($p = 0.011$) *P. maclareni*, and non significant in both U. species and *C. maclareni*.

Negative correlations (log THg vs. T_L, T_W) were observed in U. species (Table 11). Worth noting, is that while all regressed parameters except for $\delta^{15}N$ vs. T_L were negatively correlated in *P. maclareni*, T_L and T_W were negatively correlated with $\delta^{15}N$ in *C. maclareni*. Tadiso et al. (2011), also observed positive correlation and significant relationship between mercury concentrations with total length and total weight in *T. zilli, C. auratus,* and *C. gariepinus,* (same for *C. maclareni* in this study) but not in *O. niloticus* (same observation with U. species *and P. maclareni* in the present study). With reference to their study, such positive correlations are indicative of Hg concentration being influenced by both bioaccumulation and biomagnification as in *C. maclareni*.

Trewavas et al. (1972) traditionally assessed the trophic positions of the fishes of Lake Barombi Mbo by inferring feeding behaviour and stomach content analysis. But, according to Atwell et al. (1998), such methods are most often just a "snapshot" of feeding habits for a particular season, life history stage, or location and may not necessarily reflect long-term feeding habits of the aquatic biota, which can eventually influence its contaminant uptake and load (e.g. Hg in this study). Additionally, fishes are opportunistic feeders whose diets and trophic levels often change as they grow, and can also vary significantly even among individuals of the same species (Trippel & Beamish, 1993). So based on Trewavas et al. (1972), food web for fishes of Lake Barombi Mbo, the species sampled in this study

represent a subset of the food web and forage on varying food items. However, the use of stable isotope analysis provides a more conventional measure of either the simplicity or complexity of a food web structure (Stapp et al., 1999). Used as a continuous measure of trophic behaviour (Kidd et al., 1995a), the nitrogen isotope ($\delta^{15}N$) serves as a food web descriptor and reflects variations in the underlying food web structure with top predators having the highest ^{15}N enrichment relative to assimilated food (Cabana & Rasmussen, 1994; Zanden et al., 1999). These observations are consistent with the results of this study, as *C. maclareni* exhibited the highest $\delta^{15}N$ and occupied the highest trophic level of all three fish species. Isotope values support Trewavas et al. (1972) trophic positioning of *C. maclareni* as the top level predator of Lake Barombi Mbo. Though there was a significant difference in mean $\delta^{13}C$ values for all the fish species, none of them exhibited a wide variation in $\delta^{13}C$ values (Fig. 14). This could suggest foraging within the same pelagic confine and sharing the same carbon source.

4.8. Biomagnification of mercury

The mean trophic index values for the fish species was 6.6 6 ‰ $\delta^{15}N$ in U. species, 8.4 ‰ $\delta^{15}N$ in *P. maclareni* and 9.9 ‰ $\delta^{15}N$ in *C. maclareni.*

The regression equation:

(Log THg (mg/kg) = - 2.49 + 0.0893 $\delta^{15}N$) . . . **(1)**

depicting trophic biomagnification of THg, revealed an overall biomagnification rate of 0.0893 per $\delta^{15}N$ (‰) **.**

There was a significant difference in the biomagnification rate of the sampled fish species of Lake Barombi Mbo (n=54, r^2 = 0.322, p=0.000) and *C. maclareni* had the highest Hg uptake rate (0.0335) (Table 11 & Fig. 17). Although, the biomagnification rate depicted by the relationship between log (THg), mg/kg ww) and ($\delta^{15}N$, ‰) was significant in *P. maclareni* (n=19, slope=-0.0882, r^2=0.324, p=0.011), there was no significant relationship in U. species (n=20, slope=-0.0007, r^2=0.000, p=0.949) and *C. maclareni* (n=15, slope = 0.0335, r^2=0.002, p=0.621) respectively (Table 11). There was also no evidence of biomagnification with trophic position in U. species (Log (THg) (mg/kg) = - 2.03 - 0.0007 $\delta^{15}N$) and *P. maclareni* (Log THg (mg/kg) = - 0.836 - 0.0882 $\delta^{15}N$), but, *C. maclareni* showed evidence of biomagnification with trophic position (Log THg (mg/kg) = - 1.96 + 0.0335 $\delta^{15}N$). However, the significant linear relationship (n=54, r^2 = 0.322, p = 0.000) between trophic index, $\delta^{15}N$ and THg in fish species (Fig. 17), indicated an overall biomagnification of Hg. The biomagnification rate (0.0893) in this study did not represent the entire food web and neither was it within 0.12 to 0.26 per $\delta^{15}N$, ‰ , reported in some African lakes (Campbell et al., 2003b, 2004;

Kidd et al., 2003) nor within the world wide range (0.11- 0.35 per $\delta^{15}N$, ‰) referred from several studies by Sharma et al (2008).

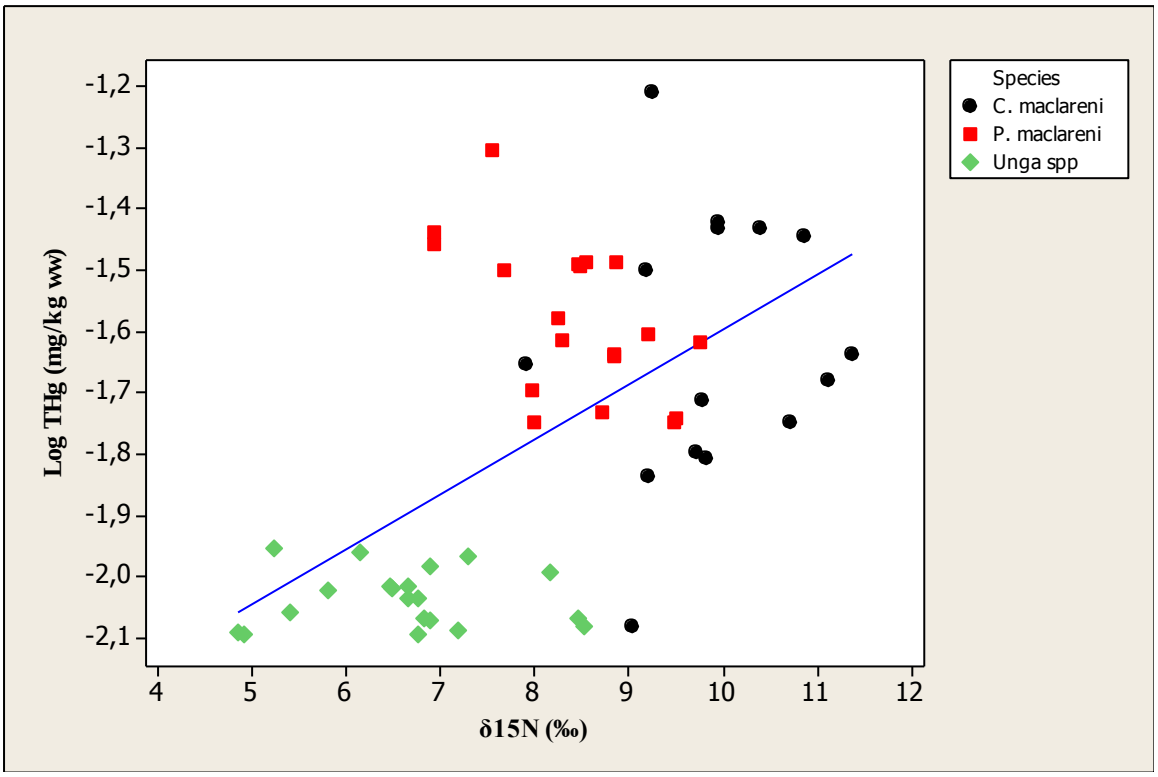


Figure 17. Relationship between trophic level, determined by log-transformed mercury concentrations (log [THg], mg/kg ww) and stable isotope ratios of nitrogen ($\delta^{15}N$, ‰) in fish from Lake Barombi Mbo, Cameroon.

The negative correlation between log (THg) and $\delta^{13}C$ in *P. maclareni*, may be indicative of an effect due to source diet on mercury load as suggested similarly for roach according to Sharma et al. (2008) and tilapia by Desta (2007). The concentration of Hg in *C. maclareni* was very low but had a significant relationship with fish size (length and weight) as also reported for *C. gariepinus* though with no significant relationship to fish length (Desta et al., 2007). The low mercury levels in fish suggest shift in feeding habits associated with low Hg preys, rapid growth and probably rapid Hg depuration. Kidd (2005) confirms that, fish that eat other fish rather than insects or plants are more heavily contaminated. However, the main prey of *C. maclareni* determined from stomach content analysis was mostly aquatic may fly larvae (Trewavas et al., 1972). In this study, the mean Hg concentration of the top level catfish *C. maclareni* (0.0266±0.0136) was very low and similar to levels measured (especially in catfish *C. gariepinus*) from other African lakes (Black et al., 2011; Campbell et al., 2003c, 2006; Desta et al., 2007; Kidd, 2005; Kidd et al., 2004; Tadiso et al., 2011).

4.9. Risk Assessment

World Health Organization (WHO) has given advice to both Cd and Hg on consumption based on Provisional Tolerable Weekly Intake (PTWI) related to body weight and groups at risk (pregnant women and children). WHO (2012), recommend that the level should be to 1.6 μg/kg body weight/week in order to sufficiently protect the developing foetus from neurotoxic effects. The foetus is exposed to Hg through contaminated food eaten by the pregnant mother. This new recommendation changes the prior recommendation for a dietary limit of 3.3 μg/kg body weight/week. With dietary exposure assumed to account for total Hg exposure in humans through fish consumption, a total daily intake (TDI) for a 70 kg person can be estimated to be 16 μg/day ((1.6 μg/kg body weight/week * 70 kg) / 7 days). A PTWI of 1.6 μg/kg body weight/week, means that a person of 70 kg body weight (bw) should not consume more than 560 g of fish muscle if the Hg concentration in that fish is 0.2 mg/kg ww. The mean Hg concentration (0.0093 mg/kg) in U. species which is the most consumed fish from Lake Barombi Mbo, and 0.0266 mg/kg in *C. maclareni* was well below the marketing limit of the European Union (0.5 mg/kg) (FAO/WHO, 2003) and recommended guideline for safe fish consumption (0.2 mg/kg) (WHO, 1990). Thus, a person of 70 kg will have to consume 11760 g muscle of U. species or 4200 g muscle of *C. maclareni* a week to attain 0.2 mg/kg ww of Hg.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

The findings of the present study showed that the concentrations of investigated trace metals in water were below ambient water quality criteria for acute and chronic levels recommended by EPA, CCME, South Africa Water Quality Guidelines and WHO guidelines for aquatic life protection. However, BCF analyses showed evidence of trace metal uptake from water and subsequent bioaccumulation in gills and liver of fish sampled. Accumulation sequence for trace metals in fish tissues sampled varied between species with minor differences between trace metals. The high accumulation of Al, Mn and Sr on gills of the three fish species, indicates that they are bioavailable and probably high in Lake Barombi Mbo. With *P. maclareni* having the highest gill-Al concentration (140 μg/g dw) and given that Al causes ion regulation disturbances affecting growth and survival, this may be a reason why *P. maclareni* is on the IUCN red list for critically endangered species. Total Hg concentration in fish muscles revealed concentrations lower than WHO recommended guidelines of 0.2 mg/kg for groups vulnerable to mercury toxicity and 0.5 mg/kg European Union marketing limit. Also**,** coupled PTWI U. species and *C. maclareni* consumption as protein source will not pose a risk of mercury exposure to people vulnerable to mercury toxicity. In addition, isotopic ratio analyses showed mercury biomagnification along the food web of Lake Barombi Mbo, with *C. maclareni* at the top of the food chain. These findings indicate that though some trace metals may accumulate in tissues of fish at worrisome levels, the general water quality of the lake suggests that trace metals do not pose an immediate threat to the biota. Based on results obtained, this study may provide baseline information on the environmental quality of the lake for future research work.

Finally, it is important that the environmental quality of the lake be monitored constantly especially for the metals Al, Mn, and Sr, which seemed to indicate bioavailability and high presence in Lake Barombi Mbo. This may contribute in preserving the already threatened endemic cichlids especially *P. maclareni* which accumulated most of the metals investigated, in highest concentrations. Also, future work may focus on linking trace metals in tissues with food type ingested by different fish species of Lake Barombi Mbo, Cameroon.

REFERENCES

Adeyeye, E. I., Akinyugha, N.J., Fesobi, M.E., Tenabe, V.O. 1996. "Determination of some metals in Clarias gariepinus (Cuvier and Valenciennes), Cyprino Carpio (L) and Oreochromis niloticus (L) fishes in a Polyculture Freshwater Pond and their Environments." *Aquacult*. **47**, 205-214.

Agah, H., Leermakers, M., Elskens, M., Fatemi, S.M.R., Baeyens, W. (2009). "Accumulation of trace metals in the muscles and liver tissues of five fish species from the Persian Gulf." *Environ. Monit. Assess.* **157**, 499-514.

Alabaster, J.S., and Lloyd, R. 1982. *Water quality criteria for freshwater fish*. 2nd ed. Butterworths, London, pp. 361.

Almeida, J.A., Novelli, E.L.B., Dal-Pai-Silva, M., and Alves-Júnior, R. 2001. Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. *Environ. Pollut*. **114**(2), 169-175.

AMAP. 1998. Assessment report: Arctic pollution issues. Arctic Monitoring and Assessment Programme, Oslo.

Amlund, H., Lundebye, A., and Berntssen, M.H.G. 2007. Accumulation and elimination of methylmercury in Atlantic cod (*Gadus morhua* L.) following dietary exposure. *Aquat. Toxicol*. **83**, 323-330.

Anderson, J.W., and D'Apollonia, S. 1978. Aquatic Animals. *In*: F.W. Fifield and P.J. Haines. ed., Environmental Analytical Chemistry. Blackwell Science Ltd, 2000, pp. 360-392.

Atwell, L., Hobson, K.A., and Welch, H.E. 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. *Can. J. Fish. Aquat. Sci.* **55**, 1114–1121.

Babiarz, C.L., Hurley, J.P., Hoffmann, S.R., Andren, A.W., Shafer, M.M., and Armstrong, D.E. 2001. Partitioning of total mercury and methylmercury to the colloidal phase in freshwaters. *Environ. Sci. Technol*. **35**, 4773-4782.

Baker, J., and Schofield, C. 1982. Aluminium toxicity to fish in acidic waters. *Water Air Soil Pollut*. **18**, 289.

Beaumont, M. W., Butler, P.J., and Taylor, E.W. (1995). "Exposure of brown trout, Salmo trutta, to sub-lethal copper concentrations in soft acidic water and its effects upon sustainedswimming performance." *Aquat. Toxicol.* **33**, 45-63.

Bendell-Young, L.I., Harvey, H.H., and Young, J.F. 1986. Accumulation of cadmium by white suckers, Catastromus commersoni in relation to fish growth and lake acidification. *In:* M.S. Lwanga, F. Kansiime, P. Denny, and J. Scullion (2003). Heavy metals in Lake Georgia, Uganda, with relation to metal concentrations in tissues of common fishes. *Hydrobiologia*. **499**, 83-93.

Benes, P., Cejchanova, M., and Havlik, B. 1985. Migration and speciation of lead in river system heavily polluted from a smelter. *Water Res*. **19**, 1-6.

Berntssen, M.H.G., Aatland, A., and Handy, R.D. 2003. Chronic dietary mercury exposure causes oxidative stress, brain lessions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. *Aquat. Toxicol*. **65**, 55-72.

Biney, C., Amuzu, A.T., Calamari, D., Kaba, N., Mbome, I.L., Naeve, H., Ochumba, P.B.O., Osibanjo, O., Radegonde, V., and Saad, M.A.H. 1994. Review of heavy metals. CIFA Technical Paper 25, Rome: FAO, pp. 33-60.

Black, F.J., Bokhutlo, T., Somoxa, A., Maethamako, M., Modisaemang, O., Kemosedile, T., Cobb-Adams, C., Mosepele, K., and Chimbari, M. 2011. The tropical African mercury anomaly: Lower than expected mercury concentrations in fish and human hair. *Sci. Total. Environ*. **409**, 1967-1975.

Bury, N.R., Walker, P.A., and Glover, C.N. 2003. Nutritive metal uptake in teleost fish. *J. Exp. Biol*. **206**, 11-23.

Cabana, G., and Rasmussen, J.B. 1994. Modelling food chain structure and contaminant biomagnification using stable nitrogen isotopes. *Nature* (Lond.). **372**, 255–257.

Campbell, P.G., 1995. Interactions between trace metals and aquatic organisms: A critique of the free-ion activity model. *In*: Franklin, N.M., Stauber, J.L., Markich, S.J., and Lim, R.P. 2000. pH-dependent toxicity of copper and uranium to a tropical freshwater alga (Chlorella sp.). *Aquat. Toxicol*. **48**, 275–289.

Campbell, L.M., Osano, O., Hecky, R.E., and Dixon, D.G. 2003c. Mercury in fish from three rift valley lakes (Turkana, Naivasha and Baringo), Kenya, East Africa. *Environ. Pollut.* **125**, 281-286.

Campbell, L.M., Osano, O., Hecky, R.E., Dixon, D.G., and Chapman, L.J. 2006. Food web structure and mercury transfer in two contrasting Ugandan highland crater lakes (East Africa). *Afr. J. Ecol*. **44**, 337-346.

CCME (Canadian Council of Ministers of the Environment). 2007. Canadian Water Quality Guidelines for the Protection of Aquatic Life: Summary table. *In*: Canadian Environmental Quality Guidelines, 1999. Winnipeg: Canadian Council of Ministers of the Environment.

CCREM (Canadian Council of Resource and Environment Ministers). 1987. Canadian Water Quality Guidelines. Prepared by the task force on water quality guidelines. Government of Canada, Winnipeg.

Chan, H.M., Scheuhammer, A.M., Ferran, A., Loupelle, C., Holloway, J., and Weech, S. 2003**.** Impacts of Mercury on Freshwater Fish-Eating Wildlife and Humans. *Human Ecological Risk Assess.* **9** (4).

Chapman, G.A. 1978. Toxicities of cadmium, copper and zinc to juvenile stages of chinook salmon and steelhead. *Trans. Am. Fish. Soc*. **107**, 841-847.

Chau, Y. K., Wong, P.T. S., Kramer, O., Bengert, G.A., Cruz, R.B., Kinrade, J.O., Lye, J., and vanLoon, J.C. 1980. Occurence of tetramethyllead compounds in the aquatic environment. *Bull. Environ. Contam. Toxicol*. **24**, 265-269.

Christie, G.L. 2000. Speciation. *In*: F.W. Fifield and P.J. Haines. ed., Environmental Analytical Chemistry. Blackwell Science Ltd, 2000, pp. 309-325.

Davison, W., Buffle, J., and de Vitre, R. 1988. Direct polarographic determination of O_2, Fe(II), Mn(II), S(-II) and related species in anoxic waters. *Pure Appl. Chem*. **60**, 1535-1540.

Demayo, A., Taylor, M. C., and Taylor, K. W. 1982. Toxic effects of lead and lead compounds on human health, aquatic life, wildlife plants, and livestock. *Crit. Rev. Environ. Contr*. **1**, 257-305.

Desta, Z. 2007. Food web structure and mercury transfer pattern in the fish community of Lake Awassa, Ethiopia. In: T.M. Tadiso, R. Borgstrøm, and B.O. Rosseland. 2011. Mercury concentrations are low in commercial fish species of lake Ziway, Ethiopia, but stable isotope data indicated biomagnification. *Ecotoxicol. and Environ Safety.* **74**, 953-959.

Desta, Z., Borgstrøm, R., Rosseland, B.O., and Dadebo, E. 2007. Lower than expected mercury in piscivorous African Sharptooth catfish *Clarias gariepinus* (Burchell). *Sci. Total Environ.* **376**, 134-142.

Driscoll, C.T., and Schecher, W.D. 1990. The chemistry of aluminium in the environment. *J. Environ. Geochem. and Health* **12** (1/2), 28-49.

Driscoll, C.T., Fuller, R.D., and Simone, D.M. 1988. Longitudinal variations in trace metal concentrations in northern forested ecosystem. *J. Environ. Qual*. **17**, 101-107.

Eisenreich, S.J. 1980. Atmospheric input of trace metals to Lake Michigan. *Water Air Soil Pollut.* **13,** 187-301.

Eisler, R. 1988a. Lead hazards to fish, wildlife, and invertebrates: A synoptic review. Biological Report 85 (1.14). U.S. Department of the interior, Fish and Wildlife Service.

Eisler, R. 1986. Chromium hazards to fish, wildlife, and invertebrates: A synoptic review. Biological Report 85 (1.2). U.S. Department of the Interior, Fish and Wildlife Service.

Eisler, R. 1998. Copper hazards to fish, wildlife, and invertebrates: A synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR--1997-0002. pp. 98.

Erickson, R.J., Benoit, D.A., Mattson, V.R., Nelson, H.P.Jr., and Leonard, E.N. 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. *Environ. Toxicol. Chem.* **15**, 181-193.

EPA, 1980. Water Quality Criteria Documents: Availability. United States Environmental Protection Agency, Washington, DC.

EPA, 1985. Water Quality Criteria Documents: Availability. United States Environmental Protection Agency, Washington, DC.

EPA, 1998. Water Quality Criteria Documents: Availability. United States Environmental Protection Agency, Washington, DC.

EPA, 2001. Water Quality Criteria Documents: Availability. United States Environmental Protection Agency, Washington, DC.

EPA, 2004. Water Quality Criteria Documents: Availability. United States Environmental Protection Agency, Washington, DC.

EPA, 2005. Online series. National Recommended Water Quality Criteria. http://water.epa.gov/scitech/swguidance/standards/current/index.cfm

Evans, D.W., Dodoo, D.K., and Hanson, P.J. 1993. Trace metal concentrations in fish liver: Implications of variations with fish size in pollution monitoring. *Mar. Pollut. Bullet*. **26**(6), 329-334.

Ewers, U., and Schlipkoter, H-W. 1991. Intake, distribution and excretion of metals and metal compounds in humans and animals. *In*: Lwanga, M.S., Kansiime, F., Denny, P., and Scullion, J. (2003). Heavy metals in Lake Georgia, Uganda, with relation to metal concentrations in tissues of common fishes. *Hydrobiologia*. **499**, 83-93.

Exley, C., Chappell, J.S., and Birchall, J.D. 1991. A mechanism for acute aluminum toxicity in fish, *In*: Moiseenko,T.I., Sharowa, O.N. (2006). Physiological mechanisms of degradation of fish populations in acidified water bodies.*Russian Journal of Ecology*. **37**(4), 257-263.

FAO/WHO, 2003. Summary and conclusions of the sixty first meeting of the joint FAO/WHO Expert Committee on Food Additives (JECFA), Vol. 2003. Rome: FAO/WHO.

Farag, A. M., Woodward, D. F., Goldstein, J. N., Brumbaugh, W., and Meyer, J. S. 1998. Concentration of metals associated with mining in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River Basin, Idaho. *Arch. Environ. Contam. Toxicol*. **34**, 119-127.

Farag, A. M., Thomas, M., Marty, G. D., Easton, M., Harper, D. D., Little, E. E., and Cleveland, L. 2006. The effect of chronic chromium exposure on the health of chinook salmon (*Oncorhynchus tshawytscha*). *Aquat. Toxicol*. **76**, 246-257.

Fitzgerald, W.F., Engstrom, D.R., Mason, R.P., and Nater, E.A. 1998. The case for atmospheric contamination in remote areas. *Environ. Sci. Technol.* **32**(1), 1-7.

Franklin, N.M., Stauber, J.L., Markich, S.J., and Lim, R.P. 2000. pH-dependent toxicity of copper and uranium to a tropical freshwater alga (Chlorella sp.). *Aquat. Toxicol*. **48**, 275–289.

Frieden, E. 1972. The chemical elements of life. *Sci. Am*. **227**, 52-60.

Förstner, U., and G.T.W. Wittman, 1979. Metal pollution in the aquatic environment. Springer Verlag, Berlin.

Gensemer, R.W., and Playle, R.C. 1999. The bioavailability and toxicity of aluminium in aquatic environments. *Crit. Rev. Environ. Sci. Technol*. **29**, 315-450.

Gey Van Pittius M, Van Vuren JH, Du Preez HH. 1992. Effects of chromium during pH change on blood coagulation in *Tilapia sparrmanii* (Cichlidae) *Comp. Biochem. Physiol. C*. **101**(2), 371–374.

Giesy, J.P.Jr., and Wiener, J.G. 1977. Frequency distributions of trace metals in five freshwater fishes. *Trans. Am. Fish. Soc*. **106** (4), 393-403.

Gounot, A-M. 1994. Microbial oxidation and reduction of manganese: Consequences in groundwater and applications. FEMS *Microbiol. Rev*. **14**(4), 339-349.

Hall, B.D., Bodaly, R.A., Fudge, R.J.P., Rudd, J.W.N., and Rosenberg, D.M. 1997. Food as the dominant pathway of methylmercury uptake by fish. *Water Air Soil Pollut.* **100,** 13-24.

Hammerschmidt, C.R., Sandheinrich, M.B., Wiener, J.G., and Rada, R.G. 2002. Effects of dietary methylmercury on reproduction of fathead minnows. *Environ. Sci. Technol.* **36,** 877-883.

Handy, R.D. 1992. Dietary exposure to toxic metals in fish. *Arch. Environ. Contam. Toxicol*. **22**, 74-85.

Handy, R.D. 1994. The accumulation of dietary aluminium by rainbow trout, *Oncorhynchus mykiss*, at high exposure concentrations. *J. Fish Biol*. **42**, 603-606.

Heath, A.G. 1987. Water Pollution and Fish Physiology. CRC press, 245 pp. Florida, USA..
effects of mercury on survival, behaviour, bioaccumulation and ionic pattern in the catfish (*Clarias lazera*). *Comp. Biochem. Physiol*. **87C**: 303-308.

Hem, J.D. 1985. Manganese. Study and interpretation of the chemical characteristics of natural water. USGS water-supply paper 2254. pp. 84-88.

Hodson, P. V., U. Borgmann, and H. Shear. 1979. Toxicity of copper to aquatic biota. *In*: R. Eisler. 1998. Copper hazards to fish, wildlife, and invertebrates: A synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR--1997-0002. pp. 98.

Holcombe, G.W., Benoit, D.A., Leonard, E.N., and McKim, J.M. 1976. Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Bd. Can.* **33**, 1731-1741.

Ikingura, J.R., and Akagi, H. 2003. Total mercury and methylmercury levels in fish from hydroelectric reservoirs in Tanzania. *Sci. Total Environ*. **304**, 355–368.

Kalay, M., and Canli, M. 2000. Elimination of essential (Cu, Zn) and non-essential (Cd, Pb) metals from tissues of a freshwater fish Tilapia zilli. *Turk J. Zool***. 24,** 429-436

Kidd, K. 2005. Mercury in fish from African lakes. *Nat. Res. Forum*. **29**, 177-178.

Kidd, K., and Batchelar, K. 2012. Mercury. *In*: C.M. Wood, A.P. Farrel, and C.J. Brauner, ed., *Homeostasis and Toxicology of Non-Essential Metals*, Fish Physiology, Vol. 31B. Academic Press, 2012, pp. 238-284.

Kidd, K.A., Hesslein, R.H., Fudge, R.J.P., and Hallard, K.A. 1995*a*. The influence of trophic level as measured by $\delta^{15}N$ on mercury concentration in freshwater organisms. *Water Air Soil Pollut*. **80**, 1011–1015.

Kidd, K.A., Bootsma, H.A., Hesslein, R.H., Lockhart, L., and Hecky, R.E., 2003. Mercury concentrations in the food web of Lake Malawi, East Africa. *Journal of Great Lakes Research,* **29**(2), 258–266.

Kidd, K.A., Stern, G., and Lemoalle, J., 2004. Mercury and other contaminants in fish from Lake Chad (Africa). *Bull. Env. Contam. Toxicol*. **73**(2), 249–256.

Khangarot, B.S., Tripathi, D.M. 1991. Changes in humoral and cell-mediated immune responses and in skin and respiratory surfaces of catfish, *Saccobranchus fossils,* following copper exposure. *Ecotoxicol. Environ. Safety.* **22,** 291-308.

Khunert, P. M., and Khunert, B. R. 1976. The effect of in vivo chromium exposure on Na/K- and Mg-ATPase activity in several tissues of the rainbow trout (*Salmo gairndneri). Bull. Environ. Contam. Toxicol.* **15**, 383-390.

Khunyakari, R.P., Vrushali, T., Sharma, R.N., and Tare, V. 2001. Effects of some Trace Heavy Metals on Poecilia reticulate, *Journal of Environmental Biology*. **22** (2), 141- 144.

Knox, D., Cowey, C.B., Adron, J.W. 1981. The effect of low dietary manganese intake on rainbow-trout (*Salmo gairdneri*). *British Journal of Nutrition*. **46**(3), 495–501.

Kroglund, F., Rosseland, B.O., Teien, H.C., Salbu, B., Kristensen, T., and Finstad, B. 2008. Water quality limits for Atlantic salmon (*Salmo salar* L.) exposed to short term reductions in pH and increased aluminium simulating episodes. *Hydrol. Earth Syst. Sci.* **12**, 491-507.

Krumschnabel, G., and Nawaz, M. 2004. Acute toxicity of hexavalent chromium in isolated teleost hepatocytes. Aqua. Toxicol. **70**, 159–67.

Kumar, P., and Singh, A. 2010. Cadmium toxicity in fish: An overview. GERF *Bulletin of Biosciences*. **1**(1), 41-47.

Kroglund, F., Rosseland, B.O., Teien, H.C., Salbu, B., Kristensen, T., and Finstad, B. 2008. Water quality limits for Atlantic salmon (Salmo salar L.) exposed to short term reductions in pH and increased aluminium simulating episodes. *Hydrol. Earth Syst. Sci.* **12**, 491-507.

Landler, L. 1988. Speciation of metals in water, sediments and soil systems. Springer, Heidelberg. *In*: Siepak, J., Walna, B., Drzymala, S. (eds). Speciation of aluminium released under the effect of acid rain. *Polish Journal of Environmental Studies*. **8**(1), 55-58.

Langard, S., and Norseth, T. 1979. Chromium. *In*: L. Friberg, G.F. Nordberg, and V.B. Vouk, ed., *Handbook on the Toxicology of Metals,* Amsterdam. pp. 383-397. Elsevier/North Holland Biomedical Press, 1979, pp. 383-397.

Lawrence, S.G., Holoka, M.H., Hunt, R.V., and Hesslein, R.H. 1996. Multi-year experimental additions of cadmium to a lake epilimnion and resulting water column cadmium concentrations. *Can. J. Fish. Aquat. Sci*. **53**, 1876-1887.

Lewis, M. 1976. Effects of low concentrations of manganous sulfate on eggs and fry of rainbow trout. Progressive Fish-Culturist, **38**(2), 63–65.

L'Her Roux, L., Le Roux, S., Appriou, P. 1998. Behaviour and speciation of metallic species Cu, Cd, Mn and Fe during estuarine mixing. *Mar. Pollut. Bulletin*. **36**(1), 56–64.

Liebscher, K., and Smith, H. 1968. Essential and non-essential trace elements. *Arch. Environ. Health*. **17**, 881-890.

Limbong, D., Kumampung, J., Rimper, J., Arai, T., and Miyazaki, N. 2003. Emissions and environmental implications of mercury from artisanal gold mining in north Sulawesi, Indonesia. *Sci. Total Environ*. **302**, 227-236.

Luoma, S.N. 1983. Bioavailabilty of trace metals to aquatic organisms-a review. *Sci Total Environ*. **28**, 1-22.

Lwanga, M. S., Kansiime, F., Denny, P., and Scullion, J. (2003). "Heavy metals in Lake Georgia, Uganda, with relation to metal concentrations in tissues of common fishes." *Hydrobiologia.* **499**, 83-93.

Lydersen, E., Löfgren, S., and Arnesen, R.T. 2002. Metals in Scandinavian Surface Waters: Effects of Acidification, Liming, and Potential Reacidification. *Crit. Rev. Environ. Sci. Technol*. **32**(2), 72-295.

Mager, E.M. 2012. Lead. *In*: C.M. Wood, A.P. Farrel, and C.J. Brauner, ed., *Homeostasis and Toxicology of Non-Essential Metals*, Fish Physiology, Vol. 31B. Academic Press, 2012, pp. 186-225.

Malik, N., Biswas, A. K., Qureshi, T. A., Borana, K., Virha, R. 2010. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. *Environ. Monit. Assess.* **160**, 267-276.

Manly, R. 2000. Biological indicators. *In:* F.W. Fifield and P.J. Haines. ed., Environmental Analytical Chemistry. Blackwell Science Ltd, 2000, pp. 280-306.

Masresha, A.E., Skipperud, L., Rosseland, B.O., Zinabu, G.M., Meland, S., Teien, H-C., and Salbu, B. 2011. Speciation of selected trace elements in three Ethiopian Rift Valley lakes (Koka, Ziway, and Awassa) and their major inflows. *Sci. Total Environ*. **409** (19), 3955-3970.

McGeer, J.C., Szebedinszky, C., McDonald, D.G., and Wood, C.M. 2000a. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout. 1: Ionoregulatory disturbance and metabolic costs. *Aquat. Toxicol.* **50,** 231-243**.**

McGeer, J.C., Nigoyi, S., and Smith, D.S. 2012. Cadmium. *In*: C.M. Wood, A.P. Farrel, and C.J. Brauner, ed., *Homeostasis and Toxicology of Non-Essential Metals*, Fish Physiology,Vol. 31B. Academic Press, 2012, pp. 126-168.

Mishra, A. K., and Mohanty, B. 2009. Chronic exposure to sublethal hexavalent chromium affects organ histopathology and serum cortisol profile of a teleost, *Channa punctatus* (Bloch). *Sci. Total Environ*. **407**, 5031-5038.

Moiseenko, T.I., Sharowa, O.N. 2006. Physiological mechanisms of degradation of fish populations in acidified water bodies. *Russian Journal of Ecology* **37**(4), 257-263.

Monette, M. Y., McCormick, S. D. 2007. Impacts of short-term acid and aluminum exposure on Atlantic salmon (*Salmo salar*) physiology: A direct comparison of parr and smolts. *Aqua Toxicol.* **86**, 216–226.

Namminga, H.N., and Wilhm, J. 1976. Effect of high discharge and an oil refinery clean up operation on heavy metals in water and sediments in skeleton creek. Proceedings of the Oklahoma Academy of Science. **56**, 133–138.

Nealson, K.H., Tebo, B.M., and Rosson, R.A. 1988. Occurrence and mechanisms of microbial oxidation of manganese. *In*: Laskin, A.I., ed., Advances in applied microbiology. Vol. 33. London. Academic Press Inc, 1988, pp. 279-368.

Noor El Deen, A.I.E., Mona, M.I., Mohamed, A.E., and Omima, A.A. 2010. Comparative studies on the impact of humic acid and formalin on ectoparasitic infestation in Nile tilapia Oreochromis niloticus. *Nature and Science*. **8**, 121-125.

Nsikak, U.B., Joseph, P. E., Akan, B.W., and David, E. B. 2007. Mercury accumulation in fishes from tropical aquatic ecosystems in the Niger Delta, Nigeria. *In*: N. Malik, A.K. Biswas, T.A. Qureshi, K. Borana, and R. Virha. 2010. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. *Environ. Monit. Assess*. **160**, 267–276.

Nyberg, P., Andersson, P., Degerman, E., Borg, H., and Olofsson, E. 1995. Labile inorganic manganese — An overlooked reason for fish mortality in acidified streams? *Water, Air Soil Pollut.* **85**(2), 333–340.

Okada, I.A., Sakuma, A.M., Maio, F.D., Dovidemskas, S., and Zenebon, O. 1997. Evaluation of lead and cadmium in milk due to environmental contamination in Paraiba Valley region of South Eastern Brazil. Revista-de-Saude-Publication. **31**, 140-143.

Olsson, P.E. and Hogstrand, C. 1987. Subcellular distribution and binding of cadmium to metallothionein in tissues of rainbow trout after exposure to ^{109}Cd via the water. *Environ. Toxicol. Chem*. **6**, 867-874.

Olowu, R.A., Ayejuyo, O.O., Adewuyi, G.O., Adejoro, I.A., Denloye, A.A.B., Babatunde, A.O., and Ogundajo, A.L. 2010. Determination of Heavy Metals in Fish Tissues,Water and Sediment from Epe and Badagry Lagoons, Lagos, Nigeria. *E-J. Chem*. **7(**1**)**, 215-221.

Pelgrom, S.M.G.J., Lock, R.A.C., Balm, P.H.M., and Wendelaar Bonga, S.E. 1995. Integrated physiological response of tilapia, *Oreochromis mossambicus*, to sublethal copper exposure. *Aquat. Toxicol*. **32**, 303-320.

Peuranen, S., Keinänen, M., Tigerstedt, C., and Vuorinen, P.J. 2003. Effects of temperature on the recovery of juvenile grayling (Thymallus thymallus) from exposure to Al + Fe. *Aquat. Toxicol*. **65**, 73-85.

Pickering, Q.H., and Gast, M.H. 1972. Acute and chronic toxicity of cadmium to the fathead minnow (*Pinephales promelas*). *J. Fish. Res. Bd Can*. **29**, 1099-1106.

Poléo, A.B.S., Lydersen, E., and Muniz, I.P. 1991. The Influence of Temperature on Aqueous Aluminum Chemistry and Survival of Atlantic Salmon (Salmo salar, L.) Fingerlings. *In*: T.I. Moiseenko and O.N. Sharowa (2006). Physiological mechanisms of degradation of fish populations in acidified water bodies. *Russian Journal of Ecology*. **37**(4), 257-263.

Pratap, H.B., and Wendelaar Bonga, S.E. 1993. Effect of ambient and dietary cadmium on pavement cells, chloride cells, and Na^+/K^+-ATPase activity in the gills of the freshwater teleost *Oreochromis mossambicus* at normal and high calcium levels in the ambient water. *Aquat. Toxicol*. **26**(1-2), 133-149.

Praveena, S. M., Radojevic, M., Abdullah, M. H. and Avis, A. Z. 2007. Factor-cluster analysis and enrichment study of mangrove sediments – An example from Mengkabong Sabah. *In*: F.E. Olubunmi and O.E. Olorunsola (2010). Evaluation of the Status of Heavy Metal Pollution of Sediment of Agbabu Bitumen Deposit Area, Nigeria. *European Journal of Scientific Research*. **41**(3), 373-382.

Ramlal, P.S., Bugenyi, F.W.B., Kling, G.W., Nriagu, J.O., Rudd, J.W.M. and Campbell, L.M. 2003. Mercury concentrations in water, sediment and biota from Lake Victoria, East Africa. *Journal of Great Lakes Research*. **29**, 283–291.

Reid, G.M. 1995. Threatened fishes of the world: *Pungu maclareni* (cichlidae). *Environ. Biol. Fishes*, **43**, 362.

Reid, S.D. 2012. Molybdenum and Chromium. *In*: C.M. Wood, A.P. Farrel, and C.J. Brauner, ed., *Homeostasis and Toxicology of Essential Metals*, Fish Physiology, Vol. 31A. Academic Press, 2012, pp. 376-406.

Rogers, J.T., and Wood, C.M. 2004. Characterization of branchial lead-cadmium interaction in the freshwater rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol*. **207**, 813-825.

Rogers, J.T., Richards, J.G., and Wood, C.M. 2003. Ionoregulatory disruption as the acute toxic mechanism for lead in the rainbow trout (*Oncorhynchus mykiss). Aquat. Toxicol. **64**, 215-234.*

Rogers, J.T., Patel, M., Gilmour, K.M., and Wood, C.M. 2005. Mechanisms behind Pb- induced disruption of Na+ and Cl^- balance in rainbow trout (*Oncorhynchus mykiss). Am. J. Physiol. Regul. Integr. Comp. Physiol. **289**, R463.R472.*

Romeo, M., Siau, Y., Sidoumou, Z., and Gnassia-Barelli, 1999. Heavy metal distribution in different fish species from the Mauritania coast. *In*: A. Rauf, M. Javed, and M. Ubaidullah (2009). Heavy metal levels in three major carps (*Catla catla, Labeo rohita* and *Cirrhina mrigala*) from the River Ravi, Pakistan. *Pakistan Vet. J*. **29**(1), 24-26.

Rosseland, B.O., Eldhuset, T.O., and Staurnes, M. 1990. Environmental Effects of Aluminum, *Environ. Geochem. Health*. **12**, 17–27.

Rosseland, B.O., Blakar, L.A., Bulger, A., et al. 1992.The mixing zone between limed and acid river waters: Complex aluminum chemistry and extreme toxicity for salmonids, *Environ. Pollut.* **78**, 3–8.

Rosseland, B. O. and Staurnes, M. 1994. Physiological mechanisms for toxic effects and resistance to acidic water: An ecophysiological and ecotoxicological approach. Pp. 227-246. In: Steinberg, C. E.W. &Wright, R. W. (eds.) Acidification of Freshwater Ecosystems: Implications for the future. John Wiley & Sons, Ltd. ISBN 0-471-94206-5.

Rosseland, B.O., Massabaua, J.-C., Grimalt, J., Hofer, R., Lackner, R., Raddum, G., Rognerud, S., and Vives, I. 2001. Fish Ecotoxicology: The European Mountain Lake Ecosystem Regionalisation, DiaGnostic and Socio-economic Evaluation (EMERGE)Fish Sampling Manual for Live Fish. Norwegian Institute of Water Research, Oslo < http://www.mountain-lakes.org/emerge/methods/29.pdf>.

Rosseland, B.O., Rognerud, S., Collen, P., Grimalt, J., Vives, I., Massabuau, J-C., Hofer, R., Lackner, R., Fjellheim, A., Harriman, R., and Pina, B. 2007. Brown trout in Lochnagar. Population and contamination by metals and inorganic micropollutants. *In*: Rose, N. (Ed): Lochnagar: The Natural History of a Mountain Lake. Developments in Paleoenvironmental Research (DPER) Volume 12, page 247-279. ISBN 1-4020-3900-X, 2007.

Rytuba, J. 2003. Mercury from mineral deposits and potential environmental impact. *Environ. Geol.* **43**, 326-338.

Salbu, B., and Oughton, D.H. 1995. Trace element in natural waters. *In*: H.C. Teien, B. Salbu, F. Kroglund, B.O. Rosseland (2004). Transformation of positively charged aluminium-species in unstable mixing zones following liming. *Sci. Total Environ*. **330** (1-3), 217-232.

Sayer, M.D.J., Reader, J.P., and Morris, R. 1989. The effect of calcium concentration on the toxicity of copper, lead and zinc to yolk-sac fry of brown trout, *Salmo trutta* L., in soft acid water. *J. Fish Biol*. **35**, 323-332.

Schlindler, D.W. 1988. Effects of acid rain on freshwater ecosystems. *Science*. **239**, 149-157.

Settle, D.M., and Patterson, C.C. 1980. Lead in albacore: guide to lead pollution in Americans. *Science.* **207**, 1167-1176.

Sharma, C.M., Borgstrøm, R., Huitfeldt, J.S., and Rosseland, B.O. 2008. Selective exploitation of large pike Esox lucius- Effects on mercury concentrations in fish populations. *Sci. Total Environ*. **399**, 33-40.

Shaw, T.L., and Brown, V.M. 1974. The toxicity of some forms of copper to rainbow trout. *Water Res*. **8**, 377-382.

Skeaff, J.M., Dubreuil, A.A.,and Brigham, S.I. 2002. The concept of persistence as applied to metals for aquatic hazard identification. *Environ. Toxicol. Chem.* **21,** 2581-2590.

South Africa Water Quality Guidelines. 1996. South Africa Water Quality Guidelines. Vol. 7. Aquatic Ecosystems (2nd ed.). Department of Water Affairs and Forestry, Water Quality Mangement, Pretoria.

Spear, P.A., and Pierce, R.C. 1979. Copper in the aquatic environment. Chemistry, distribution and toxicology. National Research Council of Canada, Publ. No. NRCC 16454.

Sprague, J. B. 1985. Factors that modify toxicity. In: Rand, G., and Petrocelli, S.R. (eds.) Fundamentals in aquatic toxicology. pp. 124–163. Hemisphere, Washington, DC., USA.

Spry, D.J., and Wiener, J.G. 1991. Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. *Environ. Pollut.* **71**, 243-304.

Stubblefield WA, Brinkman SE, Davies PH, Garrison TD (1997) Effects of water hardness on the toxicity of manganese to developing brown trout (*Salmo trutta*). *Environ.Toxicol. Chem.* **16**(10):2082–2089.

Svobodova, Z., B. Vykusova, and J. Machova. 1994. The effects of pollutants on selected haematological and biochemical parameters in fish. pp. 39-52. *In:* R Muller and R. Lloyd (eds). Sublethal and chronic effects of pollutants on freshwater fish. FAO. Blackwell Science Ltd.

Tadiso, T.M., Borgstrøm, R., and Rosseland, B.O. 2011. Mercury concentrations are low in commercial fish species of lake Ziway, Ethiopia, but stable isotope data indicated biomagnification. *Ecotoxicology and Environ Safety.* **74**, 953-959.

Teien, H. C., Salbu, B., Krogland, F., Rosseland, B. O. 2004. Transformation of positively charged aluminium species in unstable mixing zones following liming. *Sci. Total Environ.* **330**, 217-232.

Teien, H.C., Kroglund, F., Salbu, B., and Rosseland, B.O. 2006. Gill reactivity of aluminium-species following liming. *Sci. Total Environ*. **358**, 206-220.

Thaker, J., Chhaya, J., Nuzhat, S,. and Mittal, R. 1996. Effects of chromium(VI) on some ion-dependent ATPases in gills, kidney and intestine of a coastal teleost *Periophthalmus dipes*. *Toxicol*. **112**, 237–244.

Thornton, I. 1995. *Metals in the Global Environment: Facts and Misconceptions.* International Council on Metals and the Environment (ICME), Ottawa, http://www.icmm.com/library

Towill, L.E., Shriner, C.R., Drury, J.S., Hammons, A.S., and Holleman, J.W. 1978. *Reviews of the Environmental Effects of Pollutants: III. Chromium.* Report 600/1-78-023. United States Environmental Protection Agency, Washington, DC.

Trewavas, E., Green, J., and Corbet, S.A. 1972. Ecological studies on crater lakes in West Cameroon: Fishes of Barombi Mbo. *Proc. Zool*. Soc. Lond. **167**, 41-95.

Türkmen, A., Türkmen, M., Tepe, Y., and Akyurt, I. 2005. Heavy metals in three commercially valuable fish species from Iskenderum Bay,Northern East Mediterranean Sea, Turkey. *Food Chem*. **91**(1), 167-172.

Van Hoof, F., and Van San, M. 1981. Analysis of Cu, zinc, cadmium and chromium in *Platichthys flesus* L. and its effect on plasma electrolyte concentrations. *J. Fish. Biol*. **20**, 491-500.

VanLoon, G.W., and Duffy, S. J. 2011. ed. Environmental Chemistry: A global perspective. Oxford University Press. pp. 545.

Van der Putte, I., Lubbers, J., and Kolar, Z. 1981. Effect of Ph on uptake, tissue distribution and retention of hexavalent chromium in rainbow trout (*Salmo gairdneri*). *Aquat. Toxicol*. **1**, 3-18.

Van der Putte, I., Brinkhorst, M. A., and Koeman, J. H. 1981a. Effect of pH on the acute toxicity of hexavalent chromium to rainbow trout (*Salmo gairdneri*). *Aquat. Toxicol*. **1**, 129-142.

Van der Putte, I., Laurier M. B. H. M., and Van Eijk G. J. M. 1982. Respiration and osmoregulation in rainbrow trout (*Salmo gairdneri*) exposed to hexavalent chromium at different pH values. Aquat Toxicol. **2**, 99–112.

Velz, C,L. 1984. Applied stream sanitation (ed.,). New York, Wiley.

Vincent, S., Ambrose, T., Cyrill, L., Selvanaygam, M. 1995. Biochemical responses of the Indian Major carp, *Catla catla* (Ham.) to chromium toxicity. *Indian J. Environ Health*. **37**, 192–196.

Voegborlo, R.B., and Akagi, H. 2007. Determination of mercury in fish by cold vapour atomic absorption spectrometry using an atomic mercury analyzer. *Food Chemistry*, **100** (2), 853-858.

Ward, N. I. 2000. Trace elements. *In*: F.W. Fifield and P.J. Haines. ed., Environmental Analytical Chemistry. Blackwell Science Ltd, 2000, pp. 360-392.

Wepener, V., Van Vuren, J.H.J., Du Preez, H.H. 1992. Effect of manganese and iron at a neutral and acidic pH on the hematology of the banded tilapia (*Tilapia sparrmanii*). *Bullet. Environ. Contam. Toxicol*. **49**, 613–619.

Westcott, K., and Kalff, J. 1996. Environmental factors affecting methylmercury accumulation in zooplankton. *Can. J. Fish. Aquat. Sci.* **53**, 2221-2228.

WHO, 1990. World Health Organization. IPCC Environmental Health Criteria 101: Methylmercury. International Programme of Chemical Safety. Geneva, Switzerland.

WHO, 2006. Guidelines for Drinking Water Quality, 2nd edn. Vol. 2. Health Criteria and other supporting information. World Health Organization, Geneva.

WHO 2012. World Health Organization. UN Committee recommends new dietary intake limits for mercury. http://www.who.int/mediacentre/news/notes/2003/np20/en/

Wiener, J.G., Krabbenhoft, D.P., Heinz, G.H., and Scheuhammer, A.M. 2003. Ecotoxicology of mercury. *In*: C.M. Wood, A.P. Farrel, and C.J. Brauner, ed., *Homeostasis and Toxicology of Non-Essential Metals*, Fish Physiology, Vol. 31B. Academic Press, 2012, pp. 238-284.

Wilson, R.W. 2012. Aluminium. *In*: C.M. Wood, A.P. Farrel, and C.J. Brauner, ed., *Homeostasis and Toxicology of Non-Essential Metals*, Fish Physiology, Vol. 31B. Academic Press, 2012, pp. 68-104.

Wilson, R.W., and E.W. Taylor. 1993. The physiological responses of freshwater rainbow trout, *Oncorhynchus mykiss*, during acutely lethal copper exposure. *J. Comp. Physiol. B.* **163**, 38-47.

Wong, P.T.S., Chau, Y.K., Kramer, O., and Bengert, G.A. 1981. Accumulation and depuration of tetramethyllead by rainbow trout. *Water Research*. **15**, 621-625.

Wren, C.D., and Stephenson, G.L. 1991. The effect of acidification on the accumulation and toxicity of metals to freshwater invertebrates. *Environ. Pollut*. **71**(2-4), 205-241.

Xun, L., Campbell, N.E.R., and Rudd, J.W.M. 1987. Measurements of specific rates of net methylmercury production in the water column and surface sediments of acidified and circumneutral lakes. *Can. J. Fish Aquat. Sci.* **44**, 750-7.

Yamamoto, H., Satoh, S., Takeuchi, T., Watanabe, T. 1983. [Effects on rainbow trout of deletion of manganese or trace elements from fish meal diet. *Bullet. Japanese Soc. Sci. Fish.* **49**(2), 287–293.

Zanden, M., Vander, J., and Rasmussen, J.B. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. *Ecol*. **80**, 1395-1404.

APPENDIX

APPENDIX 1: Triplicate readings for water parameters at different locations in field.

Location	pH	Temperature (℃)	TDS (mg/l)	EC (mS/m)
Littoral 1	7.62	30.8	20	5
Littoral 1	7.60	30.6	20	4
Littoral 1	7.51	30.6	20	4
Littoral 2	7.58	30.5	20	4
Littoral 2	7.52	30.3	20	4
Littoral 2	7.49	30.6	20	4
Littoral 3	7.50	30.7	20	4
Littoral 3	7.50	30.6	20	4
Littoral 3	7.49	30.3	20	4
Littoral 4	7.33	30.5	20	4
Littoral 4	7.33	30.6	10	4
Littoral 4	7.31	30.3	20	4
Littoral 5	7.31	30.7	20	4
Littoral 5	7.28	30.5	20	4
Littoral 5	7.29	30.6	20	4
Littoral 6	7.28	30.6	20	4
Littoral 6	7.29	30.3	20	4
Littoral 6	7.65	30.4	20	4
Littoral 7	7.65	30.3	20	4
Littoral 7	7.63	30.5	20	4
Littoral 7	7.60	30.6	20	4
Littoral 8	7.61	30.3	20	4
Littoral 8	7.61	30.5	20	4
Littoral 8	7.60	30.5	20	4
Littoral 9	7.61	29.8	20	4
Littoral 9	7.61	30.2	20	4
Littoral 9	7.65	30.1	20	4
Littoral 10	7.62	30.2	20	4
Littoral 10	7.48	29.4	20	4
Littoral 10	7.44	29.6	20	4
In lake 11	7.18	29.2	20	4
In lake 11	7.05	29.6	20	4
In lake 11	7.04	29.6	20	4
In lake 12	7.08	29.5	20	4
In lake 12	7.05	29.2	20	4
In lake 12	7.04	29.0	20	4

APPENDIX 2: Electrical conductivity (EC) and total dissolved solid (TDS) values at different locations of the lake.

Date	Locations					
03/01/12	Littoral		In lake		Outlet	
	EC mS/cm	TDS g/L	EC mS/cm	TDS g/L	EC mS/cm	TDS g/L
	0.04	0.02	0.04	0.02	0.05	0.02
	0.04	0.02	0.04	0.02	0.05	0.02
	0.04	0.02	0.04	0.02	0.04	0.02
	0.04	0.02	0.04	0.02	0.04	0.02
	0.04	0.02	0.04	0.02	0.04	0.02
	0.04	0.02	0.04	0.02	0.04	0.02
	0.04	0.02	0.04	0.02	0.04	0.02
	0.04	0.02	0.04	0.02	0.04	0.02
	0.04	0.02	0.04	0.02	0.04	0.02
	0.04	0.02	0.04	0.02	0.04	0.02

APPENDIX 3: pH and Temperature of composite water samples from different locations of the lake

Sample No	GPS Coordinates	GPS Elevation (m)	Barometric reading (mmHg)	Altitude (m)	pH	Temperature (°C)
1	4°08′59.8N	408	731	239±5.0	7.28	30.5
	9°17′05.8E				7.29	30.3
					7.31	30.4
					7.30	30.4
2	4°08′59.8N	408	730	249±5.0	7.23	29.8
	9°17′05.8E				7.19	29.4
					7.20	29.7
					7.21	29.2
3	4°08′59.8N	408	730	255±5.0	7.48	30.4
	9°17′05.8E				7.50	30.2
					7.47	30.0
					7.43	29.9
4	4°39′08.3N	332	729	260±5.0	7.59	30.6
	9°24′32.7E				7.60	30.5
					7.63	30.6
					7.61	30.3

APPENDIX 4: Sizes of individual fish species sampled from Lake Barombi Mbo, Cameroon.

Year 2011	No	Species	Length (cm)	Weight (g)
12/12/11	1	Unga	15.5	85.6
12/12/11	2	Unga	16.0	94.4
12/12/11	3	Unga	18.5	90.2
12/12/11	4	Unga	20.8	107.4
12/12/11	5	Unga	18.6	91.4
13/12/11	6	Unga	18.4	89.8
13/12/11	7	Unga	17.4	87.0
13/12/11	8	Unga	17.4	88.3
13/12/11	9	Unga	14.5	77.7
14/12/11	10	Unga	18.1	104.3
14/12/11	11	Unga	15.4	92.5
14/12/11	12	Unga	11.9	53.0
14/12/11	13	Unga	9.3	49.9
15/12/11	14	Unga	13.4	62.0
15/12/11	15	Unga	14.0	64.5
15/12/11	16	Unga	13.5	62.8
15/12/11	17	Unga	14.8	72.0
15/12/11	18	Unga	13.4	62.0
16/12/11	19	Unga	13.9	65.4
16/12/11	20	Unga	17.5	100.0

Year 2011	No	Species	Length (cm)	Weight (g)
16/12/11	1	*Pungu*	10.0	28.1
16/12/11	2	*Pungu*	9.5	23.0
19/12/11	3	*Pungu*	9.2	19.9
19/12/11	4	*Pungu*	10.3	29.8
19/12/11	5	*Pungu*	10.4	30.1
19/12/11	6	*Pungu*	8.4	16.6
22/12/11	7	*Pungu*	10.3	30.7
22/12/11	8	*Pungu*	8.8	17.9
22/12/11	9	*Pungu*	9.3	21.5
23/12/11	10	*Pungu*	8.5	16.9
23/12/11	11	*Pungu*	10.1	29.6
23/12/11	12	*Pungu*	10.5	31.1
27/12/11	13	*Pungu*	10.6	31.1
27/12/11	14	*Pungu*	10.0	30.2
27/12/11	15	*Pungu*	8.8	16.6
27/12/11	16	*Pungu*	10.7	31.2
27/12/11	17	*Pungu*	9.1	21.4
27/12/11	18	*Pungu*	10.0	30.5
27/12/11	19	*Pungu*	9.6	24.3

Year 2011	No	Species	Length (cm)	Weight (g)
16/12/11	1	*Clarias*	23.2	154.0
16/12/11	2	*Clarias*	30.0	192.0
19/12/11	3	*Clarias*	28.9	187.0
19/12/11	4	*Clarias*	25.3	172.4
19/12/11	5	*Clarias*	27.3	185.0
21/12/11	6	*Clarias*	30.6	194.0
21/12/11	7	*Clarias*	29.1	190.0
21/12/11	8	*Clarias*	25.1	170.5
21/12/11	9	*Clarias*	23.5	155.0
21/12/11	10	*Clarias*	25.0	168.0
22/12/11	11	*Clarias*	24.0	159.0
22/12/11	12	*Clarias*	25.0	167.9
23/12/11	13	*Clarias*	24.2	158.1
23/12/11	14	*Clarias*	37.5	254.0
23/12/11	15	*Clarias*	25.2	171.9

APPENDIX 5: ICP-MS and ICP-OES concentration of trace metals in water sample fractions from Lake Barombi Mbo, Cameroon.

Water Fractions	Al	Cr	Mn	Co	Ni	Sr	Cd	Ba	Pb	U	Fe	Ni	Zn
	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L
LBM Raw Water 1	4,854	0,273	2,083	0,041	1,202	39,418	0,01	13,5	0,1	0,002	0,0201		0,00
LBM Raw Water2	6,088	0,411	2,815	0,030	0,510	48,707	0,00	16,5	0,0	0,001	0,0197		0,00
LBM Raw Water 3	6,597	0,299	2,612	0,031	0,388	40,880	0,00	14,0	0,0	0,000	0,0208		0,00
LBM Raw Water 4	6,458	0,484	0,064	0,014	0,791	0,016	0,01	0,0	0,8	0,001	0,0185		0,00
LBM Chelex filtrate 1	3,615	0,266	0,432	0,012	0,334	0,083	0,00	0,3	0,0	0,000	0,01	0,01	0,00
LBM Chelex filtrate 2	3,532	0,348	0,480	0,012	0,358	0,055	0,00	0,3	0,0	0,000	0,01	0,01	0,00
LBM Chelex filtrate 3	2,983	0,171	0,398	0,015	0,542	0,085	0,00	0,3	0,1	0,001	0,01	0,01	0,00
LBM Chelex filtrate 4	1,172	0,173	0,126	0,013	0,503	33,798	0,00	10,9	2,9	0,002	0,01	0,01	0,01
LBM Chelex filtrate 1'	2,545	0,144	0,295	0,009	0,242	0,026	0,00	0,2	0,0	0,000	0,01	0,01	0,00
LBM Chelex filtrate 2'	2,9	0	0,4	0,0	0,3	0,06	0,01	0,2	0,0	0,000	0,01	0,01	0,00
LBM Chelex filtrate 3'	5,50	0,22	0,41	0,01	0,32	0,07	0,00	0,29	0,04	0,000	0,02	0,01	0,00
LBM Chelex filtrate 4'	3,90	0,26	0,28	0,01	0,27	0,03	0,00	0,20	0,04	0,000	0,01	0,01	0,00

APPENDIX 6: Mercury and IRMS analyses in fish muscle tissue.

Code	Species	Tot - Hg (mg/kg ww)	Log THg (mg/kg ww)	Length (cm)	Weight (g)	δ15N	δ13C	N, %	C, %
U-1	*U. species*	0,008	-2,08	15,5	85,6	8,5	-31,4	14,5	48,2
U-2	*U. species*	0,009	-2,02	16	94,4	5,8	-33,9	13,0	45,6
U-3	*U. species*	0,008	-2,09	18,5	90,2	4,8	-33,1	11,6	39,0
U-4	*U. species*	0,008	-2,07	20,8	107,4	6,9	-33,2	12,5	45,6
U-5	*U. species*	0,008	-2,09	18,6	91,4	4,9	-33,0	12,2	39,1
U-6	*U. species*	0,008	-2,09	18,4	89,8	7,2	-33,2	14,1	48,3
U-7	*U. species*	0,010	-1,99	17,4	87,0	8,2	-31,5	13,9	49,9
U-8	*U. species*	0,009	-2,07	17,4	88,3	8,5	-32,2	14,2	49,1
U-9	*U. species*	0,009	-2,06	14,5	77,8	5,4	-33,4	12,9	43,2
U-10	*U. species*	0,009	-2,07	18,1	104,3	6,8	-33,5	12,9	46,6
U-11	*U. species*	0,009	-2,04	15,4	92,5	6,8	-31,6	12,6	41,8
U-12	*U. species*	0,011	-1,96	11,9	53,0	6,1	-33,6	13,7	46,8
U-13	*U. species*	0,010	-1,99	9,3	49,9	6,9	-32,4	14,2	45,6
U-14	*U. species*	0,010	-2,02	13,4	62,0	6,6	-33,1	13,6	45,5
U-15	*U. species*	0,010	-2,02	14	64,6	6,5	-33,4	13,5	46,1
U-16	*U. species*	0,010	-2,02	13,5	62,8	6,5	-33,2	13,6	44,6
U-17	*U. species*	0,008	-2,09	14,8	72,1	6,8	-33,3	13,9	47,9
U-18	*U. species*	0,009	-2,04	13,4	62,0	6,7	-33,5	13,8	49,0
U-19	*U. species*	0,011	-1,95	13,9	65,5	5,2	-33,1	12,5	39,1
U-20	*U. species*	0,011	-1,97	17,5	100,0	7,3	-32,4	13,7	46,0
P-1	*P. maclareni*	0,025	-1,61	10	28,1	9,2	-28,9	14,5	45,8
P-2	*P. maclareni*	0,023	-1,64	9,5	23,1	8,8	-31,2	14,0	48,4
P-3	*P. maclareni*	0,032	-1,49	9,2	19,9	8,9	-29,5	14,1	44,9
P-4	*P. maclareni*	0,036	-1,44	10,3	29,9	6,9	-38,6	14,2	47,3

cont'd

P-5	*P. maclareni*	0,049	-1,31	10,4	30,2	7,5	-35,6	13,1	40,0
P-6	*P. maclareni*	0,032	-1,50	8,4	16,6	7,7	-30,5	13,6	47,2
P-7	*P. maclareni*	0,024	-1,61	10,3	30,7	8,3	-29,7	13,9	44,7
P-8	*P. maclareni*	0,018	-1,75	8,8	18,0	9,5	-28,1	13,9	45,9
P-9	*P. maclareni*	0,032	-1,49	9,3	21,5	8,5	-29,8	14,1	46,6
P-10	*P. maclareni*	0,020	-1,69	8,5	16,9	8,0	-27,5	14,2	46,8
P-11	*P. maclareni*	0,032	-1,49	10,1	29,6	8,5	-30,5	12,8	46,7
P-12	*P. maclareni*	0,018	-1,74	10,5	31,2	9,5	-28,7	13,9	42,4
P-13	*P. maclareni*	0,033	-1,49	10,6	31,2	8,5	-28,4	13,5	40,5
P-14	*P. maclareni*	0,035	-1,46	10	30,2	6,9	-28,8	13,3	41,2
P-15	*P. maclareni*	0,026	-1,58	8,8	16,7	8,3	-31,3	12,9	44,8
P-16	*P. maclareni*	0,023	-1,64	10,7	31,2	8,8	-31,3	13,4	47,2
P-17	*P. maclareni*	0,019	-1,73	9,1	21,5	8,7	-29,2	13,9	46,5
P-18	*P. maclareni*	0,024	-1,62	10	30,5	9,8	-28,9	14,2	47,3
P-19	*P. maclareni*	0,018	-1,75	9,6	24,3	8,0	-26,9	13,5	42,5
N-1	*C. maclareni*	0,015	-1,84	23,2	154,1	9,2	-33,7	12,6	49,5
N-2	*C. maclareni*	0,038	-1,42	30	192,1	9,9	-30,2	13,3	47,5
N-3	*C. maclareni*	0,036	-1,45	28,9	187,0	10,8	-29,2	14,5	47,1
N-4	*C. maclareni*	0,019	-1,71	25,3	172,4	9,8	-32,4	14,0	47,3
N-5	*C. maclareni*	0,037	-1,43	27,3	185,0	10,4	-30,0	14,3	45,8
N-6	*C. maclareni*	0,032	-1,50	30,6	194,0	9,2	-31,6	11,0	44,0
N-7	*C. maclareni*	0,037	-1,43	29,1	190,0	9,9	-30,9	12,0	47,3
N-8	*C. maclareni*	0,016	-1,80	25	170,5	9,7	-31,7	14,0	47,6
N-9	*C. maclareni*	0,023	-1,64	23,5	155,0	11,4	-30,4	14,5	47,5
N-10	*C. maclareni*	0,016	-1,81	25	168,1	9,8	-34,3	9,8	53,3
N-11	*C. maclareni*	0,022	-1,65	24	159,0	7,9	-32,1	11,9	44,1

cont'd

N-12	*C. maclareni*	0,021	-1,68	25	168,0	11,1	-31,6	13,6	49,8
N-13	*C. maclareni*	0,008	-2,08	24,2	158,2	9,0	-33,4	13,2	46,5
N-14	*C. maclareni*	0,062	-1,21	37,5	254,1	9,2	-29,6	13,1	43,7
N-15	*C. maclareni*	0,018	-1,75	25,2	172,0	10,7	-31,7	14,2	49,3

APPENDIX 7: ICP-MS concentration of trace metals in digested gills and liver of fish species sampled from Lake Barombi Mbo.

Tissue	**Species**	**Dry weight of sample**	**Al**	**Cr**	**Mn**	**Co**	**Cu**	**Sr**	**Cd**	**Pb**	**U**
		g	**µg/L**	**µg/L**	**µg/L**	**µg/L**	**µg/L**	**µg/L**	**µg/L**	**µg/L**	**µg/L**
gill U1	*U. species*	0,101	5,075	0,230	407,623	2,104	1,036	339,443	0,008	0,067	0,013
gill U2	*U. species*	0,0926	14,854	0,135	189,245	1,891	1,055	325,192	0,014	0,125	0,015
gill U3	*U. species*	0,066	33,395	0,288	385,268	4,576	2,109	636,976	0,015	0,283	0,037
gill U4	*U. species*	0,1049	19,804	0,204	342,304	5,096	1,670	628,475	0,017	0,178	0,044
gill U5	*U. species*	0,1737	6,063	0,568	127,359	1,792	0,834	194,642	0,006	0,127	0,013
gill U6	*U. species*	0,1148	18,919	0,233	168,580	1,880	0,918	287,765	0,006	0,122	0,018
gill U7	*U. species*	0,1251	18,064	0,151	180,800	2,043	0,755	367,615	0,011	0,127	0,028
gill U8	*U. species*	0,1388	6,174	0,458	210,692	2,101	0,687	362,913	0,010	0,121	0,026

gill U9	*U. species*	0,0638	20,922	0,145	209,471	1,639	0,931	284,800	0,013	0,120	0,019
gill U10	*U. species*	0,141	5,120	0,101	143,848	1,813	1,015	253,691	0,010	0,072	0,018
gill U11	*U. species*	0,0877	5,216	0,103	421,679	2,108	0,902	323,562	0,015	0,100	0,019
gill U12	*U. species*	0,0275	66,698	0,415	167,538	1,687	0,823	314,655	0,010	0,113	0,015
gill U13	*U. species*	0,0121	93,295	12,558	145,259	1,596	0,816	362,931	0,019	0,102	0,013
gill U14	*U. species*	0,0455	19,764	0,288	156,144	1,841	0,937	257,981	0,008	0,152	0,018
gill U15	*U. species*	0,0563	41,504	0,503	121,468	1,431	0,857	299,185	0,009	0,145	0,019
gill U16	*U. species*	0,0497	33,162	0,437	161,443	1,752	0,914	289,262	0,006	0,338	0,017
gill U17	*U. species*	0,0614	28,677	0,267	193,514	1,787	0,929	296,608	0,008	0,189	0,018
gill U18	*U. species*	0,0418	27,399	0,429	161,158	1,835	0,853	298,965	0,007	0,149	0,030
gill U 19	*U. species*	0,0547	60,922	0,583	164,984	1,637	0,973	290,869	0,009	0,142	0,021
gill U20	*U. species*	0,1016	5,833	0,142	207,960	1,723	0,829	314,467	0,009	0,178	0,022
gill P1	*P. maclareni*	0,0092	127,048	1,603	82,691	1,851	0,086	463,896	0,039	0,407	0,053
gill P2	*P. maclareni*	0,0079	71,458	0,441	84,972	1,889	1,173	439,594	0,027	0,730	0,039
gill P3	*P. maclareni*	0,0079	73,937	4,571	86,250	1,792	-0,320	392,284	0,004	2,248	0,021
gill P4	*P. maclareni*	0,0057	34,878	0,015	60,563	1,289	-1,077	372,375	0,013	0,188	0,061
gill P5	*P. maclareni*	0,014	34,957	2,207	47,096	1,626	0,234	332,764	0,015	0,238	0,026
gill P6	*P. maclareni*	0,0055	45,279	0,956	77,723	1,664	-1,434	395,591	0,020	0,244	0,037
gill P7	*P. maclareni*	0,0109	226,836	0,680	78,968	1,326	2,688	375,744	0,028	0,863	0,031
gill P8	*P. maclareni*	0,0064	350,792	1,850	102,681	1,848	2,881	327,228	0,046	0,370	0,058
gill P9	*P. maclareni*	0,0095	47,189	0,191	103,814	2,023	1,812	425,551	0,036	0,410	0,015
gill P10	*P. maclareni*	0,0053	21,180	1,150	80,479	1,299	-1,780	428,793	0,010	0,125	0,052
gill P11	*P. maclareni*	0,0101	25,156	-0,027	62,883	1,042	-0,151	391,305	0,013	0,324	0,035
gill P12	*P. maclareni*	0,0088	25,851	0,067	150,533	1,740	0,144	493,090	0,025	0,362	0,037
gill P13	*P. maclareni*	0,0112	118,513	0,546	93,217	3,031	0,121	540,349	0,029	0,310	0,041

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gill P14	*P. maclareni*	0,0113	866,549	0,187	86,628	2,018	0,221	458,464	0,020	0,242	0,028
gill P15	*P. maclareni*	0,0079	47,973	0,115	72,298	1,093	-1,017	256,420	0,014	0,163	0,017
gill P16	*P. maclareni*	0,0138	261,030	1,362	105,100	1,313	0,736	315,693	0,023	0,333	0,033
gill P17	*P. maclareni*	0,0062	178,745	0,496	117,536	1,235	-0,382	311,578	0,023	0,308	0,030
gill P18	*P. maclareni*	0,0094	26,000	0,109	103,049	2,023	-0,316	514,792	0,041	0,398	0,029
gill P19	*P. maclareni*	0,0077	73,204	0,618	76,285	1,722	0,037	411,242	0,022	0,186	0,036
gill N1	*C. maclareni*	0,0392	40,861	0,444	57,540	0,165	0,615	171,206	0,071	0,029	0,001
gill N2	*C. maclareni*	0,0441	23,543	0,210	59,749	0,306	1,142	317,763	0,039	0,082	0,001
gill N3	*C. maclareni*	0,0621	19,499	0,229	60,488	0,440	1,342	220,673	0,056	0,075	0,002
gill N4	*C. maclareni*	0,0488	24,540	0,151	84,852	0,187	0,654	206,961	0,069	0,041	0,000
gill N5	*C. maclareni*	0,0395	8,932	0,111	84,923	0,279	0,614	211,688	0,082	0,023	0,000
gill N6	*C. maclareni*	0,0742	1,770	0,077	79,953	0,198	0,369	228,647	0,016	0,032	0,001
gill N7	*C. maclareni*	0,0627	127,011	0,515	62,603	0,318	1,240	219,429	0,089	0,080	0,003
gill N8	*C. maclareni*	0,0491	40,372	0,187	50,217	0,200	1,079	210,527	0,064	0,039	0,001
gill N9	*C. maclareni*	0,0376	7,788	0,191	119,166	0,243	0,589	182,623	0,149	0,030	0,000
gill N10	*C. maclareni*	0,0625	13,002	0,110	51,619	0,158	1,141	143,499	0,066	0,030	0,000
gill N11	*C. maclareni*	0,058	2,337	0,089	38,131	0,151	1,435	114,922	0,039	1,096	0,000
gill N12	*C. maclareni*	0,0404	47,675	0,646	73,904	0,235	1,212	212,322	0,152	0,042	0,001
gill N13	*C. maclareni*	0,0763	11,748	0,117	54,858	0,162	1,214	161,956	0,041	0,025	0,000
gill N14	*C. maclareni*	0,1175	88,578	0,387	48,469	0,443	0,638	308,800	0,036	0,053	0,002
gill N15	*C. maclareni*	0,0567	81,415	0,463	75,785	0,261	1,568	214,216	0,119	0,050	0,002
liver U1	*U. species*	0,0057	153,757	0,677	196,945	6,463	1027,455	1,841	0,912	0,042	0,019
liver U2	*U. species*	0,0094	209,966	0,858	56,277	9,066	1459,617	1,324	1,716	0,194	0,059
liver U3	*U. species*	0,0516	159,121	3,144	182,346	42,457	7908,616	16,528	10,636	0,067	0,090
liver U4	*U. species*	0,0634	208,176	3,732	184,321	89,181	########	6,321	20,256	0,107	0,190

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liver U5	*U. species*	0,0378	103,899	8,453	122,164	41,547	6409,351	3,423	8,066	0,052	0,072
liver U6	*U. species*	0,0233	182,148	1,318	118,927	29,557	3037,063	2,300	3,077	0,044	0,035
liver U7	*U. species*	0,0251	122,928	1,767	85,870	24,415	1740,326	2,962	5,340	0,049	0,094
liver U8	*U. species*	0,0142	121,127	1,375	88,986	25,108	1337,288	2,705	5,296	0,064	0,083
liver U9	*U. species*	0,0213	57,637	1,891	213,250	25,540	3041,843	5,039	4,575	0,047	0,037
liver U10	*U. species*	0,0380	132,813	25,509	78,518	12,131	2119,696	1,500	3,082	0,056	0,038
liver U11	*U. species*	0,0041	26,122	0,295	136,846	4,966	395,612	0,952	1,348	0,004	0,015
liver U14	*U. species*	0,0195	50,715	0,553	29,741	31,731	2118,257	1,894	3,665	0,062	0,030
liver U15	*U. species*	0,0120	18,542	0,264	35,393	5,936	760,787	16,779	1,527	0,020	0,016
liver U17	*U. species*	0,0287	63,416	1,761	66,998	19,446	2580,319	2,702	3,782	0,035	0,041
liver U18	*U. species*	0,0063	98,430	1,020	27,325	9,558	1153,146	0,531	1,064	0,022	0,014
liver U19	*U. species*	0,0356	372,945	1,781	36,237	3,172	260,388	30,167	11,659	6,421	0,012
liver U20	*U. species*	0,0406	180,478	1,158	96,517	17,697	1474,859	7,790	15,094	0,691	0,038
liver P2	*P. maclareni*	0,0056	37,018	7,325	4,048	14,803	18,159	0,836	1,147	0,074	0,034
liver P3	*P. maclareni*	0,0088	44,689	0,653	6,297	28,035	10,743	1,318	2,260	1,638	0,022
liver P5	*P. maclareni*	0,0029	24,817	1,472	1,387	7,120	4,486	0,716	0,754	0,061	0,011
liver P6	*P. maclareni*	0,0006	108,650	0,472	2,579	2,170	1,542	0,666	0,113	0,026	0,002
liver P8	*P. maclareni*	0,0080	93,285	0,589	13,341	16,375	5,400	45,878	2,132	0,174	0,053
liver P9	*P. maclareni*	0,0078	39,019	0,539	5,628	19,480	6,835	0,483	2,224	0,059	0,023
liver P10	*P. maclareni*	0,0010	12,888	0,335	1,259	7,451	0,572	0,279	0,393	0,052	0,004
liver P11	*P. maclareni*	0,0550	550,769	5,953	42,628	26,976	73,302	5,897	13,151	0,882	0,033
liver P12	*P. maclareni*	0,0010	19,955	0,835	7,788	6,840	0,563	15,560	2,178	0,045	0,028
liver P13	*P. maclareni*	0,0035	44,311	2,930	5,105	20,353	6,006	0,517	2,454	0,095	0,026
liver P14	*P. maclareni*	0,0010	5,847	0,156	0,952	4,669	2,316	0,132	0,582	0,005	0,001
liver P15	*P. maclareni*	0,0098	329,309	0,829	45,420	9,619	7,264	52,291	1,200	0,086	0,016

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liver P16	*P. maclareni*	0,0032	22,454	0,204	3,205	6,884	4,098	0,293	0,812	0,052	0,005
liver P17	*P. maclareni*	0,0017	11,398	0,238	8,499	3,837	0,601	29,497	0,531	0,052	0,003
liver P18	*P. maclareni*	0,0032	24,188	0,301	3,549	15,589	6,037	0,838	2,470	0,053	0,012
liver P19	*P. maclareni*	0,0052	73,531	0,482	4,550	20,663	7,683	0,801	1,171	0,067	0,010
liver N1	*C. maclareni*	0,0253	271,702	1,326	23,190	0,874	199,320	5,215	13,207	0,175	0,000
liver N4	*C. maclareni*	0,0144	158,778	1,633	24,961	0,596	71,957	4,607	10,754	0,117	0,000
liver N5	*C. maclareni*	0,0326	268,786	0,750	27,688	1,454	269,604	8,576	18,504	0,302	0,000
liver N6	*C. maclareni*	0,0477	346,631	0,897	35,547	1,300	50,392	9,896	3,142	0,355	0,002
liver N7	*C. maclareni*	0,0450	140,144	0,819	46,804	2,016	93,282	8,967	18,713	0,496	-0,001
liver N8	*C. maclareni*	0,0324	102,516	0,433	25,485	2,397	82,940	6,658	22,899	0,096	-0,002
liver N9	*C. maclareni*	0,0272	164,234	1,165	45,288	1,053	130,859	6,249	34,115	0,685	0,002
liver N12	*C. maclareni*	0,0123	125,306	0,931	21,905	0,802	127,686	5,772	25,252	0,230	0,008
liver N13	*C. maclareni*	0,0236	88,390	0,917	24,427	1,149	144,215	3,739	9,853	0,153	-0,001
liver N14	*C. maclareni*	0,1399	319,491	2,267	89,657	15,790	143,271	23,112	40,346	0,983	0,004
liver N15	*C. maclareni*	0,0036	73,766	0,145	6,732	0,223	6,205	1,118	1,980	0,067	0,005

For obtaining concentration of trace metals in gills and liver based on dry weight (µg/g d.w.),

Concentration (µg/g d.w) = (measured ICP-MS value in digested tissue) / (100 * dry weight of tissue)

Measured value is divided by 100 because samples were digested in 10 ml instead of 1000 ml.

APPENDIX 8: Concentration of trace metals in gills and liver based on dry weight (µg/g d.w).

Tissue	Species	Al	Cr	Mn	Co	Cu	Sr	Cd	Pb	U
		µg/g d.w	µg/g d.w	µg/g d.w	µg/g d.w	µg/g d.w	µg/g d.w	µg/g d.w	µg/g d.w	µg/g d.w
gill U1	*U. species*	0,502	0,023	40,359	0,208	0,103	33,608	0,001	0,007	0,001
gill U2	*U. species*	1,604	0,015	20,437	0,204	0,114	35,118	0,001	0,013	0,002
gill U3	*U. species*	5,060	0,044	58,374	0,693	0,320	96,512	0,002	0,043	0,006
gill U4	*U. species*	1,888	0,019	32,631	0,486	0,159	59,912	0,002	0,017	0,004
gill U5	*U. species*	0,349	0,033	7,332	0,103	0,048	11,206	0,000	0,007	0,001
gill U6	*U. species*	1,648	0,020	14,685	0,164	0,080	25,067	0,001	0,011	0,002
gill U7	*U. species*	1,444	0,012	14,452	0,163	0,060	29,386	0,001	0,010	0,002
gill U8	*U. species*	0,445	0,033	15,180	0,151	0,049	26,146	0,001	0,009	0,002
gill U9	*U. species*	3,279	0,023	32,832	0,257	0,146	44,639	0,002	0,019	0,003
gill U10	*U. species*	0,363	0,007	10,202	0,129	0,072	17,992	0,001	0,005	0,001
gill U11	*U. species*	0,595	0,012	48,082	0,240	0,103	36,894	0,002	0,011	0,002
gill U12	*U. species*	24,254	0,151	60,923	0,613	0,299	114,420	0,004	0,041	0,006
gill U13	*U. species*	77,103	10,379	120,049	1,319	0,675	299,943	0,016	0,085	0,011
gill U14	*U. species*	4,344	0,063	34,317	0,405	0,206	56,699	0,002	0,033	0,004
gill U15	*U. species*	7,372	0,089	21,575	0,254	0,152	53,141	0,002	0,026	0,003
gill U16	*U. species*	6,672	0,088	32,483	0,353	0,184	58,202	0,001	0,068	0,003
gill U17	*U. species*	4,670	0,044	31,517	0,291	0,151	48,308	0,001	0,031	0,003
gill U18	*U. species*	6,555	0,103	38,555	0,439	0,204	71,523	0,002	0,036	0,007
gill U 19	*U. species*	11,138	0,107	30,162	0,299	0,178	53,175	0,002	0,026	0,004
gill U20	*U. species*	0,574	0,014	20,469	0,170	0,082	30,951	0,001	0,018	0,002
gill P1	*P. maclareni*	138,096	1,742	89,882	2,012	0,094	504,235	0,043	0,442	0,058
gill P2	*P. maclareni*	90,453	0,558	107,559	2,392	1,484	556,448	0,034	0,924	0,050

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gill P3	*P. maclareni*	93,592	5,786	109,177	2,268	-0,405	496,563	0,005	2,846	0,027
gill P4	*P. maclareni*	61,189	0,027	106,251	2,262	-1,890	653,290	0,022	0,329	0,107
gill P5	*P. maclareni*	24,969	1,576	33,640	1,161	0,167	237,689	0,010	0,170	0,019
gill P6	*P. maclareni*	82,325	1,738	141,315	3,026	-2,608	719,257	0,037	0,444	0,068
gill P7	*P. maclareni*	208,106	0,624	72,448	1,216	2,466	344,719	0,025	0,792	0,029
gill P8	*P. maclareni*	548,112	2,891	160,439	2,887	4,502	511,294	0,071	0,578	0,091
gill P9	*P. maclareni*	49,672	0,201	109,278	2,130	1,908	447,949	0,038	0,432	0,016
gill P10	*P. maclareni*	39,962	2,169	151,847	2,451	-3,358	809,044	0,019	0,236	0,097
gill P11	*P. maclareni*	24,907	-0,027	62,261	1,031	-0,149	387,430	0,013	0,321	0,034
gill P12	*P. maclareni*	29,376	0,076	171,060	1,977	0,163	560,330	0,028	0,412	0,042
gill P13	*P. maclareni*	105,815	0,487	83,229	2,706	0,108	482,454	0,026	0,277	0,037
gill P14	*P. maclareni*	766,857	0,166	76,662	1,785	0,196	405,720	0,017	0,214	0,025
gill P15	*P. maclareni*	60,726	0,146	91,516	1,383	-1,288	324,583	0,018	0,206	0,022
gill P16	*P. maclareni*	189,152	0,987	76,159	0,951	0,533	228,763	0,017	0,241	0,024
gill P17	*P. maclareni*	288,298	0,800	189,575	1,992	-0,616	502,546	0,037	0,497	0,048
gill P18	*P. maclareni*	27,659	0,116	109,627	2,152	-0,336	547,651	0,043	0,424	0,031
gill P19	*P. maclareni*	95,071	0,803	99,072	2,236	0,048	534,080	0,028	0,242	0,046
gill N1	*C. maclareni*	10,424	0,113	14,679	0,042	0,157	43,675	0,018	0,007	0,000
gill N2	*C. maclareni*	5,339	0,048	13,549	0,069	0,259	72,055	0,009	0,019	0,000
gill N3	*C. maclareni*	3,140	0,037	9,740	0,071	0,216	35,535	0,009	0,012	0,000
gill N4	*C. maclareni*	5,029	0,031	17,388	0,038	0,134	42,410	0,014	0,008	0,000
gill N5	*C. maclareni*	2,261	0,028	21,500	0,071	0,155	53,592	0,021	0,006	0,000
gill N6	*C. maclareni*	0,239	0,010	10,775	0,027	0,050	30,815	0,002	0,004	0,000
gill N7	*C. maclareni*	20,257	0,082	9,984	0,051	0,198	34,997	0,014	0,013	0,000
gill N8	*C. maclareni*	8,222	0,038	10,227	0,041	0,220	42,877	0,013	0,008	0,000
gill N9	*C. maclareni*	2,071	0,051	31,693	0,065	0,157	48,570	0,040	0,008	0,000

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gill N10	*C. maclareni*	2,080	0,018	8,259	0,025	0,183	22,960	0,011	0,005	0,000
gill N11	*C. maclareni*	0,403	0,015	6,574	0,026	0,247	19,814	0,007	0,189	0,000
gill N12	*C. maclareni*	11,801	0,160	18,293	0,058	0,300	52,555	0,038	0,010	0,000
gill N13	*C. maclareni*	1,540	0,015	7,190	0,021	0,159	21,226	0,005	0,003	0,000
gill N14	*C. maclareni*	7,539	0,033	4,125	0,038	0,054	26,281	0,003	0,005	0,000
gill N15	*C. maclareni*	14,359	0,082	13,366	0,046	0,276	37,781	0,021	0,009	0,000
liver U1	*U. species*	269,75	1,19	345,52	11,34	1802,55	3,23	1,60	0,07	0,03
liver U2	*U. species*	223,37	0,91	59,87	9,65	1552,78	1,41	1,83	0,21	0,06
liver U3	*U. species*	30,84	0,61	35,34	8,23	1532,68	3,20	2,06	0,01	0,02
liver U4	*U. species*	32,84	0,59	29,07	14,07	2240,63	1,00	3,19	0,02	0,03
liver U5	*U. species*	27,49	2,24	32,32	10,99	1695,60	0,91	2,13	0,01	0,02
liver U6	*U. species*	78,18	0,57	51,04	12,69	1303,46	0,99	1,32	0,02	0,02
liver U7	*U. species*	48,98	0,70	34,21	9,73	693,36	1,18	2,13	0,02	0,04
liver U8	*U. species*	85,30	0,97	62,67	17,68	941,75	1,91	3,73	0,04	0,06
liver U9	*U. species*	27,06	0,89	100,12	11,99	1428,10	2,37	2,15	0,02	0,02
liver U10	*U. species*	34,95	6,71	20,66	3,19	557,81	0,39	0,81	0,01	0,01
liver U11	*U. species*	63,71	0,72	333,77	12,11	964,91	2,32	3,29	0,01	0,04
liver U14	*U. species*	26,01	0,28	15,25	16,27	1086,29	0,97	1,88	0,03	0,02
liver U15	*U. species*	15,45	0,22	29,49	4,95	633,99	13,98	1,27	0,02	0,01
liver U17	*U. species*	22,10	0,61	23,34	6,78	899,07	0,94	1,32	0,01	0,01
liver U18	*U. species*	156,24	1,62	43,37	15,17	1830,39	0,84	1,69	0,03	0,02
liver U19	*U. species*	104,76	0,50	10,18	0,89	73,14	8,47	3,28	1,80	0,00
liver U20	*U. species*	44,45	0,29	23,77	4,36	363,27	1,92	3,72	0,17	0,01
liver P2	*P. maclareni*	66,10	13,08	7,23	26,43	32,43	1,49	2,05	0,13	0,06
liver P3	*P. maclareni*	50,78	0,74	7,16	31,86	12,21	1,50	2,57	1,86	0,02
liver P5	*P. maclareni*	85,58	5,08	4,78	24,55	15,47	2,47	2,60	0,21	0,04

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liver P6	*P. maclareni*	1810,84	7,87	42,98	36,17	25,71	11,10	1,89	0,43	0,03
liver P8	*P. maclareni*	116,61	0,74	16,68	20,47	6,75	57,35	2,66	0,22	0,07
liver P9	*P. maclareni*	50,02	0,69	7,22	24,97	8,76	0,62	2,85	0,08	0,03
liver P10	*P. maclareni*	128,88	3,35	12,59	74,51	5,72	2,79	3,93	0,52	0,04
liver P11	*P. maclareni*	100,14	1,08	7,75	4,90	13,33	1,07	2,39	0,16	0,01
liver P12	*P. maclareni*	199,55	8,35	77,88	68,40	5,63	155,60	21,78	0,45	0,28
liver P13	*P. maclareni*	126,60	8,37	14,59	58,15	17,16	1,48	7,01	0,27	0,07
liver P14	*P. maclareni*	58,47	1,56	9,52	46,69	23,16	1,32	5,82	0,05	0,01
liver P15	*P. maclareni*	336,03	0,85	46,35	9,81	7,41	53,36	1,22	0,09	0,02
liver P16	*P. maclareni*	70,17	0,64	10,02	21,51	12,81	0,92	2,54	0,16	0,01
liver P17	*P. maclareni*	67,05	1,40	49,99	22,57	3,53	173,51	3,12	0,31	0,02
liver P18	*P. maclareni*	75,59	0,94	11,09	48,71	18,87	2,62	7,72	0,17	0,04
liver P19	*P. maclareni*	141,41	0,93	8,75	39,74	14,78	1,54	2,25	0,13	0,02
liver n1	*C. maclareni*	107,39	0,52	9,17	0,35	78,78	2,06	5,22	0,07	0,00
liver N4	*C. maclareni*	110,26	1,13	17,33	0,41	49,97	3,20	7,47	0,08	0,00
liver N5	*C. maclareni*	82,45	0,23	8,49	0,45	82,70	2,63	5,68	0,09	0,00
liver N6	*C. maclareni*	72,67	0,19	7,45	0,27	10,56	2,07	0,66	0,07	0,00
liver N7	*C. maclareni*	31,14	0,18	10,40	0,45	20,73	1,99	4,16	0,11	0,00
liver N8	*C. maclareni*	31,64	0,13	7,87	0,74	25,60	2,06	7,07	0,03	0,00
liver N9	*C. maclareni*	60,38	0,43	16,65	0,39	48,11	2,30	12,54	0,25	0,00
liver N12	*C. maclareni*	101,87	0,76	17,81	0,65	103,81	4,69	20,53	0,19	0,01
liver N13	*C. maclareni*	37,45	0,39	10,35	0,49	61,11	1,58	4,17	0,06	0,00
liver N14	*C. maclareni*	22,84	0,16	6,41	1,13	10,24	1,65	2,88	0,07	0,00
liver N15	*C. maclareni*	204,91	0,40	18,70	0,62	17,24	3,11	5,50	0,19	0,01

APPENDIX 9: Certified and Information values for DOLT-4.

Element	Mass Fraction (mg/kg)
Arsenic	9.66±0.62
Cadmium	24.3±0.8
Copper	31.2±1.1
Iron	1833±75
Lead	0.16±0.04
Mercury	2.58±0.22
Nickel	0.97±0.11
Selenium	8.3±1.3
Silver	0.93±0.07
Zinc	116±6
CH_3Hg (as Hg)	1.33±0.12
Information value for DOLT-4	
Na	6800
Mg	1500
Al	200
K	9800
Ca	680
V	0.6
Cr	1.4
Co	0.25
Sr	5.5
Mo	1
Sn	0.17

APPENDIX 10: Descriptive statistics for mercury accumulation in muscles between fish species.

For pairwise comparison if intervals do not include zero (0), then, it means they are significantly different. ($p < 0.05$).

One-way ANOVA: THg (mg/kg w.w) versus Species

```
Source   DF         SS         MS      F      P
Species   2  0,0039789  0,0019895  26,62  0,000
Error    51  0,0038121  0,0000747
Total    53  0,0077911

S = 0,008646   R-Sq = 51,07%   R-Sq(adj) = 49,15%


                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level   N      Mean     StDev  --+---------+---------+---------+-------
N      15  0,026574  0,013590                          (-----*-----)
P      19  0,027368  0,008190                           (-----*-----)
U      20  0,009254  0,001007  (----*-----)
                                --+---------+---------+---------+-------
                                0,0070    0,0140    0,0210    0,0280

Pooled StDev = 0,008646
```

Grouping Information Using Fisher Method

```
Species   N      Mean  Grouping
P        19  0,027368  A
N        15  0,026574  A
U        20  0,009254     B

Means that do not share a letter are significantly different.


Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of Species

Simultaneous confidence level = 87,93%


Species = N subtracted from:

Species      Lower     Center      Upper
P        -0,005201   0,000794   0,006789
U        -0,023248  -0,017320  -0,011391

Species     +---------+---------+---------+---------
P                          (----*----)
U             (----*----)
            +---------+---------+---------+---------
         -0,024    -0,012     0,000     0,012


Species = P subtracted from:

Species      Lower     Center      Upper
U        -0,023675  -0,018114  -0,012554

Species     +---------+---------+---------+---------
U             (----*----)
            +---------+---------+---------+---------
         -0,024    -0,012     0,000     0,012
```

N/B:

One-way ANOVA is used to test whether accumulation of mercury (Hg) is dependent on fish species and consequently trophic level in the present study.

However , pairwise comparison between the various fish species indicates differences. If the intervals do not include zero (0), then, it means they are significantly different. Thus, accumulation of Hg will be different for the species at different trophic levels.

From the above data display, I can conclude that *P. maclareni* (P) and *C. maclareni* (N) are significantly different from U. species (U) in their accumulation of mercury.

The scatter plot diagram of weight vs length for all three species showed high correlation.

U. species (U): R^2= 0,836; *P. maclareni* (P): R^2= 0,947; *C. maclareni* (N): R^2= 0,968

It is important to note that, high correlation here implies that both variables can play the same role in a model, or that the variation of weight is highly explained by variation in length.

In U. species, variation in total mercury (THg) concentration is not significantly explanatory by either length or weight (R^2_L=30,7% > R^2_W=22,8%).

In *P. maclareni*, variation in THg concentration is weakly explained by both length and weight (R^2_L=7,6% > R^2_W=7.1%).

In *C. maclareni*, much of the variation in THg concentration is significantly explanatory by the length and weight (R^2_L=83,5% > R^2_W=83,7%).

Where, R^2_L= R_Square for length; R^2_W= R_Square for weight.

APPENDIX 11: Significant differences for stable isotopes in muscle of fish species sampled

One-way ANOVA: δ15N versus Species

```
Source   DF       SS      MS      F      P
Species   2   93,460  46,730  54,29  0,000
Error    51   43,894   0,861
Total    53  137,355

S = 0,9277   R-Sq = 68,04%   R-Sq(adj) = 66,79%
```

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
N	15	9,878	0,913	(---*---)
P	19	8,432	0,802	(--*---)
U	20	6,614	1,043	(--*---)

```
                        --------+---------+---------+---------+-
                              7,2       8,4       9,6      10,8

Pooled StDev = 0,928
```

Grouping Information Using Fisher Method

Species	N	Mean	Grouping
N	15	9,8781	**A**
P	19	8,4324	**B**
U	20	6,6144	**C**

Means that do not share a letter are significantly different.

Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of Species

Simultaneous confidence level = 87,93%

Species = N subtracted from:

Species	Lower	Center	Upper	----+---------+---------+---------+-----
P	-2,0890	-1,4457	-0,8024	(---*---)
U	-3,8998	-3,2636	-2,6275	(---*---)
				----+---------+---------+---------+-----
				-3,2 -1,6 -0,0 1,6

Species = P subtracted from:

Species	Lower	Center	Upper	----+---------+---------+---------+-----
U	-2,4146	-1,8179	-1,2212	(---*--)
				----+---------+---------+---------+-----
				-3,2 -1,6 -0,0 1,6

One-way ANOVA: δ13C versus Species

Source	DF	SS	MS	F	P
Species	2	71,08	35,54	10,09	0,000
Error	51	179,63	3,52		
Total	53	250,71			

S = 1,877 R-Sq = 28,35% R-Sq(adj) = 25,54%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
N	15	-31,526	1,497	-+---------+---------+---------+--------
P	19	-30,190	2,766	(-------*-------)
U	20	-32,891	0,744	(------*-------)
				(------*------)
				-+---------+---------+---------+--------
				-33,6 -32,4 -31,2 -30,0

Pooled StDev = 1,877

Grouping Information Using Fisher Method

Species	N	Mean	Grouping
P	19	-30,190	**A**
N	15	-31,526	**B**
U	20	-32,891	**C**

Means that do not share a letter are significantly different.

```
Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of Species

Simultaneous confidence level = 87,93%


Species = N subtracted from:

Species   Lower  Center   Upper     +---------+---------+---------+---------
P         0,034   1,335   2,637                       (------*-----)
U        -2,652  -1,365  -0,078            (-----*------)
                                   +---------+---------+---------+---------
                                -4,0      -2,0       0,0       2,0


Species = P subtracted from:

Species   Lower  Center   Upper     +---------+---------+---------+---------
U        -3,907  -2,700  -1,493     (-----*------)
                                   +---------+---------+---------+---------
                                -4,0      -2,0       0,0       2,0
```

APPENDIX 12: Significant statistical relationships between metal concentration and tissue size of fish species.

Unga species

Gills

Regression Analysis: Al versus Total length (cm)

```
The regression equation is
Al = 123 - 6,19 Total length (cm)


Predictor            Coef  SE Coef      T      P
Constant           123,20    22,24   5,54  0,000
Total length (cm)  -6,190    1,404  -4,41  0,000


S = 16,8241   R-Sq = 51,9%   R-Sq(adj) = 49,3%


Analysis of Variance

Source          DF       SS      MS      F      P
Regression       1   5502,7  5502,7  19,44  0,000
Residual Error  18   5094,9   283,0
Total           19  10597,6
```

Regression Analysis: Al versus Total weight (g)

```
The regression equation is
Al = 111 - 1,05 Total weight (g)


Predictor            Coef  SE Coef      T      P
Constant           110,76    16,78   6,60  0,000
Total weight (g)  -1,0523   0,2052  -5,13  0,000
```

S = 15,4672   R-Sq = 59,4%   R-Sq(adj) = 57,1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	6291,4	6291,4	26,30	**0,000**
Residual Error	18	4306,2	239,2		
Total	19	10597,6			

Regression Analysis: Co versus Total weight (g)

The regression equation is
Co = 0,057 + 0,0257 Total weight (g)

Predictor	Coef	SE Coef	T	P
Constant	0,0568	0,9366	0,06	0,952
Total weight (g)	0,02574	0,01145	2,25	**0,037**

S = 0,863154   R-Sq = 21,9%   R-Sq(adj) = 17,6%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	3,7631	3,7631	5,05	**0,037**
Residual Error	18	13,4106	0,7450		
Total	19	17,1738			

Regression Analysis: Co versus Total length (cm)

The regression equation is
Co = - 1,02 + 0,201 Total length (cm)

Predictor	Coef	SE Coef	T	P
Constant	-1,015	1,052	-0,97	0,347
Total length (cm)	0,20057	0,06640	3,02	**0,007**

S = 0,795708   R-Sq = 33,6%   R-Sq(adj) = 30,0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	5,7770	5,7770	9,12	**0,007**
Residual Error	18	11,3967	0,6332		
Total	19	17,1738			

Regression Analysis: Cr versus Total length (cm)

The regression equation is
Cr = 9,58 - 0,555 Total length (cm)

Predictor	Coef	SE Coef	T	P
Constant	9,579	3,101	3,09	0,006
Total length (cm)	-0,5550	0,1957	-2,84	**0,011**

S = 2,34517   R-Sq = 30,9%   R-Sq(adj) = 27,0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	44,239	44,239	8,04	0,011
Residual Error	18	98,997	5,500		
Total	19	143,236			

Clarias maclareni

Gills

Regression Analysis: Co versus Total length (cm)

The regression equation is
Co = - 0,258 + 0,0189 Total length (cm)

Predictor	Coef	SE Coef	T	P
Constant	-0,2582	0,1215	-2,13	0,053
Total length (cm)	0,018870	0,004471	4,22	**0,001**

S = 0,0635321   R-Sq = 57,8%   R-Sq(adj) = 54,6%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0,071888	0,071888	17,81	**0,001**
Residual Error	13	0,052472	0,004036		
Total	14	0,124361			

Regression Analysis: Co versus Total weight (g)

The regression equation is
Co = - 0,262 + 0,00287 Total weight (g)

Predictor	Coef	SE Coef	T	P
Constant	-0,2622	0,1235	-2,12	0,053
Total weight (g)	0,0028663	0,0006852	4,18	**0,001**

S = 0,0638542   R-Sq = 57,4%   R-Sq(adj) = 54,1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0,071355	0,071355	17,50	**0,001**
Residual Error	13	0,053006	0,004077		
Total	14	0,124361			

Regression Analysis: Sr versus Total length (cm)

The regression equation is
Sr = - 92,2 + 11,2 Total length (cm)

Predictor	Coef	SE Coef	T	P
Constant	-92,25	64,46	-1,43	0,176
Total length (cm)	11,166	2,372	4,71	**0,000**

S = 33,7094 R-Sq = 63,0% R-Sq(adj) = 60,2%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	25172	25172	22,15	**0,000**
Residual Error	13	14772	1136		
Total	14	39945			

Regression Analysis: Sr versus Total weight (g)

The regression equation is
Sr = - 90,0 + 1,67 Total weight (g)

Predictor	Coef	SE Coef	T	P
Constant	-89,95	67,26	-1,34	0,204
Total weight (g)	1,6700	0,3732	4,47	**0,001**

S = 34,7786 R-Sq = 60,6% R-Sq(adj) = 57,6%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	24220	24220	20,02	**0,001**
Residual Error	13	15724	1210		
Total	14	39945			

Liver

Regression Analysis: Co versus Total weight (g)

The regression equation is
Co = - 0,490 + 0,00574 Total weight (g)

Predictor	Coef	SE Coef	T	P
Constant	-0,4901	0,3794	-1,29	0,229
Total weight (g)	0,005742	0,002092	2,75	**0,023**

S = 0,186303 R-Sq = 45,6% R-Sq(adj) = 39,5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0,26157	0,26157	7,54	**0,023**
Residual Error	9	0,31238	0,03471		
Total	10	0,57395			