TROPHIC TRANSFER OF MERCURY IN FISH SPECIES FROM LAKE PHEWA, POKHARA, NEPAL

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(Photo: B. O. Rosseland)

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Preface

This thesis represents an output of a two years master study in the Department of Ecology and Natural Resource Management (INA) at the Norwegian University of Life Sciences (UMB).

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Summary

This study represents the first attempt to quantify concentrations of mercury and its trophic transfer along the food chain, in one of the lakes in Nepal, Lake Phewa. Stomach content analysis and stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) were used as an index of trophic level and carbon source and were used to study bioaccumulation and biomagnifications of mercury in Nile Tilapia (*Oreochromis niloticus*), African magur (*Clarias gariepinus*), Sahar (*Tor putitora*) and Chuche Baam (*Mastacembelus armatus*) . Metal concentration in water and in the gill and liver of these fish species were examined. Muscles samples of the largest among each species were analyzed to identify the concentration of Persistant Organic Pollutants (POPs). The diet of *O. niloticus* were dominated by aquatic plants, regardless the size, whereas in other three species, aquatic insects, crustacea, fish were dominated in the diet. The mean and maximum value of total mercury (THg mg kg^{-1} w.w.) concentrations were 0.071 and 0.32 for *C. gariepinus*, 0.031 and 0.081 for *O. niloticus*, 0.116 and 0.21for *T. putitora* and 0.079 and 0.22 for *M. armatus.* The relationship between THg and total length and total weight were positive and significant in *C. gariepinus* and *M. armatus.* Age determination could not be done for any of the fish species. For none of the species, the value of $\delta^{15}N$ and $\delta^{13}C$ correlates with the total length of the fish, possibly because of few isotope analysis. The relationship between log THg and $\delta^{15}N$ were with the present analysis, not significant for any of the species indicating no biomagnification. Since the THg level in all the fish species was consistently low, it will not pose any harm to the fish consuming community. In the food chain, *M. armatus* hold the top position and *O. niloticus* the bottom. Metal analysis of water, gill and liver showed the exposure of fish species to various trace metals. Except for high levels of manganese (Mn), the values were comparable to levels found in other lake and river system in Europe. Metabolites of DDT and endosulfan sulfate were found in the muscle sample of fish, but at levels of low concern by the present regulations.

The present study represents only 4 out of nearly 30 species so far registered in Lake Phewa. Further studies on Hg and pesticides in the most important fish species in Lake Phewa, as well as in the other lakes in the region, should be facilitated to improve risk assessment and enable a proper management in relation to pollutants in the fish populations of the lakes.

1. Introduction

Mercury (Hg) is released to the atmosphere from both natural and anthropogenic sources, and spread to areas far away from the emission source, and thereby has a global concern (Morel et al. 1998; Bergan et al. 1999; Chen et al. 2005).

Mercury occurs in the earth's crust, especially in the form of mineral cinnabar which is released in the atmosphere naturally as a result of volcanic activities, weathering of rocks, forest fires and anthropogenically during mining, combustion of fossil fuel, manufacturing processes and waste incineration (UNEP 2008; Clarkson 1997). Among three inorganic forms of mercury that exist in the atmosphere, gaseous elemental mercury contributes more than 95% (Slemr et al. 1985; Tokos et al.1998). In the gaseous form, mercury has a small deposition velocity and can stay in the atmosphere for up to 18 months, thereby being transported throughout the troposphere. By both dry and wet deposition mercury is returned to the earth surface, and rain or snow washes it to the water bodies (Slemr et al. 1985; Tokos et al. 1998; UNEP 2008). Within the lakes or water bodies, bacterial methylation of inorganic mercury to methyl mercury (MeHg) takes place, converting it to a bioavailable form (Gilmour et al. 1992).

Mercury is also a substance that may accumulate in the organism through bioaccumulation, but also biomagnify through the food chain (Clarkson 1997; Downs et al. 1998; Morel et al. 1998; Rognerud et al. 2002). Mercury and associated compounds are among the most toxic substances in the aquatic ecosystem, being highly toxic to animals and humans (Morel et al. 1998).

The main route of mercury exposure to humans is by the consumption of fish from mercury polluted water (Counter and Bauchanan 2004; Durrieu et al. 2005). Methylmercury is neurotoxic with pronounced effects on fetuses and neonates, as it can cross the placenta and damage the fetus directly (Counter and Bauchanan 2004). Methylmercury can also be transported through breast milk to the children (Counter and Bauchanan 2004; Sakatomo et al. 2002). Laboratory experiments on fish, piscivorous birds and mammals reflected pronounced changes in physiological, neurological, immunological, reproductive, histological and behavioral patterns on exposure to methylmercury (Fjeld et al. 1998; Scheulhammer et al. 2007). The dominant pathway of methylmercury to fish is by the consumed food (Hall et al. 1997), but uptake of

mercury can also be possible through the gills (Atwell et al. 1998). Methyl mercury bioaccumulates in the fish muscle tissues, and since MeHg biomagnify it correlates well with the trophic position (Morel et al. 1998; Adams & Onorato 2005; Rognerud et al. 2002). Due to the bioaccumulation, size and age of the fish is generally positively correlated with the concentration of mercury (Campbell et al. 2003a; Desta et al. 2006, Rognerud et al. 2002). In some fast growing fish species biodilution of mercury is also evident, resulting in lower mercury concentration in piscivorous fish species than expected from their trophic position (Desta et al. 2007a; Sharma et al. 2008).

Concentration of mercury in fish is influenced by the contamination level of lake water and sediments, but food web structures and biological factors such as longevity and growth can be important variables to explain the observed variables (Cabana and Rasmussen, 1996). Traditionally, food web structure was explained by the analysis of stomach contents, which gives the information of recent feeding habit (Atwell et al. 1998; Rosseland et al. 1999). To compliment the gut content technique, stable isotopes of nitrogen (^{15}N and ^{14}N) and carbon (^{13}C and ^{12}C) are used since these isotopes integrate a longer temporal scale of feeding, assimilation and growth (Kling et al. 1992; Yoshioka et al. 1994), and may also be used to quantify bioaccumulation of mercury in the aquatic ecosystem (Peterson & Fry 1987; Rognerud et al. 2002; Rosseland et al. 2007).

The nitrogen pool in the body tissue of an organism, consisting of ^{15}N and ^{14}N isotopes, is enriched with ^{15}N by an average of 3 to 5 ‰ relative to its dietary ^{15}N, as lighter ^{14}N is selectively eliminated through excretion (Peterson & Fry 1987). So in the tissues, concentration of $\delta^{15}N$ ($^{15}N/^{14}N$) increases consistently with increase in trophic position (Peterson & Fry 1987; Campbell et al.2003c). However the absolute value of $\delta^{15}N$ cannot be used to compare $\delta^{15}N$ values of organisms in different lakes, as baseline $\delta^{15}N$ values can vary from one place to another (Atwell et al. 1998). In the temperate region, the fractionation of ^{15}N per increase in trophic level was found to be 3.4 ‰ (Post 2002; Vander Zanden & Rasmussen 2001), while recent studies in the tropical region showed much lower fractionation, about 1.6 $^{0}/_{00}$ (Kilham et al. 2009). Since mercury increases with the increase in trophic level, the concentration correlates with $\delta^{15}N$, and

the slope of the regression of log transformed mercury concentrations against $\delta^{15}N$ values shows the biomagnification rate of mercury within the foodweb (Kidd et al. 1995; Atwell et al.1998).
The stable isotopes of carbon is a means of tracing the carbon flow, especially in identifying plant carbon sources of organisms at all trophic levels, $\delta^{13}C$ ($^{13}C/^{12}C$) in animals feeding on the same food source are similar to each other, and only an enrichment with around 1 ‰ occurs as a function of biomagnifications through their food source (Peterson and Fry 1987; Campbell et al. 2003c). The value of $\delta^{13}C$ may vary due to the differences in photosynthetic enzymatic fixation, growth rates, levels of CO_2 and pH, and $\delta^{13}C$ value also shows photosynthetic pathways for primary productivity (Hecky & Hesslein 1995). C_4 plants(grasses, freshwater algae), converting atmospheric CO_2 into compounds with four carbon atoms, have more $\delta^{13}C$ values while C_3 plants (mostly terrestrial plants), converting atmospheric CO_2 into compounds with three carbon atoms have low $\delta^{13}C$ values (Peterson & Fry 1987; Hecky & Hesslein 1995). Thus difference in $\delta^{13}C$ values may reveal different carbon source (terrestrial or aquatic source) and also littoral production or pelagic production since $\delta^{13}C$ value at the base of the littoral food web tends to be enriched with ^{13}C relative to the pelagic food web (France 1995).

Nepal is a landlocked country, located in and at the base of Himalayas, and sandwiched between two giants, China to the north and India to the east, west and south. Till now, no studies related to mercury pollution have been published from Nepal and mercury pollution has not been considered as a problem by the Government of Nepal. This is mainly due to the fact that industrial release of mercury has not yet been recorded locally in Nepal. On the other hand, the natural global release of mercury has been estimated to be about to 400 to 1300 tons per year from the ocean and 500 to 1000 tons per year from land, and a lot is added from reemission of mercury to the atmosphere (UNEP 2008). Similarly, anthropogenic release of mercury was estimated to be about 1930 tons per year in 2005, and two third of this release was contributed by Asia, with China standing in the first position and India in the third, globally (UNEP 2008). Since mercury is a global pollutant, its contamination has been seen in so-called pristine and "pollution free" lakes, proved by the presence of higher concentration of Hg in piscivorous Arctic charr (*Salvelinus alpinus)* in Lake Arresjøen, an Arctic lake at Svalbard in the Northern Europe (Rognerud et al. 2002). Pacyna & Keeler (1995) advocated that, long range transport of mercury is the major route of mercury contamination in the Arctic.

Together with mercury, other components of pollutants from burning of fossil fuel and other anthropogenic releases enter Arctic and Alpine areas, like other heavy metals and organic compounds (OC), especially persistant organic pollutants (POPs) (Rognerud et al. 2002; Rosseland et al. 2007). If the pollution contains acidifying components, metals might be mobilized from the catchment and ends into streams and lakes. By studying the levels of bioavailable metals in gills (reflecting bioavailability linked to i.e. the Biotic Ligand Model, BLM (Niogi and Wood, 2004), and bioaccumulation of metals in organs like liver and kidney, as well as POPs in liver or muscle tissue, one might get a picture of the pollution level in these lakes (Rognerud et al. 2002; Rosseland et al. 2007).

During the wet season or during rainfall, the concentration of mercury deposition is relatively higher, showing precipitation as a major factor in depositing mercury (Guentzel et al. 2001). Nepal located at the base of Himalayas, receives much precipitation, and can thus be contaminated with pollutants transported through long range transport. Pokhara is one of the most famous touristic cities of Nepal, with natural and cultural richness, which welcomed 230,799 foreign tourists in 2010 (My Republica, 2011, accessed online). There are eight lakes in and around Pokhara Valley and these lakes fulfill about 40-50 % of the fish demand within the areas (pers. comm. Jay Dev Bista, Head of the Phewa Fishery Research Centre, Pokhara, Nepal). To provide baseline data for mercury contamination in fish from this region, fish from Lake Phewa were collected choosing Tilapia (*Oreochromis niloticus*), African Magur (*Clarius gariepinus*), Chuche Baam (*Mastacembelus armatus*) and Sahar (*Tor putitora*) as the sample species representing supposed different levels in a trophic food chain.

The objectives were:

- to analyze the water quality and compare metal levels in water and gill tissue
- to analyze the concentration of heavy metals in the liver and compare levels to other alpine lakes
- by screening for POPs in fish muscle of the largest specimen of each species, evaluate if levels indicate a need for further investigations
- to examine the concentration of mercury in fish species at different trophic levels,
- to determine the bioaccumulation of mercury with respect to length and weight of the fish species,

- to analyse trophic position and trophic transfer (biomagnifications) of mercury in the fish species
- if concentrations of mercury exceed the international recommendations for population at risk (especially pregnant women and children), give recommendations for the utilization of fish species from Lake Phewa.

2. Material and Methods

2.1. Study Area

Lake Phewa, the second largest lake in Nepal and the largest lake in Pokhara Valley ($28^0$7-$28^0$12'N – $84^0$7'-$84^0$19'E), is a stream fed regulated dam, representing a subtropical mountain lake of Nepal (Figure 1a and 1b). The lake is lying at an altitude of 742m asl. It covers an area of 5.23 km^2, has 39.32 X 10^6 m^3 of water volume, with average depth of 7.5 m, and a maximum depth of 24 m (Rai et al. 1995). The streams Harpan Khola and Andheri Khola are the main inlets, along with many seasonal streams. The outlet river is diverted for irrigation and hydropower generation (Gurung et al. 2005). The catchment area is 110 km^2 catchment (Rai et al. 1995), and Ferro (1980) estimated that Lake Phewa receives as much as 10 times higher water volume to the lake from its catchments area during the monsoon (June-September).

Figure 1a. Map of the study area (Source: Google map, 2011). The two stations (A & B) for sampling of water is shown.

Figure 1b. Lake Phewa, Pokhara, Nepal (Photo: S. Basnet)

The land use pattern around the lake is varied, with a river channel zone in the eastern side of the lake shore, silt trap zone on the western side, agricultural land with dense urban areas on the northern side and forested areas, with sparse settlement on the southern side. The watershed of the lake consists of forested areas (44%), agricultural land (39%), urban and watershed area (5%), pasture and barren land (5%), lake area (4%) and shrub land (3%) (DSC, 1994). The human population of the Lake Phewa watershed accounts 0.14 million, with an annual growth rate of 7.4 % (CBS, 1995). The watershed is rich in biodiversity, with 7 vegetation types, 104 species of birds, 34 species of mammals, 16 fish species, 14 reptile and 6 amphibian species (IUCN, 1995). In addition 39 aquatic macrophytes, including 23 hydrophytes and 16 helophytes are found in the lake (Shrestha and Janauer, 2001). Gurung et al. (2005) describes the presence of 28 fish species, while recent studies have shown the presence of 30 species within the lake (unpublished report, pers comm. Jay Dev Bista) (Table 1).

Table 1. Fish species and their contribution in the catch record from Lake Phewa, Pokhara, Nepal

S.N	Scientific name	Local name	Contribution
1	*Tor putitora* (Hamilton)	Sahar	Low
2	*Tor tor* (Hamilton)	Sahar	__
3	*Acrossocheilus hexagonolepis* (McClelland)	Katle	Low
4	*Cirrihina reba* (Hamilton)	Rewa	Medium
5	*Mastacembelus armatus* (Lacepede)	Chuche bam	Low
6	*Xenentodon cancila* (Hamilton)	Dhunge bam	Medium
7	*Channa gaucha* (Hamilton)	Bhoti	Low
8	Channa striatus (Bloch)	Bhoti	Low
9	*Barilius barna* (Hamilton)	Lam Fageta	High
10	*Barilius bola* (Hamilton)	Fageta	High
11	*Barilius vagra* (Hamilton)	Fageta	High
12	*Barilius bendelisis* (Hamilton)	Fageta	High
13	*Mystus bleekeri* (Hamilton)	Junge	Low
14	*Puntius sophore* (Hamilton)	Bhitte	High
15	*Puntius sarana* (Hamilton)	Kande	High
16	*Puntius titius* (Hamilton)	Bhitte	High
17	*Puntius ticto* (Hamilton)	Bhitte	High
18	*Psilorynchus pseudochenesis*(Menon and Dutta)	Tite	Low
19	*Cirrhinus mrigala* (Hamilton)	Naini	Low
20	*Catla catla* (Hamilton)	Bhakur	Low
21	*Labeo rohita* (Hamilton)	Rohu	Medium
22	*Aristichthys nobilis* (Richardson)	Bighead carp	High
23	*Hypothalmichthys molitrix* (Valenciennes)	Silver carp	High
24	*Ctenopharyngodon molitrix* (Valenciennes)	Grass carp	Low
25	*Cyprinus carpio* (L.)	Common carp	Low
26	*Garra annaldalei* (Hora)	Buduna	Low
27	*Nemachelius rupicola* (McClelland)	Gadela	Low
28	*Clarias batrachus* (L.)	Magur	Low
29	*Clarias gariepinus* (Burchell)*	African Magur	High
30	*Oreochromis niloticus* (Linnaeus)*	Tilapia	High

Source: Gurung et al. (2005), and pers. comm. Jay Dev Bista (2010)

*Exotic species in Lake Phewa, (*Clarias gariepinus* and *Oreochromis niloticus* have been recorded in the commercial catches since 2001 and 2003, respectively)

The region and its watershed experience intense monsoon rainfall events (Figure 2), and probably it is one of the highest rainfall receiving watersheds of Nepal (IWMP, 1991).

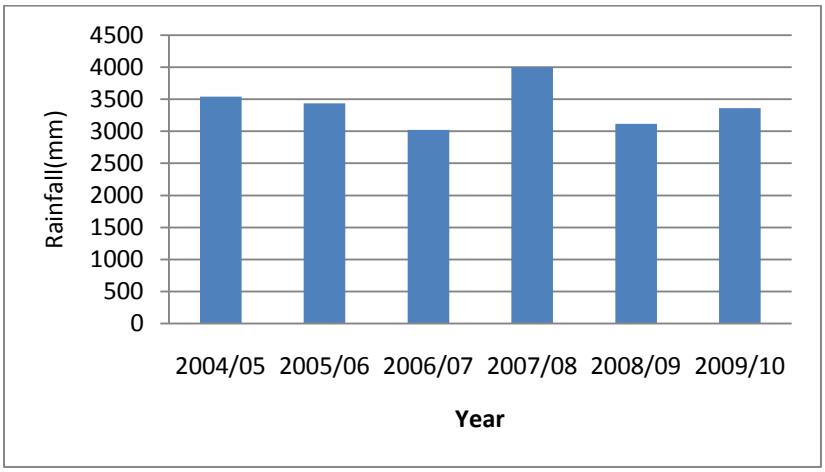


Figure 2. Yearly precipitation record for the Pokhara region (MoEnv, 2010)

Water temperature of the lake ranges between 15 ^{0}C and 32 ^{0}C (Figure 3). The lake has a high pH, ranging between 6 and 10 (Figure 4).

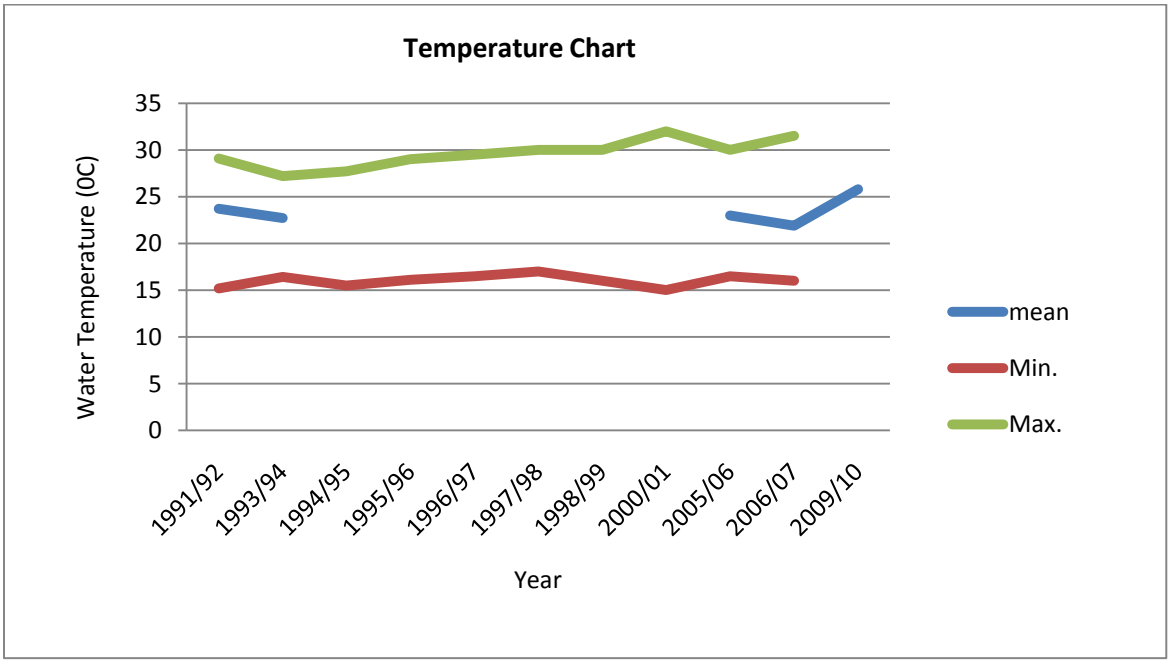


Figure 3. Surface water temperature of Lake Phewa (NARC, 2010)

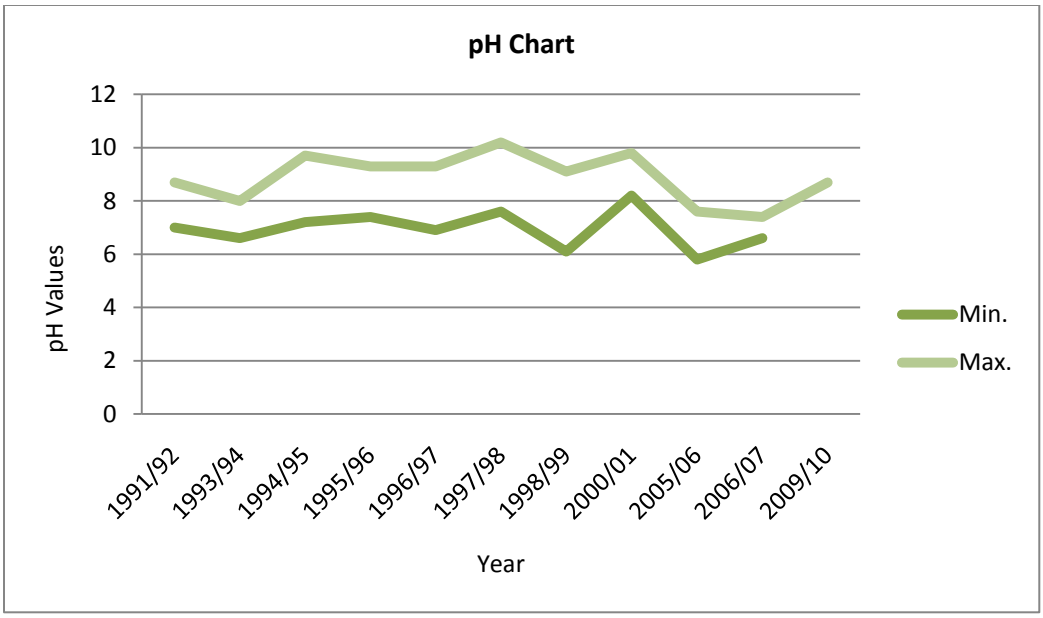


Figure 4. Annual minimum and maximum pH of Lake Phewa (NARC, 2010)

The lake water is used for production of hydroelectricity, irrigation, fishery, boating, and swimming, as well as serving the hotels and restaurants in the area. Gurung et al. (2005) described that about 1700 wooded and other small boats were operating in the lake for touristic purposes, but the number has increased to about 2000 since then (pers. comm. Jay Dev Bista).

Lake Phewa is influenced by encroachment, like pollution, heavy siltation, invasive species and eutrophication. Silt and sediments transported by the streams Harpan Khola, Andheri Khola, Seti canal and other seasonal streams are deposited in the lake every year. According to DSC (2002), the siltation rate was in the range 175,000-225,000 m^3 during the period of 1990-1994 and at this rate the terminal silt trap portion or the lake will be separated from the main lake by the next 20-25 years and the lake will be "dead" by the next 135-175 years, with 80 % loss of its water volume.

Similarly, sewages from the city areas carried by the Seti canal and other seasonal canals, as well as solid wastes by the tourists (Figure 5), have heavily polluted the lake water and the lake area. Addition of agricultural runoffs, soaps and detergents during bathing and washing clothes in the lake has also altered the lake chemistry. Organic runoffs from the watershed area, agricultural wastes and chemical fertilizers etc have changed the lake chemistry resulting in eutrophication of

the lake (Rai, 2000; Shrestha and Janauer 2001). Shrestha and Janauer (2001) described the changes in the lake from oligotrophic in 1970s, to mesotrophic in 1980s and to eutrophic by 1990s. Similarly, Rai (2000) also supports the changing trend of the lake towards rapid eutrophication, but due to the seasonal heavy rainfall in the catchment area, the lake productivity varies considerably throughout the year.

Figure 5. Pollution coming into the Lake Phewa from the Seti Canal (Photo: S. Basnet)

2.1.1. Fishery Management in Lake Phewa

From the very past, the ethnic community called "Pode or Jalari" was depended on fishing in the lake. Fish from the lake and river has been their only source of income. To organize the entire fishermen and their stake holders, an organization named "Matsya Byawasai Sangathan, Kaski" was registered in 1996. Its sister organization named "Phewa Matshya Byabasai Samiti (PMBS)", which means Fishers committee of Phewa has been operating in Phewa lake. This "PMBS" has an active committee board of 9 members, assigned the role as President, Vice President, secretary, treasurer and other members. To operate as a commercial fisherman in the lake, one needs to be from the Jalari community, they need to be a member of the committee, and need to follow the rules and regulation of the committee. Until 2010, there were 90

households being members of the fishers committee involved in the lake for fishery or cage culture.

For fishing in the lake they use gill nets, cast nets and hook and line. To have a gillnet, they need a permission from Phewa Fishery Research Center, and need to pay Rs.15 per gillnet every year (US$1=Rs.70). The community holds more than 4000 gillnets, and the number is increasing every year. About 400-500 gillnets of different mesh size, each exceeding 20 m in length, were in daily operation, but the number depends on the season.

2.1.2. Sustainable Fishery

To make sure their profession will continue for generations, PMBS has set up some regulations. Though any one can enjoy fly-fishing in the lake, catching of fish by gill nets can only be done by the Jalari people. They can set their gill nets in the evening, but have to pull then the next morning. If they are found setting gill nets in the day time, they are fined with Rs. 5000. Some places near Ratna Mandir, Barahi Mandir and around Phewa Fishery Research Centre, fishing is prohibited.

As the lake turned eutrophic, water hyacinth (*Eichhornia* spp.) an exotic species, is a problem along with other non degradable pollutants like plastics. The members of PMBS are involved in cleaning (removing water hyacinth, plastic bags, and bottles) and conservation of the lake. They also buy fingerlings from Phewa and Begnas fishery research centre, mostly of carps, which are released in the lake every year.

Apart from this, they also monitor the illegal fish killings by using poison, electricity and explosives in the littoral area of the lake as well as in the river that feed the lake. Especially the *Tor* species, a native species, are migratory species which ascend upward to the river from the lake during breeding season. Monitoring of the breeding grounds and providing awareness about the conservation of the breeding ground are also part of their major role.

PMBS has also a role in the management of marketing the fish captured in the lake, as well as fish produced in cage cultures in the lake. Most of the fish caught in the lake and from the cages are brought to the fish market centre “Khapaudi”, where the species and weight are recorded, and

sold according to the rates for different fish species. The fishermen need to pay tax for their catch. If the weight is less than 3 kilos, they have to pay tax Rs. 2 per kilo, while above this, they have to pay Rs. 10 per kilo. Out of this tax collected, Rs.1 per kilo goes as the government tax and rest is collected in PMBS's account. This amount is used in buying fingerlings, arranging training programs, and providing loans to the fishers group from Jalari community.

2.2. Sampling

Samplings for this study include fish, aquatic plants, zooplankton and water samples, and performed from September to December 2010.

2.2.1. Selected fish species

I chose four fish species commonly appearing in the daily catches that supposedly represented different trophic levels. Fish from cage cultures were not used. With proper investigation, questionnaire and observation, I chose Tilapia (*Oreochromis niloticus*), African Magur (*Clarius gariepinus*), Chuche Baam (*Mastacembelus armatus*) and Sahar (*Tor putitora*) as my sample species.

Tilapia (*O. niloticus*) is a cichlid fish native to Africa, occurring in a wide variety of freshwater habitats like rivers, lakes, sewage canals and irrigation channels (Bailey, 1994). It is omnivorous when young, while in later stages it consumes large quantities of phytoplankton (Teferi et al. 2000). This exotic species was introduced to Nepal in 1988, by Government of Nepal, for the purpose of aquaculture and research (FAO, 2004). In lake Phewa, source and date of introduction of tilapia is not known but it started coming into catch record from 2003 (Pers. comm. Jay Dev Bista). Today tilapia is the most important species in the catches.

Figure 6. Tilapia, *Oreochromis niloticus* captured in Lake Phewa (Photo: S. Basnet)

African Catfish (Figure 7) inhabits lakes, ponds, rivers and swamps, and due to its pseudo lungs, long body shapes and capacity to produce large amount of mucus, it can survive for a long time in stagnant water and even out of water (Vitule et al. 2006). It is an opportunistic feeder with remarkable array of feeding adaptation, and found to feed on detritus, filamentous algae, zooplankton, macrophytes, aquatic and terrestrial insects, fish, nematodes, arthropods, mollusks, crustaceans, birds, reptiles and amphibians (Yalcin et al. 2001, Desta et al. 2007). *C. gariepinus* was introduced in Nepal unofficially in 1996-97 by fry traders from India and Bangladesh, and its culture has been expanding since early 2000s (FAO, 2011), and it has been recorded since 2001 (Pers. comm. Jay Dev Bista). High catch rate, good size and good fillet, has made this species important for the fishing community around Lake Phewa.

Figure 7. African catfish *Clarias gariepinus* captured in Lake Phewa (Photo: S. Basnet)

The spiny eel, *Mastacembelus armatus* (Figure 8), is a common teleost in Asia. It is economically important, and the demand exceeds the supply, because of its palatability and being nutritious (Serajuddin et.al. 1998). Its active predatory and carnivorous habit is described by well developed dentition, absence of gill rakers, strongly built stomach, short intestine and dominance of animal prey in the gut contents, including shrimps and other crustaceans, along with aquatic insects, fish, mollusks, and aquatic vegetation (Serajuddin et.al. 1998). This fish species is believed to have medicinal value to cure arthritis, so demand of this fish is very high (Pers comm. local people).

Figure 8. *Mastacembelus armatus* captured in Lake Phewa (Photo: S. Basnet)

Golden Mahseer (*Tor putitora*) (Figure 9), a popular large freshwater game fish of mountain rivers and lakes in most Trans-Himalayan countries, may attain a weight of about 45 kg (Shrestha 1997). It is an opportunistic feeder which feeds on a wide variety of food, such as algae and other plant matter, insect larvae, mollusks and fish (Shrestha 1997, 1999)

Figure 9. *Tor putitora* captured in Lake Phewa, November 2010 (Photo: S. Basnet)

2.2.2. Fish Sampling

For the sampling of fish, I used European gillnets of mesh size 16, 22.5, 29, and 29 mm (measured from knot to knot). These gillnets were not effective in catching the selected species, so fish samples were also bought from the local fishermen. As soon as possible after the fish were landed, it was brought to Phewa Fishery Research Centre, where a "clean lab" had been established. Total length (from snout to the caudal fin) and total weight of the specimens were measured to the nearest cm and gram, respectively. The fish were then dissected according to the EMERGE Protocol (Rosseland et al. 2002). New aluminium foil was used as a cover on the dissecting bench, for dissection of each fish. All the dissecting instruments were cleaned with the lake water, and new surgical blades were set in the scalpel for each fish. Plastic Ziploc bags, scale envelopes and plastic vials were properly marked with a distinct code for each fish. Fish samples representing different length and weight were chosen for dissection of organs.

Gill samples (second gill arch on the right side) were collected in pre-weighted plastic vials. Scales, otoliths and operculum bone were taken out from each specimen, cleaned and put in scale envelopes which were stored in small Ziploc bags. Stomach contents were collected in plastic vials and added 80 % ethanol. Liver samples were collected in a piece of aluminium foil, wrapped properly and marked, and then were placed in the pre marked Ziploc bag. Muscle samples in between the dorsal and adipose fin and above the dorsal line were collected, after removing the skin and without any contamination. Muscle tissue was divided into three parts and then freed from the inner side. Muscle samples closer to the tail was collected for Hg, the middle one for stable isotope analysis and the upper and bigger one for POPs. Each sample was packed in a separate aluminium foil, marked, and put inside the pre marked Ziploc bag. Then all the samples of a fish were again placed in a bigger Ziploc bag marked with the fish code, fish name, date and location and then the samples were placed in the freezer. When returning to Norway, the frozen samples were put in an ice box with freezing elements, and transported to Norway, where they were stored in freezer at UMB, until the analysis.

2.2.3. Sampling of aquatic plants

Aquatic plants were sampled for the analysis of stable isotopes. Floating plants were handpicked whereas submerged plants were collected with the help of local fisherman (Figure 10a & 10b).

Long bamboo rod was used to collect the submerged plants that they use to feed the fish in their cage. The plants were kept inside the plastic bag and were frozen and transported to Norway for analysis.

Figure 10a. Collecting aquatic plants in Lake Phewa (Photo: Bjørn Olav Rosseland)

Figure 10b. Submerged plant samples from lake Phewa (Photo: Bjørn Olav Rosseland)

2.2.4. Water sampling

Water samples were collected from two different location of the lake, one from the northern side and the other from the southern side (Figure 1a (Site A & B). *In-situ* filtration (Figure 11) was carried out to obtain the filtered water samples. For the sampling, 0.45µm membrane filters (Millipore, d: 45µm) and ion exchange resin, Chelex 100 resin was used with 50-100µm mesh size. Water was collected in a 50 ml glass tube, capped and noted. Time to fill 30 ml and 50 ml was recorded. And all the water samples were placed in a cool and dark place until they were transported to Norway for further analysis.

Figure 11. *In-situ* water filtration in Lake Phewa at site (A), autumn 2010 (Photo: Roshan Raut)

2.3. Laboratory analysis

2.3.1. Stomach content analysis

Analysis of stomach contents was carried out in the Laboratory at Aquatic Ecology Centre, Kathmandu University, Dhulikhel Nepal and the lab at Khwopa College, Bhaktapur, Nepal. Out of the 105 fish sampled, 52 had empty stomachs, and all except one specimen of Tor had empty stomach. Stomach contents were examined using a dissection microscope. Many of the fish stomachs had nearly fully digested food.

2.3.2. Analysis of mercury and stable isotopes

For the analysis of mercury and stable isotopes, 64 fish samples representing 20 samples each from *Clarias gariepinus* and *Mastacembelus armatis*, 10 from *Oreochromis niloticus* and 14 from *Tor putitora* species. These were chosen by selecting representative of different length and weight classes.

2.3.3. Mercury Analysis

Analysis of mercury was carried out in the Environmental Chemistry Section of the Department of Plant and Environmental Sciences (IPM), Norwegian University of Life Sciences (UMB). THg concentrations (mg Hg kg^{-1} wet weight, w.w.) have been analysed using Perkin-Elmer model FIMS 400 Flow Injection Model System. Dissolution of fish muscles for total mercury determination was done by Anton Paar microwave oven. Calibration of the equipment was done by plotting calibration curves using the measurement values of four different synthetic standards. The curves were linear, and calibration was rechecked after every five samples. Also included were blanks as well as DORM-2 (piked dogfish, *Squalas acanthias* L.), certified reference material from the National Research Council of Canada, Ottawa, to control the accuracy of the method (see Desta et al. 2006).

2.3.4. Stable isotope analysis

Muscle samples for the analysis of stable isotopes of nitrogen and carbon were homogenized in distilled water with the help of a blender. Then the solutions of samples were transferred in glass container and were freeze dried. The puffy samples were then transferred to tin capsules and weighted (0.08-0.120gm), and packed as small balls. Isotopic ratios of nitrogen and carbon were determined by combusting the samples in a Flash Elemental Analyzer (EA), separating the combustion gases (CO_2 and N_2) with a Poraplot Q column and transferring them to a Finnigan Delta^{Plus} XP continuous-flow isotope ratio mass spectrometer (CF-IRMS). These analyses were carried out at the Isotope Laboratory, Norwegian University of Life Sciences (see Desta et al. 2006; Desta et al. 2007; Sharma et al. 2008). The stable isotopic ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$) were expressed as delta values, as parts per thousand ($^{0}/_{00}$) difference from a standard using the following formula:

$\delta X\ (^{0}/_{00}) = [(R_{(sample)}-R_{(standard)})/R_{(standard)}]x1000$

"X" represents ^{15}N or ^{13}C and "R" is the ratio between heavy and light isotope, and standard is a primary standard, i.e. atmospheric air for nitrogen and VPDB (Vienna Pee Dee Belemnite) for carbon. Standard material included the International Atomic Energy Agency (IAEA) (IAEA-N_1 and IAEA-$N_2$for nitrogen and IAEA-CH_6 for carbon) external reference standards and also the in-house standard (trout, *Salmo trutta*). All standards were measured at the beginning of each run, and once after every ten samples within a run during the routine of stable isotope analysis.

2.3.5. Age determination

The age of *O. niloticus*, *C. gariepinus*, *M. armatus* and *T. putitora* were determined by counting the macrozones on otoliths, scales and operculum bones. Small and thin otoliths were read as a whole under a binocular microscope (Leica 40x). The bigger otoliths were cut into half through the centre and burnt carefully till it turned brown, placed in propandiol to make the zones more clear and read under the binocular microscope (Christensen 1964; Borgstrøm et al. 1992). Slides of scales were made and observed under the microscope whereas operculum bone were observed under the microscope after clearing it with propandiol and the zones were counted.

It was very difficult to count the macrozones in otoliths, scales and operculum bones. Accordingly I was not convinced with the aging, and after advice from one of the supervisors (R. Borgstrøm) further analysis regarding aging was stopped.

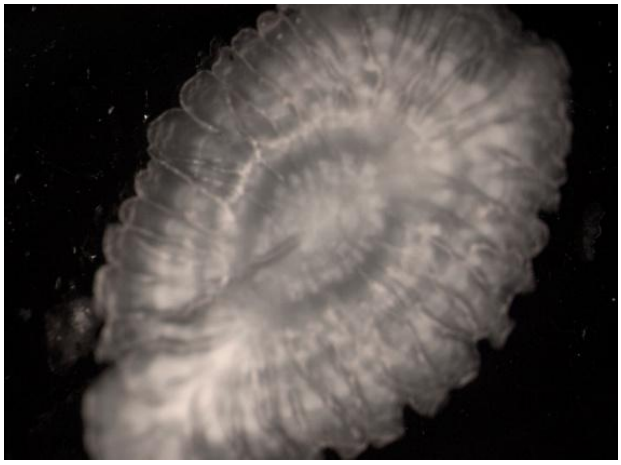


Figure 12. Otolith of *Oreochromis niloticus* from Lake Phewa. Zones can be seen, but it is impossible to determine number of annuli (Photo: S. Basnet)

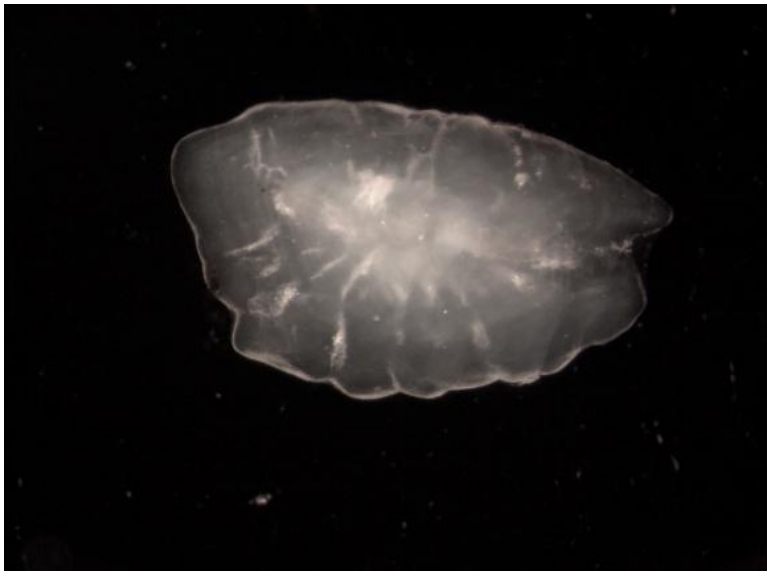


Figure 13. Otolith of *Tor putitora* from Lake Phewa (Photo: S. Basnet)

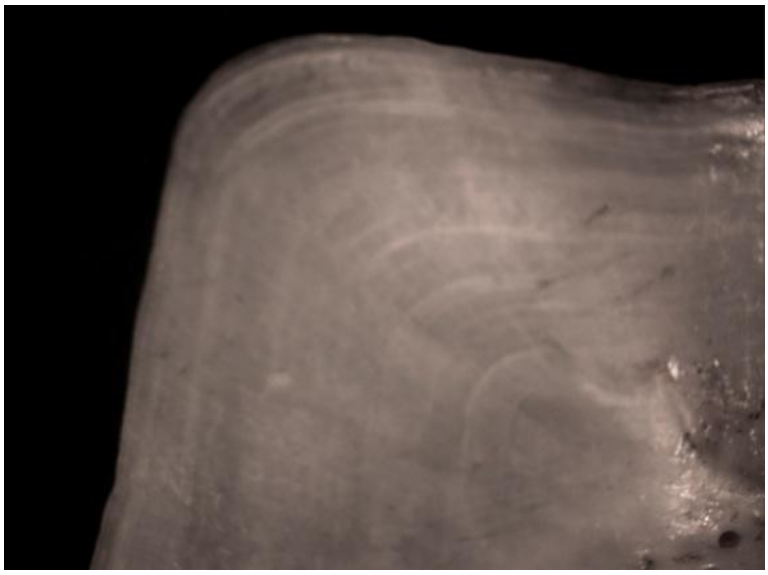


Figure 14. Operculum of *Tor putitora* from Lake Phewa. It is impossible to decide what is an annulus. (Photo: S. Basnet).

2.3.6. Analysis of POPs

POPs analysis was carried out at the Laboratory of Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Norway. Samples were analyzed for OC pesticides and PCBs by gas chromatography coupled to mass spectrometry (details in Sharma et al. 2009).

2.3.7. Water chemistry analysis

The inductively coupled plasma – optical emission spectrometer (ICP-OES) was used to analyze major cations (Na, K, Mg, Si, Ca) and ion chromatography was used to analyze major anions (Cl, NO_3, SO_4) in water. TOC- analyzer was used to measure total organic carbon (TOC) in water. Inductively coupled plasma mass spectroscopy (ICP-MS) was used to investigate the levels of trace metals in water sample.

2.3.8. Analysis of metal concentration in gills and liver samples

For the analysis of metals, 25 liver samples representing 14 samples from *T. putitora*, 6 from *O. niloticus* and 5 from *C. gariepinus* were selected. Similarly, 5 gill samples of *T. Putitora* and *O. niloticus* respectively, were selected for metal analysis.

Gill samples, stored in pre-weighted plastic vials, and liver samples were freeze dried, weighted and transferred to the contamination free plastic container. The samples were then mixed with 2.5ml of 5% Ultra Pure HNO_3, 0.25ml of internal standard and were diluted to 50 ml with MQ-water. Then the mixtures were placed inside the Ultraclave for digestion. All samples were analysed using ICP-MS and reported as µg/g gill or liver dry weight (dw).

For accuracy, blank samples were analysed every single time ICP-MS was used. Internal standard was also added in each gill and liver sample that controlled the digestion and dilution procedure. Similarly, sample reference material DOLT 4 (dogfish's liver) was used to compare the calibration procedure.

2.4. Statistical analysis

Hg concentrations were regressed against total length and total weight. Stable isotopes of C (δ^{13} C) and N ((δ^{15} N) were regressed against fish length to test the shift in trophic position and carbon source. Log transferred Hg concentrations were regressed against $\delta^{15}N$ values to determine biomagnifications of Hg between different trophic position. Stable isotope of nitrogen ($\delta^{15}N$) value was regressed against ($\delta^{13}C$) to find the trophic positions in food chain. Statistical significance was accepted at $p \leq 0.05$. Regression analysis of the concentration of every element, with total length and weight, were performed. All statistical tests were done using MINITAB 15, and MS- EXCEL.

3. Results

3.1. Sample size

The length and weight of the sixty four samples, representing 20 from *C. gariepinus*, 10 from *O. niloticus*, 14 from *T. putitora* and 20 from *M. armatus* (Figure 15).

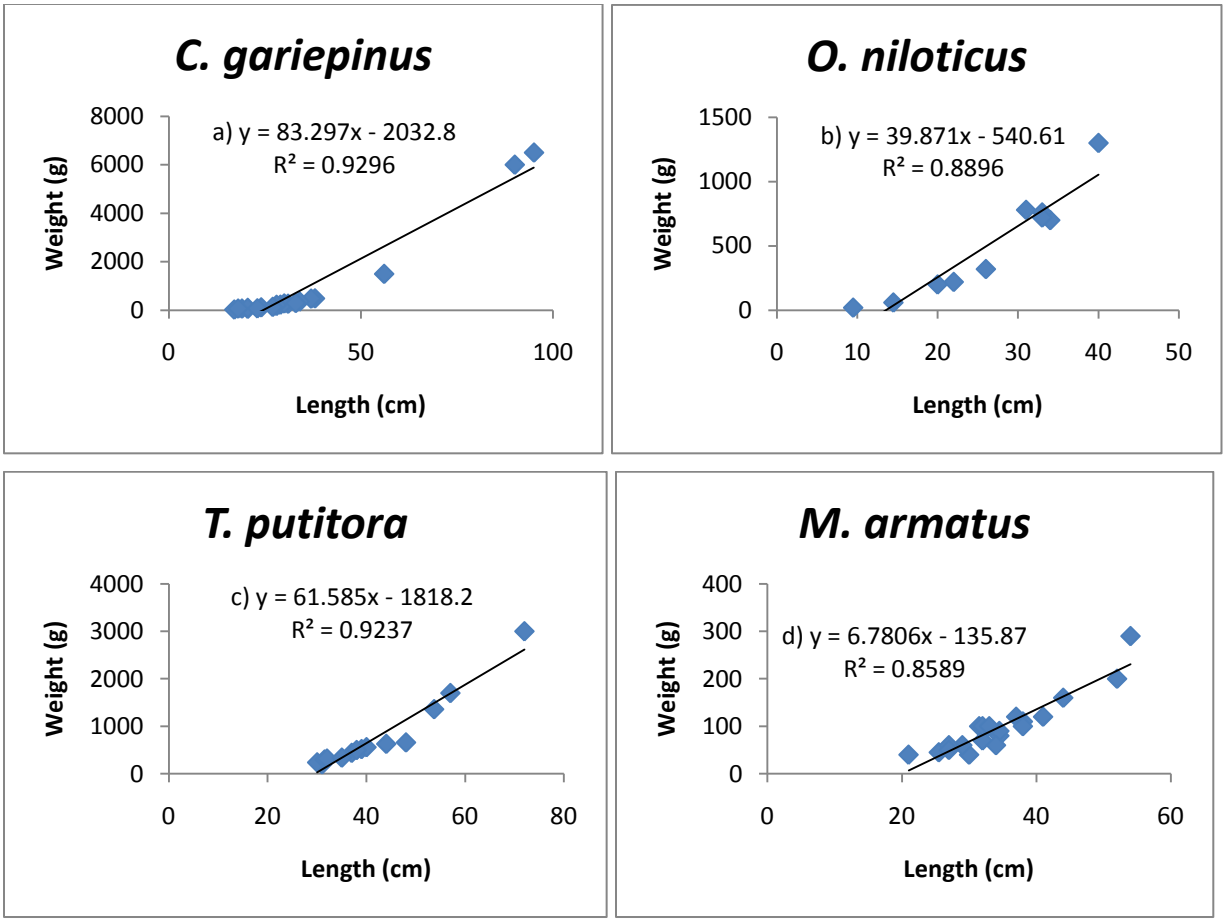


Figure 15. Distribution of 20 samples from *C. gariepinus*, 10 from *O. niloticus*, 14 from *T. putitora* and 20 from *M. armatus* according to length and weight of the individual specimens.

3.2. Fish diet

Large differences in stomach contents were observed between the species (Table 2). Aquatic plants dominated by volume in *O. niloticus*. Plants were also found in the stomach contents of *M. armatus* and *C. gariepinus*, but these species had also insects and fish as major diet groups (Table 2).

In the two length-classes of *O. niloticus*, the smallest one, 14-21 cm, had eaten insects and silt along with aquatic plants (Figure 16), while length class (21 – 35 cm), had eaten aquatic plants only. The diet of *M. armatus* consisted of many invertebrate groups, odonata, diptera, arachnida, crustacea, hymenoptera and orthoptera as well as fish and small proportion of aquatic plants and feathers. The gut content of length class 21-33 and 33-54 cm are shown in figure 17 and 18 respectively. Similarly, irrespective of the length class 17-30 cm and 30-95 cm, *C. gariepinus* showed a varied diet, with insects (odonata, coleoptera, diptera, hemiptera, hymenoptera, orthoptera, lepidoptera and zygoptera), crustaceans, gastropods, fish, frog and some proportions of aquatic plants (Figure 19 and 20). The gut content in the single sample of *T. putitora* consisted of Odonata (45%) and fish (55%).

Table 2. Number of examined stomachs, number of empty stomachs, and major items identified from each fish species from Lake Phewa, sampled in autumn 2010.

S.N	Species	No. of samples	Empty stomachs	Major Contents
1	*O. niloticus*	31	14	Aquatic plants
2	*M. armatus*	29	13	Insects, fish, plants
3	*C. gariepinus*	31	9	Insects, fish , frog, aquatic plants
4	*T. putitora*	14	13	Insects, fish

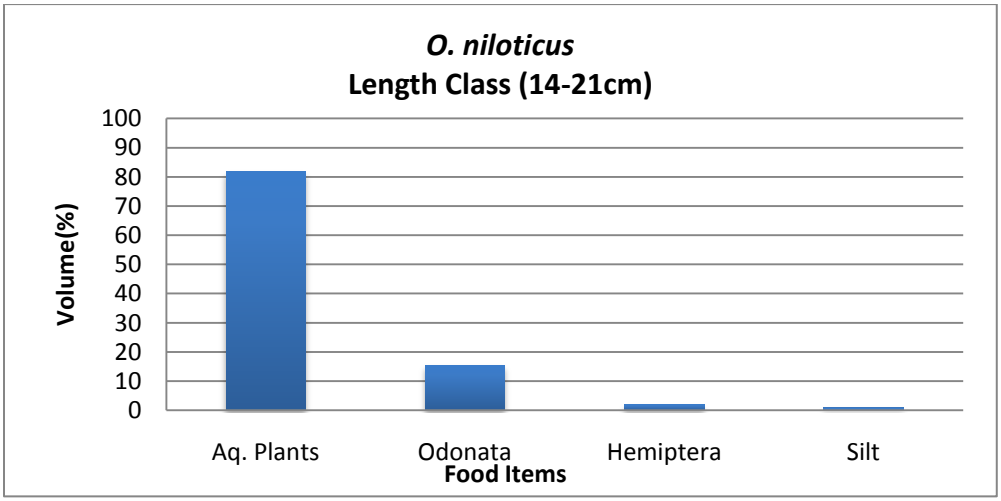


Figure 16. Percentage (by volume) food items consumed by *Oreochromis niloticus*, length class 14-21 cm, sampled in Lake Phewa during autumn 2010.

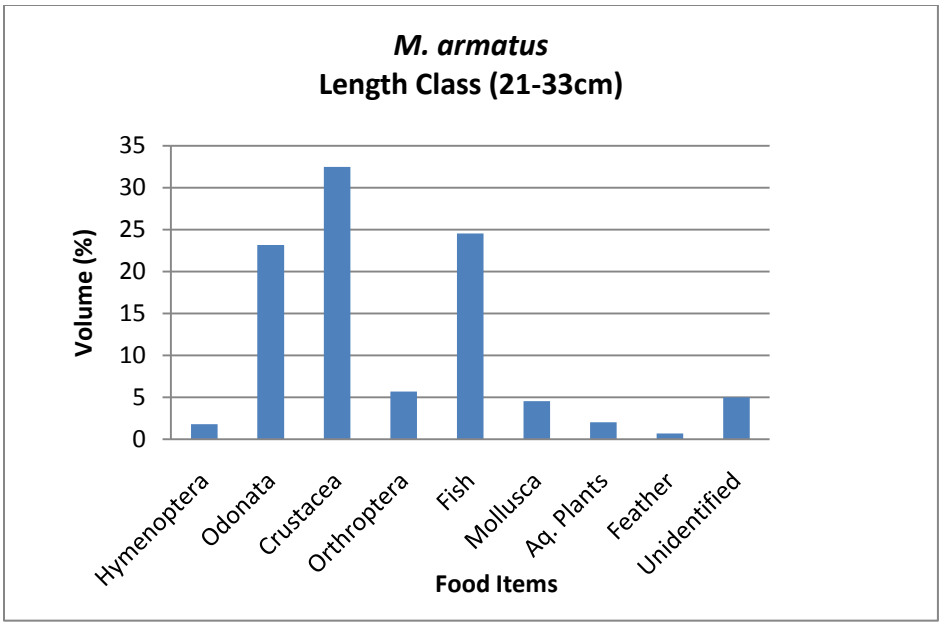


Figure 17. Percentage by volume of food items consumed by length class 21-33 cm of *Mastacembelus armatus* sampled in Lake Phewa during autumn 2010.

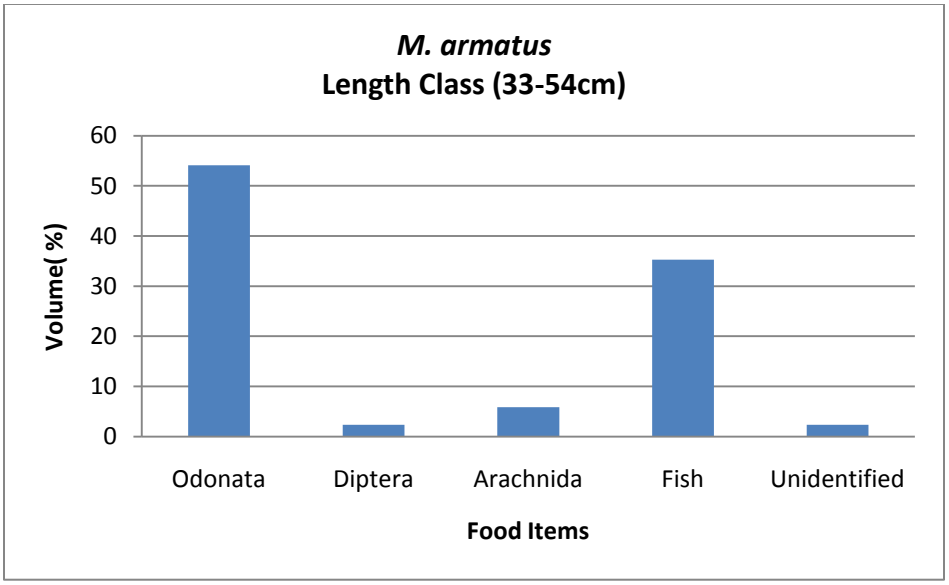


Figure 18. Percentage (by volume) of food items consumed by length class 33-54 cm of *Mastacembelus armatus* sampled in Lake Phewa during autumn 2010.

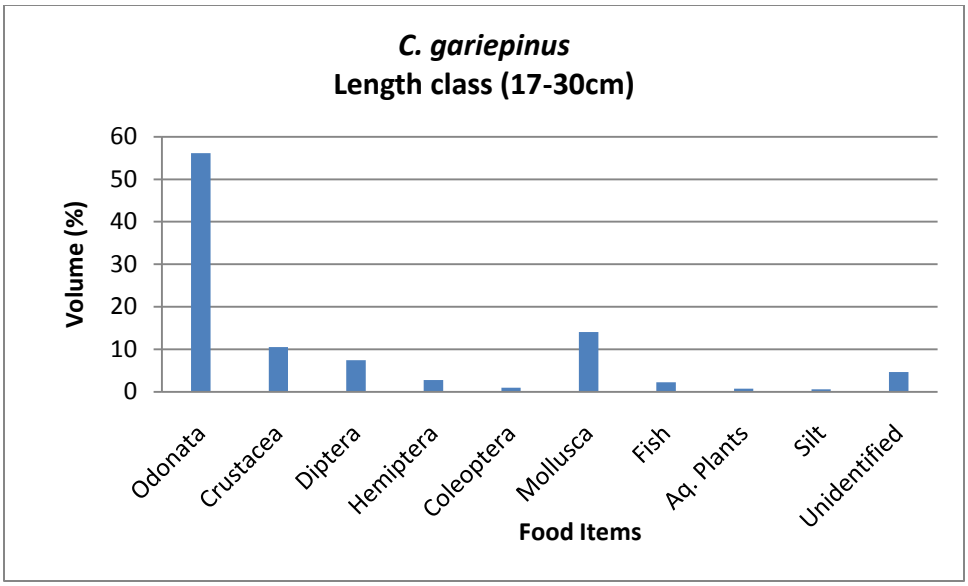


Figure19. Percentage (by volume) of food items consumed by length class 17-30 cm of *Clarias gariepinus* sampled in Lake Phewa during autumn 2010.

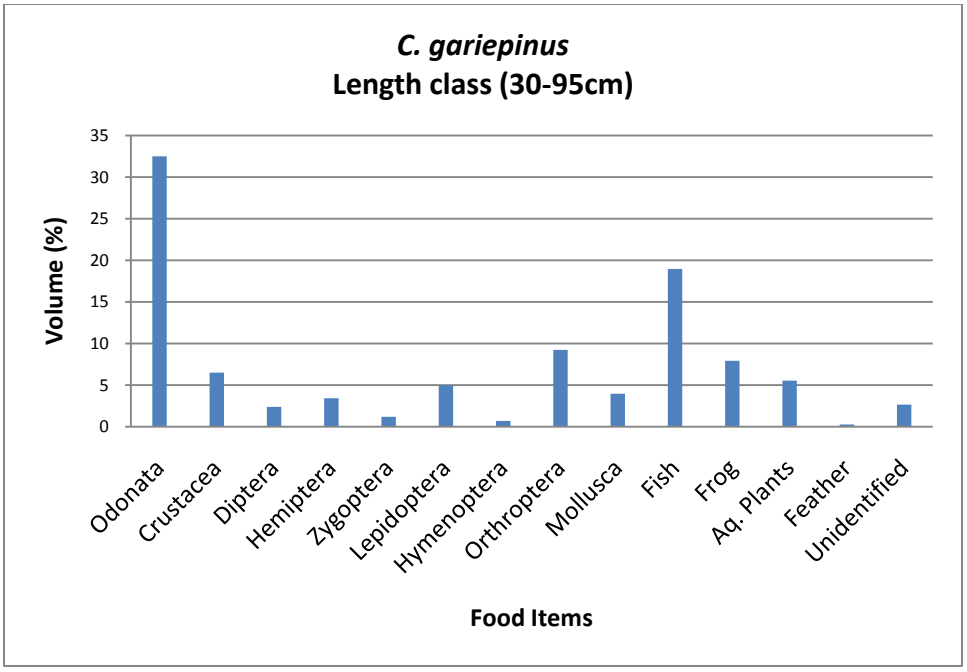


Figure 20. Percentage (by volume) of food items consumed by length class 30-95 cm of *Clarias gariepinus* sampled in Lake Phewa during autumn 2010.

3.3. Total mercury concentration in muscle tissue

The mean values of THg concentrations (mg kg^{-1}, ww) in the muscles of C. *gariepinus*, *O. niloticus*, *T. putitora*, and *M. armatus* were 0.07 ± 0.062, 0.030 ± 0.020, 0.115 ± 0.050 and 0.080 ± 0.049 respectively (Table 3). For *C. gariepinus* the highest concentration of mercury was 0.31 mg THg kg^{-1} ww and the lowest was 0.024 mg THg kg^{-1} ww. In *O.niloticus*, the highest was 0.081 mg THg kg^{-1} ww and the lowest was 0.006 mg THg kg^{-1} ww. Similarly for *T. putitora* and *M. armatus*, the highest concentration of mercury was found to be 0.21 mg THg kg^{-1} ww and 0.22 mg THg kg^{-1} ww and the lowest to be 0.050 mg THg kg^{-1} ww and 0.023 mg THg kg^{-1} ww respectively.

Table 3. Mean (±SD) of THg concentrations(mg kg-1, ww), N (sample size), length (cm), weight (g) of *C. gariepinus, O. niloticus, T. putitora, and M. armatus* sampled in Lake Phewa, Pokhara, Nepal, during September to December 2010.

Species	N	Length (cm)	Weight (g)	Tot. Hg (mg/kg w.w.)
C. gariepinus	20	34.9 ± 21.6	874.2 ± 1867.3	0.07 1± 0.062
O. niloticus	10	26.3 ± 9.6	508.0 ± 408.0	0.031±0.021
T. putitora	14	42.0 ± 12.0	769.2 ± 770.6	0.116±0.049
M. armatus	20	34.7 ± 8.2	99.7 ± 60.4	0.079±0.049

The relationship between mercury concentration (THg) and total length (TL) were positive and significant for C. *gariepinus* (linear regression, p=0.000) and *M. armatus* (linear regression, p=0.000) (Table 4, Figure 21). No significant relationship was seen between mercury concentration and total length in *O. niloticus* (linear regression, p=0.787) and *T. putitora* (linear regression, p=0.166), while THg concentrations showed a decreasing trend with increasing length for *O. niloticus*. The correlation coefficient (R^2) between THg and length and weight of fish was used to calculate the way of variation of THg with these parameters. The total length explained from 1 to 54.9% of the variations in mercury concentrations in the four species (Figure 21).

Similarly, the relationship between mercury concentration and total weight (TW) (Table 4, Figure 22) were found to be positive and significant in *C. gariepinus* (linear regression, p=0.000) and *M. armatus* (linear regression, p=0.000). There was no significant relationship between

mercury concentration and total weight in *O.niloticus* (linear regression, p=0.588) and *T. putitora* (linear regression, p=0.136).Total weight explained 3.8 to 60.8% of the variation in mercury concentrations in the four species (Table 4).

Table 4. Regression analysis of mercury concentrations (mg kg^{-1}, ww) against total length of fish (TL, cm) and total weight (TW, g). For each regression, the sample size (N), intercept, slope, R^2 (%), R^2-adjusted (%) and P values are given. Bold numbers indicate significant regression for p - value at α =0.05.

Species	Regression	N	Intercept	Slope	R^2 (%)	R^2-adjusted(%)	P-value
C. gariepinus	THg vs TL	20	-0.004	0.002126	54.90	52.400	**0.000**
	THg vs Tw	20	0.048	2.59E-05	60.80	58.600	**0.000**
O. niloticus	THg vs TL	10	0.036	-0.00021	1.00	0.000	0.787
	THg vs Tw	10	0.036	-9.9E-06	3.80	0.000	0.588
T. putitora	THg vs TL	14	0.048	0.00161	15.30	8.300	0.166
	THg vs Tw	14	0.095	2.69E-05	17.60	10.700	0.136
M. armatus	THg vs TL	20	-0.067	0.004205	50.60	47.900	**0.000**
	THg vs Tw	20	28.287	0.000591	53.40	50.800	**0.000**

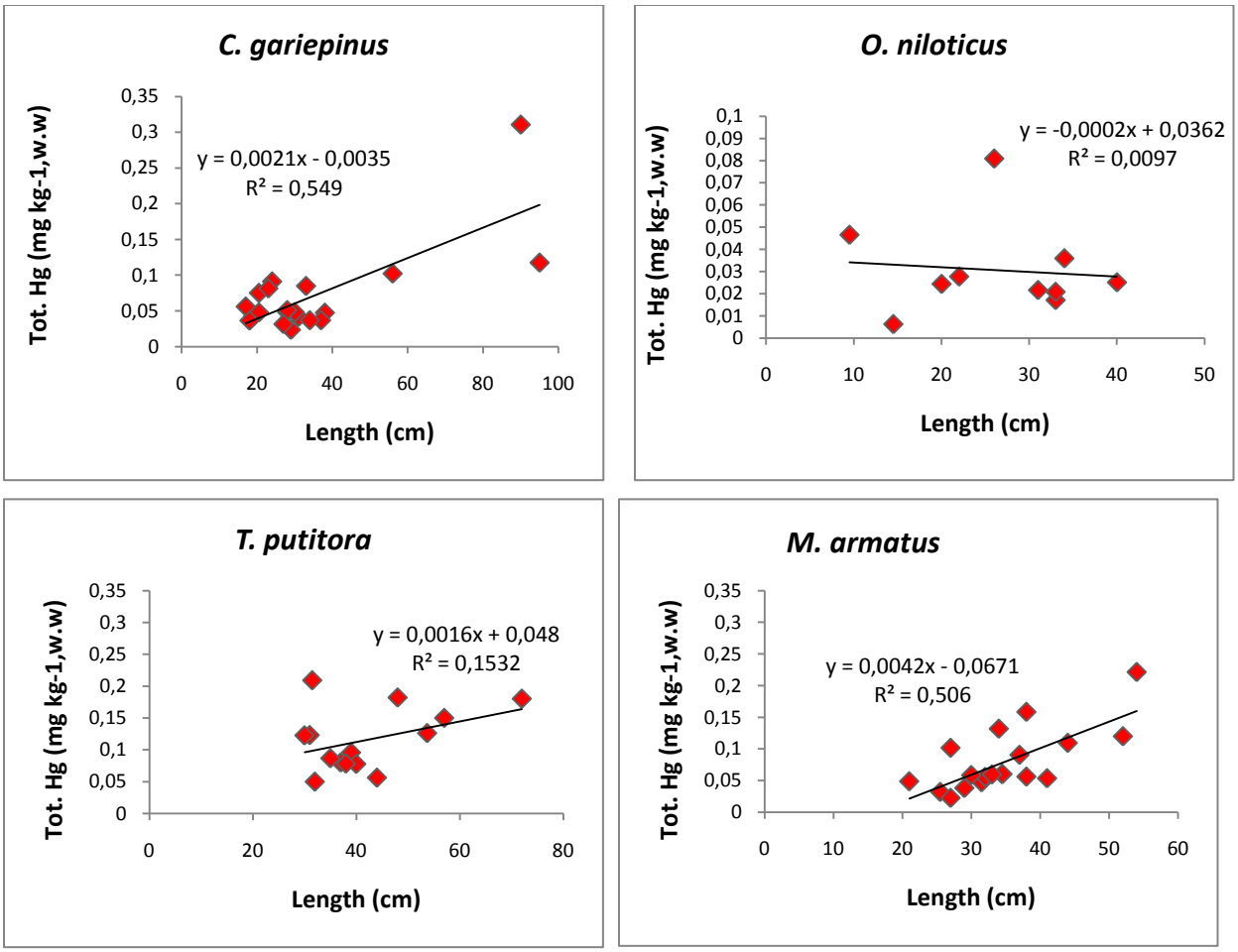


Figure 21. Mercury concentrations (mg THg kg^{-1} ww) versus total length (cm) for C. *gariepinus*, *O. niloticus*, *T. putitora*, and *M. armatus* from Lake Phewa, Pokhara, Nepal.

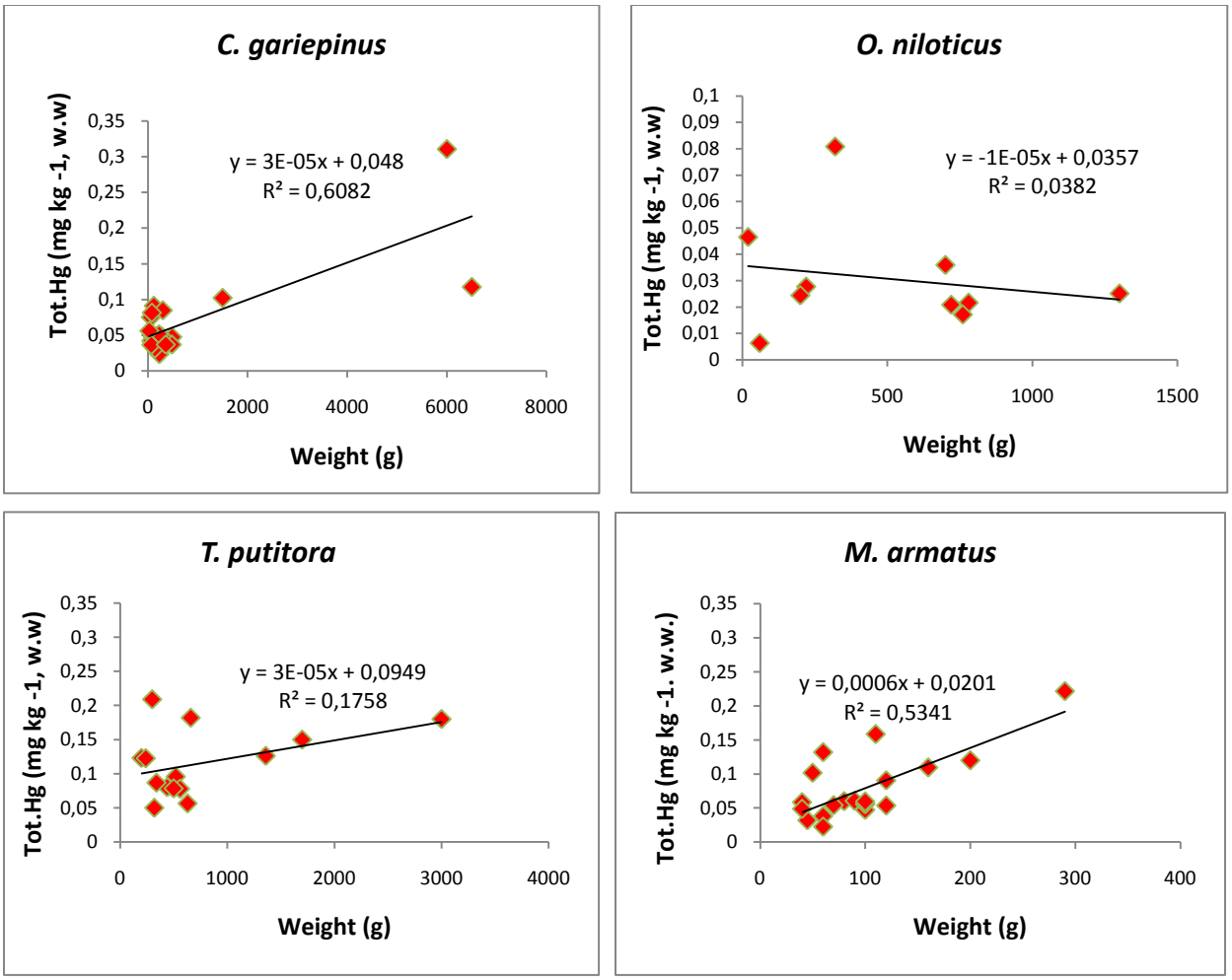


Figure 22. Mercury concentrations (mg THg kg^{-1} ww) versus total weight (g) for C. *gariepinus*, *O. niloticus*, *T. putitora*, and *M. armatus* from Lake Phewa, Pokhara, Nepal.

3.4. Stable isotopes of nitrogen and carbon

The instrumentation for analyses of stable isotopes at IPM had several problems in 2011. This has resulted in only 21 specimen analyses of individual fish samples and no analysis of the primary producers.

The available stable isotope data were regressed against fish size to interpret the dietary shift statistically. The values for δ^{15} N ranged from 5.837 ‰ to 12 ‰ for *C. gariepinus* (N=6), 7.63 ‰ to 9.01 ‰ in *O. niloticus* (N=4), 6.52 ‰ to 9.394 ‰ in *T. putitora* (N=4) and 9.506 ‰ to 13.361 ‰ in *M. armatus* (N=7). There were no significant relationship between δ^{15} N and total length for any of the species (Table 5, Figure 23).

The δ^{13} C values for *C.gariepinus* ranged between -18.407 ‰ to -31.838 ‰, *O. niloticus* ranged from -22.145 ‰ to -23.23 ‰, for *T. putitora* ranged from -17.55 ‰ to -24.734‰ and that for *M. armatus* ranged from -21.505 ‰ to -29.592‰. The linear regression between δ^{13} C and total length were not significant for any of the species (Table 5, Figure (24).

Table 5.Linear regression analysis (sample size (N), intercept, slope, r^2 (%) and p value) between Log THg concentrations (mg Hg kg^{-1} w.w.) and stable isotopes of nitrogen (δ^{15} N‰) and linear regression of stable isotopes of nitrogen and carbon against length for *C. gariepinis, O. niloticus, T. putitora* and *M. armatus* from Lake Phewa, Pokhara, Nepal.

Species	Linear regression	N	Intercept	Slope	r^2 (%)	P- value
C. gariepinus	Log THg versus δ^{15}N	6	-1.314	0.006	0.040	0.900
	δ^{15}N versus Length	6	9.363	-0.020	6.700	0.621
	δ^{13}C versus Length	6	-20.582	-0.120	53.600	0.098
O. niloticus	Log THg versus δ^{15}N	4	-2.618	0.123	28.000	0.470
	δ^{15}N versus Length	4	9.615	-0.039	25.300	0.497
	δ^{13}C versus Length	4	-20.858	-0.052	57.100	0.244
T. putitora	Log THg versus δ^{15}N	4	-0.197	-0.093	65.700	0.190
	δ^{15}N versus Length	4	8.694	-0.023	10.000	0.685
	δ^{13}C versus Length	4	-23.344	0.047	8.700	0.704
M. armatus	Log THg versus δ^{15}N	7	-1.548	0.041	2.700	0.725
	δ^{15}N versus Length	7	10.007	0.034	7.900	0.541
	δ^{13}C versus Length	7	-29.062	0.151	33.100	0.176

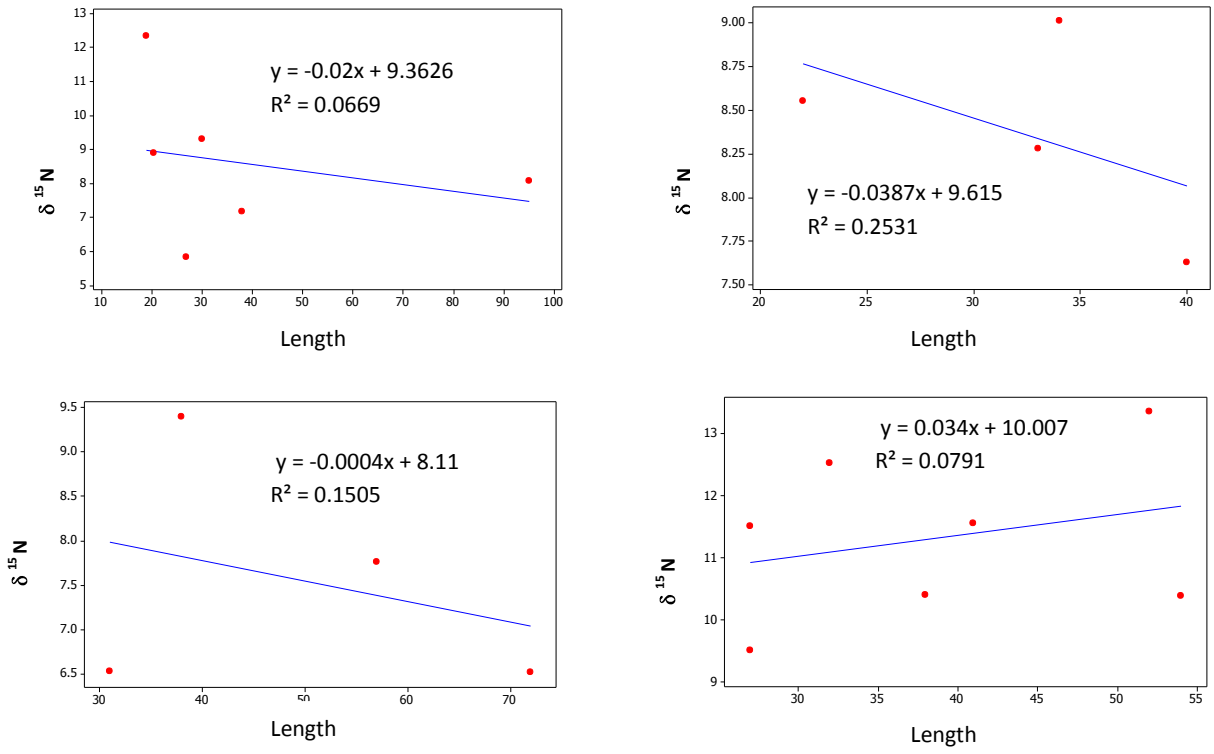


Figure 23. Relationship between stable isotope of nitrogen (δ^{15} N) and total length (cm), *Clarias gariepinus* (upper left), *Oreochromis niloticus* (upper right), *Tor putitora* (lower left) and *Mastacembelus armatus* (lower right).

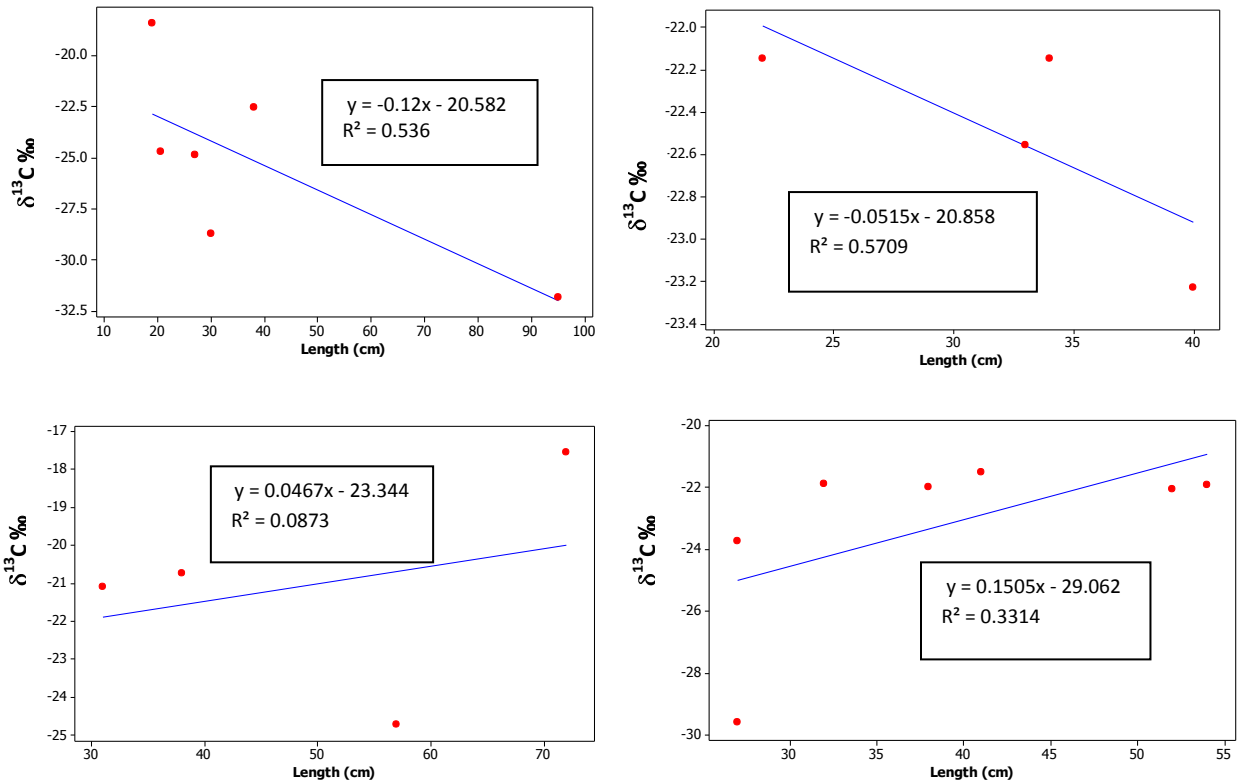


Figure 24. Relationship between stable isotope of nitrogen (δ^{13} C) and total length (cm), *Clarias gariepinus* (upper left), *Oreochromis niloticus* (upper right), *Tor putitora* (lower left) and *Mastacembelus armatus* (lower right).

Calculation of THg biomagnification within the fish species were done by regressing Log transferred THg (Log THg) against δ^{15} N values (Table5, Figure 25).

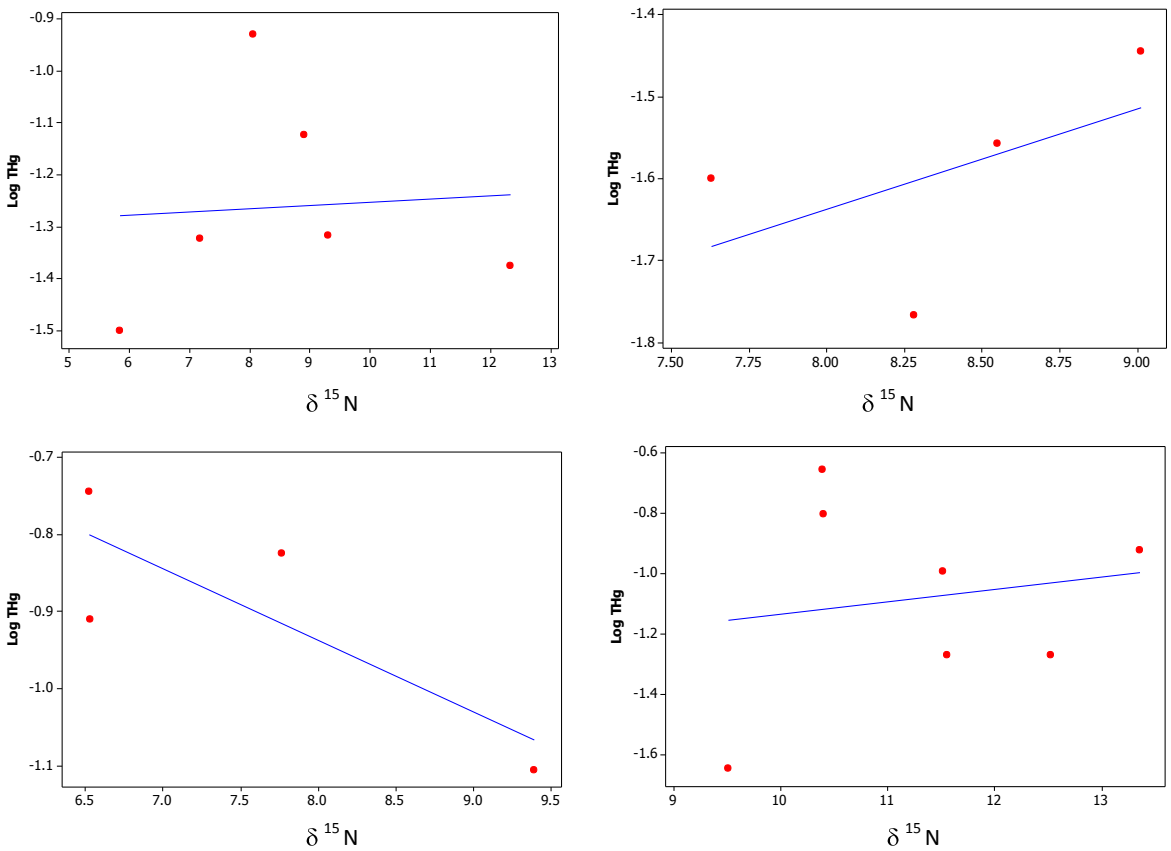


Figure 25. Relationship between Log transferred (Log THg) and stable isotope of nitrogen (δ^{15} N), *Clarias gariepinus* (upper left), *Oreochromis niloticus* (upper right), *Tor putitora* (lower left) and *Mastacembelus armatus* (lower right).

The food web structure in Lake Phewa was analyzed by plotting trophic level (δ^{15} N) against carbon source (δ^{13} C) (Figure 26). The isotopic ratio is highest for *M. armatus* and lowest for *T. putitora* (Figure 26)

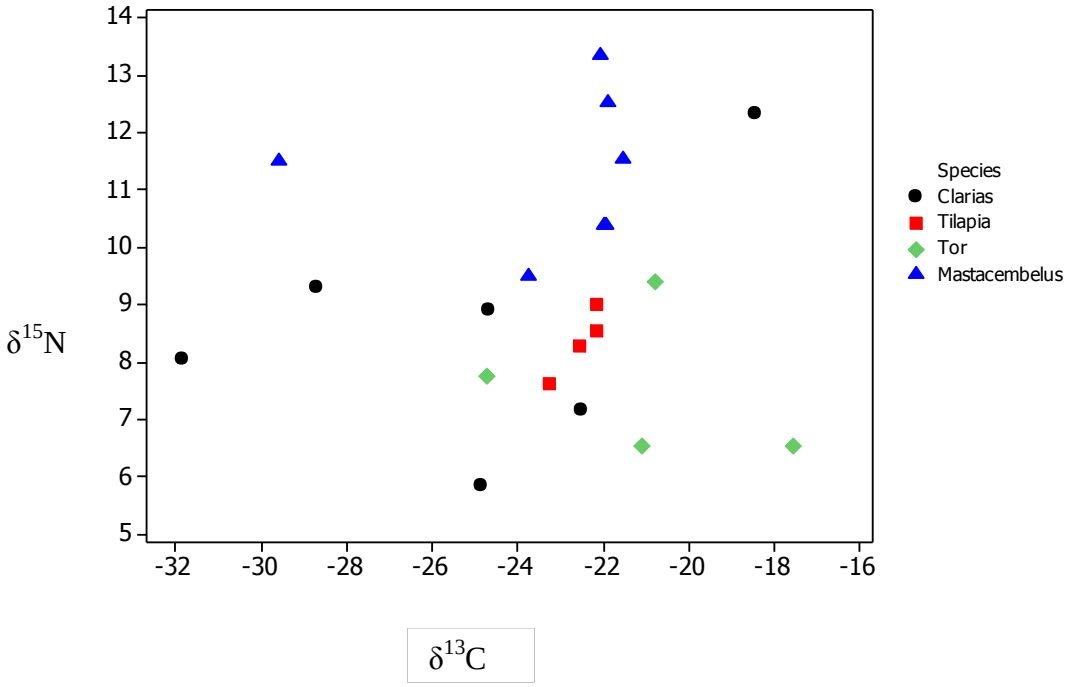


Figure 26. The relationship between $\delta^{15}N$ (trophic level) and $\delta^{13}C$ (carbon source) for all fish samples from Lake Phewa, Pokhara Nepal.

3.5. POPs in muscle samples

Of the many organic compounds analysed for, only DDts and Endosulfan sulfate were detected by the methods used by Bioforks (Appendix 1).The concentration of DDTs and Endosulfan sulfate from the largest specimen of each species are shown in Table 6.

Table 6.Concentration of POPs in the largest specimen of the four fish species from Lake Phewa, Pokhara, Nepal. Length (cm), weight (g) and concentration of DDE-pp, DDD-pp and Endosulfan-sulfate are given in ug/kg,w.w.

Species	Length (cm)	Weight (g)	DDE-pp(ug/kg)	DDD-pp (ug/kg)	Endosulfan-sulfate (ug/kg)
O. niloticus	40	1300	1.8	<1,0	3.76
C.gariepinus	95	6500	11.6	4.51	28.5
M. armatus	54	290	8.1	<1,0	<1,0
T. putitora	72	3000	7.6	1.32	4.72

3.6. General water quality

The water sampled on23/11/2010 and 29/11/2010 had water temperature 24^{0}C, and a pH of 7.09, conductivity 68 mS/m with the concentration of major cations and anions as described in the Table 7.

Table 7. General water chemistry, Lake Phewa, Pokhara Nepal.

Temperature (°C)	pH	Conductivity (mS/m)	TOC (mg/L)	SO_4 (mg/L)	NO_3 (mg/L)	Cl (mg/L)	Ca (mg/L)	K (mg/L)	Mg (mg/L)	Na (mg/L)	Si (mg/L)
24	7.09	68	0.8	1.3	0.3	1.1	10.3	3.9	1.2	1.7	2.9

3.7. Water chemistry analysis ICP-MS

Trace metal concentration in the filtered water sample (<0.45µm) was analysed with ICP-MS and the total concentration of trace metals were determined (Figure 27.).

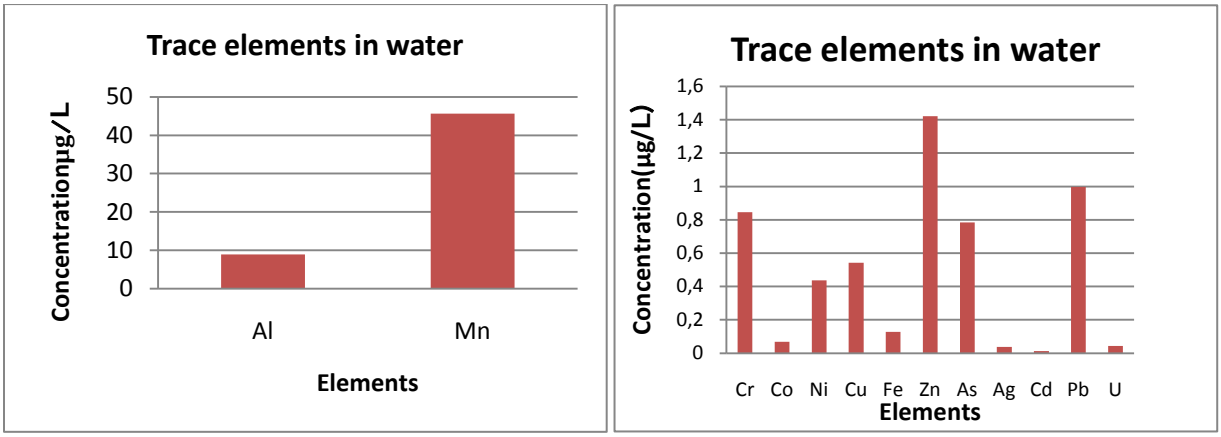


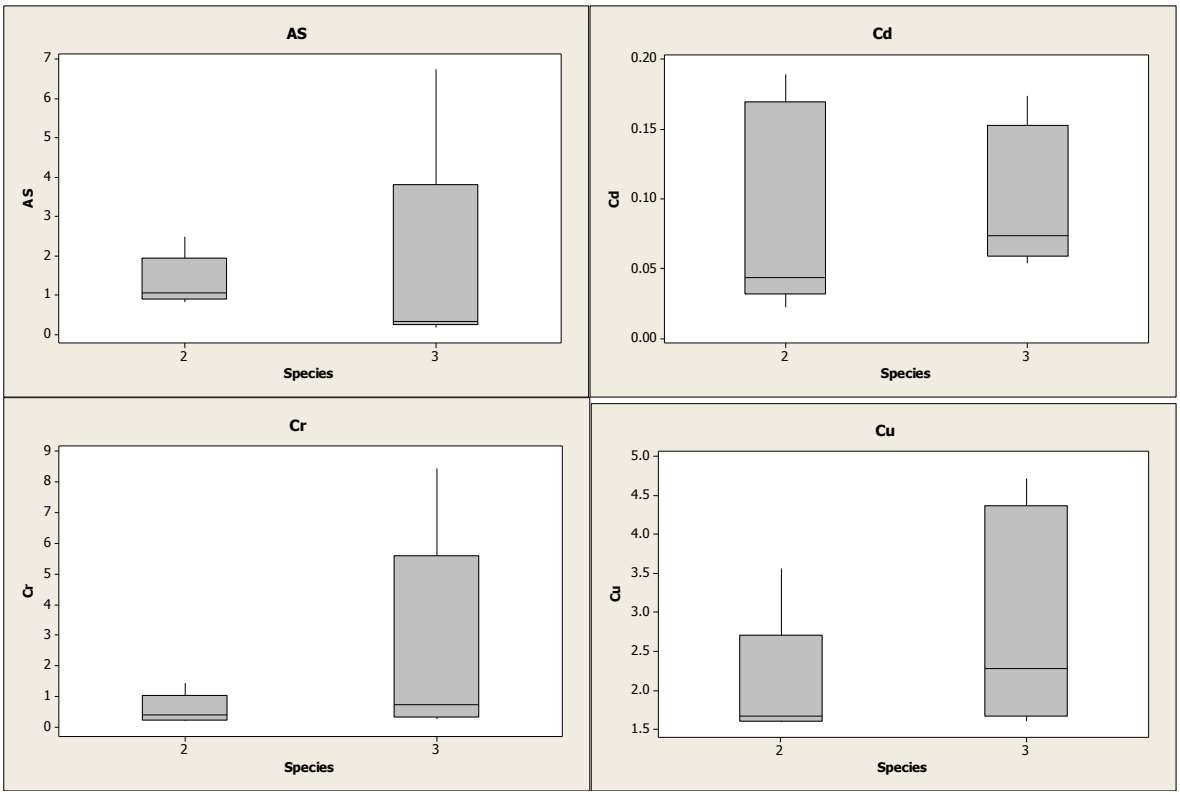
Figure 27. Concentration of trace metals in water from Lake Phewa, Pokhara, Nepal.

3.8. Trace metals in gills

Table 7 shows the mean concentration of trace metals in five gills of *O. niloticus* and *T. putitora* respectively. Significant difference was observed in the concentration of Mn and Ni among the two species (Figure 27). Likewise by using factor analysis the metals As, Cd, Cr,Cu, Mn, Ni, Pb, Se, Zn were found to follow each other in the gills. (Figure 28).

Table 7. Trace metals in gills for 5 individuals (N=5) of *O. niloticus* and *T. putitora* expressed as µg/g gill dry weight (d. w.), Average ±SD

	O. niloticus (N=5)	*T. putitora* (N=5)
AS	1.33 ± 0.67	1.68 ± 2.83
Cd	0.09 ± 0.08	0.10 ± 0.05
Cr	0.57 ± 0.51	2.50 ± 3.46
Cu	2.06 ± 0.84	2.86 ± 1.14
Mn	52.80 ± 27.21	146.02 ± 77.46
Ni	1.57 ± 0.29	3.47 ± 1.96
Pb	2.02 ± 2.20	6.98 ± 9.19
Se	2.59 ± 0.88	2.76 ± 1.05
Zn	71.36 ± 15.89	117.76 ± 70.28



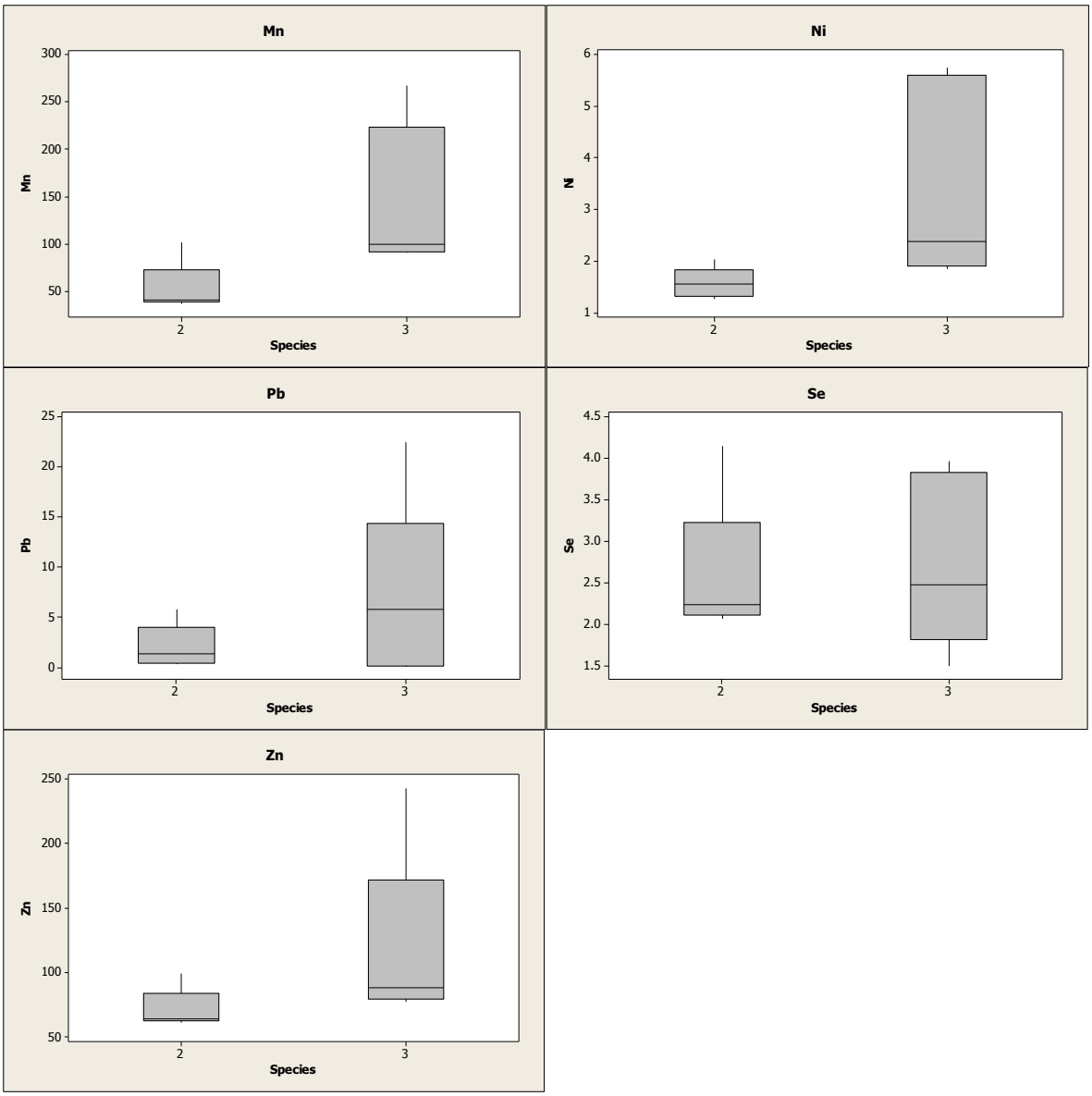


Figure 27. Box plots for the concentration (µg/g gill d.w.) of different trace elements in gills of *O. niloticus* (2), and *T. putitora* (3) from Lake Phewa, Pokhara Nepal. There were significant differences for Mn and Ni between the two species.

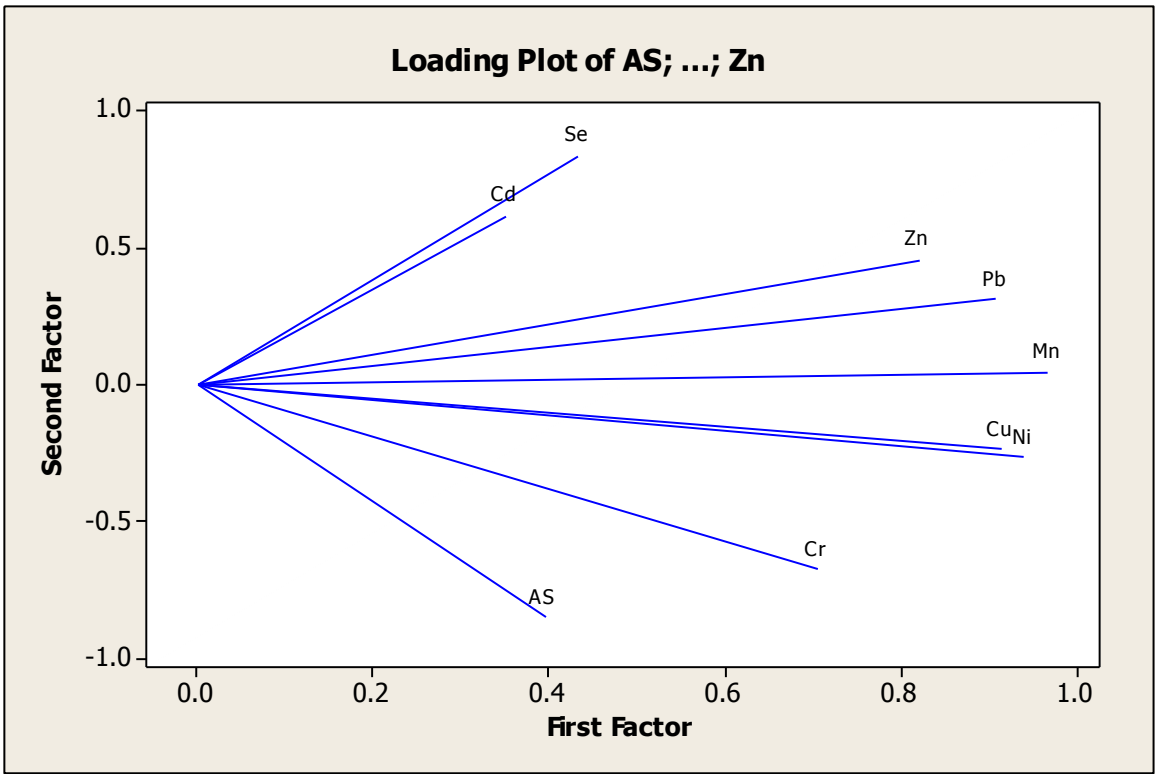


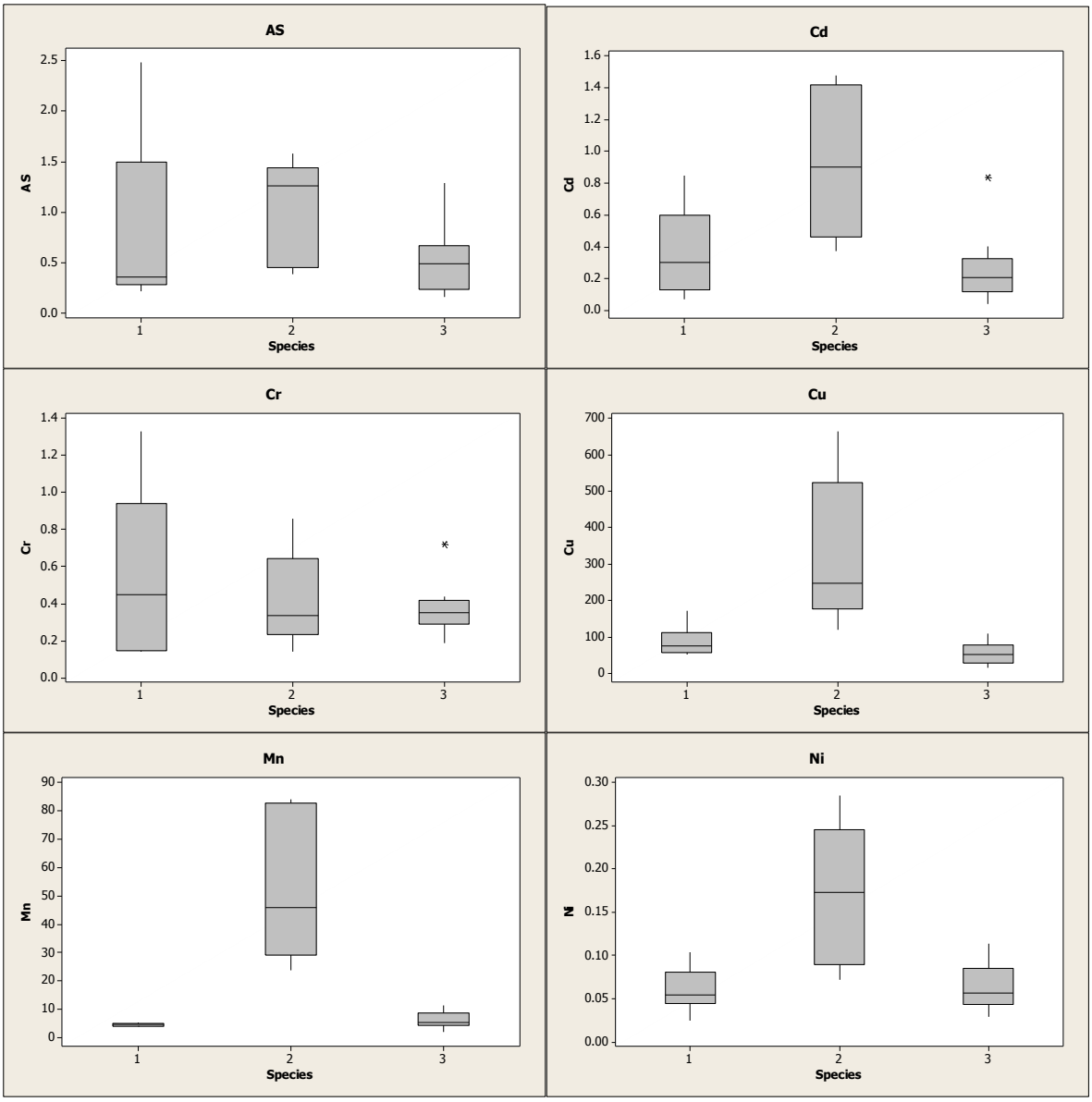
Figure 28. Factor analysis plot for metals in gills of *O. niloticus* and *T. putitora* for Lake Phewa, Pokhara, Nepal.

3.9. Trace metals in Liver

The concentration of trace metals in the liver of *C. gariepinus*, *O. niloticus* and *T. putitora* were obtained from ICP-MS analysis and are presented in table 8. Significant difference was observed among the species in relation to concentration of Cd, Cu, Mn, Ni, Pb and Se (Figure 29). Trace metals Mn, Ni, Cu, Cd were found to follow each other while Zn, Se, Pb and Cr were found to follow each other. Zn and As showed the opposite trend (Figure 30).

Table 8. Trace metals in liver as µg/g liver dry weight (d. w.), Average ± SD

	C. gariepinus (N=6)	*O. niloticus* (N=5)	*T. putitora* (N=14)
AS	0.81 ± 0.89	1.01 ± 0.53	0.50 ± 0.30
Cd	0.36 ± 0.28	0.93 ± 0.48	0.25 ± 0.20
Cr	0.56 ± 0.46	0.42 ± 0.27	0.36 ± 0.13
Cu	86.44 ± 43.98	328.75 ± 209.19	54.03 ± 28.68
Mn	4.46 ± 0.53	53.79 ± 27.49	6.04 ± 2.82
Ni	0.06 ± 0.03	0.17 ± 0.08	0.06 ± 0.02
Pb	0.90 ± 1.48	0.38 ± 0.15	0.12 ± 0.05
Se	25.24 ± 13.16	7.74 ± 2.75	7.60 ± 2.96
Zn	135.84 ± 50.19	83.20 ± 18.01	142.59 ± 48.67


AS
AS
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
Species
Cd
Cd
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
1
2
3
Species
Cr
Cr
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
1
2
3
Species
Cu
Cu
700
600
500
400
300
200
100
0
1
2
3
Species
Mn
Mn
90
80
70
60
50
40
30
20
10
0
1
2
3
Species
Ni
Ni
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1
2
3
Species

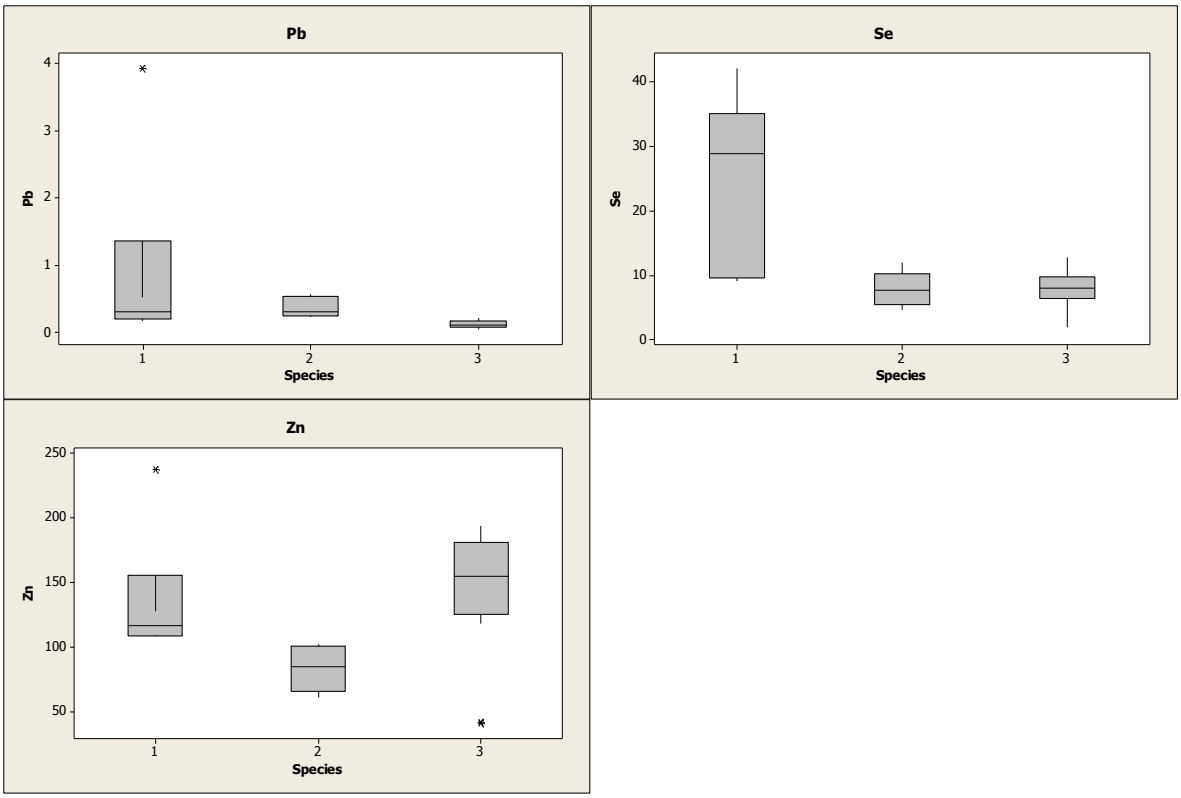


Figure 29. Box plots for different trace elements in liver of *C. gariepinus* (1), *O. niloticus* (2) and *T. putitora* (3), from Lake Phewa, Pokhara, Nepal.

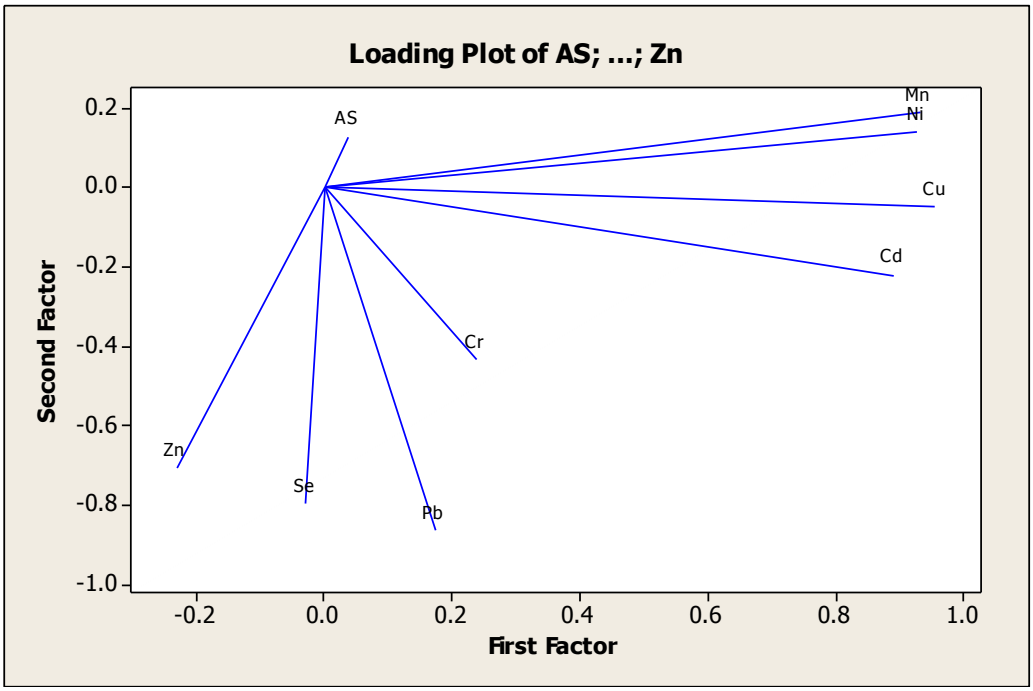


Figure30. Factor analysis for different trace elements in liver of *C. gariepinus* (N=6), *O. niloticus* (N=5) and *T. putitora* (N=14) from Lake Phewa, Pokhara, Nepal.

4. Discussion

4.1. Fish samples

Due to the problems of catching fish with the gillnets taken from UMB, the fish used in this study had to be bought at the fish market at Khapaudi, Pokhara. This means that the fish have been captured at different sites in Lake Phewa, depending on where the individual fishermen have their permission to fish. We therefore have no control of any local efforts, if any of the fish species or specimen is localized to certain areas in the lake with higher pollution levels.

Also, due to problems of ageing, we could only use size as an indicator of exposure and accumulation of pollutants in the individual fish. The size groups sampled, however, seems to cover at least the size groups from which the population in Pokhara will be exposed to, when buying and eating the fish from the fish market.

4.2. Diet

The diet of *O. niloticus* was fairly dominated with aquatic plants whereas the diet of *C. gariepinus, M. armatus* and *T. putitora* were dominated by aquatic insects and fish.

In case of the upper length classes of *O. niloticus* aquatic plants were the major diet which is comparable to the stomach content of *O. niloticus*, in Lake Awassa (Desta et al., 2007b), where up to 80% was contributed by blue green algae. Similar studies made in Lake Koka and Lake Ziway (Berger, 2009) showed the dominance of blue green algae and diatoms. Presence of aquatic insects in the diet of *O. niloticus* with lower length class in the present study showed similarity with the studies made by Desta (2007a), where zooplankton dominated the diet of juveniles of *O. niloticus*, in Lake Awassa. The diet of *C. gariepinus* in Lake Phewa was dominated by aquatic insects, fish with some plant matters, mollusks and frogs. This is comparable to the findings of Tadiso (2007), from Lake Ziway, where aquatic insects were the main diet for *C. gariepinus*. Desta et al. (2007a), however, found fish as the most important diet for *C. gariepinus* in Lake Awassa, which is comparable to the present study. Similar result was found by Gade (2009) where aquatic insects comprised the major diet along with fish eggs, mollusks, detritus, macrophytes and fish, in *C. gariepinus* from Lake Koka and Lake Ziway, with the result of present study. Being an opportunistic feeder (Goudswaard and Witte, 1997),

its feeding habit varies according to the variations in the availability of food items (Desta et al. 2007a). The diet of *M. armatus* comprised of aquatic arthropods with odonates and crustaceans as major food items along with fish, mollusks and some aquatic plants. Serajuddin et al. (1998), found shrimps, dipteran larvae, earthworm and minor carps with some aquatic vegetation as their major diet. The single gut content in *T. putitora* was with aquatic insects and fish and showed its carnivore nature. Malhotra (1982) (cited in Bista et.al 2002) described this species as a carnivore. In contrast to this Nautiyal and Lal (1984) found insect matter (81.4%), plant matter (15.9%) and other items including fish (1.6%) in the fingerlings of *T. putitora* from their natural habitat.Based on only one sample in the present study, it is impossible to conclude what is the dominating food source for *T. putitora* in Lake Phewa. This difference in diet from present study is possibly due to very small sample size in study. Most of the fish had nearly fully digested food which might be caused by the time-lag between the times of catch, purchasing and dissection, as most of the fish were alive while purchasing.

4.3. Mercury concentrations

The concentrations of THg in the studied fish species from Lake Phewa were consistently low, with averages of mercury concentration in different species, ranging from 0.031 to 0.116 mg kg^{-1}w.w. Although Lake Phewa with its elevation742 m.a.s.l. is not among the highest located lakes in Nepal, it is still comparable to most of the mountain and Arctic lakes studied in Europe by Rognerud et al. (2002) and Rosseland et.al.(2007). The levels in Lake Phewa are within the same range as found by Rognerud et al. (2002), who described that the mean concentration of THg ranging from 0.021 to 0.179 mg kg^{-1} w.w.. Rosseland et al. (2007) described that Hg concentration in the muscles of brown trout in Lochnagar ranging from 0.04 to 0.23 mg kg^{-1}w.w., levels which can be compared to the concentration of our present result. Not much data about the concentration of mercury in fish were available from the high altitude lakes of South Asian region including Nepal. Studies made by Holsbeek et al. (1997), in Bangladesh freshwater anadromous fish showed the lower level of mercury in different fish species (ranged from 0.002 to 0.430 mg kg^{-1} w.w.), which they explained due to the absence of mining and industrial input. As there are no mining or industry having Hg as point pollution source to Lake Phewa, the main source of Hg is through the long range transportation, one reason which corresponds to the present study site with no industrial input and mining of Hg in the lake. Excluding one specimen

each of *C. gariepinus*, *T. putitora* and *M. armatus*, the THg concentrations in all fish species were low and did not approach the WHO recommended guidelines level (0.2 mg kg^{-1}) designed to protect at risk-groups (WHO, 1990). As one of the two largest *C. gariepinus* (weight 6000 g) had a THg concentration of 0.31 mg kg^{-1} w.w., while the even larger specimen of 6500 g , had as low as 0.12 mg kg^{-1} w.w., indicate the importance of individual food preferences within the species.

4.4. Mercury concentration in relation to fish size and isotope ratios

Recent studies on the relationship between mercury concentration and fish size (length and weight) confirmed to have positive relationships (Brabo et al. 2000; Rognerud et al. 2002; Campbell et al. 2003a, 2003b, 2003c, 2003d, 2004, 2006; Adams and Onorato, 2005; Desta et al. 2006). Present study showed to have a positive and significant correlation of THg concentration with fish length and weight, in case of *C. gariepinus* and *M. armatus*. Whereas, for *O. niloticus* and *T. putitora*, no such relation was found. In case of *O. niloticus*, THg level showed to be decreasing slightly with increasing length and weight. Similar decreasing trend was seen in *O. niloticus*, in African lakes (Campbell et al. 2003d; Desta 2007; Tadiso et al. 2011). Campbell et al. (2003d) suggested growth dilution or depuration of THg as an explanation, while Tadiso et al. (2011) and Desta (2007) explained the phenomenon as an effect of changing food habits from zooplankton in the younger size classes to a dominance of aquatic algae and plants as they grew older. The latter explanation ("eating down the food chain") seemed more convincing in case of Lake Phewa.

No positive correlation of stable isotope, $\delta^{15}N$ and $\delta^{13}C$ with the fish length was found in the studied species from Lake Phewa, which shows that each species have reach "their own" trophic position and consumed the prey from the same basic carbon source, regardless of their size.

The fractionation of $\delta^{15}N$ was found to be 3.4‰ (Vander Zanden & Rasmussen 2001; Post 2002), per trophic level in temperate region while Kilham et al. (2009) showed it to be much lower of about 1.6‰ per trophic level. The values of $\delta^{15}N$ in the studies species of Lake Phewa ranged from 5.83 (*C. gariepinus*) to 13.36 (*M. armatus*). No analyses of the primary producers are abailable but the number of trophic levels in the fauna of Lake Phewa is approximated to be

at least three. The values of $\delta^{15}N$ for *C. gariepinus* in Lake Phewa, were ranged from 5.83 to 12.32 which shows a difference of at least two trophic levels within the species if the differences between trophic levels were close to 3%. Tadiso (2007) showed a difference of at least one trophic level between the smallest and largest *C. gariepinus*. The $\delta^{15}N$ values for *O. niloticus* ranged from 7.63 to 9.01, which showed a non statistical significant (due to few samples) tendency of shift to a lower trophic positions with increases size, as expected by the change in food preferences towards aquatic algae and plants with increased size. Although , unable to to compare levels due to lack of primary producer signals from Lake Phewa, the mean $\delta^{15}N$ value for *O. niloticus* (8.59±2.21) had a mean value similar for the same species in Lake Awassa (8.76±1.15, Desta 2007). The range of $\delta^{15}N$ values for *T. putitora* and *M. armatus* were 6.52 to 9.39‰ and 9.50 to 13.36‰ respectively. These values showed no shift in trophic position for any of the species.

The $\delta^{13}C$ values for *C. gariepinus* ranged from -31.8 to -18.4. The variations were large which indicates a wide range of habitats and food resources used by the species. Tadiso (2007) found $\delta^{13}C$ values ranging from -27 to -20, in Lake Ziway. The $\delta^{13}C$ values for *O. niloticus* ranged from -23.23 to -22.14 which showed to shift in the habitat and resource utilization. This result can be compared to the findings of Tadiso (2007) with $\delta^{13}C$ values ranging from -25.3 to -21.03 with little or no tendency in the shift of carbon source. Similarly the $\delta^{13}C$ values for *T. putitora* and *M. armatus* ranged from -24.73 to -17.55 and -29.59 to -21.74 respectively. Both species showed a wide range of habitat and resource utilization in Lake Phewa.

The scatter plot of $\delta^{15}N$ and $\delta^{13}C$ showed *M. armatus* at the highest trophic position and *O. niloticus* at the lowest trophic position. This is in agreement with the concentration of THg which was comparatively higher in *M. armatus* than in *O. niloticus*. Diet and trophic position of the fish determines the Hg concentration in fish (Kidd et al. 2004; Desta et al. 2006), with highest concentration in piscivorous species with higher trophic positions. The one large *C. gariepinus* had typically fish in stomach, and also the third highest value for $\delta^{15}N$.

The linear relationship between Log THg and $\delta^{15}N$ was not significant in Lake Phewa for all the species indicating no biomagnification in the species. However, this could be the result of too few analyses of the stable isotopes of nitrogen.

4.5. POPs in the fish muscles

Many of the POPs analysed for by Bioforsk, had too low levels to in relation to the methods used, with only one sample per species, the result are only indicative to see if further analyses should be recommended in fish from Lake Phewa. However, concentration of DDT metabolites (DDE-pp) was found as 1.8 µg/kg w.w in *O. niloticus* to 11.6 µg/kg w.w. in *C. gariepinus*. Similarly DDD-pp was less than 1.0 µg/kg w.w. in *O. niloticus* and *M. armatus* while the highest value was 4.51 µg/kg w.w. for *C. gariepinus*. Similar studies on high altitude lakes by Rognerud et al. (2002), found the concentration of DDE- pp, in piscivorous Arctic charr, to be slightly higher, up to 20586 pg/g w.w. (20 µg/g w.w.), compared to the values of present data from Lake Phewa. Sharma et al. (2009), found the concentration of DDT metabolites to be in an average of 609.85ng/g (609 µg/kg w.w.), in piscivorous pike in Lake Årungen, which was much higher than the present data from Lake Phewa. The reason for the presence of DDT metabolites in the fish species of Lake Phewa, may be due to both locally and long range transported sources. In Nepal, these pesticides were used to eradicate malaria for more than two decades since 1958 (Rottenberg, 2004, Subedi, 2001) and thus can have the local source.

Likewise the concentration of endosulfan-sulfate was found to be much higher in *C. gariepinus* (28.5µg/kg w.w.), in *M. armatus* (1.0 µg/kg w.w). The value was less than the acute reference dose proposed by USEPA (2002) which is 0.15mg/kg/day and higher than the average daily intake referred as chronic reference dose was 0.006mg/kg (USEPA, 2002). This would be reached by eating 0.21 kg fillet of the most contaminated specimen analyzed from Lake Phewa.

4.6. Water quality and trace metals in water, gill and liver of fish

Surface of water temperature was 24 ^{0}C, with pH 7.09 and conductivity 68 ms/m. This result can be compared to the results from Rai (2000) and NARC (2010), with the water temperature ranging from 15.5^0 to 27.9 ^{0}C and pH from 7.2 to 9.7 in Lake Phewa at similar time of the year. The concentration of Ca, Mg, Na and K were 10.3, 1.2, 1.7 and 3.9 mg/L respectively, which were higher than the values found in the studies made by Johnes et al. (1989), where the concentrations were 7, 1, 1.5 and 0.6 mg/L for Ca, Mg, Na and K respectively. Similarly, concentration of Cl and SO_4 were found to be 1.1 and 1.3 mg/L respectively, which is higher than 0.7 and 0.2 mg/L values observed by Johnes et al. (1989).

For trace metals in water Al, Mn and Sr were found in higher concentration than other metals with a concentration of 8.9, 45.6 and 26.0µg/L, respectively in the filtered samples.
The concentration of Al is low, representing no problems for even very sensitive fish like salmonides in this neutral and high Ca waters (Rosseland and Staurnes, 1994). Yancheva (2009) found the concentration of Mn much lower than present study (0.3±0.05 µg/L). as for the other metals in the present study, with concentration for Cd, Pb, As, Ni, found to be 0.04, 1.00, 0.78, 0.44 µg/L respectively, they are comparable to the findings of Yancheva (2009), where the concentration for Cd (0.1±0.05 µg/L), Pb (0.24±0.14 µg/L), As (1±0.1 µg/L) and Ni (1±0.1 µg/L) are within the range or Lake Phewa water concentration.

In gills, the concentration of trace metals (in µg/g gill d.w.) for *O. niloticus* and *T. putitora* were Cu (2.06 and 2.86 µg/g), Zn (71.36 and 117.76 µg/g), Pb (2.02 and 6.98 µg/g), Ni (1.57 and 3.47 µg/g), As (1.33 and 1.68 µg/g), Cr (0.57 and 2.50 µg/g), Mn (52.80 and 146.77 µg/g), Co (0.88 and 0.83) and Cd (0.09 and 0.10 µg/g). The differences in gill metal concentration between the two species might be due to species differences in gill tissue/cartilage tissue in the gill arches. It could also reflect the differences in habitat concentration at catch, but we have no information about where the individual fish were caught, and the respective water quality at that site.

There exist few data on gill and liver metals in literature, so the results from lake Phewa will be compared to gills of Atlantic salmon (*Salmo salar*) in the acidified but limed lake Storelva in Southern Norway (Yancheva 2009), and brown trout (*Salmo trutta*) from the slightly acidified Lochnagar in Scotland (Rosseland et al. 2007). The liver metal value will be compared to the study of Yancheva (2009).

The concentration of gill Cu was comparable to the finding of Yancheva (2009) who found from 2-4 µg/g dw on Atlantic salmon, and Rosseland et al. (2007) who found the concentration in brown trout to be 2 µg/g dw. Yancheva (2009) found the concentration of Zn ranged between 688 and 833 µg/g dw, much higher than the present result from Lake Phewa. The concentration of Zn was also higher in brown trout from Lochnagar with 394.5 µg/g dw (Rosseland et al. 2007). The concentration of gill Pb in Lake Phewa was comparable to the brown trout from

Lochnagar, with mean concentration of 19.5 µg/g dw. The concentration of Cd was found to be ranged between 5 to 8 µg/g dw in River Storelva (Yancheva, 2009) while Rosseland et al. (2007) found it to be in 10.5 µg/g dw from brown trout in Lochnagar, much higher than in the present study. For the concentration of Ni, Cr and Mn, Rosseland et al. (2007) found it to be in a concentration of 1.5, 3 and 21 µg/g dw respectively. The Concentration of Mn in our study was much higher (52-157 µg/g dw), which probably reflected the high Mn concentration in the lake water. Yancheva (2009) found Co in the gills with concentration ranged between 0.4 – 0.79 µg/g dw, values not unlike the results from Lake Phewa (0.88-0.83 µg/g dw).

For *O. niloticus*, *T. putitora*, and *C.gariepinus* the average concentration of trace metals in the liver were Cd (0.93, 0.25 and 0.36 µg/g dw), Cu (328.75, 54.03 and 86.44 µg/g dw), Zn (83.24, 142.59, 135.84 µg/g dw), Pb (0.38, 0.12 and 0.90 µg/g dw), As (1.01, 0.50, 0.81 µg/g dw), Ni (1.68, 0.06 and 0.06 µg/g dw), Cr (0.42, 0.36 and 0.56 µg/g dw), Mn (53.78, 6.04 and 4.46 µg/g dw) and Co (6.47, 0.17 and 0.77 µg/g dw) respectively. Similar studies by Yancheva (2009) found the highest mean concentration for Cd as 5.9±1 µg/g dw, Cu concentration was 71±90 µg/g dw, Zn concentration as 15±41 µg/g dw from fresh water, Pb to be in the concentration of 1.54±1.53 µg/g dw, As in the concentration 0.7±0.5 µg/g dw, Ni in the concentration 4±1,99 µg/g dw, and Cr , Mn and Co in the concentration 0.3±0,3 µg/g dw, 1±3 µg/g dw and 5±4 µg/g dw respectively.

4.7. Conclusion

The concentration of total mercury (THg) in the muscle tissue in four important fish species in Lake Phewa, Nepal , namely Nile Tilapia (*Oreochromis niloticus*), African Magur (*Clarias gariepinus*), Chuche Baam (*Mastacembelus armatus*) and Sahar (*Tor putitora*), demonstrated that the THg levels increased with increasing size in case of *C. gariepinus* and *M. armatus* while THg level decreased with increasing size in *O. niloticus*. The level of THg is lower than the EUs present regulation for trade and consumption in all the four species, and the levels will by todays knowledge not represent a human health risk for the fish consumption population. Analyses of stable isotopes ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) demonstrated that Chuche Baam was on the top of the food chain, while Nile Tilapia held the lowest trophic position. Analysis of trace metal concentration in water, gills and liver of fish, demonstrated levels comparable with other

international studies, where only manganese (Mn) was found in concentrations close to critical levels for fish. A pilot study analyzing only muscle concentration of persistant organic pollutants (POPs), including pesticides, in the single largest fish specimen of each species, demonstrated metabolites of DDTs (DDE and DDD) and Endosulfan sulfate. The levels, however, did not represent any threat to consumers based on today's safe levels set by World Health Organization (WHO).

The present study represents 4 out of nearly 30 species so far registered in Lake Phewa.Further studies on Hg and pesticides in the most important fish species in Lake Phewa, as well as in the other lakes in the region, should be facilitated to improve risk assessment and enable a proper management in relation to pollutants in the fish populations of the lake.

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ANNEX I

Muscle samples analysed for POPs (Bioforsk), in the fish samples from Lake Phewa, Pokhara, Nepal, concentrations in µg/kg w.w.

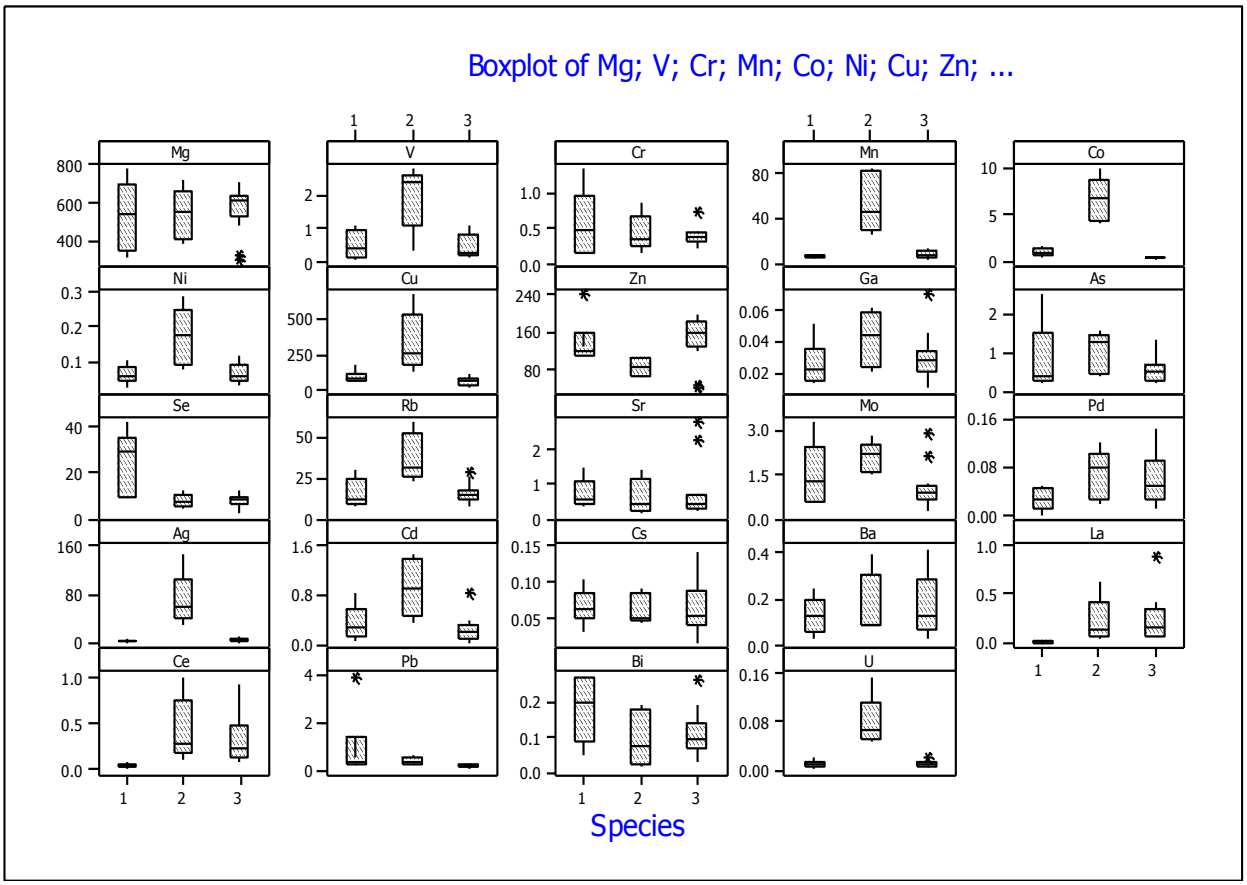
Pesticides	PH 36 (Tilapia)	PH 48 (Clarias)	PH 89 (Mastacembelus)	PH 104 (Tor)
HCH-alfa	<1,0	<1,0	<1,0	<1,0
HCB	<1,0	<1,0	<1,0	<1,0
HCH-beta	<1,0	<1,0	<1,0	<1,0
Lindan	<1,0	<1,0	<1,0	<1,0
Heptachlor	<2,0	<2,0	<2,0	<2,0
Aldrin	<2,0	<2,0	<2,0	<2,0
Chlorpyrifos	<1,0	<1,0	<1,0	<1,0
Heptachlorepoxid	<3,0	<3,0	<3,0	<3,0
Chlordane-trans	<1,0	<1,0	<1,0	<1,0
Chlordane-cis	<1,0	<1,0	<1,0	<1,0
DDE-op	<1,0	<1,0	<1,0	<1,0
Endosulfane-alfa	<2,0	<2,0	<2,0	<2,0
DDE-pp	1.8	11.6	8.1	7.6
Dieldrin	<3,0	<3,0	<3,0	<3,0
DDD-op	<1,0	<1,0	<1,0	<1,0
Endrin	<4,0	<4,0	<4,0	<4,0
Endosulfane-beta	<2,0	<2,0	<2,0	<2,0
DDD-pp	<1,0	4.5	<1,0	1.3
DDT-op	<1,0	<1,0	<1,0	<1,0
Endosulfane-sulfate	3.8	28.5	<1,0	4.7
DDT-pp	<1,0	<1,0	<1,0	<1,0
Metoxychlor	<1,0	<1,0	<1,0	<1,0
PCB 28	<1,0	<1,0	<1,0	<1,0
PCB 52	<1,0	<1,0	<1,0	<1,0
PCB 101	<1,0	<1,0	<1,0	<1,0
PCB 118	<1,0	<1,0	<1,0	<1,0
PCB 153	<1,0	<1,0	<1,0	<1,0
PCB 138	<1,0	<1,0	<1,0	<1,0
PCB 180	<1,0	<1,0	<1,0	<1,0

ANNEX II.

Trace metals in liver as µg/g liver dry weight (d. w.), Average ± SD

	C. gariepinus (N=5)	*O. niloticus* (N=5)	*T. putitora* (N=14)
Mg	526.74±182.72	531.88±136.68	552.92±120.24
V	0.45±0.42	1.95±0.98	0.42±0.36
Cr	0.56±0.46	0.42±0.27	0.35±0.13
Mn	4.46±0.53	53.79±27.49	6.15±2.79
Co	0.77±0.45	6.48±2.44	0.16±0.08
Ni	0.06±0.03	0.17±0.08	0.07±0.02
Cu	86.44±43.98	328.75±209.19	50.71±28.44
Zn	135.84±50.19	83.20±18.01	138.50±46.97
Ga	0.03±0.01	0.04±0.02	0.03±0.01
As	0.81±0.89	1.01±0.53	0.51±0.29
Se	25.24±13.16	7.74±2.25	7.17±3.41
Rb	15.94±8.94	37.14±14.75	14.10±4.52
Sr	0.72±0.43	0.62±0.53	0.74±0.78
Mo	1.52±1.06	2.07±0.53	1.02±0.71
Pd	0.03±0.02	0.07±0.04	0.06±0.04
Ag	2.83±1.78	70.37±45.81	5.01±3.21
Cd	0.36±0.28	0.93±0.48	0.23±0.20
Cs	0.07±0.02	0.06±0.02	0.06±0.02
Ba	0.13±0.08	0.18±0.14	0.19±0.13
La	0.01±0.01	0.23±0.24	0.19±0.13
Ce	0.03±0.02	0.43±0.36	0.27±0.16
Pb	0.90±1.48	0.38±0.15	0.09±0.10
Bi	0.18±0.10	0.09±0.08	0.11±0.06
U	0.01±0.01	0.08±0.04	0.01±0.00

Annex II contd……



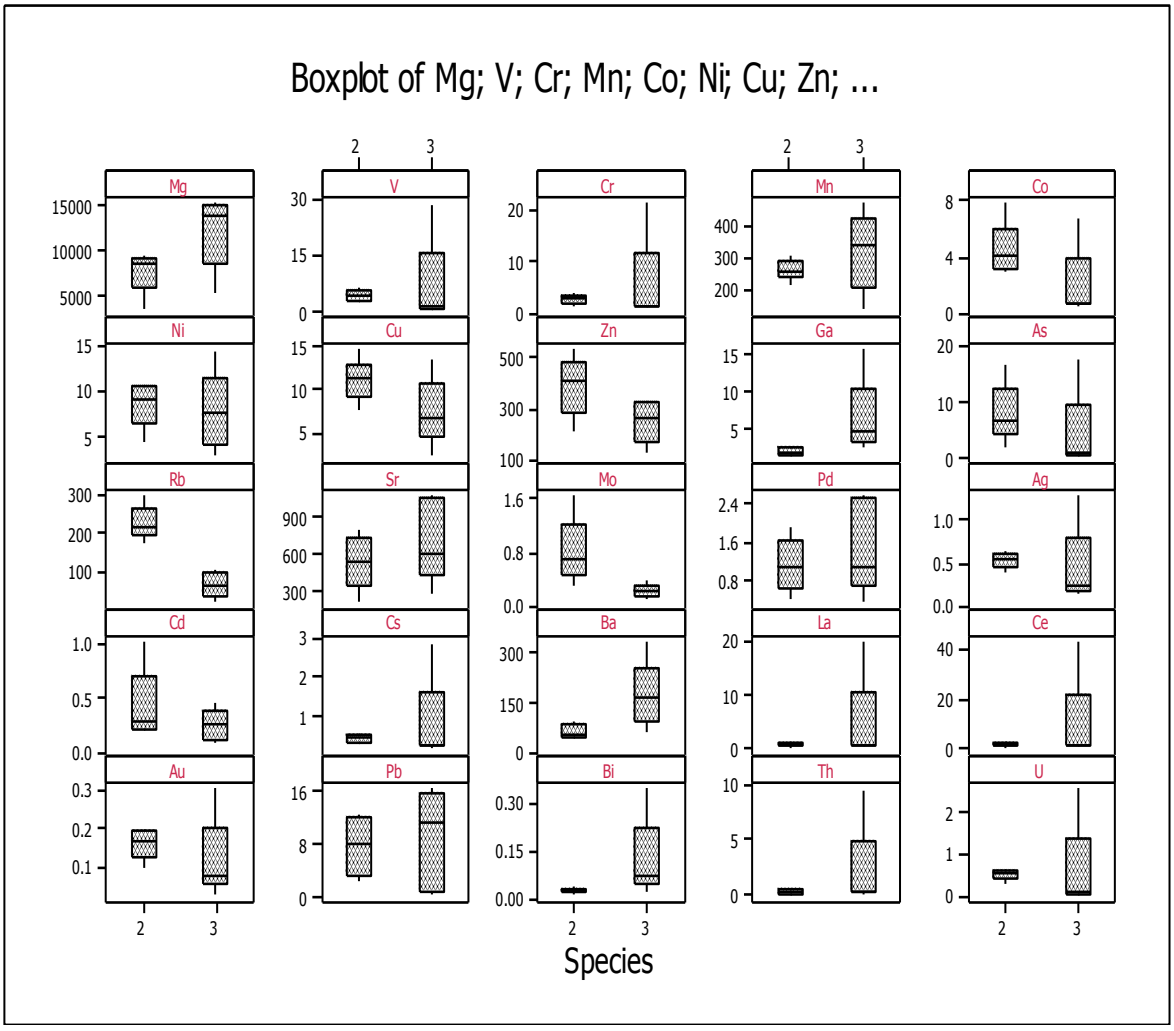
Box plots for different trace elements in liver of *C. gariepinus* (1), *O. niloticus* (2) and *T. putitora* (3), from Lake Phewa, Pokhara, Nepal.

ANNEX III

Trace metals in gills for 5 individuals (N=5) of *O. niloticus* and *T. putitora* expressed as µg/g gill dry weight (d. w.), Average ±SD

	O. niloticus (N=5)	*T. putitora* (N=5)
Mg	7555.35±2319.24	12282.14±4117.32
V	3.82±1.38	6.53±12.26
Cr	2.52±0.92	5.28±8.96
Mn	257.90±33.99	314.88±124.82
Co	4.40±1.98	1.86±2.77
Ni	8.56±2.57	7.71±4.28
Cu	11.14±2.57	7.37±3.97
Zn	386.17±118.93	247.10±83.21
Ga	1.75±0.49	6.14±5.44
As	7.92±5.32	4.16±7.48
Rb	226.25±45.63	61.74±32.20
Sr	529.61±218.93	721.69±346.81
Mo	0.80±0.50	0.22±0.11
Pd	1.12±0.56	1.50±0.96
Ag	0.53±0.10	0.43±0.47
Cd	0.42±0.34	0.25±0.15
Cs	0.40±0.08	0.78±0.16
Ba	58.89±22.40	168.92±102.59
La	0.59±0.36	4.34±8.66
Ce	1.26±0.75	9.32±18.75
Au	0.16±0.04	0.12±0.11
Pb	7.74±4.48	8.78±7.73
Bi	0.02±0.01	0.12±0.13
Th	0.24±0.16	2.02±4.12
U	0.49±0.13	0.57±1.13

Annex III contd….



Box plots for the concentration (µg/g gill d.w.) of different trace elements in gills of *O. niloticus* (2), and *T. putitora* (3) from Lake Phewa, Pokhara Nepal.