

ASSESSMENT OF HUMAN HEALTH RISK FOR LEAD, COPPER, NICKEL, MERCURY AND ZINC IN FISH COLLECTED FROM LAKE GERIYO, ADAMAWA STATE.

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ABSTRACT

Fishes are constantly exposed to aquatic system and exposure of metals through fish consumption may lead to health risks, especially for high-fish consumption populations. This study determined levels of Pb, Cu, Hg, Ni, and Zn in muscle tissues of fish from lake geriyo wetland and estimated the health risk posed by fish ingestion. The levels of heavy metals were below the permissible limits issued by JECFA of FAO/WHO. The tolerable intake of As, Cu, Hg, Ni, and Zn as PTWI (Provisional Tolerable Weekly Intake) and PTDI (Provisional Tolerable Daily Intake) was calculated and presented. To estimate the human health risk, the target hazard quotient (THQs), was calculated, THQs for individual metals were lower than USEPA guideline value of 1. However, the hazard index of lead, copper, mercury, nickel and zinc mixture for *Clarrias* was marginally high. The target cancer risk (TR) of lead and nickel for intake of the *Clarrias*, *nilotica* and *Tilapa* was 1.5×10^{-4} and 5.8×10^{-4} , 7.7×10^{-5} and 3.0×10^{-4} , 4.7×10^{-5} and 5.4×10^{-4} , respectively, with the average of 8.6×10^{-5} and 4.7×10^{-4} , respectively.

Keywords: Fish muscle, lead, copper, mercury, nickel, zinc, health risk, tolerable intake, HI, THQs, TR

INTRODUCTION

Metals can enter into the food web through direct consumption of water or organisms, or through uptake processes, and be potentially accumulated in edible fish and other wildlife (Paquin, *et al.*, 2003). Although Fishes are major part of the human diet because, it has high protein content, low saturated fat and also contains omega fatty acids known to support good health (Dural, *et al.*, 2007), but there is a growing concern that metals accumulated in fish muscle tissues may represent a health risk, especially for populations with high fish consumption rates (Liao and Ling, 2003; Burger and Gochfeld, 2009; Diez, *et al.*, 2009). Metals like Cu, Fe, Mn and Zn have normal physiological regulatory activities in organisms (Hogstrand and Haux, 2001), but some other metals like arsenic, cadmium, mercury, nickel, and lead exhibit toxic effects on organisms (Mason, 1991). For instance, metals like arsenic (As) has been associated to various systemic effects like cardiovascular diseases, skin disorders, and neurotoxicity; nickel compounds (nickel sulphate and combinations of nickel sulphides and oxides) has showed an increased risk of lung and nasal cancer in humans (IARC, 1990), and mercury (Hg) have been implicated as various causes for severe neurological damage to humans (Liu, *et al.*, 2008; Diez, *et al.*, 2009). These health concerns become of greater issue when we consider susceptible populations such as young children or women of child bearing age. As fishes are constantly exposed to pollutants in contaminated water, they could be used as excellent biological markers of heavy metals because non essential metals are also taken up by fish and are accumulated in their tissues (Canli and Atli, 2003). Consumption of these contaminated fishes showed the risk potential for human (USEPA, 2000; Storelli, 2008; Michael, *et al.*, 2011; Imar and Carlos, 2011;). Therefore, studies have been taken worldwide on the contamination of different fish species to determine their heavy metal contamination and human health risk (Laar, *et al.*, 2011; Anim, *et al.*, 2011; Kumar, *et al.*, 2010; Mallick, *et al.*, 2010; Mol, *et al.*, 2010; Bhattacharyya, *et al.*, 2010; Lin, 2009; Roach, *et al.*, 2008; Cheung, *et al.*, 2008). In this study we emphasized measurement of As, Cu, Hg, Ni, and Zn in muscle tissues of fish from Kolkata wetland and estimated the health risk posed by fish ingestion.

MATERIALS AND METHOD

Site Description and sampling

Lake Geriyo is a natural lake located between longitude 12° 25' and 12° 29' East of Greenwich and latitude 9° 8' and 9° 17' North of the Equator (Mahmud, 2013) outside Yola town on the North-East of Nigeria. Yola is the headquarters of Adamawa State of Nigeria and covers a total land mass of 10,900 km² with a population of 600, 000 (NPC, 2006). Majority of the inhabitants are farmers. Yola is also the commercial hub the state. Since the state creation it has been exposed to rural-urban migration. Due to this exodus, population growth superseded the infrastructural development. This growth has influenced the land use pattern within the surroundings of the Lake area with the creation of an urban waste dumpsite (Plate 2.1). Lake Geriyo (Figures 2.1) is a shallow Lake with a mean depth of about 3 meters covering an area of about 250 hectares in size with intensive irrigation farming around it.

The area lies within the hot climatic Northern Guinea Savannah zone of Nigeria with distinct dry and wet seasons. It has an average annual rainfall of about 988.99 mm and 185.9 m above the sea level. Atmospheric temperature range between 20°C to 42°C and the weather is characterized by cold and dusty conditions from December to January (Harmattan) followed by intense heat between March and April (high temperature). The rainy season runs from May through September while the dry season start in November and terminate in April (UBRBDA, 1985).

The Lake Geriyo irrigation scheme was introduced in 1978 by the Upper Benue River Basin Development Authority (UBRBDA) along the Banks of River Benue and the Lake Geriyo. It has a potential irrigable land area of about 1500 ha with 1200 ha already developed in year for the cultivation of rice and vegetables. The source of water for the irrigation purpose is the Lake and River Benue. The water is delivered to the farmlands through direct pumping. The project provides employment to about 1,500 farming family and employment to about 6000 workers and the map of the study area showing irrigation site is shown in (Fig 2.1).

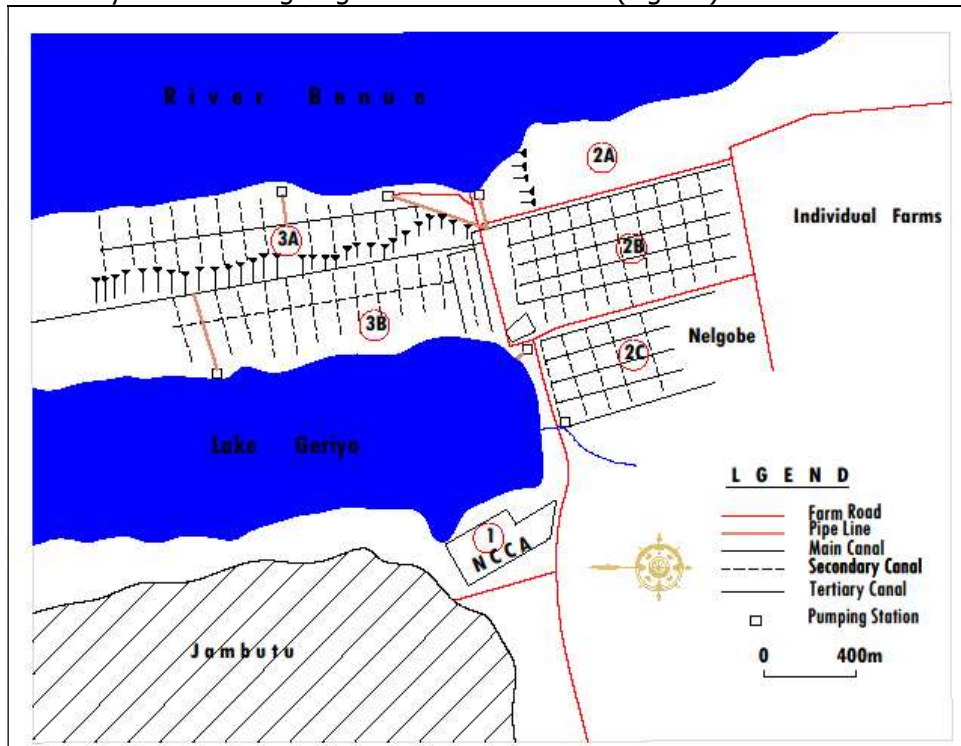


Figure 2.1: Map of the study area showing irrigation site

The lake over flows its banks in the wet season because it receives inflow of waters originating from river Benue and from the urban waste dumpsite at the surrounding of the Lake (Plate. 3.1). Others include run off from irrigation farm lands, which may contain herbicides, pesticides, organic manure, and fertilizers. The other uses of the lake water include communal livestock feeding, fishing, domestic and household washing by the inhabitants of the immediate local community including the fishermen.



Plate 2.1: Urban waste dumpsite in the study area

Fish samples were caught using Malian nets set overnight with the help of the local fishermen at the lake basin during the two seasons (Radujevic and Bechlin, 1999). Five fishes of each species mentioned were collected. They were identified by a specialist from the department of fisheries, Modibbo Adama University (MAU) Yola. The fish samples were washed with the lake water to remove sticking contaminant at the sampling site. Fish samples were labeled, they were preserved using ice and transported to the laboratory. All the samples were stored at -20°C prior to pre-treatment and analysis.

Pre-treatment of Sample

The frozen Samples were thoroughly washed with Milli-Q water after removing the scales, and muscle portion, which was taken for further processing. Muscle tissue was oven dried at 110°C , powdered with pestle and mortar, and was stored until chemical analysis. Heavy metals were analyzed after digesting the homogenized samples in a mixture of concentrated nitric and perchloric acid. Digestion was carried out after 0.5 gm homogenized powdered sample was placed in a Teflon beaker and digested with a few drops of sodium chloride solution (30%) and a 10 ml mixture (1:5) of concentrate Nitric acid (65%) and concentrated Perchloric acid (70%). The free chlorine developed loosens the chemical bonds in organic compounds after gentle heating (at $70\pm 5^{\circ}\text{C}$) in a water bath for 12 hrs and destroy the organic matter in order to transfer the metals into the solution. The solution was transferred to a volumetric flask (50 mL), and then filled with 0.01 N of HNO_3 to make a 50 mL of final solution. The digested solutions were centrifuged and the supernatant was analyzed.

2.3 Instrumental Analysis

Determination of copper, nickel and zinc was carried out using Flame Atomic Absorption Spectrometry (FAAS, Thermo, UK). AAS coupled to Hydride generator (HG) was used to analyze the total amount of mercury (cold vapor mode) and arsenic (heating mode). Background corrections were applied whenever required during the analysis and the method of standard additions was used to compensate for matrix effects. Performance of the instrument was checked by analyzing the standard reference material solutions (Merck NJ, USA) concurrently to check the precision of the instrument. After appropriate dilutions of stock standard solutions, a five level calibration curve was prepared. Samples were analyzed in triplicate. The values obtained from the sample were corrected for final digestion volume and sample weight was taken. The detection limit for As, Cu, Hg, Ni and Zn was 0.002, 0.02, 0.001, 0.025 and 0.01 ppm, respectively. Duplicate method blanks were processed and analyzed alongside the samples to check any loss or cross contamination. A certified reference material (SW 8022) was processed along with samples to determine the accuracy of the method and the results were compared to the acceptable limits (Table 1). The recovery of the studied heavy metals was ranged between 97-109±2-9 percent. The results were reported on dry weight basis as $\mu\text{g g}^{-1}$ (dry wt.).

Table1: Heavy metals concentration ($\mu\text{g g}^{-1}$ dry wt.) in Certified Reference Material (SW-8022)

Metals	Reference value	*Measured value	Recovery (%)	S D ($\pm\%$)
Lead	14	13	97	3
Copper	71	73	103	2
Mercury	26	27	104	4
Nickel	160	174	109	9
Zinc	289	312	108	8

* Average of three replicate

Data Processing

In this study, metal concentrations for all the species were calculated by application of Excel 2003 (Microsoft Inc., USA) and correlation coefficients were on the basis of Student's t test to a $p < 0.05$ level of significance.

Health Risk Estimation for Fish Consumption

To estimate the human health risk from consuming metal contaminated fish, the Target hazard quotient (THQ) was calculated as per US EPA Region III Risk-Based Concentration Table (USEPA 2011). The THQ is an estimate of the risk level (noncarcinogenic) due to pollutant exposure. The input parameters used in the health risk estimation for fish intake from Kolkata wetlands were given in Table 2. The equation used for estimating THQ was as follows:

$$\text{THQ} = (\text{M}_c \times \text{IR} \times 10^{-3} \times \text{EF} \times \text{ED}) / (\text{RfD} \times \text{BW}_a \times \text{AT}_n)$$

Where, THQ is the target hazard quotient, M_c is the metal concentration in fish ($\mu\text{g g}^{-1}$), IR is the fish ingestion rate (g day^{-1}), EF is the exposure frequency (day year^{-1}) or number of exposure events per year of exposure, ED is the exposure duration, total for adult (year), RfD is the reference dose ($\mu\text{g g}^{-1} \text{day}^{-1}$), BW_a is the body weight, adult (kg), and AT_n is the averaging time, noncarcinogens (day year^{-1}). The hazard index (HI) from THQs can be expressed as the sum of the hazard quotients (USEPA, 2011).

$\text{HI} = \text{THQ}_{\text{As}} + \text{THQ}_{\text{Cu}} + \text{THQ}_{\text{Hg}} + \text{THQ}_{\text{Ni}} + \text{THQ}_{\text{Zn}}$ Where HI is the hazard index; THQ_{As} is the target hazard quotient for As intake; THQ_{Cu} is the target hazard quotient for Cu intake; THQ_{Hg} is the target hazard quotient for Hg intake; THQ_{Ni} is the target hazard quotient for Ni intake; and THQ_{Zn} is the target hazard quotient for Zn intake.

Target cancer risk (TR) was used to indicate carcinogenic risks. The method to estimate TR was also provided in USEPA Region III Risk-Based Concentration Table (USEPA, 2011). The model for estimating TR was shown as follows:

$$\text{TR} = (\text{M}_c \times \text{IR} \times 10^{-3} \times \text{CPSo} \times \text{EF} \times \text{ED}) / (\text{BW}_a \times \text{AT}_c)$$

Where TR is the target cancer risk; M_c is the metal concentration in fish ($\mu\text{g g}^{-1}$), IR is the fish ingestion rate (g day^{-1}), CPSo is the carcinogenic potency slope, oral (mg/kg bw-day^{-1}); ATc is the averaging time, carcinogens (day years^{-1}). Since Cu, Hg and Zn do not cause any carcinogenic effects, so, TR value for intake of As and Ni was calculated to indicate the carcinogenic risk.

Table 2: Summary statistics of input parameters in the health risk estimation

Symbol	Description	Unit	Value
M_c	metal concentration	$\mu\text{g g}^{-1}$	Presented in Table 3
IR	Fish ingestion rate	g day^{-1}	19.5
EF	exposure frequency	days year^{-1}	287
ED	Exposure duration	years	30
RfD	reference dose	$\mu\text{g g}^{-1} \text{ day}^{-1}$	As= 3×10^{-4} , Hg= 3×10^{-4} , Cu= 0.04 , Ni= 0.02 , Zn= 0.3
BW _a	body weight (adult)	Kg	56
AT _n	Averaging time noncarcinogens	days	10950
AT _c	Averaging time carcinogens	days	25550
CPSo	Carcinogenic potency slope, oral	$\mu\text{g g}^{-1} \text{ day}^{-1}$	As=1.5, Ni=1.7 (nickel subsulfide) Cu, Hg & Zn= no value

Exposure Duration: The exposure duration is defined as the exposure frequency of 365 d/yr for 30 yr (i.e., 10,950 d). The averaging time and number of fish consumed are required to provide input for an estimate of human health risk from exposure through fish ingestion. An averaging time of 365 d/yr for 70 yr (i.e., ATc = 25,550 d) was used to characterize lifetime exposure for cancer risk calculation and an averaging time of 365 d/yr for 30 yr (i.e., ATn = 10,950 d) was used in characterizing noncancer risk (USEPA, 2011).

Fish Ingestion: Fish consumption patterns was described by Speedy (2003) and Little, *et al.*, (2002), which was based on fish consumption rate, consumption frequency and weeks of consumption for residents in Kolkata, India. Speedy (2003) stated the average fish ingestion rates of 19.5 g day^{-1} . Little *et al.*, (2002) calculated fish ingestion frequency of 5.5 – 6.5 meals per week and majority of peoples takes 5.5 meals per week. Based on this data we used the ingestion rate and ingestion frequency as 19.5 g day^{-1} and $287 \text{ days year}^{-1}$, respectively.

Body Weight: We used a 56 kg body weight for an average Indian adult, as suggested by Shukla, *et al.*, (2002).

Toxicity Factors: The reference dose (RfD) and carcinogenic potency slope factor (CPS) used for health risks (TR and THQ) evaluation as provided by USEPA (USEPA, 2011). The cancer slope factors for ingested arsenic and nickel (subsulfide) are 1.50 and 1.7, respectively. RfD is the reference dose (As= 3×10^{-4} , Cu= 0.04 , Hg= 3×10^{-4} , Ni= 0.05 , Zn= $0.3 \mu\text{g g}^{-1} \text{ day}^{-1}$) (USEPA, 2011) that is likely to be without appreciable risk of deleterious effects during a lifetime.

Acceptable Risk Distribution: The acceptable risk distribution was assigned by constraints on percentiles. The lower end of the range of acceptable risk distribution is defined by a single constraint on the 95th percentile of risk distribution that must be equal or lower than 10^{-6} for carcinogens (TR) and may be up to 10^{-4} in some circumstance. The health protection standard of lifetime risk for THQ is 1 (USEPA, 2011).

RESULTS AND DISCUSSION

3.1 Concentrations

Concentration range and mean of lead, copper, mercury, nickel and zinc in muscle tissue of fish samples from lake geriyo Wetlands were presented in Table 3. The concentration in fishes varies considerably among the species. This was possibly due to differences in metabolism and feeding patterns of the fishes. In this study, the fish individuals had metal levels below the guidelines stipulated by international agencies (FAO/WHO JECFA, USFDA, EC Directives, China, Hong Kong and India) (Table 4).

The concentration of lead was ranged from BDL to 1.22 $\mu\text{g g}^{-1}$ with the mean of $0.63 \pm 0.05 \mu\text{g g}^{-1}$. The highest concentration, $1.07 \pm 0.05 \mu\text{g g}^{-1}$ was recorded in *Clarrias*, with the lowest, $0.35 \pm 0.08 \mu\text{g g}^{-1}$ been recorded in *Tilapia* (Table 3). Lead levels in the literature have been reported in the range of 0.24-2.13 $\mu\text{g g}^{-1}$ in market fish from South China (Cheung, *et al.*, 2008), 0.62-1.37 $\mu\text{g g}^{-1}$ in fish caught from river Presa, Corsica, France (Foata, *et al.*, 2009), 0.76 ± 0.03 - $0.93 \pm 0.19 \mu\text{g g}^{-1}$ and $0.97 \pm 0.78 \mu\text{g g}^{-1}$ in fish from Taiwan reported by Lin, (2009) and Kar, *et al.*, (2011), respectively, 0.16-0.28 $\mu\text{g g}^{-1}$ in fish from Euphrates, Turkey (Mol, *et al.*, 2010). Shah, *et al.*, (2009) reported the higher concentration (2.12-5.2 $\mu\text{g g}^{-1}$) of lead in different freshwater fish from Manchar Lake, Sindh, Pakistan. The concentrations of lead in fish samples from lake geriyo wetlands were below the stipulated guideline values (0.5 - $2.3 \mu\text{g g}^{-1}$), except Chinese guideline value of $0.5 \mu\text{g g}^{-1}$ (Table 4).

Table 3: As, Cu, Hg, Ni and Zn levels ($\mu\text{g g}^{-1}$ dry wt.) in selected fishes from lake Geriyo

Metal	Statistics	Fish species (N)			All samples
		<i>Clarrias</i> (9) (32)	<i>Oreochromis nilotica</i> (11)	<i>Tilapia zilli</i> (12)	
Pb	Range	0.82-1.22	BDL-1.02	BDL-0.73	BDL-1.22
	Mean $\pm$ SE*	1.07 ± 0.05	0.56 ± 0.14	0.35 ± 0.08	0.63 ± 0.05
	Median	1.10	0.78	0.24	0.70
	CV**	14.55	79.57	76.02	42.02
Cu	Range	2.10-5.20	4.95-8.91	3.97-7.51	2.10-8.91
	Mean $\pm$ SE	3.29 ± 0.38	6.44 ± 0.41	5.30 ± 0.31	5.13 ± 0.21
	Median	3.01	6.45	5.17	5.13
	CV	34.69	20.93	22.35	23.10
Hg	Range	0.14-0.51	0.14-0.70	0.15-0.72	0.14-0.72
	Mean $\pm$ SE	0.26 ± 0.05	0.42 ± 0.07	0.46 ± 0.05	0.39 ± 0.04
	Median	0.19	0.49	0.45	0.30
	CV	56.51	54.44	44.78	52.17
Ni	Range	2.60-5.40	BDL-6.44	BDL-8.50	BDL-8.50
	Mean $\pm$ SE	3.74 ± 0.26	1.95 ± 0.66	3.49 ± 0.97	3.03 ± 0.66
	Median	3.50	2.30	2.50	3.05
	CV	21.19	112.82	107.70	124.00
Zn	Range	22.88-41.00	34.03-71.98	31.71-92.70	22.88-
	Mean $\pm$ SE	29.21 ± 2.40	50.70 ± 2.88	61.07 ± 5.06	92.70
	Median	29.09	47.00	58.03	48.55 ± 3.46
	CV	24.63	18.88	32.04	46.60 40.31

*Std Err=standard deviation/ $\sqrt{n}$, **CV=coefficient of variation

The observed concentrations of Cu ranged between 2.10 to 8.91 $\mu\text{g g}^{-1}$, with the highest mean concentration, 6.44 ± 0.41 in *Oreochromis nilotica*. However, the lowest mean concentration (3.29 ± 0.38) was observed in *Clarrias* (Table 3). Other workers reported the copper levels in the literature in the range of 0.38-3.16 $\mu\text{g g}^{-1}$ in Fish from Lake Macquarie, New South Wales, Australia

(Roach, *et al.*, 2008), $7.12 \pm 3.3 \mu\text{g g}^{-1}$ in Fish from sewage fed lake of Karnataka, India (Puttaiah and Kiran, 2008), $0.06\text{--}0.35 \mu\text{g g}^{-1}$ in market fish from South China (Cheung, *et al.*, 2008), $0.13\text{--}0.57 \mu\text{g g}^{-1}$ in freshwater Fish from Lithuania (Staniskiene, *et al.*, 2009), $2.09 \pm 0.40 \mu\text{g g}^{-1}$ in fish muscles from Taiwan (Lin, 2009), $0.02\text{--}0.17 \mu\text{g g}^{-1}$ in meat of freshwater fish from West Pomerania, Poland (Magdalena, *et al.*, 2009), $0.10\text{--}2.78 \mu\text{g g}^{-1}$ in fish from Euphrates, Turkey (Mol, *et al.*, 2010), $7.59 \mu\text{g g}^{-1}$ in fish collected from Pennar estuary, India (Ravanaiah and Murthy, 2010), $0.40\text{--}0.59 \mu\text{g g}^{-1}$ in fish from freshwater lake of Bhopal, India (Malik, *et al.*, 2010), $45.60\text{--}110.56 \mu\text{g g}^{-1}$ in fish samples from Densu River, Ghana (Anim, *et al.*, 2011). The observed concentration of Cu in analyzed samples of fish species collected from Kolkata wetlands was below the guidelines (Table 4).

Fish obtained methylated mercury through dietary uptake, which could be influenced by size, diet, ecological and environmental factors. It is stored in fish protein matrices covalently bound to sulfhydryl groups, usually as methyl mercury (MeHg). At present a maximum level of 0.5 mg/kg applies to fishery products, with the exception of certain listed fish species for which 1 mg/kg applies (EFSA, 2004). The concentration of mercury in muscle of fish from lake geriyo wetlands varied from 0.14 to $0.72 \mu\text{g g}^{-1}$ with a mean of $0.39 \pm 0.04 \mu\text{g g}^{-1}$. The highest mean concentration was observed in *Tilapia* ($0.46 \pm 0.05 \mu\text{g g}^{-1}$), and lowest concentration was in *Clarias* ($0.26 \pm 0.05 \mu\text{g g}^{-1}$) (Table 3). Our results are in agreement with results reported by Bhattacharyya *et al.*, (2010) for fish from wetlands and sewage fed aquaculture. Other studies in the literature reported the Hg concentration, in the range of $0.07\text{--}0.34 \mu\text{g g}^{-1}$ in market fish from South China (Cheung, *et al.*, 2008), $0.03\text{--}0.96 \mu\text{g g}^{-1}$ in Fish from Lake Macquarie, New South Wales, Australia (Roach, *et al.*, 2008), $0.01\text{--}0.19 \mu\text{g g}^{-1}$ in meat of freshwater fish from West Pomerania,

Poland (Magdalena, *et al.*, 2009), $0.33 \mu\text{g g}^{-1}$ in fish collected from Pennar estuary, India (Ravanaiah and Murthy, 2010), $0.08\text{--}0.14 \mu\text{g g}^{-1}$ in fish muscles from freshwater lake of Bhopal, India (Malik, *et al.*, 2010), $\text{ND}\text{--}0.65 \mu\text{g g}^{-1}$ in fish from Euphrates, Turkey (Mol, *et al.*, 2010), $0.74 \pm 0.66\text{--}1.77 \pm 0.39 \mu\text{g g}^{-1}$ in fish from Cape Fear River watershed, North Carolina, USA (Michael, *et al.*, 2011). Although, in some samples from lake geriyo wetlands, levels of Hg were higher than guidelines but average concentrations were below the guideline values for all the fish samples (Table 3, Table 4).

Table 4: Level of As, Cu, Hg, Ni and Zn in fish muscle ($\mu\text{g g}^{-1}$): comparison with guideline values

Country	Pb	Cu	Hg	Ni	Zn	Reference
Present study	0.63	5.13	0.39	3.03	48.55	
Guidelines						
China	0.5	50	0.3			CNSMD, 2001
Hong Kong	2.3		0.5			HKG, 1987
India	1.1	30	0.5		50	FSSAI, 2011
USFDA			1.0	70-80		USFDA, 1993
International criterion	2	15	0.5		60	Summers <i>et al.</i> , 1995; HC, 2007
European Comm.			0.5 – 1.0		40-100	EC, 2001
FAO/WHO	1.4	30	0.5		50	WHO, 1983

Nickel is essential for normal growth and reproduction in animals and human beings, but shows carcinogenic effect

when consumed in high amount. The concentrations of Nickel in the samples analysed in this study ranged between BDL to $8.50 \mu\text{g g}^{-1}$ with the average concentration of $3.03 \pm 0.66 \mu\text{g g}^{-1}$. The highest average concentration ($3.74 \pm 0.26 \mu\text{g g}^{-1}$) was measured in *Clarias* and the lowest concentration ($1.95 \pm 0.66 \mu\text{g g}^{-1}$) measured in *Oreochromis nilotica* (Table 3). Nickel concentration reported in the literature by other workers was in the range of $0.04\text{--}0.36 \mu\text{g g}^{-1}$ in market fish from South China (Cheung, *et al.*, 2008), $1.07 \pm 0.25 \mu\text{g g}^{-1}$ in fish from sewage fed lake of Karnataka, India (Puttaiah and Kiran, 2008), $0.12\text{--}10.66 \mu\text{g g}^{-1}$ in freshwater Fish from Lithuania

(Staniskiene, *et al.*, 2009), 0.03-0.13 $\mu\text{g g}^{-1}$ in meat of freshwater fish from West Pomerania, Poland (Magdalena, *et al.*, 2009), 0.21-0.67 $\mu\text{g g}^{-1}$ in fish muscles from freshwater lake of Bhopal, India (Malik, *et al.*, 2010), BDL-0.54 $\mu\text{g g}^{-1}$ in fish from Cape Fear River watershed, North Carolina, USA (Michael, *et al.*, 2011), <0.010.84 $\mu\text{g g}^{-1}$ in fish samples from Densu River, Ghana (Anim, *et al.*, 2011). The recommended dietary allowances for nickel have not been established but, estimated maximum guideline set by USFDA for Ni is 70-80 $\mu\text{g g}^{-1}$. Thus the concentrations of Ni in all the samples were below the stipulated guideline values (Table 4).

Zinc is an essential trace metal for both animals and humans. A deficiency of zinc is marked by retarded growth, loss of taste and hypogonadism, leading to decreased fertility (Sivapermal, *et al.*, 2007). The concentration of Zn in fish samples from lake geriyo wetland were ranged between 22.88 to 92.70 $\mu\text{g g}^{-1}$ with an average of $48.55 \pm 3.46 \mu\text{g g}^{-1}$ (Table 3). The concentration of zinc in the literature have been reported in the range of 2.67-19.1 $\mu\text{g g}^{-1}$ in market fish from South China (Cheung, *et al.*, 2008), $37.68 \pm 11.91 \mu\text{g g}^{-1}$ in Fish from sewage fed lake of Karnataka, India (Puttaiah and Kiran, 2008), 14.0-75.4 $\mu\text{g g}^{-1}$ in fish from Lake Macquarie, New South Wales, Australia (Roach, *et al.*, 2008), $37.98 \pm 6.49 \mu\text{g g}^{-1}$ in fish muscles from Taiwan (Lin, 2009), 10.03-22.0 $\mu\text{g g}^{-1}$ in freshwater fish from Lithuania (Staniskiene, *et al.*, 2009), 2.8-6.8 $\mu\text{g g}^{-1}$ in fish from West Pomerania, Poland (Magdalena, *et al.*, 2009), 10.27-19.74 $\mu\text{g g}^{-1}$ in fish from Euphrates, Turkey (Mol, *et al.*, 2010), 0.48-1.88 $\mu\text{g g}^{-1}$ in fish muscles from freshwater lake of Bhopal, India (Malik, *et al.*, 2010), 10.49 $\mu\text{g g}^{-1}$ in fish collected from Pennar estuary, India (Ravanaiah and Murthy, 2010), 2.19-5.86 $\mu\text{g g}^{-1}$ in fish from Cape Fear River watershed, North Carolina, USA (Michael, *et al.*, 2011), 18.92-30.04 $\mu\text{g g}^{-1}$ in fish samples from Densu River, Ghana (Anim, *et al.*, 2011). Comparing our observed values with guideline values (Table 4), results showed that the values of Zn are lower than guideline values (Table 4).

Accumulations of metals were generally found to be species specific and which may be related to their feeding habits and the bio-concentration capacity. However, the efficiency of metal uptake from contaminated water and food may differ in relation to ecological needs, metabolism, and the contamination gradients of water, food, and sediment, as well as salinity and temperature (Mason, 2000).

Generally, no significant correlation ($p < 0.05$) was found among Ni, Hg, and Zn concentrations. However, Ni concentrations in muscle tissues of fishes were significantly correlated ($p < 0.05$) with As ($r^2 = 0.519$), Cu was well correlated with Hg ($r^2 = 0.392$), and Zn ($r^2 = 0.351$), and a positive correlation ($r^2 = 0.372$) between Hg and Zn was observed.

3.2 Metal Intake (Estimated PTDI and PTWI)

Foods having toxic metals could present a toxic hazard for the consumer which is dependent on the metal concentration in food and amount of food consumed. Hazard consists of determining the toxicological properties related to a specific substance (Kuhnlein and Chan, 2000). The 'tolerable intake' is widely used to describe 'safe' levels of intake; and can be expressed on either a daily basis (TDI or tolerable daily intake) or a weekly basis (TWI or tolerable weekly intake). The tolerable intake of heavy metals as PTWI (Provisional Tolerable Weekly Intake), are set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA). PTWI is the maximum amount of a contaminant to which a person can be exposed per week over a lifetime without an unacceptable risk of health effects. The estimated tolerance daily intake (ETDI) and estimated tolerance weekly intake (ETWI) in this study were calculated and presented in Table 5. Intake estimates were expressed as per unit body weight ($\mu\text{g/kg b.w./day}$ or weekly).

Table 5: Estimated dietary intake ($\mu\text{g/kg b.w./day}$ or week) of Pb, Cu, Hg, Ni and Zn of fish species

Fish samples	Lead		Copper		Mercury		Nickel		Zinc	
	D*	W**	D	W	D	W	D	W	D	W
<i>Clarrias</i>	0.30	2.09	0.92	6.41	0.07	0.51	1.04	7.28	8.12	56.82

<i>Oreochromis nilotica</i>	0.16	1.10	1.79	12.53	0.12	0.83	0.54	3.80	14.09	98.63
<i>Tilapia</i>	0.10	0.67	1.47	10.31	0.13	0.89	0.97	6.79	16.97	118.79
All samples	0.17	1.22	1.43	9.98	0.11	0.76	0.84	5.90	13.49	94.43
Guideline (FAO/WHO, FSA)	2.14	14.98	700	3500	0.23	1.61	5	35	1000	7000

*Estimated daily intake, ** Estimated weekly intake

Lead: Lead is acutely toxic to humans and animals. The inorganic lead forms are more hazardous to humans than the organic ones. It has been established that fish and seafood can accumulate sizeable quantities of organic lead from their environment. The JECFA has established a PTWI of 14.98 µg/kg b.w./week for As, which is equivalent to 2.14 µg/kg b.w./day for inorganic arsenic. The daily intake of arsenic by humans reflects generally the quantities of seafood in the diet in which arsenic occurs mainly in the organic form. The ETDI and ETWI for arsenic ranged from 0.10 to 0.30 µg /kg b.w./day (mean, 0.17 µg /kg b.w./day) and 0.67 to 2.09 µg /kg b.w./week (mean, 1.22 µg /kg b.w./day), respectively (Table 5). Based on guideline data (Table 5), the observed lead intake from fish muscle tissues were much lower and pose no adverse effects to the consumers.

Copper: Copper is present in all animals and plants and is an essential nutrient for both animals and humans.

Although copper is an essential trace element, high levels of intake can cause symptoms of acute toxicity. The

Recommended Daily Allowance (RDA) of copper for adults is 0.9 milligrams (mg). The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for copper of 3500 µg/kg body weight/week which was equivalent to 700 µg/kg body weight/day (PTDI). In our study the ETDI and ETWI for copper from consumption of lake geriyowetland fish was 1.43 (µg/kg b.w./day) and 9.98 (µg/kg b.w. /weekly), respectively (Table 5). As we can see, the estimated PTWI of copper in this study is far below the established PTWI.

Mercury: Mercury is an environmental contaminant that is present in fish and seafood products largely as methylmercury. The toxicokinetics of mercury is associated with its chemical form. The organic form, methylmercury (MeHg) is more hazardous than other forms. The target organ of methylmercury (MeHg) is the brain and can cause neurological changes in adults, because of the ability of MeHg to easily cross the blood–brain and placental barriers (Diez 2008). Aquatic organisms possess a remarkable capacity to turn inorganic mercury into organic compounds (MeHg), thus rendering mercury more easily transferable throughout the aquatic food chain. The JECFA established a Provisional Tolerable Weekly Intake (PTWI) based on two epidemiological studies that investigated the relationship between maternal exposure to mercury and impaired neurodevelopment in their children. In June 2003, the FAO/ WHO Joint Expert Committee on Food Additives (JECFA) revised its Provisional Tolerable Weekly Intake (PTWI) for methylmercury to 1.6 µg/kg body weight, whereas it was previously 3.3 µg/kg body weight. The observed ETDI and ETWI of Hg from this study were 0.11 µg/kg b.w./day (range, 0.07-0.13 µg/kg b.w./day) and 0.76 µg/kg b.w./week (range, 0.51-0.89 µg/kg b.w./week) from fish consumption (Table 5), and therefore, were not considered to pose adverse effects to consumers.

Nickel: IARC and European Commission Working Group of Specialized Experts has proposed the nickel sulphate in Mutagenic Category 3, with the risk phrase R68 “Possible risk of irreversible effect” and Group 1: Human carcinogens (IARC, 1990; EC, 2004). Studies indicated that nickel sulphate and combinations of nickel sulphides and oxides encountered in the nickel refining industry were responsible for cancer in humans. Nickel binds to albumin, histidine and α2-macroglobulin and is widely distributed in the organs. The JECFA has established a PTWI of 35 µg/kg b.w./week for nickel, which is equivalent to 5 µg/kg b.w./day. The ETDI and ETWI in this study for nickel ranged from 0.54-1.04 µg /kg b.w./day (mean, 0.84 µg /kg b.w./day) and 3.80 to 7.28 µg /kg b.w./week

(mean, 5.90 $\mu\text{g/kg b.w./day}$), respectively (Table 5). Based on guideline data from Joint FAO/WHO Expert Committee on Food Additives (Table 5), the observed nickel intake from fish muscle were much lower and indicates no adverse effects to the consumers.

Zinc: Zinc is essential for human health. Although humans can handle proportionally large concentrations of zinc, too much zinc can cause eminent health problems, such as stomach cramps, skin irritations, vomiting, nausea and anemia. Very high levels of zinc can damage the pancreas and disturb the protein metabolism, and cause arteriosclerosis. The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for zinc of 7000 $\mu\text{g/kg body weight/week}$ which is equivalent to 1000 $\mu\text{g/kg body weight/day}$. The ETDI and ETWI of zinc calculated for fish consumption from lake geriyo wetlands and they ranged between 8.12-16.97 $\mu\text{g/kg body weight/day}$ and 56.82-118.79 $\mu\text{g/kg body weight/week}$ with a mean of 13.49 $\mu\text{g/kg b. w./day}$ and 94.43 $\mu\text{g/kg b. w./week}$, respectively (Table 5). The estimated ETDI and ETWI of zinc in this study are below the established PTDI and PTWI indicated no health risk to the consumers.

3.3 Target Hazard Quotients (THQs) and Target Cancer Risk (TR)

Risk assessments are based on assumptions. The U.S. EPA Region III Risk-Based Concentration Table (US EPA, 2011) present methods for estimating the target cancer risk (TR) and the non-cancer risk (THQs). The risk associated with the carcinogenic effects of target metal is expressed as the excess probability of contracting cancer over a lifetime of 70 years. The USEPA established the acceptable guideline values for THQs and TR 1 and 10^{-6} - 10^{-4} , respectively. The theoretical and estimated lifetime target hazard quotients (THQs) for lead, copper, mercury, nickel and zinc; and target cancer risk (TR) for lead and nickel to humans due to exposure to these from consumption of fish from lake geriyo wetlands, were calculated and presented in Table 6. HQ higher than 1, implies that the estimated exposure exceed the USEPA reference dose for the contaminant of interest. The THQs of lead, Copper, Mercury, Nickel and Zinc were 0.45, 0.03, 0.28, 0.01 and 0.03, respectively. The observed values of THQs were lower than the safe standard 1 (USEPA, 2011). Although, the THQs of lead, copper, mercury, nickel and zinc are lower than standard but, ΣTHQs of these metals (HI) was near to the standard 1 in *Clarrias* and it was 1.01, indicates that ingestion of more amount of *Clarrias* frequently may result in noncarcinogenic risk in consumers in future. The risk was mainly contributed by lead, which may cause chronic noncarcinogenic effects. The HI value of copper, mercury, nickel and zinc sum for other species is lower than the standard 1, demonstrated that ingestion of these fish from lake geriyo wetlands does not result in overexposure of these chemicals. Thus, no adverse effect poses to the health of the consumers.

The average value of target cancer risk (TR) was calculated for Pb and Ni only, since Cu, Hg and Zn did not show carcinogenicity. The average value of TR for lead and nickel was 8.6×10^{-5} (range, 4.7×10^{-5} , *Tilapia* to 1.5×10^{-4} , *Clarrias*) and 4.7×10^{-4} (range, 3.0×10^{-4} , *Oreochromis nilotica* to 5.8×10^{-3} , *Clarrias*), respectively. Comparing the TR values with guideline values, indicates that fish from lake geriyo wetlands are safe for human consumption but, may have the probability of contracting cancer due to Pb and Ni exposure over a long lifetime of 70 years or more in future.

CONCLUSION

In this study, the selected fish individuals had metal levels below the guideline values established by different environmental agencies. Nonetheless, the estimation of noncarcinogenic risk (THQs) conducted in this study showed that adverse health effects may not occur when considering different fish consumption patterns. However, target cancer risk (TR) due to As and Ni exposure through fish consumption may have the probability of contracting cancer over a long lifetime in future. More intensive study is needed in order to determine the metals in fishes from the study area, and not only to report levels of contaminants but also had important to compare them with health criteria values to easily understood by the general population. Furthermore, it is well known that fish may contain a variety of bioaccumulative organic chemical contaminants such as dioxins/furans, chlorinated pesticides, and polychlorinated biphenyls (PCBs) that are a health concern. Therefore,

in addition to metal studies, other chemical organic contaminants of concern must be evaluated in edible fish from lake geriyo wetlands.

REFERENCES

- Anim, A.K., Ahialey, E.K., Duodu, G.O., Ackah, M. and Bentil, N.O. (2011). Accumulation profile of heavy metals in fish samples from Nsawam, along Densu River, Ghana. *Res. J. Environ. Earth Sci.*, 3(1): 56-60.
- Bhattacharyya, S., Chaudhuri, P., Dutta S. and. Santra, S. C. (2010). Assessment of total mercury level in fish collected from east Calcutta wetlands and Titagarh sewage fed aquaculture in West Bengal, India. *Bull. Environ. Cont. Toxicol.*, 84 (5): 618-622.
- Burger, J., Gochfeld, M. (2009). Perceptions of the risks and benefits of fish consumption: individual choices to reduce risk and increase health benefit. *Environ. Res.*, 109:343-349.
- Canli, M., Atli, G. (2003). The relationships between heavy metal (Cd,Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fishes species. *Environmental Pollution*, 121:129-136.
- Cheung, K. C., Leung, H. M., Wong, M. H. (2008). Metal concentrations of common freshwater and marine fish from the Pearl River Delta, South China. *Arch. Environ. Contam. Toxicol.*, 54:705-715.
- CNSMD (China National Standards Management Department). (2001). Safety qualification for agricultural product for non-environmental pollution aquatic products. GB 18406.4-2001. Beijing, China
- Diez, S. (2008). Human health effects of methylmercury exposure. *Rev. Environ. Contam. Toxicol.*, 198, doi:10.1007/978-0-387-09646-9.
- Diez, S., Delgado, S., Aguilera, I., Astray, J., Perez, G. B., Torrent, M., Sunyer, J., Bayona, J.M. (2009). Prenatal and early childhood exposure to mercury and methylmercury in Spain, a high-fish-consumer country. *Arch. Environ. Contam. Toxicol.*, 56:615-622.
- Dural, M., Goksu, M.Z.I., Ozak, A.A. (2007). Investigation of heavy metal levels in economically important fish species captured from the Tuzla Lagoon. *Food Chem.*, 102:415-421
- EFSA (European Food Safety Academy) Journal. (2004).Vol., 34: 1-14, at: <http://www.efsa.eu.int>.
- EKW (East Kolkata Wetlands). (2010). Published by: East Kolkata Wetlands Management Authority and Wetlands International- South Asia, Vol. 1.
- EU (European Commission) (2001). Commission Regulation as regards heavy metals, Directive 2001/22/EC, no: 466/2001,
- EC (European Commission) (2004). Working Group of Specialized Experts in the fields of Carcinogenicity and Mutagenicity. Nickel. Summary Record. European Chemicals Bureau.
- Foata, J., Quilichini, Y., Torres, J., Pereira, E., Spella, M. M., Mattei, J., Marchand, B. (2009). Comparison of Arsenic and Antimony Contents in Tissues and Organs of Brown Trout Caught from the River Presa Polluted by Ancient Mining Practices and from the River Bravona in Corsica (France): A Survey Study. *Arch. Environ. Contam. Toxicol.*, 57:581-589.

- FSA (Food Standard Agency). (2006). Metals and other elements in processed fish and fish products: Food Survey Information Sheet 08/06, <http://www.food.gov.uk/science/surveillance>
- FSSAI (Food Safety Standards Authority of India). (2011). Food Safety and Standards (contaminants, toxins and residues) regulations, 2011, available at: <http://www.fssai.gov.in>.
- HC (Health Canada). (2007). Canadian standards for various chemical contaminants in foods, Ottawa, Ontario. <http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php>.
- Hogstrand, C. and Haux, C. (2001). Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionein. *Comp. Biochem. Physiol.*, 100: 137.
- HKG (Hong Kong Government). (1987). Food Adulteration (Metallic Contamination) Regulations. Laws of Hong Kong 2, Chapter 132.
- IARC (International Agency for Research on Cancer). (1990). Monographs on the evaluation of carcinogenic risks to humans. Vol., 49, Chromium, Nickel and welding. IARC, Lyon, France.
- Imar M.R., Carlos, J. R.S. (2011). Metal Levels in Fish Captured in Puerto Rico and Estimation of Risk from Fish Consumption. *Arch. Environ. Contam. Toxicol.*, 60:132–144.
- Kar, S., Maity, J. P Jean, J. S., Liu, C.C., Liu, C. W., Bundschuh, J., & Lu, H.Y. (2011). Health risks for human intake of aquacultural fish: Arsenic bioaccumulation and contamination. *J. Environ. Sci. Health, Part A*, 46 (11):1266-1273
- Kuhnlein, H.V. Chan, H.M. (2000). Environment and contaminants in traditional food systems of Northern Indigenous peoples. *Ann. Rev. Nutr.*, 20, 595–626.
- Kumar, B., Senthil Kumar, K., Priya, M., Mukhopadhyay, D. P., Shah, R. (2010). Distribution, partitioning, bioaccumulation of trace elements in water, sediment and fish from sewage fed fish ponds in eastern Kolkata, India. *Toxicological & Environmental Chemistry*, 92 (2): 243-260
- Laar, C., Fianko, J.R., Akiti, T.T., Osa, S. and Brimah, A.K. (2011). determination of heavy metals in the blackchin tilapia from the Sakumo Lagoon, Ghana. *Res. J. Environ. Earth Sciences* 3(1): 8-13.
- Liao, C.M., Ling, M.P. (2003). Assessment of human health risks for arsenic bioaccumulation in tilapia (*Oreochromis mossambicus*) and large-scale mullet (*Liza macrolepis*) from blackfoot disease area in Taiwan. *Arch. Environ. Toxicol.*, 45:264–272.
- Lin, Ming-Chao. (2009). Risk Assessment on Mixture Toxicity of Arsenic, Zinc and Copper Intake from Consumption of Milkfish, *Chanos chanos* (Forsskal), Cultured Using Contaminated Groundwater in Southwest Taiwan. *Bull. Environ. Contam. Toxicol.*, 83:125–129.
- Little, D.C., Kundu, N., Mukherjee, M. and Barman, B.K. 2002. Marketing of fish in peri-urban Kolkata. Institute of Aquaculture, University of Stirling. <http://www.dfid.stir.ac.uk/dfid/nrsp/kolkata.htm>
- Liu, J., Goyer, R.A., Waalkers, M.P. (2008). Toxic effects of metals. In: Klaassen CD (ed) Casarrett and Doull's toxicology. The basic science of poisons. McGraw-Hill, New York, pp 931–979.

- Mason, C. F. (1991). *Biology of Freshwater Fishes*. Longman Science and Technology, New York.
- Mason, R.P., Laporte, J.M., Andres, S. (2000). Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium and cadmium by freshwater invertebrates and fish. *Arch. Environ. Conta. Toxicol.*, 38:283–297.
- Magdalena, L. K., Pokorska, K., Protasowicki, M., Rajkowska, M., Wechterowicz, Z. (2009). Content of selected essential and toxic metals in meat of freshwater fish from West Pomerania, Poland. *Pol. J. Food Nutr. Sci.*, 59 (3): 219-224
- 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.
- Michael, A. M., Matthew, R. M., Michael, F. E. W. (2011). Elevated levels of metals and organic pollutants in fish and clams in the Cape Fear River watershed. *Arch. Environ. Conta. Toxicol.*, 61:461–471.
- Mol, S., Ozden, O., Ahmet Oymak, S. (2010). Trace metal contents in fish species from Ataturk Dam Lake (Euphrates, Turkey). *Turkish J. Fisheries Aquatic Sciences*, 10: 209-213.
- Paquin, R.R., Farley, K., Santore, R.C., Kavvadas, C.D., Mooney, K.G., Winfield, R.P., Wu, K.B., Di Toro, D.M. (2003). Metals in aquatic systems: a review of exposure, bioaccumulation, and toxicity models. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, pp 61–90
- Puttaiah, E. T. and Kiran, B. R. (2008). Heavy metal transport in sewage fed lake of Karnataka, India. In: Sengupta, M. and Dalwani, R. (ed.), *Proceedings of TAAL 2007: The 12th World lake conference*: 347-354.
- Ravanaiah, G. and Narasimha Murthy, C. V. (2010). Accumulation Of Heavy Metals In Selected Tissues Of Fish, *Tilapia Mossambica* Collected From The Pennar Estuary. *Life Science Bulletin*, 7(2): 217-222.
- Roach, C., Maher, W., Krikowa, F. (2008). Assessment of metals in fish from lake Macquarie, New South Wales, Australia. *Arch. Environ. Conta. Toxicol.*, 54:292–308.
- Shah, A. Q., Kazi, T. G., Arain Md. B. , Jamali, Md. Khan , Afridi, H. Imran, Jalbani, N., Baig, J. A., Kandhro, G. A. (2009). Accumulation of arsenic in different fresh water fish species potential contribution to high arsenic intakes. *Food Chemistry*, [112 \(2\)](#):520-524.
- Shukla, H. C., Gupta, P. C., Mehta, H. C., Hebert, J. R. (2002). Descriptive epidemiology of body mass index of an urban adult population in western India. *J. Epidemiol. Community Health*, 56:876-880
- Sivapermal, P., Sankar, J.V. and Nair Viswanathan, P.G. (2007). Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-à-vis international standards. *Food Chem.*, 102:612-620.
- Staniskiene, B., Matusevicius, P. and Urbonavicius, A. (2009). Distribution of Heavy Metals in Muscles of Fish: Concentrations and Change Tendencies. *Environ. Res. Engineering and Management*, 2(48): 35-41
- Storelli, M, M, (2008). Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls

(PCBs) via seafood consumption: estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food Chem. Toxicol.*, 46:2782–2788.

Speedy, A. W. (2003). Global production and consumption of animal source foods. *J. Nutr.*, 133: 4048-4053.

Summers, J.K., Paul, J.F., Robertson, A. (1995). Monitoring the ecological condition of estuaries in the United States. *Toxicol. Environ. Chem.*, 49:93–108.

UNICEF, 2009. www.unicef.org/infobycountry/india_statistics.html

USEPA (United States Environmental Protection Agency). (2000). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis 3rd Edition, Office of Science and Technology of Water, Washington, DC EPA 823-B-00-007: 1-200.

USEPA (2011). USEPA Regional Screening Level (RSL) Summary Table: November 2011. Available at: <http://www.epa.gov/regshwmd/risk/human/Index.htm>, last update: 6th December, 2011.

USFDA, (1993). Food and drug administration, Guidance document for nickel in shell fish. DHHS/PHS/ FDA/CFSAN/Office of seafood, Washington D.C.

WHO (World Health Organisation). (1982). Toxicological evaluation of certain food additives: copper, zinc. WHO food additives series, No. 17

WHO (1989). Toxicological evaluations of certain food additives and contaminants: arsenic. 33rd Report of the JEFCA, WHO food additive series, No. 24

WHO, (1993). 41st Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 837. Geneva.

WHO. (1999). Joint FAO/WHO. Expert committee on food additives. In: Summary and conclusions, 53rd meeting, Rome, 1–10 June.

WHO. (2003). Joint FAO/WHO. Expert committee on food additives. In: Summary and conclusions, 61st meeting: methyl mercury. <http://www.who.int/pcs/jecfa/Summary61.pdf>.

WHO TDI for nickel, available at. <http://www.environment-agency.gov.uk/commondata>.