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Assessment of heavy metals and estimation of human health risk in Tilapia fish from Naik Lake of Nagpur, India

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Gills and muscles of Tilapia fish from Naik lake of Nagpur city were estimated for heavy metals (Zinc, Lead, Nickel, Copper and Cadmium) using inductively coupled plasma optical emission spectrometry (ICP-OES). Results showed higher concentrations of heavy metals in gills than in muscles of tilapia fish. Trends of heavy metal concentrations in gills and muscles of tilapia fish were found Zn > Pb> Ni> Cu >Cd. Highest lead and cadmium levels in muscles of the fish were 83±0.07 µg/g dw and 13±0.21 µg/g dw respectively. These levels were above the maximum permissible limits of Food and Agriculture Organization (FAO)/World Health Organisation (WHO). The results confirmed that tilapia fish from Naik lake are not safe for human consumption. Further, the health risks related to Zn, Pb, Ni, Cu and Cd were assessed based on target hazard quotients (THQ). The health risks from lead and cadmium were found to be the highest among all heavy metals in the study. THQ values of lead and cadmium were 4.0108, and 2.450, 0.818 and 2.57, 1.53 and 0.513 for person who eat tilapia fish from Naik lake five times, three times and once a week respectively. Maximum allowable daily consumption rate (CR_{lim}) for lead and cadmium in tilapia fish from Naik Lake was 0.0015 kg/day and 0.0024 kg/day respectively. Highest allowable weekly (CR_{wm}) and monthly (CR_{mm}) consumption rates for lead and cadmium in tilapia fish from Naik Lake were 0.0105 kg/week , 0.0168 kg/week and 0.198 kg/month, 0.318 kg/month respectively. Above these values of highest allowable daily, weekly and monthly consumption rates for lead and cadmium are unsafe for inhabitant who eats tilapia fish from Naik lake. Health risk assessment of lead and cadmium in tilapia fish from Naik lake suggests that consumption of fish from Naik lake might be unsafe for humans. Regular assessment of heavy metals in fish in Naik lake must be conducted to assure food safety.

Key words: Health risk estimation, heavy metals, tilapia fish, allowable daily consumption, target hazard quotients.

INTRODUCTION

During the past few years, toxic metal contamination increased in lakes due to anthropogenic activities (Canli and Atli, 2003; Wang et al., 2010). Heavy metals are transferred into sediments, phytoplankton, zooplankton, aquatic weed and fish and ultimately to human beings (Ikem and Egiebor, 2005). Fish is an important food

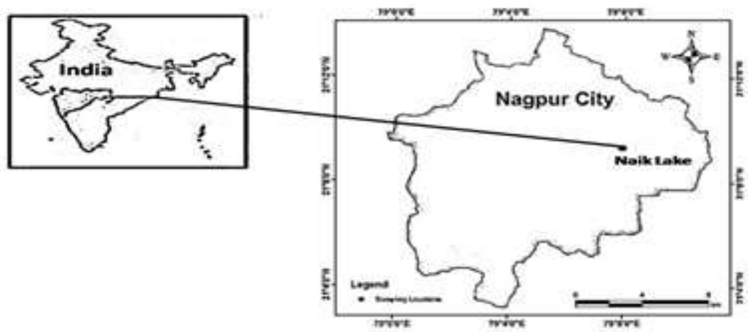

Figure 1. Location of Naik lake of the Nagpur city, Maharashtra, India.

source for humans and contain high levels of protein and omega-3 fatty acids, amino acids, vitamins and minerals like Ca, Fe, Cu and Zn (Aremu and Ekunode, 2008). Tilapia fish is common among all species of freshwater fish and most–suitable for farming in large quantity. Tilapia is considered the most traded fish worldwide (Kevin, 2008). Heavy metals contamination in fish is now global concern. Heavy metal contamination is not only threat to fish but also pose human health risks due to fish consumption.

Small amounts of copper, iron, manganese and zinc are important for human physiological activities (Celik and Oehlenschläger, 2004) but, high consumption of these metals causes health risks (Guérin et al., 2011). Excessive Cu, Fe and Mn in the human body can induce unpairing of electrons. These unpaired electrons contribute in redox reactions and catalyze the initiation of free radical reactions. This ability of electrons causes the toxicity of Cu, Fe and Mn in the human body (Storelli, 2008). Some metals like arsenic, cadmium, mercury and lead are toxic, even in small amounts (Mallin et al., 2011; Fraga, 2005). The exposure of lead causes renal failure, liver damage, coma and even death. The exposure of cadmium causes hypertension, kidney dysfunction, tumour formation and poor reproduction. Worldwide, many studies have been carried out on the health risk from fish consumption. However, very few studies on the health risk from fish consumption have been done in India (De et al., 2010; Rejomon et al., 2010). No studies on the health risk from fish consumption are available for central India.

This study focuses on Naik lake located in Nagpur city of central India. Human activities in the vicinity of Naik lake contributing to pollution such as domestic sewage, idol immersion and waste discharge. These activities resulted into total degradation of the lake system (Zade and Sitre, 2012). Human population is at risk of heavy metal pollution when fish is ingested from Naik lake. Therefore, evaluation of health risk from consumption of fish is essential. The aim of the study was to assess the selected heavy metals (Zn, Pb, Cu, Ni, Cd) in gills and muscles of the tilapia fish from Naik lake. The other important aim of the study is to determine the health risks of selected heavy metals due to the consumption of tilapia fish. The target hazard quotient (THQ) is used for estimation of health risks from the consumption of fish.

MATERIALS AND METHODS

Study sites

Naik lake is a natural freshwater ecosystem in Nagpur city, India (Figure 1), situated at latitude of 21°9'43"N and longitude 79°6'44"E in a densely populated area of Nagpur, India where fishing is carried out.

Sample collection

Tilapia (*Oreochromis mossambicus*) fish samples were collected from Naik lake in Nagpur, India using a drag net. Mean total lengths and total weights of fish samples were measured 19.83±3.21cm and 64.28±4.87g respectively. Fish samples were washed using ion free water and kept in clean isolated polyethylene bags. Samples were placed on ice immediately and taken to the laboratory, where they were deep frozen at −20°C until when they were prepared for digestion and analysis.

Fish samples preparation and heavy metal analysis of by ICP-OES

Heavy metals such as Zn, Pb, Cu, Ni and Cd were selected for the study. Samples were digested and analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) Perkin

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Table 1. Microwave digestion program used for Tilapia Fish (Source: USDA 2008).

Steps	Temperature	Time	Power
1	25-96 C	20 min	1000 W
2	96 C (Hold)	30 min	1000 W
3	180 C	10 min	1000 W
4	180 C (Hold)	10 min	1000 W

Table 2. Summary of the operational parameter settings used for the ICP-OES (Perkin Elmer, Optima 4100DV).

Characteristics	Instrument condition
RF generator	Fully solid-state generator; Operating frequency-40 MHz
RF power	Adjustable power from 750 to 1300 watts
Spray chamber	Scott type
Nebulizer	Cross Flow
Plasma gas flow	15.0 L/min
Auxiliary gas flow	L/min
Nebulizer gas flow	0.60 L/min

Table 3. Measured and reference values of metals concentrations.

Metal	Measured values	Reference values	Recovery in %	S D (±%)
Cadmium	0.89	0.82±0.16	93	7
Copper	3.89	4.04±0.33	97	3
Lead	1.11	1.19±0.18	94	6
Zinc	139	137±13	101	1
Nickel	0.89	0.93±0.12	96	4

Elmer, Optima 4100DV according to the method reported in USDA (2008). All glasswares were rinsed using ion free water for the analysis. Fish samples were thawed to room temperature and boneless muscles and gills were removed from the fish with sterilized surgical blades and scissors. Fish samples were then oven dried at 80°C in acid washed petri dishes up to a constant weight for 48 h. Fish samples were kept in desiccators for cooling. The removed fish muscle and gills were then homogenized with mortar and pestle and ground to a fine powder. Moisture content was calculated for individual fish samples. A 0.5 g of fine powdered muscle and gill material of each sample was digested in triplicate using closed-vessel microwave digestion, Milestone model Start D, Italy. Fish muscles and gills digested in nitric acid, were subjected to a four steps microwave digestion program (Table 1).

After digestion, 2 ml of 30% hydrogen peroxide was added to digests to reduce vapours of nitric acid and accelerate the organic substance digestion by increasing the temperature (Dig-Acids, 2001). Blanks were prepared for the authentic estimation of analysis. The digested fish samples were diluted with 50 ml ion-free water in acid washed standard flasks and each fish sample filtered through 0.45 µm Whatman filter paper (Millipore, Germany). Digests were determined using ICP-OES for all selected heavy metals and operational parameter settings were applied for the Perkin Elmer, Optima 4100DV were given in Table 2.

Multi-elemental standard solution (Merck) were used for the standardization and prepared by diluting stock solutions of 1000 mg/L (Mohammad et al., 2013). The certified standard reference material for fish (SRM 2976) from the National Institute of Standards and Technology, USA (NIST) was used and recoveries of the selected heavy metals ranged from 93 to 101 ±1-7 % of the certified values (Table 3). The levels of heavy metals in fish were expressed as µg/g dry weight (dw).

Determination of human health risk

To determine the health risk from consumption of tilapia fish of Naik lake, data from muscle samples were used. The daily intake of heavy metals was estimated on the basis of concentration of heavy metals in the muscle samples of the captured fish is presented by

Table 4. Heavy metal concentrations in gills and muscles of Tilapia fish in Naik lake (mean value±SD).

Metals	Concentrations in gills (µg/g dw)	Concentrations in muscles (µg/g dw)
Cadmium	22±0.26	13±0.21
Copper	75±0.23	39±0.14
Lead	153±0.12	83±0.07
Zinc	437±0.30	232±0.10
Nickel	77±0.09	67±0.025

Equation 1.

$$THQ = \left(\frac{EFr \times ED \times FiR \times C}{RfD \times WaB \times TA}\right) 10^{-3} \quad (1)$$

Where, THQ is the Target Hazard Quotient; EFr is the Exposure Frequency (365 days/year); ED is the Exposure duration (64.19 year); equivalent to the overall life expectancy in India (United Nations Department of Economic and Social Affairs, 2011); FiR is the Fish ingestion rate (9g/day/Indian person) (Bajaj, 2011); C is the Concentration of metal in the muscle of fish (µg/g). RfDs is the Oral reference doses (USEPA, 2000); WaB is the average body weight (65 kg) (ICMR, 2009) and TA is the average exposure time for non-carcinogens (365 days/year).

Estimation of the allowable daily consumption rate (CR_{lim})

To determine the allowable daily consumption rate of Tilapia fish of Naik lake, equation (2) was applied. Unit was expressed in kg/day.

$$CR_{lim} = \frac{RfD \times Bw}{Cm} \quad (2)$$

Where, CR_{lim} = Maximum safe daily consumption rate of fish (kg/d); RfD= Reference doses of metal (mg/kg/d); Bw=Average consumer body weight (kg); Cm= Measured concentration of chemical in the fish (mg/kg). For the estimation of the allowable daily consumption rate (CR_{lim}) equation 2 was applied and expressed in kg of fish per day. RfD values of metals are given by USEPA. RfD values is found on the basis of daily consumption of metals over a life span that would not be supposed to cause any adverse effects on health of human (USEPA 2000). Within the limits of the allowable daily consumption rates of tilapia fish has no adverse health effects on humans.

Estimation of the allowable weekly and monthly consumption rates (CR_{wm} and CR_{mm})

The allowable weekly and monthly consumption rates (CR_{wm} and CR_{mm}) were estimated using equations 3 and 4 below, and expressed in kg/week and kg/month respectively.

$$CR_{wm} = CR_{lim} \times 7 \quad (3)$$

Where, CR_{wm} =Maximum weekly consumption rate of fish (kg/week), CR_{lim} = Maximum safe daily consumption rate of fish (kg/d) over 7 days per week

$$CR_{mm} = (CR_{wm} \times Tap)MS^{-1} \quad (4)$$

Where, CR_{mm}= Maximum allowable fish consumption rate (meals month^{-1}), CR_{wm}=Maximum weekly consumption rate of fish (kg/week), T= is the average weeks in a month (taken as 4.3 week/month^{-1}), and MS=Meal size, taken as 227 g (8 oz) for an adults and 114 g (4 oz) for children (USEPA, 2000).

RESULTS AND DISCUSSION

Levels of metals in tilapia fish organs

Levels of heavy metals in gills and muscles are shown in Table 4. The trend of concentrations in gills and muscles of tilapia fish was Zn > Pb> Ni> Cu >Cd. Thus, results show dissimilarities in metal accumulation in gills and muscles of Tilapia fish. Concentrations of heavy metals were high in gills compared to muscles of the fish. Therefore, concentrations of heavy metals in gills reflect the concentration of heavy metals in water because gills of the fish have most direct contact with water of the lake. Heavy metals are introduced in the food chain from the direct intake of lake water or biota as well as from non-dietary routes such as the absorption by gills (Brezonik et al., 1991). Among the studied heavy metals, zinc concentrations were highest in both gills (437±0.30 µg/g dw) and muscles (232±0.10 µg/g dw) (Figure 2).

Similarly, the Zn concentrations have been reported (37.68±11.91µg/g) in sewage feed lake of Karnataka state, India (Puttaiah and Kiran, 2007). In comparison with lake of Karnataka state, the highest concentration of zinc of tilapia fish collected from present study lake was 6 times higher than the concentrations reported by Puttaiah and Kiran (2007). Lead concentrations in gills and muscles of tilapia fish from Naik lake, Nagpur city were remarkably higher (153±0.12 and 83±0.07 µg/g dw respectively) as compared to the study by Giripunje et al. (2014) which showed that the lead concentrations were 0.14±0.24 µg/g, 0.19±0.29 µg/g, 0.15±0.20 µg/gww respectively in tilapia fish of Futala, Gandhisagar and Ambazari lakes of same city. The Ni concentrations in gills and muscles from Naik lake were 77±0.09 and 67±0.025µg/g (dw). However, Ni concentration reported by other study was much lower (1.07±0.25 µg/g) in fish

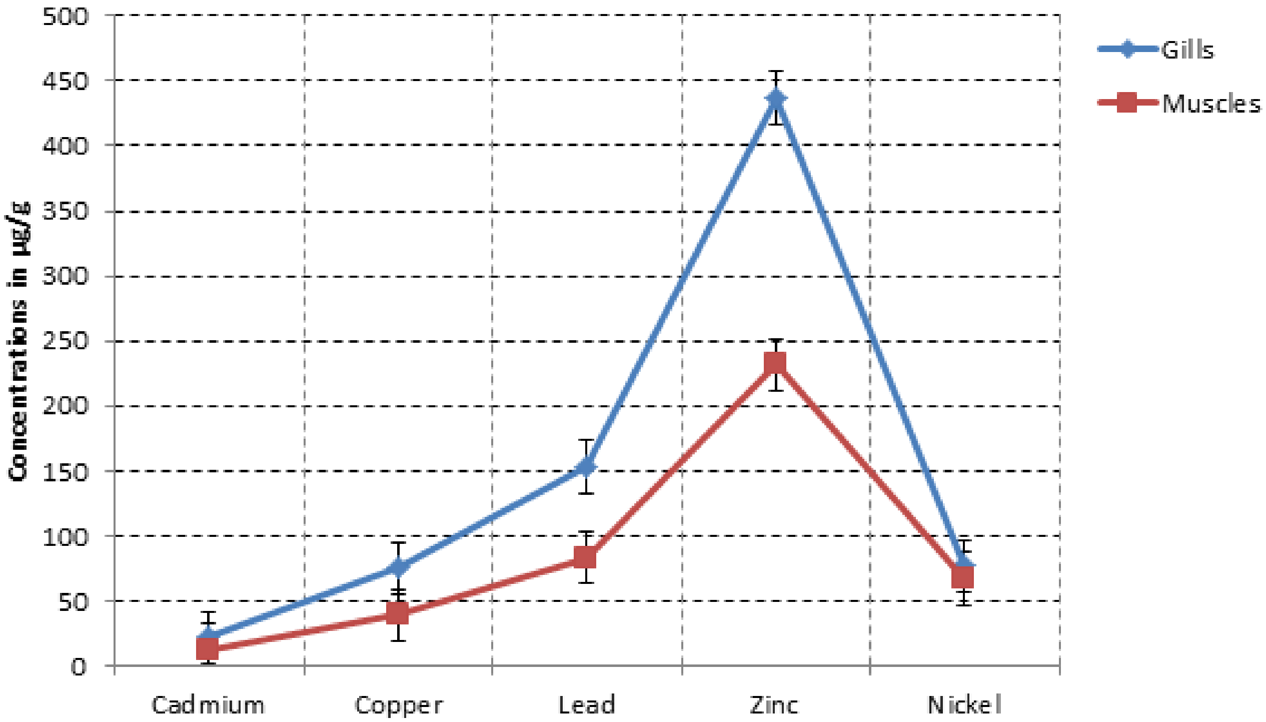

Figure 2. Heavy metals in gills and muscles of tilapia fish in Naik lake, Nagpur, India.

from the lake of Karnataka state, India (Puttaiah and Kiran, 2007).

In this study, copper concentrations in gills and muscles of tilapia fish were much higher (75±0.23 and 39±0.14 µg/g dw respectively) as compared to the study by Malik et al. (2010) which showed that the copper concentrations were 0.40 to 0.59 µg/g in fish lake of Bhopal, India. Among the studied heavy metals, cadmium concentrations in gills and muscles of fish were lowest (22±0.26 and 13±0.21µg/g dw respectively).

Similarly, Kumar et al. (2011) and Giripunje et al. (2014) reported low concentrations of cadmium in fish of lakes. In comparison with other study lake of Nagpur city, the highest Cd concentration 13±0.21µg/g dw in muscles from the present study lake was approximately 92 to 144 times higher than concentrations reported by Giripunje (2014) (0.09±0.33 to 0.14±0.51 µg/g ww, in Futala, Gandhisagar and Ambazari lakes).From Figure 2, it is clear that lead and cadmium exceeded the maximum permissible limits of FAO/WHO. This may be due to the fact that the high amount of heavy metal contaminated waste water discharged in Naik lake than other lakes in the city.

Human health risk assessment

In India, fish is one of the most important food sources because of its low cost and high nutritional values. However, water bodies in India are facing tremendous problem of contamination by toxic elements. This is considered a great concern due to the human health risk these elements pose. Inhabitants may consume toxic metals with the potential to affect their health. The potential health risk from Tilapia fish consumption posed by Zn, Pb, Cu, Ni and Cd are shown in Table 5.

Khan et al. (2008) reported that THQ values>1 indicates potential health risk from the metals. The level of exposure represents the consumption of fish five times, three times and once a week respectively. The THQ values for cadmium are 2.57, 1.53 and 0.513 for inhabitants who eat tilapia fish were five times, three times and once a week respectively (Table 5, Figure 3). THQ of Zn, Pb, Cu and Ni values for inhabitants who eat tilapia fish were five, three, one time a week ranged are also presented here. The potential health risks related to lead and cadmium are highest among all the studied heavy metals. It is clear from the estimation of health risks that the THQ values for lead and cadmium exceeded the one and THQ values are generally >1. These results indicated that the inhabitants who eat tilapia fish from Naik lake three to five times a week are at risk due to elevated Cd and Pb with potential for adverse health effects. THQ values other than lead and cadmium were all below 1 and those metals do not a pose health risk to the inhabitants (USEPA, 2011).

Table 5. Hazard quotients for intake of heavy metals from Tilapia of Naik lake.

Metal	Level of exposure (d/w)	Hazard quotient (THQ)
	5	2.57
Cadmium	3	1.53
	1	0.513
	5	0.096
Copper	3	0.057
	1	0.019
	5	4.108
Lead	3	2.450
	1	0.818
	5	0.076
Zinc	3	0.045
	1	0.015
	5	0.331
Nickel	3	0.196
	1	0.066

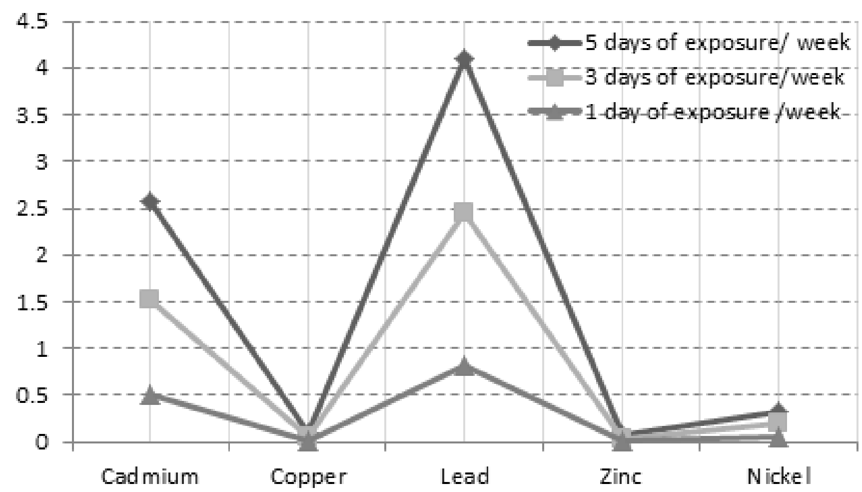

Figure 3. Hazard quotients for intake of heavy metals from Tilapia of Naik lake.

The allowable daily consumption rate (CRlim)

The allowable consumption for the one day would not essentially cause either chronic or acute health effects. However, consumption rate over a long period of time may cause a threat to human health (Jakus and Krupnick, 2002). In the view of this study, the highest allowable consumption rate was found in zinc (0.083 kg/day). The allowable consumption rates in copper, nickel, cadmium and lead were 0.065, 0.019, 0.0024 and 0.0015 kg/day respectively (Table 6). Inhabitants may ingest heavy metals which has the potential to cause adverse health effects through the consumption of tilapia fish. The daily, weekly and monthly safe consumption rates were calculated and presented in Table 7.

Highest allowable consumption rates for week and month was found in zinc (0.581 and 11.004 respectively) (Figure 4). Highest allowable consumption rates for week

Table 6. Maximum allowable daily fish consumption rate for heavy metals in Tilapia fish of Naik lake.

Metal	Metal concentrations in mg/kg	RfD (mg/kg/d)	Maximum allowable fish consumption rate (kg/d)
Cadmium	13	0.0005	0.0024
Copper	39	0.0400	0.0650
Lead	83	0.0020	0.0015
Zinc	232	0.3000	0.0830
Nickel	67	0.0200	0.0190

Table 7. Maximum allowable weekly and monthly fish consumption rate for heavy metals in Tilapia fish of Naik lake.

Metal	CR_{lim}	CR_{wm}	CR_{mm}
Cadmium	0.0024	0.0168	0.3180
Copper	0.0650	0.4550	8.6180
Lead	0.0015	0.0105	0.1980
Zinc	0.0830	0.5810	11.004
Nickel	0.0190	0.1330	2.5190

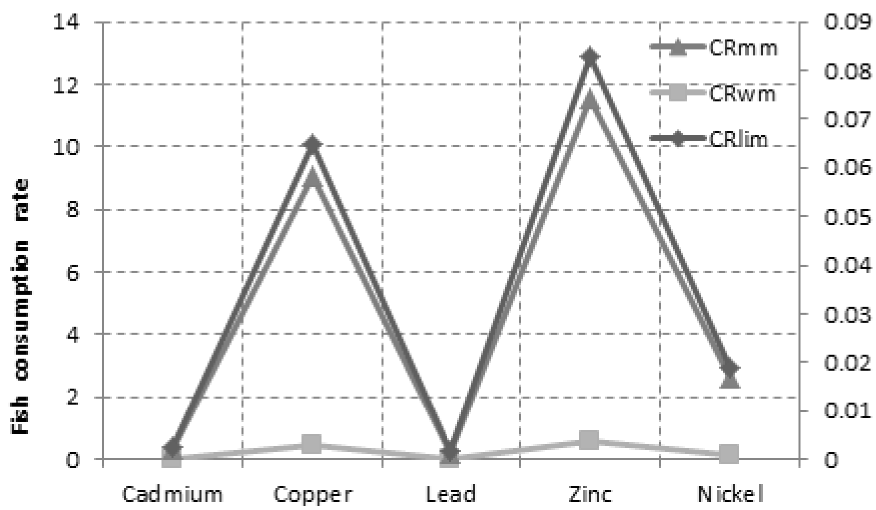

Figure 4. Maximum allowable weekly and monthly fish consumption rate for heavy metals in Tilapia fish of Naik lake.

and month in copper, nickel, cadmium and lead were 0.455 and 8.618, 0.133 and 2.519, 0.0168 and 0.318, 0.0105 and 0.198, respectively. Thus, USEPA in 2000 reported that limits of risk based consumption of fish are estimated that would expect to cause adverse health effects on inhabitants.

Conclusion

The edible part (that is, muscles) is the most important part of fish in the view of ingestion. Muscles of tilapia fish of Naik lake showed high concentrations of lead and cadmium above maximum permissible limits recommended by FAO/WHO (1999), and therefore suggest that these fish should not be consumed. The THQ values for copper, zinc and nickel indicated no risk from tilapia fish consumption for five times per week or less. Lead and cadmium showed lowest allowable consumption rates. The THQ values for lead and cadmium indicated high risk from tilapia fish consumption for most of the health risks related to fish originate in the aquatic environment. Therefore, regular and long-term assessment of heavy metals in fish in Naik lake must be conducted to assure food safety. This study serves as an initiative step to highlight the high need of remediation of heavy metals from Naik lake in the view of aquatic environment and food safety for inhabitants. The authors also suggest the need for a medical investigations and proper interventions for inhabitants.

Conflict of Interests

The authors have not declared any conflict of interests.

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