

Human Health Risk Assessment of Heavy Metals in Fish Species Collected from Catchments of Former Tin Mining

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Abstract: *In special cases, humans exposed to metals via the consumption of polluted fish may face a risk of poisoning by heavy metals. This study has been conducted to estimate the accumulation of As, Ni, Pb and Cd in the tissue, bone and gills in 10 fish species (Monopterus albus, Leiocassis poecilopterus, Mastacembelus frenatus, Mystacoleucus argenteus, Puntius schwanefeldii, Hampala microlepidota, Hemibagrus nemurus, Trichogaster trichopterus, Channa striata and Pristolepis grootii) collected from catchments of abandoned tin mine. The validation of the fish digestion method was performed by using certified reference material and ICP-MS was used to determine the metal concentrations. Although Pb and Cd were available in relatively low concentration in most of the samples; the concentrations of As, Ni in most of fish samples exceed the limits of (MFA, and WHO) standards for food. The evaluated metals were accumulated in bone and gills higher than those accumulated in tissues. The target hazard quotient (THQ) values for the Ni, Pb and Cd were below one for all fish species; however, the THQ was more than one for L. poecilopterus, M. frenatus, P. schwanefeldii, T. trichopterus, C.striata and P. grootii in case of As and for M. frenatus in case of Cd. This study suggests that the fish caught from the abandoned tin mines are dangerous and should not be eaten.*

Keywords: Risk Assessment; Target Hazard Quotient; Hazard Index; Abandoned Mining.

1. INTRODUCTION

The discharge of the mining wastes without adequate treatment; usually led to contaminate the water bodies surrounding mining area with elevated concentration of pollutants such as heavy metals [1, 2]. Several investigation studies have revealed that, the contaminants that discharge into aquatic environments are responsible for serious physical and chemical changes of the aquatic medium and negatively influenced the ecological balance of ecosystems; which in turn directly destroy the ecological environments [1-6]. Consequently, the assessment of environmental and potential health risks arising from the heavy metals contamination in former mine sites are becoming increasingly important issues.

Aquatic organisms like fish, shrimps and crabs accumulate many of contaminants in their tissues and magnify them up the food chain [7-10]. Consequently, there are several severe health hazards that threaten fish consumers by the consumption of metal accumulated fish; Minamata disaster is a good example for this case. In this regard, fish and aquatic organisms considered excellent bio-indicators to measure the abundance and availability of metals in the aquatic environments [11]. Moreover, fish are considered as fundamental biomarkers to monitor the heavy metals contamination in aquatic ecosystem for several important reasons: 1) fish occupies at a higher trophic level in an aquatic ecosystem, 2) toxicity of metals negatively affects the physical and physiological behaviour of the fish and 3) fish is an important constituent of human diet worldwide [12]. There is several factors play a key role in controlling the accumulation and toxicity of metals on aquatic organisms and fish in particular. These factors involved physico-chemical factors and biological factors. The physico-chemical factors are temperature and dissolved oxygen, hardness and alkalinity, hydrogen ion concentration pH, salinity, and suspended particulate matter and dissolved organic carbon. The biological factors are life cycle of the organism, seasonal variations, specific-species, specific organs, and contamination by food [10].

In Malaysia, there are numerous studies had investigated the growth of heavy metal contamination problem in several of aquatic environments (rivers, catchments and lakes) around Malaysia such as [2, 13-18]. Malaysia classified as one of the biggest consumers of seafood in Southeast Asia; approximately 52 kg/year is the average of Malaysian consumes of seafood with an expected increase to be at 1.68 billion kilograms in 2020. In fact, heavy metals have received great attention worldwide

by environmentalist, biologist and chemist due to their unique characteristics such as their biological significance, toxic behaviour, persistence, bioaccumulation and their tendency to be incorporated into food chains in harmful quantities [19], [20].

Assessment of heavy metals in fish from contaminated areas can be extremely important in two major aspects: a) from the public health point of view; to evaluate the potential health risks to human associated with consumption of fish from this contaminated catchments to safeguard of human health, and b) from the aquatic environment view point, to improve our knowledge on the biological status of the aquatic ecosystems as well as to improve our understanding of how the aquatic ecosystem adapts or changes according to the change in surrounding environmental conditions. Considering the above facts, the present study was undertaken to investigate and evaluate the presence of heavy metals in fish species from catchments and Kenau River flowing downstream of ex-mine area. Fish caught from some studied stations such as Kenau River are widely consumed by fishermen for sold fresh, reserved by the local people and fishermen for their own consumption or used in the aquarium trade. Consequently, close monitoring of toxic metals contamination as well as the assess the potential risks of consumption of fishes of the catchment of abandoned tin mines is recommended with a view to minimizing the risk of health of the population that depend on the Kenau river and mine catchments for their fish supply or water. Human consumes the fish tissue thus; fish muscle is chosen to evaluate the heavy metals. Gills and bones of fish are considered as a good environmental indicator of heavy metals contamination in water [21]. The significance of this investigation is to produce representative heavy metals content data, which include As, Cd, Pb and Ni for contaminated ex-mine catchments fish species. In addition to, assess the potential health risks from consumption of fish from these catchments. In the current study the (As, Cd, Pb) were chosen to be analysed in fish species due to their extremely toxic effects on the aquatic organisms and human health and are common in ex-mining area.

2. MATERIALS AND METHODS

2.1. Description of The Study Area

The Sungai Lembing abandoned mine (3°54'23"N and 103°2'30"E) is a small area that is located about 26.10 mi northwest of Kuantan in Pahang, East Coast of Peninsular Malaysia [4]. The history of tin mining activity in this city is very old where the tin was exported from Kelantan and Pahang to China during mid-13th century. The abandoned mining in Sungai Lembing is characterized by availability of a large amount of mine waste deposits, ore stockpiles, old tools, and tailings, which have become a heap around the place. Weathering processes for the heaped waste materials contributes mainly in releasing high amounts of heavy metals and toxic chemicals into surrounding aquatic environments [6]. A huge number of heavy metals and toxic chemicals from abandoned mining sites are directly discharged onto surrounding environments. Sungai Lembing mine kept its waste materials at a predetermined site near the bank of the Kenau River [4].

2.2. The Sampling, Collection, and Preparation

Table1. Physical characteristics & taxonomic classification of fish species in the catchments

Common Name	Scientific Name	Family	Feeding habitats	Status	station
Swamp eel	<i>Monopterus albus</i>	Synbranchidae	Demersal	Premature	Kenau River
Sebarau	<i>Hampala macrolepidota</i>	Cyprinidae	Benthopelagic	Premature	Kenau River
Daun	<i>Mystacoleucus argenteus</i>	Cyprinidae	Benthopelagic	Premature	Kenau River
Baung duri	<i>Leiocassis poecilopterus</i>	Bagridae	Demersal	Mature	Station 1
Lampam Sungai	<i>Puntius schwanefeldii</i>	Cyprinidae	Benthopelagic	Premature	Station 1
Spiny eel	<i>Mastacembelus frenatus</i>	Mastacembelidae	Demersal	Mature	Station 2
Baung	<i>Hemibagrus nemurus</i>	Bagridae	Benthopelagic	Mature	Kenau River
Sepat padi	<i>Trichogaster trichopterus</i>	Osphronemidae	Benthopelagic	Premature	Kenau River
Haruan	<i>Channa striata</i>	Channidae	Benthopelagic	Mature	Station 1
Indonesian leaffish	<i>Pristolepis grootii</i>	Pristolepididae	Benthopelagic	Mature	Station 1

Fish were caught using electric shocker (electrofishing). The fish samples were placed in clean polyethylene bags and kept in ice-cooled boxes for transportation to the laboratory. Fish details are shown in Table 1. The frozen fish were thawed to room temperature and scales were removed using

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stainless steel knife. The boneless tissues, gills, and bones of the fish were separated and then dried to constant weight in an oven at 80°C. Samples were pounded using a laboratory mortar to a fine powder and pestle to produce homogeneous tissues. The wet digestion method based on the [22] was used to extract metals from fish tissues, bones, gills. Samples were digested on hot sand bath at 90 °C using concentrated nitric acid (69%) and concentrated perchloric acid (HClO₄) with 3:1 ratio.

2.3. Determination of Metals

The yellowish mixture was filtered through 0.45µm pore size Whatman filter paper. Concentrations of heavy metal in the final solution were determined by using inductively-coupled plasma mass spectrometry (ICP-MS) (model ELAN 9000 Perkin Elmer ICP-MS, USA). ICP multielement standard solution of 1000 mg/L supplied by Merck was used after dilution. In order to achieve high quality results, a calibration blank and an independent calibration verification standard were analysed for every 15 samples to confirm the calibration status of the ICP-MS. Matrix interference (Blank) was < 1% for almost of elements. Metal concentrations were expressed as mg/kg dry weight of fish tissue, bone and gills. Triplicates of samples analysis yielded relative percent differences of < 5 %.

2.4. Validation Methodology

The performance of the analytical method was validated using the certified standard reference material SRM for lobster (TORT-2 Lobster Hepatopancreas Reference Material, supplied by the National Research Council Canada). Recoveries of all of the targeted elements ranged from 85.71% to 105.56% of the certified value (Table 2).

Table2. Measured and certified values for tested metals using the SRM (mg/kg dw).

Element	Fish mg/Kg± SD	SRM mg/Kg± SD	Recovery
Pb	0.30±0.10	0.35±0.13	85.71%
Ni	2.40±0.35	2.50±0.19	96 %
Cd	24.00±1.7	26.7±0.6	89.89%
As	22.80±2.3	21.6±1.8	105.56%

2.5. Health Risk Assessment for Fish Consumption

The target hazardous quotient (THQ) represents a complex parameter which is introduced by the US Environmental Protection Agency (EPA 1989). It is used commonly for the assessment of the potential of non-carcinogenic risks associated with long term exposure to contaminants, such as heavy metals from food such as fish and water. The THQ represents the ratio between the chronic daily intake of the studied metals (CDI) in (mg/kg-day) and the oral reference dose (RfD) expressed also in (mg/kg-day) Equation (1). The target carcinogenic risks (CR) can be estimated by multiplying the calculated chronic daily intake (CDI) (mg/kg-day) with the oral slope factor (mg/kg-day)⁻¹ for the carcinogenic elements Equation (2). Also, the THQ parameter does not estimate the risks; it only indicates a risk level associated with pollutants exposure [23]. As published by USEPA (2010), if the THQ value is < 1.00 that means the exposed population is supposed to be safe; however, when THQ > 1.00 there is a potential risk related to the studied metal in the exposed population. The EPA proved that when the level of carcinogenic health effects is at 10⁻⁶ for individual toxic metals, this will result in a relatively negligible cancer risks (USEPA 2010).

The general formulas that used to calculate the non-carcinogenic effects (THQ) and carcinogenic effects (CR) in this work are equations: (1) and (2), respectively

$$\text{THQ} = (\text{EFr} \times \text{ED} \times \text{IR} \times \text{C}) / (\text{RfD} \times \text{BW} \times \text{AT}) \quad (1)$$

$$\text{Cancer Risk (CR)} = \text{CDI} * \text{SF} \quad (2)$$

The EFr represents the exposure frequency which was in this study (365 days/year). This study used

365 days/year for exposure frequency, with seven days in a week and (52 weeks/year) and only one day in a week to compare between the maximum and minimum frequency of exposure from the consumption of fish from the ex-mine catchments. The ED represents the exposure duration; in this study it was (30 years) to estimate the non-carcinogens effects, and 70 years to estimate the carcinogens effects. The IR represents the daily fish ingestion rate; in this study it was 0.16 kg/day/persons for the Malaysian adults [24]. The C represents the metal concentrations (mg/kg. wet weight) in the tissues of fish samples. The metal concentrations (mg/kg dry weight) were converted into mg/kg wet weight for health risk assessments. The oral reference dose (RfD) was adopted from the USEPA, (2005). The average body weight (BW) for the Malaysian adult male is 63 kg [25] and was used in this study. The averaging time (TA) for non-carcinogens is $365\text{days/year} \times \text{ED}$.

3. RESULTS AND DISCUSSION

3.1. Distribution of Heavy Metals

A total of 24 fish belongs to eight families, 10 genera, and 10 species were caught from different catchments within the vicinity of ex-tin mine in Sg. Lembing and Kenau River downstream of the mining area. Levels of Cd, Ni, Pb and As were measured in each of fish species, tissue, bone, and gills using ICP.MS. Table 3 shows the mean concentrations of Cd, As, Ni and Pb and the corresponding mean standard deviations expressed as mg/kg dry weight were determined in the different organs (tissue, bone and gills) of the ten fish species from Sg. Lembing ex-tin mine catchments and Kenau River.

Data of heavy metals distribution in tissue, bone and gills of all evaluated fish species showed that, heavy metals were accumulated in the different organs as follows: *M. albus*; tissue - Ni > Pb > As > Cd, bone - As > Ni > Pb > Cd, gills- As > Ni > Pb > Cd: *L. poecilopterus*; tissue, bone and gills – As > Ni > Cd > Pb, *M. frenatus*; tissue- Ni > Cd > As > Pb, bone and gills – As > Ni > Pb > Cd, *M. argenteus*; tissue- Ni > Cd > As > Pb, bone and gills – As > Ni > Cd, Pb, *P. schwanefeldii*; tissue- Ni > As > Pb > Cd, bone and gills – As > Ni > Pb > Cd, *H. microlepidota*; tissue – Ni > As > Cd > Pb, bone and gills – As > Ni > Cd > Pb, *H. nemurus*; tissue – Ni > Cd > As > Pb, bone and gills – As > Ni > Cd > Pb, *T. trichopterus*; tissue – Ni > As > Cd > Pb, bone and gills As > Ni > Cd > Pb, *C. striata*; tissue, bone and gills – As > Ni > Pb > Cd, and *P. grootii*; tissue and bone - As > Ni > Cd > Pb, gills – Ni > As > Cd > Pb.

Arsenic: As is a toxic element for all living organisms including human; its toxicity depends mainly on its chemical form [8]. In present study, As had the highest concentrations and the most predominant metal in all evaluated organs of all fish species followed by Ni, Pb and Cd. As was detected in all organs of ten species of fish. *L. poecilopterus* accumulated higher amount of As in gills (97.25 ± 2.59 mg/kg), bone (77.39 ± 0.91 mg/kg) and tissue (66.75 ± 3.98 mg/kg) followed by *M. frenatus* in gills (106.86 ± 6.02 mg/kg) bone (63.57 ± 1.74 mg/kg) and tissue (0.70 ± 1.33 mg/kg). While, the lowest As concentrations were detected in *P. grootii* in gills (32.70 ± 4.09 mg/kg), bone (15.61 ± 1.22 mg/kg) tissue (11.69 ± 1.95 mg/kg) followed by *P. schwanefeldii* in gills (21.78 ± 0.59 mg/kg), bone (21.37 ± 0.39 mg/kg), and tissue (0.82 ± 0.47 mg/kg). The results of present study are in line with that of [26]. In Malaysia, As levels were range between 0.0025 to 0.83 mg/kg in fish collected from former tin mining catchment in study performed by [3]. [8], reported As concentrations ranging from 0.002 to 11.8 mg/ kg in fish from Brazil. In other survey of different fish species from China by [27], As concentration ranged between 0.88 and 4.48 mg/kg, and in Turkey, [28] reported higher mean values for As concentrations in different fish species 0.98 mg/kg to 1.74 mg/kg.

In the Malaysia Food Act (MFA), the maximum As level established for fish is 1.0 mg/kg. From this study, it can be clearly seen that, the mean concentration of As in tissues of *L. poecilopterus*, *C. striata*, and *P. grootii* were much higher than the limits of permissible levels introduced by all authorities with 66.75, 5.28 and 11.69 mg/Kg respectively; which pose a very serious threat to the health of fish consumers in this area. While As concentrations in tissues of *M. albus*, *M. frenatus*, *M. argenteus*, *P. schwanenfeldii*, *H. microlepidota*, *H. nemurus*, and *T. trichopterus* were still below the limits of permissible levels. The edible parts of fish contain approximately 85 to > 90% of the organic arsenic such as arsenocholine, dimethylarsinic acid, and arsenobetaine and only 10% of inorganic arsenic [7]. According to [28], arsenic and its compounds seemed to be mainly accumulating in muscle organs like a heart. In fish, As causes neoplastic alterations and bizarre morphological alterations in the early life stages. The immediate death can be the major result for acute exposures. Chronic exposures can lead to severe health risks [3]. Mining and its activities contribute in alteration of the biogeochemical cycles of elements. Mine tailings, AMD, and erosion from the mining areas are mainly associated with the discharge of pollutants containing toxic metals into the surrounding environments. Therefore, the aquatic environment and its organisms are considered as the straight collector of metal elements from the mining sites [1].

Nickel: In human body, Ni is believed to contribute in physiological processes as a co-factor in the absorption of iron from the intestine. Therefore, Human body needs Ni with very small amount. On the other hand, Ni classified as a moderately toxic element. According to investigations performed by the International Agency for Research on Cancer (IARC), all nickel compounds, have been considered as human carcinogens [29]. According to present study, the levels of Ni were followed levels of As; the highest level of Ni was found in *P. grootii* in gills (80.11 ± 107.18 mg/kg), bone (10.47 ± 0.67 mg/kg) and tissue (7.58 ± 1.11 mg/kg) followed by *C. striata* gills (26.55 ± 2.87 mg/kg), bone (9.11 ± 0.34 mg/kg) and tissue (4.56 ± 0.04 mg/kg), the lowest Ni concentration were determined in *P. schwanenfeldii* in gills (3.14 ± 0.05 mg/kg), bone (3.98 ± 0.22 mg/kg) and tissue (1.54 ± 0.00 mg/kg). In a study by [1] performed in India, Ni concentration was reported as 1.25–21.5 mg/kg in fish species exposed to mining waste. However, In Turkey, Ni concentrations in tissue of fish were determined as 1.1–10.2 µg/g in the study performed by [30].

Ni like other metals becomes toxic when their concentration level exceeds those required for correct nutrition. Generally, the mean concentration of Ni found in tissue of all evaluated fish species were above the critical limits set by (WHO 1985, FAO and MFA); which may causes severe health effects on fish consumers. This result is in agreement with the findings of [31], who reported the levels of Ni in some fishes (*H. forskahlii*, *H. bebe occidentalis* and *C. garipepinus*) were higher than the maximum permissible limits. In Malaysia, the acid mine drainage (AMD) is one of the major contributors in Ni contamination; due to high Ni concentrations leached from rocks, tailing and dumps of mine sites [32]. Effects of Ni on human beings include reduced lung function, lung cancer [20].

Lead: In fact, Pb is considered a non-essential element; Pb is similar to calcium in metabolism processes and in its mobilization from bone as well as its deposition in bone. Due to the large affinity of Pb for thiol and phosphate phosphate-containing ligands, it inhibits the biosynthesis of heme, thus affecting the membrane, permeability of kidney, liver and also brain cells; therefore, estimate of Pb accumulation in fish from contaminated area should be performed. Pb was not detected in all organs of *L. poecilopterus*, *M. argenteus*, *H. microlepidota*, *H. nemurus*, *T. trichopterus*, and tissue of *P. grootii*. The highest mean concentration of Pb was detected in *M. albus* in gills (4.24 ± 0.22 mg/kg), bone (1.57 ± 0.08 mg/kg) and tissue (1.49 ± 0.34 mg/kg) followed by *M. frenatus* in gills (2.14 ± 0.11

mg/kg), bone (0.98 ± 0.05 mg/kg) and tissue (0.55 ± 0.14 mg/kg). The lowest Pb levels were detected in *P. schwanefeldii* gills (0.94 ± 0.03 mg/kg), bone (1.13 ± 0.03 mg/kg) and tissue (0.19 ± 0.01 mg/kg). The mean concentration of Pb in tissue of *M. albus*, *M. frenatus*, *P. schwanefeldii*, and *C. striata* were slightly below the limits of permissible levels with 1.49, 0.55, 0.19, and 1.44 mg/Kg respectively. The values obtained for Pb in the present study are in line with that of [31, 33].

Other surveys reported Pb mean concentrations in several rivers that are in similar condition to the Kenau River under the vigorous anthropogenic influence, including mine, between 8.03–13.52 mg/kg in fish tissue from Buriganga River, Bangladesh [34], between 0.009–10.1 mg/kg in fish from Yangtze River in study performed by [35], 0.128 mg /kg in fish from Tingjiang river, China [36], between 0.015–0.039 mg/kg in fish collected from the Danube river, Croatia by [37], and between 0.03–0.41 mg/kg in fish from India [1]. Other studies reported Pb mean concentrations between 0.28–0.87 mg/kg and 0.10–0.56 mg/kg in studies performed by [30, 38] respectively, and between 0.14 and 0.39 mg/kg in fish from Iskenderun Bay [28].

Cadmium: Cd is classified as one of the major ecotoxic metals; that can cause harmful effects on physiological processes of humans, animal, aquatic organisms, and plants [39]. The levels of Cd were followed levels of Pb; the highest level of Cd was found in *P. grootii* in gills (2.85 ± 0.12 mg/kg), bone (1.52 ± 0.16 mg/kg) and tissue (0.34 ± 0.02 mg/kg) followed by *M. frenatus* gills (1.38 ± 0.02 mg/kg), bone (0.72 ± 0.02 mg/kg) and tissue (2.00 ± 0.09 mg/kg), the lowest Cd levels were detected in *L. poecilopterus* gills (0.24 ± 0.01 mg/kg), bone (0.14 ± 0.01 mg/kg) and tissue (0.08 ± 0.06 mg/kg), followed by *T. trichopterus* in gills (0.22 ± 0.03 mg/kg), bone (0.04 ± 0.02 mg/kg) and tissue (0.10 ± 0.04 mg/kg). According to [40], cadmium tends to concentrate in internal organs likes liver, kidney, and bone does not tends to accumulate in tissue (edible part) thus; Cd concentration in tissue is low. The Cd concentration that measured in all tissues of fish species were lower than the maximum recommended limit of 2.00 ppm (MFA,1993) in fish food except in tissue of *M. frenatus* had already reached the level of concern. Cd levels in fish live in Danube river that influenced by industries and mining as 0.013–0.018 mg/kg [37].

Other surveys reported Cd mean concentrations in several rivers that are under the vigorous anthropogenic influence, including mining, between 0.10–0.17 mg/kg in fish from Pearl river, China [41], between 0.004–0.85 in fees from Subarnarekha River, India [1], and between ND-2.0 mg/kg in fish from Yangtze River [35]. In a study by [42] performed in Nigeria, Cd concentration was reported as 0.79-0.98 mg/kg in fish species exposed to domestic, industrial and agricultural wastes. [28] reported the Cd levels in some fishes species were ranged between 0.01- 0.04 mg/kg. Mining activities released high concentration of cadmium into aquatic environments [17]. Cd is toxic at extremely low levels. In humans, long term exposure results in renal dysfunction and obstructive lung disease [40].

In the present study, there were some difficulties in comparison values of certain elements in the same fish species due to the lack of data. From this study, it can be clearly seen that, levels of metals in fish species were associated with the collection locations and species. Elevated concentrations of metal were recorded in fish collected from two locations, i.e., catchment in station 1, catchment in station 3. These catchments are directly related to old tin mining. Additionally, these catchments located near to the tailings of old mine. Consequently, these catchments are under the high influence of old mine and its associated tailings more than other stations. Therefore, fish samples collected from these catchments have elevated metal concentrations as compared to those collected from other locations (Kenau River). Mining and metallurgical industries are responsible for the anthropogenic alteration of the biogeochemical cycles of elements and releasing elevated concentrations of metal into the

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surrounding environments via natural leaching of mine tailings, erosion from the mining areas, and AMD [1, 10, 43]. Consequently, aquatic environments and its organisms are considered as the main recipients of the metal that discharged from the mining sites and metallurgical industries. Increasing the effluents of mining promotes levels of metal elements in the aquatic environments, which may negatively impact the quality of water and aquatic organisms [10].

Table3. Mean concentration of heavy metals (mg/kg) in the tissues, bone, and gills of fish species collected from different catchments within the vicinity of ex-tin mine in Sg. Lembing. Values are presented in means \pm S.D

Species Name	Pb mg/kg (D.w)	As mg/kg (D.w)	Ni mg/kg (D.w)	Cd mg/kg (D.w)
<i>Monopterus albus</i>				
Tissue	1.49 \pm 0.34	0.17 \pm 4.07	1.84 \pm 0.04	0.40 \pm 0.04
Bone	1.57 \pm 0.08	16.38 \pm 0.66	1.58 \pm 0.09	0.57 \pm 0.02
Gills	4.24 \pm 0.22	43.56 \pm 2.34	6.65 \pm 0.26	0.21 \pm 0.02
<i>Leiocassis poecilopterus</i>				
Tissue	< 0.0005 (μ g/L)	66.75 \pm 3.98	2.68 \pm 0.14	0.08 \pm 0.06
Bone	< 0.0005 (μ g/L)	77.39 \pm 0.91	5.22 \pm 0.17	0.14 \pm 0.01
Gills	< 0.0005 (μ g/L)	97.25 \pm 2.59	7.99 \pm 0.34	0.24 \pm 0.01
<i>Mastacembelus frenatus</i>				
Tissue	0.55 \pm 0.14	0.70 \pm 1.33	2.57 \pm 0.11	2.00 \pm 0.09
Bone	0.98 \pm 0.05	63.57 \pm 1.74	4.25 \pm 0.37	0.72 \pm 0.02
Gills	2.14 \pm 0.11	106.86 \pm 6.02	8.14 \pm 0.57	1.38 \pm 0.02
<i>Mystacoleucus argenteus</i>				
Tissue	< 0.0005 (μ g/L)	0.34 \pm 0.39	2.32 \pm 0.12	0.47 \pm 0.01
Bone	< 0.0005 (μ g/L)	48.17 \pm 1.66	5.61 \pm 0.30	0.57 \pm 0.02
Gills	< 0.0005 (μ g/L)	48.35 \pm 4.08	5.56 \pm 0.25	0.65 \pm 0.01
<i>Puntius schwanenfeldii</i>				
Tissue	0.19 \pm 0.01	0.82 \pm 0.47	1.54 \pm 0.00	0.13 \pm 0.01
Bone	1.13 \pm 0.03	21.37 \pm 0.39	3.98 \pm 0.22	0.48 \pm 0.01
Gills	0.94 \pm 0.03	21.78 \pm 0.59	3.14 \pm 0.05	0.66 \pm 0.01
<i>Hampala microlepidota</i>				
Tissue	< 0.0005 (μ g/L)	0.53 \pm 0.86	1.55 \pm 0.12	0.31 \pm 0.01
Bone	< 0.0005 (μ g/L)	43.90 \pm 7.65	3.52 \pm 0.17	0.78 \pm 0.04
Gills	< 0.0005 (μ g/L)	97.59 \pm 6.07	10.32 \pm 0.30	0.96 \pm 0.03
<i>Hemibagrus nemurus</i>				
Tissue	< 0.0005 (μ g/L)	0.38 \pm 3.37	1.79 \pm 0.13	0.70 \pm 0.04
Bone	< 0.0005 (μ g/L)	49.98 \pm 0.61	3.95 \pm 0.04	1.57 \pm 0.62
Gills	< 0.0005 (μ g/L)	104.77 \pm 3.63	7.34 \pm 0.80	1.34 \pm 0.06
<i>Trichogaster trichopterus</i>				
Tissue	< 0.0005 (μ g/L)	0.59 \pm 2.89	2.67 \pm 0.13	0.10 \pm 0.04
Bone	< 0.0005 (μ g/L)	84.57 \pm 2.26	6.48 \pm 0.34	0.04 \pm 0.02
Gills	< 0.0005 (μ g/L)	71.43 \pm 3.23	6.52 \pm 0.42	0.22 \pm 0.03
<i>Channa striata</i>				
Tissue	1.44 \pm 0.01	5.28 \pm 0.32	4.56 \pm 0.04	0.08 \pm 0.00
Bone	0.17 \pm 0.12	14.22 \pm 1.94	9.11 \pm 0.34	0.12 \pm 0.01
Gills	1.64 \pm 0.38	45.78 \pm 6.24	26.55 \pm 2.87	0.71 \pm 0.04
<i>Pristolepis grootii</i>				
Tissue	< 0.0005	11.69 \pm 1.95	7.58 \pm 1.11	0.34 \pm 0.02
Bone	0.64 \pm 0.15	15.61 \pm 1.22	10.47 \pm 0.67	1.52 \pm 0.16
Gills	2.39 \pm 3.28	32.70 \pm 4.09	80.11 \pm 107.18	2.85 \pm 0.12
Permissible Limit in Fish				
EC, 2001		0.2-0.4	-	-
USFDA, 1993		0.5	-	-
WHO, (1985, 2004)		2.0	0.1-5.0 (μ g/g)	0.5-0.6
FAO, (1983, 2004)		1.5	0.1-5.0(μ g/g)	-
MFA,1993		1	1	2

From this study, it can be clearly seen that great variations among concentrations of heavy metal in all organs and showed different affinity capabilities for accumulation. This can mainly be attributed to differences in the physiological role of each organ [44]. As a result, muscle accumulates the lowest levels of heavy metal. These results were in agreement with that of [45-48]. Additionally, the concentrations were varied between the species, all evaluated metals (Pb, Ni, Cd and As) tend to

accumulate at different levels in various species. According to [14, 44, 49], differences in heavy metal concentrations between fish species may attribute to the feeding behaviors, metabolism, biological factors, fish age, behaviour and feeding habits. The comparison of metal concentrations in the tissues, bone and gills of two different species are very difficult, due to the differences in the aquatic environments such as (omnivorous and carnivorous) and differences in age, sex, and growing rates of fish species, feeding habits and other factors. Previous study has revealed that, there were differences between the metals concentrations in evaluated species; consequently, they reported that this aspect was very important from both an ecotoxicological viewpoint and concerning public health risks associated with the consumption of these organisms [28].

The findings of the present study, heavy metal concentrations in fish species indicate that *L. poecilopterus*, *M. frenatus*, *T. trichopterus*, *H. nemurus*, and *P. grootii* seemed to be more contaminated than were other fish species, followed by *H. microlepidota*, *M. argenteus*, *C. striata*, *M. albus*, and *P. schwanenfeldii* appeared to tend to accumulate elevated concentrations of (As, Ni, Pb and Cd) demonstrating a potential as bioindicators of pollution of the former mines catchments. Depending on the different fish habitats, *L. poecilopterus*, *M. frenatus*, *T. trichopterus*, *H. nemurus* are bottom feeder fish. According to [3, 31], carnivores tend to accumulate high metal concentrations of heavy metals.

3.2. Non-carcinogenic Health Effects

The calculation of potential health risks (non-carcinogenic and carcinogenic effects) associated with the consumption of fish contaminated with (Pb, As, Ni and Cd) for an individual adult are presented in Table 4. In the non-carcinogenic health risk, the As concentrations in the fish tissues have the highest potential as a health risk for fish consumption followed by the Cd and Ni, with Pb the lowest. The As THQ from the consumption of *L. poecilopterus*, *C. striata* and *P. grootii* (seven days and one day/a week) were >1.00. In addition THQ for As from *M. frenatus*, *T. trichopterus* and *P. schwanenfeldii* tissue were higher than 1 for people who eat fish seven days per a week. This may be attributed to the high As contamination in the ex-mining area where the fish live. The THQ for other evaluated elements (Pb, Ni and Cd) were lower than 1 for people who eat fish seven and once a week; with exception (THQ) of Cd of consumption of *M. frenatus* was higher than 1 for people who eat fish seven days per week.

Table4. Shows health risks assessment for individual metal by the determination of target hazard quotients (THQ) for fish species

Species Name	EF d/w	Pb	As	Ni	Cd
<i>Monopterus albus</i>	7	0.19	0.29	0.05	0.21
	1	0.03	0.04	0.01	0.03
<i>Leiocassis poecilopterus</i>	7	0.00	> 1.00	0.07	0.04
	1	0.00	> 1.00	0.01	0.01
<i>Mastacembelus frenatus</i>	7	0.07	> 1.00	0.07	> 1.00
	1	0.01	0.17	0.01	0.14
<i>Mystacoleucus argenteus</i>	7	0.00	0.58	0.06	0.24
	1	0.00	0.08	0.01	0.03
<i>Puntius schwanenfeldii</i>	7	0.02	> 1.00	0.04	0.07
	1	0.00	0.01	0.01	0.01
<i>Hampala microlepidota</i>	7	0.00	0.90	0.04	0.16
	1	0.00	0.13	0.01	0.02
<i>Hemibagrus nemurus</i>	7	0.00	0.64	0.05	0.36
	1	0.00	0.09	0.01	0.05
<i>Trichogaster trichopterus</i>	7	0.00	> 1.00	0.07	0.05
	1	0.00	0.14	0.01	0.01
<i>Channa striata</i>	7	0.18	> 1.00	0.12	0.04
	1	0.03	> 1.00	0.02	0.01
<i>Pristolepis grootii</i>	7	0.00	> 1.00	0.19	0.17
	1	0.00	> 1.00	0.03	0.02

As a conclusion, most of THQ values for evaluated fish species are below one, that mean that intakes of heavy metals by consuming these fish is not likely to cause any appreciable health risks on the human body. While, in case of As the calculated values of THQ for *L. poecilopterus*, *C. striata* and *P. grootii* exceed the limit of one, which indicates that potential health risks in consumption to the consumers. The edible parts of fish contain approximately 85 to > 90% of the organic arsenic such as arsenocholine, dimethylarsinic acid, and arsenobetaine and only 10% of inorganic arsenic [7] and if this is used for calculation, the fish samples will not pose any potential health risks to human health. In present study, the highest values of THQ were recorded in fish samples that collected from catchments located near to the tailings and old mine area.

3.3. Carcinogenic Health Effects

Inorganic Pb and inorganic As are classified as carcinogenic elements to humans [50, 51]; which tends to accumulate mainly in the liver and kidney of human and fish. This phenomenon is attributed to the precipitation of protein by Pb, through the interaction of Pb ions with the sulfhydryl (-SH) groups of proteins (ATSDR 2007). The target carcinogenic effects derived from lead intake are calculated using Equation (2); the value of (SF) for Pb is $(8.5 \times 10^{-3} \text{ mg/kg/day})^{-1}$ (U.S. EPA, 2005). The estimated results of the carcinogenic health risks for Pb and As in evaluated fish species are presented in Table 5.

The carcinogenic risk values associated with the ingestion of Pb through consumption of *M. albus* was estimated to be 64×10^{-05} , *M. frenatus* was 2×10^{-05} , *P. schwanenfeldii* was 1×10^{-05} , and *Channa striata* was estimated to be 1×10^{-04} . According to the U.S. EPA (1989) the acceptable risk levels for carcinogenic risks range from 10^{-4} to 10^{-6} ; which means that when the level of carcinogenic health effects is at 10^{-6} for individual toxic metals, it will result in relatively negligible cancer risks (USEPA 2010). The results of the present study revealed that the potential carcinogenic risks are still within the acceptable risk levels for carcinogenic risks range which introduced by (U.S. EPA 1989).

In present study, a metalloid As was present in high levels in all organs of fish. However, As toxicity effect depends on its oxidation state thus, a full understanding of the form of this arsenic is extremely required [7]. According to [1] the most poisonous forms of arsenic to human are the inorganic forms. On the other hand, the organic forms are quite varied in their toxicity such as Mono and dimethyl arsenics have low toxicity, while the more complex Arsenobetaine, Arsenocholine and other Arsenosugars are considered inert when ingested by mammals. The major arsenic form in fish is Arsenobetaine. Generally, the health risks derived from the intake of inorganic As through consumption of fish from the Sg. Lembing ex-mine was calculated using Equation (2), the value of (SF) for Arsenic is $(1.5 \text{ mg/kg/day})^{-1}$ (U.S. EPA, 2005) [24]. The estimated results of the carcinogenic health risks for As in all fish species are presented in Table 4. The carcinogenic risk values associated with the ingesting of inorganic arsenic through the consumption of *M. albus* was 13×10^{-03} , *L. poecilopterus* was 51×10^{-01} , *M. frenatus* was 53×10^{-03} , *M. argenteus* was 26×10^{-03} , *P. schwanenfeldii* was 63×10^{-03} , *H. microlepidota* was 40×10^{-03} , *H. nemurus* was 29×10^{-03} , *T. trichopterus* was 45×10^{-03} , *C. striata* was 40×10^{-02} , and *P. grootii* was 89×10^{-02} .

The results showed the highest carcinogenic risks associated with the ingesting of inorganic arsenic through the consumption of *L. poecilopterus* was 51×10^{-01} followed by *P. grootii* was 89×10^{-02} and *C. striata* with value 40×10^{-02} and the least carcinogenic risks was calculated in *M. albus* with value 13×10^{-03} . In a comparison of the estimated results with the acceptable risk levels for carcinogenic risks, the results indicate that the carcinogenic health effects of As in all fish species were significantly highest of the acceptable risk levels for carcinogenic risks. The results of the present study revealed that the potential carcinogenic risks are highest the acceptable risk levels for

carcinogenic risks range which introduced by (U.S. EPA 1989). According to the [24], arsenic is frequently associated with, kidney, lung, skin, and bladder cancer. The determination of toxic impacts of heavy metals becomes more apparent only when long-term consumption occurs. Therefore, further assessment of heavy metals in fish from the contaminated area is necessary in order to safeguarding human health and to prevent any excessive build-up of toxic metal elements in the human food chain.

Table5. Showed the calculated results of the potential carcinogenic health risk for all fish species

Species Name	(CDI) for As	(CR)Inorganic As	(CDI)for Pb	(CR) Pb
<i>Monopterus albus</i>	0.0001	13×10^{-03}	0.0008	64×10^{-05}
<i>Leiocassis poecilopterus</i>	0.0339	51×10^{-01}	-	-
<i>Mastacembelus frenatus</i>	0.000356	53×10^{-03}	0.000282	2×10^{-05}
<i>Mystacoleucus argenteus</i>	0.00017	26×10^{-03}	-	-
<i>Puntius schwanefeldii</i>	0.000417	62×10^{-03}	97×10^{-04}	1×10^{-05}
<i>Hampala microlepidota</i>	0.000269	40×10^{-03}	-	-
<i>Hemibagrus nemurus</i>	0.000193	29×10^{-03}	-	-
<i>Trichogaster trichopterus</i>	0.00030	45×10^{-03}	-	-
<i>Channa striata</i>	0.00268	40×10^{-02}	0.0007	1×10^{-04}
<i>Pristolepis grootii</i>	0.005939	89×10^{-02}	-	-

4. CONCLUSION

The main reasons to developed present study are to provide data on accumulation of toxic and essential metals in different fish species from former tin mine catchments and surrounding aquatic environment and to, assess the potential health risks associated with consumption of fish from these catchments. From this study, it can be clearly seen that great variations among concentrations of heavy metal in all organs and showed different affinity capabilities for accumulation. Results of the present study show that the concentrations of heavy metals in the fish exceed the limits of (MFA, and WHO) standards for food As, Ni in many of samples. Most of THQ values for all evaluated fish species are under one, that mean that intakes of heavy metals by consuming these fish is not likely to cause any appreciable health risks on the human body. While, in case of As the calculated values of THQ for *L. poecilopterus*, *C. striata* and *P. grootii* exceed the limit of one, As a conclusion, more assessment studies of heavy metals pollution in ex-tin mine catchments should be performed in order to provide more useful data and help to ensure the quality of fish and safeguard of human health.

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