

# Proximate Composition and Determination of Heavy Metal Content in Indian Fish using ICP-MS after Closed Vessel Micro Wave Digestion

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**Abstract** The objective of the present study was to investigate the heavy metal content in fresh water fish collected from the outlets of twin cities of Hyderabad and Secunderabad, Andhra Pradesh, India. The heavy metal content of fresh water fish varieties was investigated using Inductively Coupled Plasma Mass Spectrophotometer (MD-ICP-MS) after microwave digestion. Heavy metal content in fresh water fish were ranged from 52-680 µg/kg for Nickel in *Rastrelliger kanagurta* and *Stromateus sinensis*, 38-690 µg/kg for Arsenic in *Rastrelliger kanagurta* and *Pomus argenteus*, 3-62 µg/kg for Cadmium in *Mastomys sibilon* and *Pomus argenteus* and 27-108 µg/kg for Lead in *Panaeus monodon* and *Rastrelliger kanagurta* respectively. Results were in accordance with recommended human daily intake values except for arsenic, which were in agreement with WHO/FAO recommended values. Since the available data for heavy metal content in fish is scanty, efforts were made to present a precise data for the same as estimated on ICP-MS. This study may provide information on important heavy metal concentrations of fresh water fish and shell fish and therefore provides an essential baseline data with which future levels may be compared and evaluated. Results were in accordance with recommended daily intake allowance by WHO/FAO.

**Keywords** Fresh Water Fish, Heavy Metals, ICP-MS, Microwave Digestion

## 1. Introduction

Fish and shellfish are one of the major sources of animal protein and have been widely accepted as a good source of nutrients for the maintenance of a healthy body. These are also increasingly marketed for their health benefits to consumers [1]. It is widely consumed in many parts of the world and valued for its omega 3 fatty acids, which is reported to reduce the risk of heart diseases, stroke and

preterm delivery [2,3,4]. It is unfortunate that we, human beings without realizing the consequences of pollution, do a lot of activities that terribly ruin the nature, resulting in the denial of healthy environment to our successors. Water contamination is one of the serious concerns that affect the marine ecosystem with high concentration of trace metals. According to Jingaram [5] the coastal or river waters are contaminated by the dumping of industrial wastages. The metals accumulated in these waters infect the humans by direct consumption of water or through consuming the affected organisms like fishes [6,7] claim that when the level of trace metal concentrations exceeds the stipulated level; it turns out to be toxic. Very recently, the work in Mc Lintock [8] has stated that the higher level of metal concentration will bring shattering effect to the ecological balance by altering the range of organisms in water.

Among a variety of substances entering the soil, inland waters and the ocean as waste products, especially substances like heavy metals like Nickel (Ni), Arsenic (As), Cadmium (Cd) and Lead (Pb) create long term problems. Not only they accumulate in organisms and circulate in food chain, also remain in the ecosystem in sediments for longer(9). The increasing levels of heavy metals in the environment, their entry into the food chain, and the overall health effects on people who consume fish are of major concern to researchers in the field of food and nutrition, because heavy metal toxicity is a result of long term, low level exposure to pollutants through air, water and numerous consumer food products. Heavy metals can be classified as potentially toxic (arsenic, cadmium, lead, mercury, etc.) probably essential (nickel, vanadium, cobalt) and essential (copper, zinc, iron, manganese etc...) [9]. Food is usually the most important path by which toxic elements enter the body e.g., fish and shellfish are known to accumulate heavy metals. Heavy metals are natural component of the earth's crust that cannot be degraded or destroyed and they occur at a wide range of concentrations and with a broad array of chemical attributes. Recent studies have shown that even low levels of Cd, Pb, As and Hg can cause health complications in humans

[10,11]. Cd causes kidney dysfunction, hyper tension, and osteoporosis and is also a potential carcinogen [12,10]. Pb toxicity is associated with naturism; a disease characterized by anaemia, hematopoietic, renal toxicity and peripheral neuropathy [13]. The most toxic form of arsenic found in food and water are the inorganic As (III) and As (V) which is classified as carcinogen by International Agency for Research on Cancer (IARC) [14]. Considering the potential toxicity of heavy metals, there has been a growing interest in determining heavy metal levels in the fresh water and marine environment and attention was drawn to the measurement of contamination levels in public food supplies, particularly in fish.

Among the Asian countries India ranks second in aquaculture, fourth largest producer of fish and one of the top leading exporters of sea foods [15]. Literature is scarce on the availability of heavy metal content of fish and shell fish in the Indian market. Therefore the present study attempts to provide information on the heavy metal levels in fresh water fish and shellfish species collected from the twin cities of Hyderabad and Secunderabad using Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS).

The data provides useful information to consumers in choose fish and shellfish, besides to complement information in Indian Food Composition database. Several reliable analytical methods like Colorimetric and Atomic Absorption Spectrophotometer are available for monitoring heavy metals in food samples, but ICP-MS is being the most sophisticated and reliable technique, fast quantitative, high sensitivity, good precision and accuracy. Isotope ratio measurements were also possible using ICP-MS and interferences relatively few in number compared to other analytical techniques such as ICP-AES. Based on the above reasons efforts were made to present a precise data on heavy metals in a large variety of fish and shell fish. It is expected

that the results of this research will assist in acquiring information about the level of toxic metals in fresh water fish.

## 2. Materials and Methods

### 2.1. Reagents

All chemicals used were analytical grade. Ultra pure water was obtained from a Millipore water system (Millipore), Ultra pure Nitric acid (HNO<sub>3</sub>, Merck) was used to digest the samples. Stock standard solutions of arsenic, cadmium, nickel and lead concentration containing 10 µg/ml in 2% HNO<sub>3</sub> was prepared [16]. The certified reference materials (CRM) were procured from National Institute of Standard Technology (NIST-8436) and European Commission (EC-804) and used for method validation.

### 2.2. Collection of Fish Sample

Different varieties of fresh water fish species (weight ranges from 0.3 to 2.0 kg) were presented in *Table-1* with their local name and scientific name. All varieties of fresh water fish were collected from local markets of twin cities of Hyderabad and Secunderabad. The targeted species were commercial fish and shellfish available to consumers in Hyderabad.

In the present study, fish and shellfish samples were collected by stratified random sampling procedure. This approach is the most suitable method for generating food composition database [17]. Collected fish samples were immediately curved in ice, kept in polystyrene boxes and transported to laboratory to sustain freshness. Upon arrival to laboratory, fish and shellfish were individually measured for their total body weight and length.

**Table 1.** List of Fresh water fish collected from markets with a narrow range of weight and length

Local name	Common Name	Scientific name	Edible portion/kg	Weight(Kg) (min-max)	Length(cm) (min-max)
Bommidayalu	Channa marulius	<i>Masto sypollon</i>	733	0.10-0.25	10-25
Ravva	Roho labeo	<i>Lebio rohita</i>	632	0.50-3.20	15-35
Chanduva	Pomfret-White	<i>Stromateus Sinensis</i>	630	0.30-1.50	13-35
Nallasandawah	Pomfret-Black	<i>Formio niger</i>	644	0.30-1.50	13-35
Jallalu	Cat fish	<i>Arius sona</i>	720	0.10-0.20	15-25
Chandamama	Silver Pomfret	<i>Stromateus sinensis</i>	621	0.05-0.15	10-25
Bangaru teega	Gold fish	<i>Cyprinus carpio</i>	587	0.50-2.00	10-30
Korramenu	Soleole	<i>Rastrelliger kanagurta</i>	698	0.50-3.50	15-35
Royya	Tiger prawn	<i>Panaeus monodon</i>	570	0.10-0.30	05-15
Royya	Scamil prawn	<i>Macrobrachium rosenbergii</i>	408	0.10-0.20	05-15

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### 2.3. Sample Preparation for Analysis

The fish samples were beheaded, gutted, washed and filleted. The primary sample of each species was used to prepare a composite sample. Special care was taken to prevent metal contamination of the samples from laboratory equipment. All laboratory-ware were soaked in 2 M HNO<sub>3</sub> for 48 h, and rinsed several times with distilled water and deionized water prior to use. Small size fish were pulverized with skin and the edible portion weight was recorded. Aliquots of edible tissue was taken for analysis of heavy metals and proximate composition and stored in a freezer at -20°C till further analysis.

### 2.4. Heavy Metal Analysis

#### 2.4.1. Microwave Digestion

The closed vessel microwave digestion system (CEM-MARS-USA) was used to digest homogenized fish samples (between 0.5 and 1.5 g) and placed in a Teflon digestion vessel with 3 ml of ultra-pure HNO<sub>3</sub> and 1 ml of Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub> - Merck). Sealed containers were placed in a microwave oven and heated according to the digestion program (program: Power 1600W (100%), Ramp time 15 minutes, Temperature 200°C, Hold time 15 minutes and cooling time 15minutes). After digestion, sample solutions were cooled to room temperature and then transferred quantitatively in to acid cleaned 25 ml standard volumetric flasks and made up to 25 ml with double distilled deionized water and prepared under the same conditions as the calibration standards in 6% (v/v) HNO<sub>3</sub>. A blank digest was carried out in the same way [16].

#### 2.4.2. Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

Nickel, Arsenic Cadmium and Lead in fresh water fish were determined using ICP-MS (PerkinElmer Élan 9000-USA). For better operating conditions the ICP-MS was adjusted to: Nebulizer gas flow 0.91 L/min, Radio frequency (RF) 1200 W, Lens voltage 1.6 V, Cool gas 13.0 L/min, and Auxiliary gas 0.70 L/min [18]. CRM samples were procured from NIST and EC and used for method standardization, standard graphs were drawn. Recovery study was also done and results were shown in Table-2. The detection ranges of heavy metals in the recovery study were from 96% to 100%. The description of the data was given as mean ± SD.

**Table 2.** Recovery study using Certified Reference Material (CRM)-mg/kg

Name of the Element	Analyzed Value	Certified Value	% of recovery
Nickel	0.163±0.08	0.17±0.08	96.0-NIST
Arsenic	0.048±0.04	0.05±0.00	98.7-EC
Cadmium	0.110±0.06	0.11±0.05	100-NIST
Lead	0.224±0.00	0.23±0.00	97.3-NIST

Values are mean±SD, n=6

NIST: National Institute of Standard Technology

EC: European Commission

#### 2.4.3. Statistical Analysis

ANOVA was employed to test the difference between means of various fishes with regard to heavy metals.

## 3. Results and Discussion

The weight and length of the available varieties of fresh water fish and shell fish were shown in Table 1. The fish varieties collected in the present study had shown variation in weights and lengths. Among the varieties studied, (10-25cm and weight is 0.10-0.25 kg, 05-15 cm and weight is 0.10-0.20 kg, 15-35 cm weight is 0.50-3.5 kg). In the previous study conducted by Rejomon [19] the length of the fish and shell fish were gradually increased depending on *Panaeus monodon* and *Stromateus sinensis* with least length and weight (05-15 cm and weight is 0.05-0.15 kg) followed by *Masto symbolon*, *Macrobrachium rosenbergii*, and *Rastrelliger kanagurta* with highest length and weight the weight of the fish. Similar findings were observed in the fish collected for heavy metal analysis in the present study.

### 3.1. Proximate Analysis

Proximate content of moisture, ash, and fat contents was determined using Association of Official Analytical Chemists [20]. Protein content ( $N \times 6.25$ ) was estimated using AOAC Kjeldahl method [21]. Proximate composition of fresh water fish was as shown in Table 3. Present study reveals that the moisture content of fresh water fish was diverse ranging from 70.19% in *Arius sona* and 78.39% in *Cyprinus carpio*. Ash content was shown in the range of 0.71% to 1.12% in *Panaeus monodon* and *Arius sona*. Total minimum and maximum content of protein was observed from 13.25% to 17.78% in *Arius sona* and *Pampus argenteus* and the level of fat content was ranged from 5.40% to 15.40% in *Labeo rohita* and *Arius sona*, respectively. These results were similar to those reported by Tuzen et al [22] for sea bass, 76.72% moisture, 1.23% ash, 19.43% protein, and 4.81% fat. In another study the proximate composition values of fresh water fish range from 65 to 78% for moisture, 1.19 to 3.92% for ash, 14 to 18% for protein, and 1.53 to 5.41% for fat [23]. Taha et al [24] reported that the ranges of moisture in fish were from 63.52 to 70.71%, of ash from 1.35 to 1.66%, of protein content from 19.81 to 20.35%, and of fat levels from 6.10 to 15.11%, respectively, whereas in another study moisture content was 74.74%, ash content was 1.53%, protein content was 18.80%, and fat content was 6.53% [25]. The proximate parameters also showed 69.91% for moisture, 1.22% for ash, 8.25% for protein, and 10.37% for fat [26]. The proximate composition values in fish were determined in the ranges of 72.22% of moisture, 1.57% of ash, 21.08% of protein, and 6.01% of fat [27]. It is known that the variations in the chemical composition of fish were closely related to nutrition, living area, fish size, catching season, and seasonal and sexual variations as well as other environmental conditions [28,29,30]. The variations in proximate parameters could be due to sexual and environmental conditions. The heavy metal content and proximate composition data in Indian marine fish was also published by the research team from same lab [31].

**Table 3.** Proximate Composition in Fresh water Fish-g%

Common Name	Scientific name	Moisture	Ash	Protein	Fat
Channa marulius	<i>Masto symbolon</i>	74.08±1.2	0.997±0.12	14.02±0.52	10.05±1.1
Roho labeo	<i>Lebio rohita</i>	76.29±0.9	1.076±0.11	16.39±0.45	5.4±0.85
Pomfret-White	<i>Pompus argenteus</i>	75.15±1.5	1.067±0.23	14.72±0.78	9.15±1.34
Pomfret-Black	<i>Pompus argenteus</i>	75.26±0.82	0.956±0.12	17.78±0.85	6.2±0.46
Cat fish	<i>Arius sona</i>	70.19±1.21	1.125±0.31	13.25±0.23	15.4±1.85
Silver Pomfret	<i>Stromateus sinensis</i>	75.01±1.43	0.778±0.09	11.71±0.13	12.55±1.23
Gold fish	<i>Cyprinus carpio</i>	78.39±1.89	0.776±0.14	15.56±0.53	5.48±0.46
Soleole	<i>Rastrelliger kanagurta</i>	73.42±1.46	1.023±0.21	15.12±0.52	10.45±1.78
Tigar prawn	<i>Panaeus monodon</i>	76.11±0.82	0.717±0.37	14.24±0.37	9.00±0.99
Scamil prawn	<i>Macrobrachium rosenbergii</i>	76.99±0.79	0.816±0.35	13.4±0.42	8.8±0.86

Values are Mean ± SD, n=6

### 3.2. Heavy Metal Analysis

Heavy metals such as Cadmium (Cd), Lead (Pb), Nickel (Ni) and metalloids such as Arsenic (As) are very stable and cannot be destroyed or degraded in their inorganic elemental form. Fresh water fish and shell fish were susceptible to accumulate these heavy metals from their aquatic environment. Shellfish in particular can be high in Cd levels. Arsenic is found in different chemical forms, where inorganic arsenic is regarded as potentially more toxic than organic species. Fish and shellfish mainly tend to accumulate organic arsenic [32]. The range of values for weight and the length of different varieties of fresh water fish and shell fish along with binomial and vernacular nomenclature are presented in *Table 1*. There was a high degree of variability in biometric values of weight and length among the available fish varieties with least length and weight of 05 to 15 cm and 0.05 to 0.15 Kg respectively recorded for *Panaeus monodon* and *Stromateus sinensis* and highest length and weight of 15 to 35 cm and 0.5 to 3.5 kg respectively been recorded for *Lebio rohita* and *Rastrelliger kanagurta*. [33] Oribhabor et al [34] Gokhan et al have reported that the length of the fresh water fish, marine fish and shell fish alter depending upon the weight of the fish. Among the 10 different fresh water fishes studied, the Nickel content ranged from 52.31 to 680 µg/kg, Arsenic content ranged from 37.85 to 689.5 µg/kg, Cadmium content ranged from 3.12 to 61.87 µg/kg and Lead content ranged from 26.56 to 108.2 µg/kg respectively. The average contents of Ni, As, Cd and Pb in all the 10 different fishes studied were 157.4, 348.5, 13.6 and 61.5 µg/kg respectively. The specific contents of Nickel, Arsenic, Cadmium and Lead quantified using MD-ICP-MS in different species of fresh water fish and shell fish were provided in *Table 4*.

### 3.3. Nickel content in Fresh Water Fish and Shell Fish

Higher concentrations of Ni was observed in fishes such as *Stromateus sinensis*, *Pompus argenteus* and *Masto symbolon* with average Ni contents of 680.1, 148.3 and 116.5 µg/kg respectively. Among 10 different fishes

evaluated median range Ni contents was noticed in *Arius sona* and *Lebio rohita* with mean Ni contents of 66.17 and 63.51 µg/kg respectively. Lower concentrations of Ni were found in *Rastrelliger kanagurta* with 52.31 µg/kg of Ni accumulation. In the present study the levels of Ni in fresh water fish and shell fish varieties were found to vary from a minimum of 52.31 µg/kg in in *Rastrelliger kanagurta* and to a maximum of 680 µg/kg *Stromateus sinensis*. Generally Ni concentration may be low in aquatic environment and is found in several oxidation states ranging from -1 to +4. However the +2 oxidation state (Ni<sup>+2</sup>) is the most common in biological systems<sup>24</sup> which was the state determined in the present study as well [3]. When compared to the values of Ni that ranged from 0.2 to 3.97 mg/kg in *Rastrelliger brachysoma* from Aegean and Mediterranean Sea [35]. Ni concentrations in fresh water fish in the present study were lower. In another similar study the concentration of Ni ranged from 0.03 to 0.069 mg/kg in muscle tissues of fish from Indian markets [36] (Sivaperumal et al and [37] Turkmen et al as well also reported that the Ni concentrations values of 0.66 to 1.59 mg/kg in muscle tissues of fish from Iskenderun bay, Mediterranean Sea; 0.009 to 0.011mg/kg in muscle tissue and 0.07 to 0.10 mg/kg in livers tissues of fish from Mediterranean region. Similarly Nawal Al-Bader [38] (FSANZ) presented the concentrations Ni that varied from 1.92 to 5.68 mg/kg in *M. merlangus* and *Mugil cephalous*. In the present study, the content of Ni varied significantly among different fish varieties (*Table 4*).

### 3.4. Arsenic Content in Fresh Water Fish and Shell Fish

The level of Arsenic toxicity largely depends on its chemical form, which means that exposure to inorganic Arsenic could be highly harmful. Determination of arsenic in biological tissues is cumbersome due to the presence of interferences, difficulties in mineralization of some organic Arsenic species such as Arsenobetaine which is a predominant arsenic species in fish tissues. The interferences in the determination of arsenic could be minimized using sophisticated techniques such as ICP-MS as compared to other techniques like ICP-AES and AAS. Higher concentrations of arsenic were found in *Pompus argenteus*

and *Arius sona* with mean contents of 689.5, 686.4 and 586.1 µg/kg respectively. Median concentrations of As was noticed in *Cyprinus carpio* and *Macrobrachium rosenbergii* with mean contents of 23.1, and 204.7 µg/kg respectively. Lower As contents of 37.85 and 42.16 µg/kg were obtained in *Rastrelliger kanagurta* and *Masto symbolon* respectively. There was a significant difference between the different fish species and the levels were found to be higher than previous reports. Similar values of Arsenic were reported in black and whitespecies of *Lates calcarifer*. [37] Turkmen et al have shown that the As levels in fish were in the range of 0.25 to 0.42 mg/kg. Reports of total Arsenic concentrations in fish and crustacean in food standards were not found, the maximum limit of As concentration being of 2 mg (inorganic As)/kg [38]. The study reveals that higher level of arsenic content is could be observed in *Pompus argenteus*. Such prominent content of Arsenic in various fish species may be attributed to the extensive upwelling occurring in the area, which brings Arsenic rich sub-surface waters to the surface.

### 3.5. Cadmium Content in Fresh Water Fish and Shell Fish

Among all the 10 fishes studied, Cadmium contents were found to higher in *Pompus argenteus*, and *Macrobrachium rosenbergii* with average contents of 61.87 and 23.77 µg/kg respectively. Lower concentrations of Cd were observed in *Masto symbolon*, *Panaeus monodon* and *Rastrelliger kanagurta* with mean Cd content of 3.12, 3.53 and 3.95 µg/kg and these fish were contained similar levels of Cd but Cd content was significantly different in *Arius sona* with 11.63 µg/kg. Mustafa et al (2002) have reported that Cd values of marine fish were found to range from 0.02 to 0.37 mg/kg in *Trigla gurnardus* and *Silonella aurata*. Another study revealed that Cd concentrations in fish from lakes in Tokat, Turkey were 0.1 to 1.2 mg/kg [21]. Cd levels ranged from 0.02 to 1.32 mg/kg in muscle tissues of fish obtained from internal markets of India [36]. Cd levels in *Mugil cephalus* and *Mugil barbatus* ranged between 0.450 to 0.900 mg/kg [39]. In another similar study the Cd levels were found to be in the range of 90 to 480 µg/kg of dry weight in fish samples of the middle Black Sea, Turkey [21]. In another study the Cd concentrations were 0.10 to 0.840 mg/kg of fish [22]. Cadmium concentrations were similar in all the fresh water fishes except *Pompus argenteus*,

*Stromateus sinensis* and *Arius sona* with minimum Cd concentration observed in *Masto symbolon*. In natural water Cadmium is rarely found [23]. Cadmium is considered to be toxic if its concentration exceeds 0.01 mg/ L both in drinking and irrigation water [24]. Cadmium with some other heavy metals lead and mercury do not have any known biological or metabolic function in human system and are potentially toxic even at trace concentrations [25]. Acute cadmium toxicity causes high blood pressure, kidney damage, destruction of testicular tissue and red blood cells [26].

### 3.6. Lead Content in Fresh Water Fish and Shell Fish

Lead is considered as a non-essential and toxic metal which also implies that it has no known function in biochemical processes. Lead induces reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults [27]. Lead contents were found to be highly variable amongst all the different 10 fish studied. *Rastrelliger kanagurta*, *Arius sona* and *Pompus argenteus* contained comparatively higher Pb concentrations with values of 108.2, 87.66 and 77.35 µg/kg respectively. Lower concentrations of Pb of 26.56 and 35.66 µg/kg were found in *Panaeus monodon* and *Macrobrachium rosenbergii* respectively. The levels of Pb in the present study were estimated to be a minimum of 26.56 µg/kg in *Panaeus monodon* and a maximum of 108.2 in µg/kg *Rastrelliger kanagurta*. [20] Mustafa et al have found that the levels of Pb in fish ranged from 0.33 to 0.86 mg/kg in *Trigla gurnardus* and *Dracunculus vulgaris* respectively. Another marine study reveals that the Pb contents were in the range between 0.33 to 0.93 mg/kg in muscles tissues of fish in Black and Aegean seas [28]. Concentrations of Lead in *Engraulis encrasicolus* and *Merlangius merlangus* fishes in Black and Aegean Seas were found to be in the range of 0.33 to 0.93 mg/kg [29]. Similarly [30] Dural et al have also reported that the Pb contents were in the range of 0.40 to 2.44mg/kg in muscle and 1.41 to 3.95 mg/kg in liver tissues of fish of Tulza Lagoon. Previous studies have demonstrated that the Pb levels were varied with different parts of fish such as flesh, guts, and gills etc. In general Pb levels were found in the order of gill>liver>flesh [31, 40].

**Table 4.** Heavy metal content in fresh water fish- µg/kg

Common Name	Scientific Name	Name of the elements			
		Nickel	Arsenic	Cadmium	Lead
Channa marulius	<i>Masto symbolon</i>	116.5±0.66	42.16±0.44	3.12±0.37	40.56±0.7
Roho labeo	<i>Lebio rohita</i>	63.51±0.72	529.1±0.55	4.36±0.25	47.22±0.6
Pomfret-White	<i>Pompus argenteus</i>	148.3±0.63	586.1±0.28	5.65±0.35	77.35±0.7
Pomfret-Black	<i>Pompus argenteus</i>	168.3±0.72	689.5±0.42	61.87±0.9	35.73±0.6
Cat fish	<i>Arius sona</i>	66.17±0.75	686.4±0.96	11.63±0.8	87.66±0.8
Silver Pomfret	<i>Stromateus sinensis</i>	680.1±0.77	66.95±0.77	12.95±0.5	87.1±0.71
Gold fish	<i>Cyprinus carpio</i>	95.25±0.84	237.1±0.42	5.25±0.49	68.8±0.84
Soleole	<i>Rastrelliger kanagurta</i>	52.31±0.65	37.85±0.49	3.95±0.21	108.2±0.9
Tigar prawn	<i>Panaeus monodon</i>	103.8±0.35	405.8±0.45	3.53±0.51	26.56±0.8
Scamil prawn	<i>Macrobrachium rosenbergii</i>	80.17±0.80	204.7±0.65	23.77±0.6	35.66±0.5

Values are Mean ± SD, n=6

The concentrations of heavy metal in fish may vary in different species and different aquatic environments [41]. Fish species have the potential to accumulate heavy metals above the biotic environment which is known as bioaccumulation. The observed variation in the pattern of heavy metal bioaccumulations in different species might be due to the difference in feeding habits, habitats and physiology, the ability of organisms to digest the metals and the concentration of such metals in the aquatic medium [42]. Fishes mainly take up the metals from the water, food sources and sediment [41]. The efficiency of metal uptake from contaminated water and food depends upon the ecological needs, metabolism, and the contamination gradient of water, food and sediment, environmental factors such as pH, salinity and temperature [43, 44].

Various anthropogenic activities such as domestic sewage, combustion, emission, mining, metallurgical activities and industrial effluents cause the heavy metal inputs into the environments. Assessment of metal contamination of fish is important to determine the safety aspects of consumption and such pertinent data is scanty. In food sources, the allowed quantity of heavy metals is defined by norms, which are based both on the WHO recommendations and local requirements. World Health Organization (WHO) recommends that daily intake of Ni and As in the inorganic form of 100 to 300 µg/kg and up to 15 µg/kg of body weight as safer limits for humans. The maximum permissible levels of Cd and Pb were 0.05 to 5.5 mg/kg and 0.5 to 6.0 mg/kg in fish respectively. As per the National Nutrition Monitoring Bureau (NNMB), National Institute of Nutrition (NIN, ICMR) an average household consumption of fish in Andhra Pradesh is 6g/CU/day [45]. The consumption of fish is very low compared to the recommended levels of WHO/FAO [32]. In this context the heavy metal levels in fresh water fish observed in the present study was below the recommended level of WHO and previous literature. The present study findings indicating that the consumption of fish available in twin cities of Hyderabad and Secunderabad may not have any adverse heavy metal toxic effects on human health.

#### 4. Conclusions

Present study was in conformation with FAO/WHO and literature values for heavy metal contents in fresh water fish. Weekly intakes of heavy metals through fish consumption were below the provisional tolerable levels as recommended by WHO/FAO. It is known that a variation in the mineral composition of fresh water foods is closely related to seasonal and biological differences (Species, size, dark/white muscle, age, sex and sexual maturity), area of catch, processing method, food source and environmental conditions. For monitoring and evaluation of the ecological conditions of fish, such fish metal accumulation pattern can be helpful. In order to develop aquaculture industry in water resources it is necessary to maintain water quality standards, and to monitor contamination levels of water sources and

that of fish regularly. The chemical nature of the metals, ionic strength and pH also play critical role variable in the accumulation patterns. This study may provide information on the important heavy metal concentrations of fresh water fish and shell fish consumed in twin cities of Hyderabad and Secunderabad, and therefore provides an essential baseline data with which future levels may be compared and evaluated.

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