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Tissue-specific trace elements concentration and human health risk assessment in three marine fish species from Visakhapatnam **to Kakinada coasts**

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Abstract

 Purpose The current study was aimed to evaluate the bioaccumulation of elements (As, Cd, Cu, Fe, Mn, Se and Zn) in the gills, liver and muscle of three distinct marine fish species, namely *Nemipterus japonicas, Upeneus vittatus and Pampus argenteus* species were collected from Visakhapatnam to Kakinada coastal waters.

Methods The fish tissues were analyzed for trace metals using energy-dispersive X-ray fluorescence (EDXRF). To ensure the quality control, reliability of the experimental validation, two certified reference materials (CRMs) procured from National Institute of Standards and Testing (NIST SRM 1577c-Bovine Liver) and European Commission-Joint Research Centre, Institute for Reference Materials and Measurements (ERMBB422-Fish muscle) were analyzed using EDXRF.

 Results The evaluated estimated daily intake (EDI) values for each metal are below the reference dose levels established by World Health Organisation (WHO). The target hazard quotient (THQ) values of both the locations of the present study are lower than one (< 1) except for arsenic and the combined target hazard quotient (CTHQ) values are higher than one (> 1) showing the presence of health risk. The cancer risk (CR) factor value for arsenic was below the acceptable lifetime carcinogenic risk (10^{-5}) . The levels of heavy metals varied significantly among three fish species and organs. The mean concentrations of As, Cd, Cu, Fe, Mn, Se and Zn were slightly higher than the Ministry of Agriculture, Fisheries and Food (MAFF), Food and Agriculture Organization (FAO), Turkish food codex and Brazilian Legislation standard levels. The findings of this study revealed that muscles possessed the lowest concentrations mostly of all the metals when compared with liver and gills. **Conclusion** The study concluded that consumption of the muscle tissue may not pose severe health risk to human health but should be consumed moderately to prevent bioaccumulation of the metals especially As.

Keywords Health risk assessment · Marine fish species · Trace elements · Tissue specific

Introduction

The consumption of fish has increased worldwide in recent decades, particularly with the awareness of its nutritional benefits on human health. Fish are enriched with vitamins, minerals, organic and inorganic micronutrients like vitamin D and selenium $[1]$. The consumption of fish containing omega-3 fatty acids are beneficial to the people suffering from Cardiovascular diseases and Type 2 diabetes [2]. Essential elements such as Fe, Cu, Mn, Se and Zn are needed in trace amounts for various biochemical functions and enzymatic reactions of living organisms [3, 4]. The essential elements can potentially become hazardous to humans if consumed in excess [5]. Nonessential elements such as As and Cd have no biological role and are potentially toxic at relatively low concentrations [6].

 In the last few decades, the rapid development of industry and agriculture has resulted in increased contaminants, which is a significant environmental hazard for fish and humans [7]. Among the contaminants, heavy metals should be highlighted due to the consequences of their bioaccumulation in aquatic ecosystems [8]. The toxicity of heavy metals has long been a concern, considering heavy metals enter the aquatic

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environment from both natural pathways (geological weathering) and a variety of anthropogenic sources (transportation, industrial, agricultural and pharmaceutical activities). The heavy metals are distributed throughout the water column, are deposited in sediments [9] and can be consumed by fish via the food web [10]. The heavy metals can have a negative impact on aquatic ecosystems, the food chain and human health because they are not removed from aquatic ecosystems by self-purification [11]. Regular monitoring of heavy metal concentrations in aquatic environments and related food chains is important to prevent toxic effects in humans consuming aquatic organisms.

Different tissues of fish species have different bioaccumulation patterns [12]. The concentrations of metals in the tissues of fish are governed primarily by the level of pollution in the water and food and so are indicative of the level of the pollution in the aquatic environment [13]. Furthermore, gender, size and seasonal changes can also affect metal accumulation in different tissues of fish [14]. The gill has a large surface area with water in fish; therefore, it absorbs right amount of metal ions [15]. The concentrations of heavy metals in gills indicate the heavy metal concentrations in the waters of aquatic environment [16]. The liver is an essential organ in heavy metal accumulation due to its role in fish metabolism [17]. The fish flesh (muscles), which is significant fish part consumed by humans, is not always a good indicator of the whole-body fish contamination. Therefore, it is important to analyze other tissues such as gills and liver for the human health risk assessment of fish in heavy metal pollution [18, 19].

 Visakhapatnam port is considered as one of the 13 major ports in India and the major harbor of Andhra Pradesh. The increased industrialization, urbanization and many heavy industries like Hindustan Petroleum, Visakhapatnam Steel Plant, Shipyard, National Thermal Power, Bharat Heavy Electricals, Naval Dockyard, etc., caused to raise contaminants in the harbor [20]. Kakinada is the another location which lies on the coast of the Bay of Bengal belonging to the state of Andhra Pradesh; being a part of Special Economic Zone (SEZ); proposed Petroleum, Chemical and Petrochemical Investment Region (PCPIR) Development in the coastal zone has been particularly rapid in the last few years resulting pollution. The effluents from industries and agricultural fields are directly or indirectly discharged into aquatic environments, which ultimately reaches marine biota and a few trace elements get biomagnified in aquatic organisms [21].

 Limited works have been carried out so far in this locations regarding concentration of heavy metals in *Nemipterus japonicas, Upeneus vittatus and Pampus argenteus* and the health risk assessment is found to be minimal [22, 23]. Therefore, the objectives of the present study have been framed as: (1) to evaluate the present status of metals (As, Cd, Cu, Fe, Mn, Se and Zn) in the three commercially important fish species *Nemipterus japonicas, Upeneus vittatus and Pampus argenteus* belonging to Visakhapatnam and Kakinada harbors along the Andhra Pradesh coast and (2) to evaluate the carcinogenic and non-carcinogenic health risk for humans through fish consumption.

Materials and methods

Sample collection and preparation

Three marine fish species, *Nemipterus japonicas*, *Upeneus vittatus* and *Pampus argenteus* , were collected at Visakhapatnam (17.6958° N, 83.3025° E) and Kakinada (16° 58′ 30″ N, 82° 16′ 44″ E) harbors located along the coast of Andhra Pradesh. The thematic map representing the sampling locations was generated using ArcGIS software and depicted in Fig. 1. For the present study, fish species *Nemipterus japonicas* $(n = 10)$, *Upeneus vittatus* $(n = 12)$ and *Pampus argenteus* $(n=9)$ were collected freshly at sampling sites from July, 2020 to June, 2021.

Fig. 1 Sampling sites of Visakhapatnam and Kakinada Coasts

Fig. 2 Fish samples collected at Visakhapatnam fishing harbor

Nemipterus japonicus Upeneus vittatus Pampus argenteus

Fig. 3 Fish samples collected at Kakinada fishing harbor

Nemipterus japonicus Upeneus vittatus Pampus argenteus

The fish species collected for the present study are displayed in Fig. 2. The fish were individually placed in precleaned polythene bags, stored in an icebox and transported to the laboratory. The total lengths (TL) of *Nemipterus japonicas* (6–8 cm, TL), *Upeneus vittatus* (5–7 cm, TL) and *Pampus argenteus* (6–8 cm, TL) were recorded. The fish were cleaned with de-mineralized water and the samples were dissected to remove the gills, liver and muscle separately without damage. The samples were kept in a deep freezer at − 20 °C overnight. Then, the samples were lyophilized at − 50 °C and pulverized using agate mortar and pestle [24]. The fine powders were packed in ziplock bags with unique identification number. One hundred and fifty mg of each sample was pressed into a pellet of thickness ~ 0.96 mm with a pelletizer by applying a pressure $\langle 150 \text{ kg/cm}^2$. Three such pellets were prepared for each sample for the analysis and their average was taken. The three pellets form of each sample was representative of the concerned or respective tissue. All the metal concentrations present in the gills, liver and muscle tissues of fish species were reported in ppm (mg/ kg) dry weight (Fig. 3).

Experimental configuration

 The experimental work was carried out in the trace elements research laboratory available at UGC-DAE (UGC-University Grants Commission; DAE-Department of Atomic Energy) Consortium for Scientific Research, Kolkata Centre by utilizing energy-dispersive X-ray fluorescence (EDXRF) spectrometer. EDXRF is one of the simplest, most accurate and economic analytical methods for the determination of wide range of elements. The elemental analysis of fish samples was carried out by using EDXRF spectrometer [Xenematrix (Ex-3600)] [25] which consists of an oil-cooled rhodium (Rh) anode X-ray tube

Table 1 The operational parameters of EX 3600 EDXRF spectrometer

Parameter	e ₁	e ₂	e ₃
Voltage (kV)	8	15	37
Current (mA)	100	35	50
Time (s)	200	600	900
Atmosphere	Air	Air	Air
Energy range (keV)	10	40	40
Throughput	Low	Low	Low
Filter	None	None	Fe

(maximum voltage 50 kV, current 100 mA). The analysis was carried out with air chamber between the source and sample, using different type of X-ray absorber filters at different times for optimum detection of elements. Analysis of X-ray energies < 10 keV was carried out using 100 mA current, 8 kV voltage and no filter; similarly X-ray energies beyond 10 keV and < 40 keV analyzed using 35 mA current, 15 kV voltage and no filter. Rest of the higher X-ray energies were analyzed by using 50 mA current, 37 kV voltage on using Iron filter. All the operational parameters are listed in Table 1.

Energy-dispersive X-ray fluorescence analysis

 The main apprehension in this technique is that both experimental sample and standard samples should have a similar matrix of elements for the construction of the calibration curve in order to avoid the matrix effect. The irradiation of all fish samples was performed by assigning a time-based program provided in the EDXRF system. The two standards procured from National Institute of Standards and Testing (NIST SRM 1577c—Bovine Liver) and European Commission-Joint Research Centre, Institute for Reference Materials and Measurements (ERMBB422-Fish muscle) were also irradiated under similar experimental conditions for the construction of calibration curves for quantitative elemental analysis in the respective samples. The generated X-ray spectra of the standards were stored in the computer. The X-ray intensities of the elements in sample spectra were calculated by using the pyMCA $5.6.7$ software [26] by integration of area of the respective X-ray peak areas using peak fitting software pyMCA. The calibration curve for each element was constructed based on the K X-ray and L X-ray intensities calculated for the respective elements present in standard samples [27]. The curves were constructed by plotting the sensitivities of the elements as a function of their atomic number. The validation of calibration curves constructed for elements present in the standards was checked through analysis of standard reference materials. The concentrations of metals in the fish samples were obtained using the following equation [26].

$$
C_i = C_i^{\text{bov}} \cdot \left(\frac{A_{\text{Ar}}^{\text{bov}}}{A_{\text{Ar}}}\right) \cdot \left(\frac{A_i}{A_i^{\text{bov}}}\right) \cdot \left(\frac{d}{d_{\text{ref}}} \frac{t_{\text{ref}}}{t}\right)
$$

where $\left(\frac{A_{\Delta x}^{\text{low}}}{A_{\Delta x}}\right)$ is the ratio of peak areas corresponding to Ar K α of the bovine liver and the sample, C_i^{box} is the certified concentration of the element present in the bovine liver and A_i^{bow} is the peak area of the element in the bovine liver spectrum while A_i is the peak area of the element present in the sample spectrum, *d* is the thickness of the sample and d_{ref} is the thickness of the bovine liver sample/pellet, and *t* is the exposure time. The spectrum consists of three portions e_1, e_2 and e_3 . The e_2, e_3 portions of individual NIST SRM 1577c (bovine liver) spectra and fish muscle sample spectra were shown in Figs. 4 and 5, respectively. The elements of interest of our study are fit into e_2 , e_3 portions of spectra with gradual increase of time. Zn and Cu concentrations were determined using the e_2 spectrum, whereas As, Cd, Fe, Se and Mn concentrations were determined using the e_3 spectrum. To ensure the quality control, recovery rates of metals were calculated in standard samples ranged from 80 to 103% [28]. The certified and measured concentrations of metals in the CRMs are listed in Table 2 .

Results and discussion

Estimation of heavy metals concentration(s) in fish tissues

 The concentrations of As, Cd, Cu, Fe, Mn, Se and Zn were determined in the gills, liver and muscle tissues of *Nemipterus japonicas, Upeneus vittatus and Pampus*

Fig. 4 Spectra of NIST SRM 1577c

argenteus collected from Visakhapatnam to Kakinada fishing harbors. The obtained concentration of metals present in fish tissues is presented in Table 3 and Table 4.

The samples were taken in triplicate for all the fish tissues and the mean and standard deviation were calculated. The standard deviation (S) was calculated as shown in below equation.

$$
S = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2}
$$

where $S =$ Sample standard deviation, $N =$ Number of observations in sample, x_i = ith observation in the sample and \overline{x} = sample mean.

 The obtained concentrations of As, Cd, Cu, Fe, Mn, Se and Zn of three fish species are higher than the maximum permissible limits established by Ministry of Agriculture, Fisheries and Food (MAFF) [29], Food and Agriculture Organization (FAO) [30], Turkish food codex [31] and Brazilian Legislation [32]. The maximum permissible levels of metals in fish established by FAO, MAFF, Turkish food

Fig. 5 Spectra of *Nemipterus japonicas* muscle

codex and Brazilian Legislation are listed in Table 5 for comparision.

Tissue-specific accumulation of elements

Variation in the accumulation of elements in the fish species of the present study is observed changing significantly among gills, liver and muscle tissues. The muscle tissues are found to contain the lowest concentrations of all the elements present in all the three fish species belonging to both Visakhapatnam and Kakinada coastal waters. Fe was found at the highest concentration in all fish species followed by Zn, Cu, Mn, As, Se and Cd in both the locations. In all fish species, the accumulation of elements in liver had the following trend: $Fe > Cu > As > Se$ and in gills $Zn > Mn > Cd$ in Visakhapatnam. In Kakinada, the accumulation of elements in liver of three fish species had the following trend: $Fe > Cu > Se$ and in gills $Zn > Mn > As > Cd$. In the present study, the concentration of metals in the muscle tissues of all fish species followed a trend of $Fe > Zn > Cu > As > Mn$ $>$ Se $>$ Cd in both the locations. The findings revealed that the concentrations of the metals present in the tissues of all fish species are in the following order: liver $>$ gills $>$ muscle. Such pattern has been observed in a number of other studies, covering a wide specter of fish species [33, 34].

 Muscle is generally considered to have a weak accumulating potential $[35]$. Gills are significant in fish, useful for pH balance, breathing, hormone production, exhibit dominant role in osmotic and ionic regulation [36] along with aquatic gas exchange besides taking oxygen from air and absorption of nutritious elements present in water; thus, on these lines, accumulation of several nutrients with the highest concentrations in the Gills can be understood [15]. Winds are also important factor in the dispersion of pollutants and suspended debris by seaward transit, which poses risks to the marine environment. Metal bioaccumulation in the liver is related to its metabolic function [37], which are chemical processes that take place within a living organism in order to maintain life. Liver serves as storage for metals, redistribution, and detoxification [38]. High accumulating ability of the liver is a result of the activity of metallothioneins, the proteins that can be bound to some metals, such as Cu and

Table 2 Certified and measured concentrations of metals in the CRMs (mg/kg dry weight)

Metal	SRM 1577c—Bovine Liver			ERMBB422—Fish muscle		
	Certified values	Measured values	Recovery $(\%)$	Certified values	Measured values	Recovery $(\%)$
As	$0.019 + 0.001$	$0.017 + 0.001$	89.4	$12.7 + 0.7$	$10.8 + 0.3$	85.1
C _d	0.097 ± 0.0014	0.078 ± 0.001	80.4	0.0075 ± 0.0018	0.0061 ± 0.001	81.3
Cu	275.2 ± 4.6	271.4 ± 6.1	98.6	1.67 ± 0.16	1.7 ± 0.13	101.7
Fe	$197.94 + 0.65$	$191.25 + 2.4$	96.6	$9.4 + 1.4$	$8.8 + 2.1$	93.6
Mn	$10.46 + 0.47$	9.87 ± 0.2	94.3	0.368 ± 0.028	$0.39 + 0.031$	103
Se	$2.031 + 0.045$	$1.9 + 0.06$	93.5	$1.33 + 0.13$	$1.2 + 0.14$	90.2
Zn	$181.1 + 1.0$	$175.3 + 4.5$	96.7	$16.0 + 1.1$	$15.6 + 2.3$	97.5

Table 3 Concentration of metals (Mean \pm SD, mg/kg dry weight) in tissues of marine fishes collected in the coastal waters of Visakhapatnam

Table 4 Concentration of metals (Mean \pm SD, mg/kg dry weight) in tissues of marine fishes collected in the coastal waters of Kakinada

Se, thus reducing their toxicity and allowing the liver to accumulate them at high concentrations [39, 40].

Fishes at different positions in the food chain accumulate different concentrations of metals. The elements concentration may also vary depending on the study area because

Table 5 Maximum permissible levels of metals in fish

Heavy metal MAFF	$[29]$ (mg/ kg)	FAO [30] (mg/kg)	Turkish Food Codex[31] (mg/kg)	Brazilian Legislation[32] (mg/kg)
As		1.0		1.0
C _d	0.2	0.5	0.1	1.0
Cu	20	30	20	
Fe		100		
Mn		$2 - 9$		
Se				0.3
Zn	50	30	50	50

higher metal levels have been seen in regions with growing populations, industries, traffic and agricultural activities. In the present study, the accumulation of heavy metals also varied among gills, liver and muscle due to variation in the concentration of metals in water, feeding habits of fish and exposure period; although some other environmental factors such as water temperature, oxygen concentration, pH, hardness and alkalinity may affect and play significant roles in metal's accumulation and toxicity to fish [41, 42]. Spawning period, size and age of fish, their life cycle and the season of capture were also found to affect bioaccumulation of metals in fish tissues $[43, 44]$.

Nonessential trace elements

Arsenic

 Aquatic ecosystems are exposed to As from a variety of sources, such as manufacturing firms, mineral or smelting operations and electricity generating stations (power plants) [45]. The production and application of arsenical defoliants and pesticides is a major source of arsenic from agriculture. As has the potential to accumulate in large quantities among the sediments found in the bottom of water courses and reservoirs, as well as in aquatic organisms [46]. As is actively metabolized in fish tissue, especially in organs like the liver, where it can decrease antibody production by weakening the fish immune system $[47]$. Chronic exposure to inorganic arsenic may have adverse effects, on the gastrointestinal tract, respiratory tract, skin, liver, cardiovascular system, hematopoietic system and the neurological system [48].

 In the present study, the highest concentration (21.4 ± 6.1 mg/kg) of As is observed in the liver of *Upeneus vittatus* while the lowest concentration $(2.5 \pm 0.3 \text{ mg/kg})$ reflected in muscle of the *Upeneus vittatus* procured from the Visakhapatnam coast. In the case of Kakinada, highest concentration $(14.3 \pm 1.8 \text{ mg/kg})$ of As is observed in the gills of *Nemipterus japonicas* , and the lowest concentration $(2.6 \pm 0.1 \text{ mg/kg})$ obtained in the muscle related to the *Nemipterus japonicas* . The concentration of As in the muscle, liver and gills of *Nemipterus japonicas, Upeneus vittatus and Pampus argenteus* exceeded the maximum permissible limits established by the Food and Agriculture Organization (FAO), and Brazilian Legislation. In the earlier study, Hong Huang et al. [49] reported that the accumulation of As in liver 0.455 ± 0.044 (mg/kg wet wt), gills 0.350 ± 0.156 (mg/kg ww) and muscle 0.249 ± 0.018 (mg/kg wet wt) in *Coilia macrognathos* species collected from Dachen fishing ground, the coast of Zhejiang Province, East China Sea. Also calculated human health risks of EDI, THO and HI values of fish indicated that fish in the Dachen fishing ground were safe to eat, but the carcinogenic risk (TR) of As was close to the critical limit (10^{-4}) . Ahmed et al. [50] reported that As $(0.73 \pm 0.03 \text{ mg/kg}$ wet wt) in the muscle tissues of *Labeo rohita* was collected from Buriganga river, Bangladesh. Also calculated the target cancer risk (TR) values suggested carcinogenic risk from As $(1.61E^{-04})$. The study concluded that the metals do not pose non-carcinogenic health hazard individually but their combined effect is potentially hazardous to human health. Both the earlier studies are similar to the present study in the result of cancer risk of As.

Cadmium

 Cadmium is a naturally occurring nonessential trace element and because of its' tendency to bio accumulate in living organisms often at harmful levels, there is cause for concern for the environment $[51, 52]$. Due to industrial uses of cadmium (batteries, electroplating, plastic stabilizers, pigment), its production, consumption and environmental emissions have drastically increased since last few decades and consequently lead to contamination of aquatic habitats [53]. Water contamination may also result from the usage of sewage sludge in farm land, fertilizer, agricultural chemicals, pesticides that contain cadmium [54]. Its accumulation in the human body may result in cancer, as well as consequences on the kidneys, lungs, liver, skeleton and reproductive system $[55]$.

 In the present study, the highest concentration $(3.3 \pm 2.9 \text{ mg/kg})$ of cadmium is observed in gills of *Nemipterus japonicas*, whereas the lowest concentration $(0.04 \pm 0.04 \text{ mg/kg})$ obtained in the muscle related to *Nemipterus japonicas* collected from Visakhapatnam. In the case of fish species procured from Kakinada, highest concentration $(2.1 \pm 0.04 \text{ mg/kg})$ of Cd is observed in the liver of *Upeneus vittatus*, while the lowest concentration $(0.01 \pm 0.01 \text{ mg/kg})$ obtained in the gills of *Upeneus vittatus*. The observed values of cadmium concentration in the *Nemipterus japonicas* (gills) of Visakhapatnam exceeded the maximum permissible limits of elements established by the (MAFF), (FAO), Turkish food codex and Brazilian Legislation. Similarly, *Upeneus vittatus* (liver) belonging to Kakinada also exceeded the maximum permissible limits.

 According to previous literature, Lakshmanan et al. [56] obtained results of Cd concentration in muscle (0.114 ± 0.14 mg/kg dry wt) in *Anchovilla commersonii* and in *Upeneus vittatus* $(0.008 \pm 0.00 \text{ mg/kg} \text{ dry wt})$ collected from Parangipettai coastal waters, Southeast Coast of India, which is similar to the present study. Kumar et al. [57] determined Cd concentration in muscle $(6.58 \pm 0.61 \text{ µg/g in})$ dry wt) in *Mugil cephalus* collected from Ennore Creek, Southeast Coast of India while Jaikumar et al. [58] the Cd concentration in muscle tissue $(5.38 \pm 0.45 \text{ µg/g})$ in *Sardinella longiceps* , collected from Ariyankuppam estuary, Puducherry coastal waters, southeast coast of India, from both the studies, the concentrations are greater than the present study.

Essential trace elements

Copper

 Copper (Cu) is an essential micronutrient and plays role in multiple enzymes and required for hemoglobin formation [59]. Furthermore, Cu is a vital element for the production of red blood cells. Neurological system disorders could result from copper deficit [60]. The widespread use of fungicides, algaecides, insecticides and sewage discharge may contribute to copper pollution [61]. However, it can be extremely toxic to intracellular mechanisms in aquatic animals at higher concentrations [62].

 In the present study, the highest concentration $(42.4 \pm 4.7 \text{ mg/kg})$ of copper is observed in the liver of *Nemipterus japonicas* while the lowest concentration is obtained in muscle $(8.4 \pm 2.7 \text{ mg/kg})$ of *Nemipterus japonicas* belonging to Visakhapatnam. In the fishes collected from Kakinada, highest concentration (46.9 ± 2.7 mg/kg) of Cu is observed in the liver of *Upeneus vittatus*, and the lowest concentration is obtained in the muscle $(8.8 \pm 1.6 \text{ mg/kg})$ of *Nemipterus japonicas*. The observed Cu concentration in *Nemipterus japonicas* (liver) collected from Visakhapatnam port exceeded the maximum permissible limit established by MAFF, FAO and Turkish food codex while the observed copper concentration in *Upeneus vittatus* (liver) belonging to Kakinada exceeded the maximum permissible limits. High levels of Cu liver tissue are usually related to natural binding proteins such as metallothioneins, which suggest that the liver plays an important role in the metabolic processes of heavy metals [37].

According to previous study, Maurya et al. [63] obtained Cu concentration in muscle (4.55 ± 1.06) , mg/g wet wt), liver $(5.89 \pm 2.03 \text{ mg/g} \text{ wet wt})$ and gills $(5.87 \pm 2.08 \text{ mg/g} \text{ wet wt})$ in *Channidae marulius* collected from Ganga river, India.

Biswas.s et al. [64] obtained values of Cu concentration in muscle $(0.42 \pm 0.8 \text{ µg/g dw})$, liver $(0.33 \pm 0.4 \text{ µg/g dw})$ and gills (0.31 ± 0.1 μg/g dw) in *Nemipterus japonicas* collected from Visakhapatnam coast, Andhra Pradesh, India. In both the studies, the concentrations are lower than the present study.

Iron

 Iron is an essential element involved in the formation of hemoglobin which transports oxygen from the lungs to the tissues [65]. It is vital for the immune system and DNA synthesis in humans. Anemia is caused by iron deficiency, which is particularly common in children and women [66]. A high level of Fe causes Parkinson's disease, Alzheimer and second type diabetes [67].

 In the present study, the concentration of Fe in *Upeneus vittatus* (liver) procured from Visakhapatnam found to be highest (905.1 \pm 63.2 mg/kg) while the lowest concentration was obtained in the muscle of *Nemipterus japonicas* $(87.5 \pm 12.3 \text{ mg/kg})$. In the case of fishes collected from Kakinada, highest concentration $(858.6 \pm 126.8 \text{ mg/kg})$ of Fe is observed in the liver of *Nemipterus japonicas* , and the lowest concentration is obtained in the muscle of *Upeneus vittatus* $(74.9 \pm 10.2 \text{ mg/kg})$. Fe concentration of *Upeneus vittatus* (liver) belonging to Visakhapatnam is found to exceed the maximum permissible limit established by FAO. *Nemipterus japonicas* (liver) of Kakinada also observed to exceed the maximum permissible limit.

According to previous literature, Kumar et al. [68] obtained Fe concentration in muscle $(60.0 \pm 13.9 \text{ µg/g dry})$ wt) in *Pampus argentius* collected from east Midnapore district of West Bengal, North East Coast of India, shows similar results with the present study. Rejomon et al. [69] obtained Fe concentration in muscle (604.75 mg/kg dw) in *Lates calcarifer* collected from the coastal waters off Kochi, India, shows higher results compared with the present study.

Manganese

 Manganese (Mn) occurs naturally in the environment and is released into water bodies through agricultural runoff, leaching and agro chemicals. Mn is required for the proper functioning of the metabolism, immunological system, bone growth and reproduction system [65]. Overexposure or overconsumption of Mn may cause neurological disorders [70]. Among fish species collected from Visakhapatnam, *Pampus argenteus* exhibited the highest concentration $(31.8 \pm 1.9 \text{ mg/kg})$ of Mn in gills while the lowest concentration was obtained in muscle $(2.3 \pm 0.2 \text{ mg/kg})$ of *Nemipterus japonicas*. In the case of fishes collected from Kakinada, highest concentration $(38.3 \pm 1.4 \text{ mg/kg})$ of Mn is observed in gills of *Pampus argenteus* , and the lowest

concentration obtained in the muscle of *Upeneus vittatus* $(5 \pm 1.0 \text{ mg/kg})$. Mn concentration present in *Pampus argenteus* (gills) collected from Visakhapatnam and Kakinada found to exceed the maximum permissible limits of elements established by FAO. The Mn concentrations in the muscle $(0.12 \pm 0.03 \text{ mg/g}$ wet wt), liver $(1.12 \pm 0.14 \text{ mg/g})$ wet wt) and gills $(7.98 \pm 1.40 \text{ mg/g}$ wet wt) of *Nemipterus japonicas* collected from the Shalateen fish landing area on the Red Sea, Egypt, were found in the earlier investigation by El-Moselhy et al. [71]. On the other hand, the Mn concentration in the muscle $(0.591 \pm 0.554 \text{ µg/g dry wt})$ and liver (3.887 ± 1.05 μg/g dry wt) in *Scomber japonicas* collected from the northwest African coast (South Atlantic Moroccan coast) studied by Afandi et al. [72] is found relative to the present study in muscle tissues.

Selenium

 Selenium is an essential trace element that regulates immune functions, thyroid hormones and functions as an antioxidant agent [59]. It prevents DNA damage and protects against oxidative lipid damage to the brain [73]. Excessive Se intake, on the other hand, can induce chronic toxicity in humans, resulting in selenosis, a condition characterized by hair and nail loss and brittleness, gastrointestinal problems, skin lesions, tooth decay and nervous system abnormalities [74].

 In the present study, highest concentration of Se is observed in liver $(11.9 \pm 1.5 \text{ mg/kg})$ of *Upeneus vittatus* and lowest concentration is obtained in the muscle of *Nemipterus japonicas* $(0.8 \pm 0.1 \text{ mg/kg})$ collected from Visakhapatnam. Highest concentration of Se is observed in liver (8.1 ± 0.9 mg/kg) of *Nemipterus japonicas* while the lowest value is obtained in muscle of *Upeneus vittatus* $(0.9 \pm 0.01 \text{ mg/kg})$ belonging to Kakinada. Se concentration of *Upeneus vittatus* (liver) belonging to Visakhapatnam and *Nemipterus japonicas* (liver) of Kakinada exceeded the maximum permissible limit established by Brazilian Legislation. According to previous literature, Subotic et al. [75] obtained Se concentrations in the muscle $(0.11 \pm 0.37 \text{ µg/g dry wt})$, liver $(0.68 \pm 0.36 \text{ µg/g dry wt})$ and gills (0.64 ± 0.48 μg/g dry wt) in *Silurus glanis* collected from Danube River, Serbia, while Se concentrations in the muscle $(2.017 \pm 1.080 \,\mu$ g/g dry wt), liver $(4.535 \pm 1.922 \,\mu$ g/g dry wt) and gills (2.061 ± 0.782 μg/g dry wt) in *Acipenser ruthenus* collected from Danube River, Serbia by Jaric et al. [19] showed relative results with the present study.

Zinc

 Zinc is an essential trace element for metabolic processes, found almost in every cell and involved in the synthesis of nucleic acids in many enzymes [76]. Furthermore, Zn is engaged in many biological processes, including the immune system, neurotransmission and cell signaling [77]. Zinc deficiency leads to complications of pregnancy and childbirth, low birth weight and poor growth in childhood, and increased infectious disease morbidity [78].

In the present study, in the case of fishes collected from Visakhapatnam, highest concentration (395.1 \pm 19.4 mg/kg) of Zn is observed in the gills of *Upeneus vittatus* while the lowest concentration is obtained in muscle of *Nemipterus japonicas* $(22.3 \pm 2.2 \text{ mg/kg})$. Among the fishes collected from Kakinada, highest concentration of Zn is observed in gills $(296.5 \pm 22.6 \text{ mg/kg})$ of *Upeneus vittatus*, and the lowest concentration is obtained in muscle of *Nemipterus japonicas* showing the value 25.7 ± 2.2 mg/kg. Observed Zn concentration of *Upeneus vittatus* (gills) belonging to Visakhapatnam and Kakinada found to exceed the maximum permissible limit established by the Ministry of Agriculture, Fisheries and Food (MAFF), Food and Agriculture Organization (FAO), Turkish food codex and Brazilian Legislation.

 The values obtained in the present study are higher than the values in the earlier studies; Canli et al. [3] obtained results pertaining to the muscle $(37.39 \pm 6.88 \text{ µg/g})$ dry wt), liver $(110.03 \pm 34.58 \text{ µg/g dry wt})$ and gills (71.21 ± 14.24 μg/g dry wt) of *Mugil cephalus* collected from northeast Mediterranean Sea while the concentration in the present study is significantly higher than the concentration present in *Clarias fuscus* $(27.8 \pm 4.65 \text{ mg/kg})$, wet wt) that collected from Pearl River Delta, China, by Leung et al. [79]. This can be attributed to the fact that Zn is present in many enzymes [51]. These results demonstrate that fishes from various geographical areas accumulate heavy metals in diverse ways. One of the reasons for variations in the metal content present in fish from different locations is regional industrial pollution and absorption by sediment [80] and the difference in feeding habits [3].

Health risk assessment

 According to United States Environmental Protection Agency (USEPA), human health risk assessment is the process of determining the nature and probability of adverse effects on people health when exposed to toxins in a contaminated environmental media, either now or in the future. Risk evaluation for heavy metals is estimated on using estimated daily intake (EDI), target hazard quotient (THQ) and daily consumption rate limit (CR_{lim}) . These risk assessment criteria were established by the EPA in the USA to evaluate potential health concerns carried on by exposure to any chemical pollutant when it happens for an extended period of time [81]. These factors include average body weight, oral reference dose (RfD) and exposure frequency and duration and cancer slope factors (CSF) or carcinogenic potency slope oral (CSF) [82] are used to estimate the cancer risk resulting from exposure to a carcinogenic or potentially carcinogenic substance. The values of reference dose [83] and cancer slope factors (oral) for heavy metals established by WHO are shown in Table 6.

Estimated daily intake

 The estimated daily intake was calculated by the following equation [88]:

$$
EDI = \frac{(E_{\rm F} \times E_{\rm D} \times F_{\rm IR} \times C_{\rm F} X C_{\rm m})}{(T_{\rm A} \times W_{\rm AB})} \times 10^{-3}
$$

where E_F and E_D are the exposure frequency (365 days/year) and the exposure duration 67 years (equivalent to the average adult life expectancy in India) [84], respectively; F_{IR} is the ingestion rate of fish $(19.5 \times 10^{-3} \text{ kg/day}$ for Indians) [85, 86]; C_F is the conversion factor to convert fresh weight to dry weight (moisture content of fish fillet equal to 79%), which is equal to 0.208 [87]; C_m is the metal concentration in the fish tissue (mg/kg dry weight basis); W_{AB} is the average body weight of an Indian adult taken as 53.5 kg [88]; and T_A is the average exposure time for non-carcinogens (equal to $E_F \times E_D$) (Table 7).

Table 6 Reference dose and carcinogenic potency slope factor (oral) for adults [82, 83]

Heavy metals	RfD (mg/kg/day)	CSF (mg/ kg bw/day)
As	0.0003	1.50
Cd	0.001	
Cu	0.040	
Fe	0.700	
Mn	0.140	
Se	0.005	
Zn	0.300	

 The EDI values (mg/kg body weight/day) of the heavy metals relating to all the three species of the Visakhapatnam are in the following sequence $Fe > Se > Cd > Cu > A$ $s > Zn > Mn$, and in the case of Kakinada results found to be in the sequence $Se > Fe > As > Cd > Cu > Mn > Zn$. In the case of Visakhapatnam, the Fe value is found to be the highest EDI value present in *Upeneus vittatus*, while the Cu value observed to be the lowest EDI value belong to *Pampus argenteus* .

 The EDI values of all the heavy metals are compared to their respective oral reference dose (RfD) values, and the results show that almost all the heavy metals are below their RfD values. Based on estimated daily intake, ingesting marine fish in the studied regions is safe for consumption.

Target hazard quotient

 The values of the target hazard quotients (THQ) and combined target hazard quotient (CTHQ) are calculated and presented in Table 8. Non-carcinogenic risk is investigated by using the target hazard quotient (THQ), which is the ratio between the estimated exposure estimated daily intake (EDI) and the oral reference dose (RfD). RfD (mg/kg bw/ day) represents an estimate of the daily oral exposure of the human population that is likely to be without an appreciable risk of deleterious effects. The RfDs are 0.0003, 0.001, 0.040, 0.700, 0.140, 0.005 and 0.300 (mg/kg bw/day) for As, Cd, Cu, Fe, Mn, Se and Zn, respectively [84]. The following equation was used to calculate the THQ [89]:

$$
THQ = \frac{EDI}{RfD}
$$

 THQ values less than one indicate that the exposed population is not at risk. A THQ of 1 indicates that the concerned receptors may experience non-carcinogenic health risk and the probability of risk increases with the rise of THQ value. The USEPA Region III risk-based concentration table provides the procedure for calculating THQ [90].

Table 7 Estimated daily intake (EDI) (mg/kg body weight/day) through consumption of two fish species collected from Visakhapatnam and Kakinada

Elements	Visakhapatnam			Kakinada		
	Nemipterus japonicas	Upeneus vittatus	Pampus argenteus	Nemipterus japonicas	Upeneus vittatus	Pampus argenteus
As	3.8×10^{-7}	1.8×10^{-7}	6.6×10^{-7}	1.9×10^{-7}	3.4×10^{-7}	7.3×10^{-7}
C _d	3.0×10^{-6}	3.7×10^{-6}	7.5×10^{-6}	3.7×10^{-6}	6.06×10^{-6}	6.8×10^{-6}
Cu	6.3×10^{-7}	$6.6 \times 10 - 7$	1.4×10^{-7}	6.6×10^{-7}	1.2×10^{-7}	1.2×10^{-7}
Fe	6.6×10^{-7}	9.7×10^{-7}	9.6×10^{-7}	7.6×10^{-7}	5.6×10^{-7}	5.9×10^{-7}
Mn	1.7×10^{-7}	2.1×10^{-7}	4.3×10^{-7}	6.2×10^{-7}	3.7×10^{-7}	4.1×10^{-7}
Se	6.06×10^{-5}	1.0×10^{-7}	7.6×10^{-5}	8.3×10^{-5}	6.8×10^{-5}	7.8×10^{-5}
Zn	1.6×10^{-7}	1.7×10^{-7}	6.0×10^{-7}	1.9×10^{-7}	3.6×10^{-7}	3.3×10^{-7}

Table 8 Estimated daily intake (THQ) (mg/kg body weight/ day) through consumption of two fish species collected from Visakhapatnam and Kakinada

 The current investigation reveals that there is a carcinogenic risk as the obtained THQ values for the arsenic (As) concentration of the Nemipterus japonicas and Pampus argenteus of Visakhapatnam are higher than (>1) . In the case of Kakinada; As concentration of *Upeneus vittatus* and *Pampus argenteus* are higher than (> 1) reflecting risk probability.

 Based on the literature, exposure to two or more pollutants might result in additive and/or interactive effects, and hence, the combined target hazard quotient (CTHQ) can be calculated. The CTHQ values provide an overview of health risks pertaining to the seven metals (As, Cd, Cu, Fe, Mn, Se and Zn) studied through fish consumption. The following equation is used to determine the CTHQ [89].

$$
CTHQ = \sum_{j=1}^{7} THQ
$$

where *j* is the index of the studied heavy metals.

 In the present study, the (CTHQ) values are higher than one (> 1) for Nemipterus japonicas and Pampus argenteus belong to Visakhapatnam while in the case of Kakinada the (CTHQ) values are higher than one (> 1) for Upeneus vittatus and Pampus argenteus. This represents there is a non-carcinogenic risk of the seven studied metals together through fish consumption from both the locations.

Cancer risk

 Cancer risk from arsenic (As) exposure over a lifetime is calculated using the cancer slope factor (CSF) provided by the USEPA [91]. The following equation is used to estimate the cancer risk [89] (Table 9).

 $CR = EDI \times CSF$

where the cancer slope factor (CSF) is expressed in mg/ kg/day, and the other parameters have already been defined

Table 9 Estimated Cancer risk due to consumption of marine fish of the present study

Element	Species	Visakhapatnam	Kakinada
As	Nemipterus japonicas	5.7×10^{-7}	2.8×10^{-7}
	Upeneus vittatus	2.7×10^{-7}	5.1×10^{-7}
	Pampus argenteus	9.9×10^{-7}	0.0

earlier. The US Environmental Protection Agency set an acceptable lifetime carcinogenic risk of 10^{-5} [90].

 The CR factor for arsenic (As) over a lifetime of exposure through contaminated fish consumption is less than the value (10^{-5}) set by USEPA, as an acceptable lifetime carcinogenic risk. The CR value of arsenic appears to be negligible.

Daily consumption limit

The daily consumption rate limit (CR_{lim}) of fish, based on the carcinogenic effect of the contaminants, was calculated by the following equation [91]:

$$
CR_{\text{lim}} = \frac{\text{ARL} \times W_{\text{AB}}}{\text{CSF} \times C_{\text{m}}}
$$

The maximum allowable daily consumption of fish has been estimated using the following calculation based on the pollutants' non-carcinogenic effects [91]:

$$
CR_{\lim} = \frac{RFD \times W_{AB}}{C_m}
$$

where CR_{lim} is the maximum allowable daily consumption of contaminated fish (kg/day), ARL indicates the maximum acceptable individual lifetime risk level; (in the present study, 10^{-5} is used [92, 93], W_{AB} is the mean value of body weight related to consumer population (kg), CSF shows the cancer slope factor; RfD stands for the oral reference dose

(mg/kg-day), and Cm is the metal concentration in the edible part of fish (mg/kg) [94].

 The maximum permitted consumption level of heavy metal-contaminated fish in terms of meals per month can be calculated using equation [93], in which the maximum allowable daily consumption in terms of kilograms is converted to meal consumption per month; while taking meal size into consideration.

$$
CR_{mm} = \frac{CR_{lim} \times T_{ap}}{MS}
$$

Table 10 Estimated CR_{lim} (Carcinogenic and Non-Carcinogenic) due to consumption of fish from Visakhapatnam

where CR_{mm} is the maximum allowable consumption rate (meals/month), T_{ap} is the average time period $[365.25 \text{ day}/12 \text{ months} = 30.44 \text{ (day/months)}$, and MS is the meal size $(0.227 \text{ kg fish/meals})$ [93].

Based on the results presented in Tables 10, 11, daily consumption rate limits of contaminated fish based on non-carcinogenic effects in Visakhapatnam ranges from 0.0018 to 3.25 kg/day and in Kakinada, the range found to vary from 0.001 to 1.4 kg/day. Based on the carcinogenic effect of As, the daily consumption rate of contaminated fish is 0.0001 kg/day from both the locations.

The meal size of fish consumption per month has been estimated based on carcinogenic and non-carcinogenic effects, and the results are shown in Tables 10, 11. Considering the non-carcinogenic effect, the meal size based on contaminated fish related to Visakhapatnam (As, Cd, Cu, Fe, Mn, Se and Zn) ranges from 0.24 to 436.6, while in the case of Kakinada, it ranges from 0.2 to 200 meals per month. With regard to the carcinogenic effect of As, the meal size ranges from 0.009 to 0.01 and 0.004 to 0.01 meals per month concerned to Visakhapatnam and Kakinada, respectively.

Conclusion

 In the present study, the concentrations of metals As, Cd, Cu, Fe, Mn, Se and Zn are quantified in the muscle, liver and gills tissues of *Nemipterus japonicas* , *Upeneus japonicas and Pampus argenteus* collected from Visakhapatnam and Kakinada coasts. The health risk assessment has been evaluated with regard to the muscle of all the three species collected from two locations. These metals mean concentrations are slightly higher than the permissible limit of FAO, the MAFF, Turkish Food Codex and the Brazilian Legislation.

Carcinogenic) due to

Kakinada

The estimated daily intake of the metals examined from fish consumption is below the provisional tolerable daily intake. The THQ values of all the fishes of the present study are less than one (< 1) except arsenic and the CTHO values are higher than one (> 1) showing a presence of health risk. The obtained cancer risk (CR) factor due to arsenic is within the acceptable limit related to carcinogenic risk (10^{-5}) . The health risk assessment reveals that exposure to the evaluated metals pose a risk to human health, who consume the studied fish from both the locations because CTHQ values are greater than 1. Based on the obtained results, among the considered metals, the main risk for human health can be related to the accumulation of arsenic (As) in fish muscle. Because of the possibility of accumulation to this metal to unsafe levels, it is recommended to continue the monitoring of heavy metal concentrations in the considered fish species, *Nemipterus japonicas, Upeneus japonicas and Pampus argenteus* collected from Visakhapatnam and Kakinada coasts.

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