

ABSTRACT

Worldwide increase in consumption of fishes as food has immense therapeutic and nutritional benefits. Heavy metal contamination of marine water ecosystem occurring due to urban and industrial waste discharge, agricultural runoff and accidental spills of toxic chemicals is globally a rising concern and possesses a huge threat for human health due to their regular feeding habits. Humans consume heavy metal accumulated fish as a dietary uptake and are vulnerable to certain health risks. An attempt was made in the present investigation to monitor heavy metals concentration namely Mn, Ni, Co and Cu in different tissues (Gills, Muscle and Liver) of three selected edible fishes of Visakhapatnam namely Indian Mackerel (*Rastrelliger kanagartha*), Yellowtail Scad (*Atule mate*) and Pink Perch (*Nemipterus japonicas*) in the vicinity of Visakhapatnam coast. The extent of metal concentration in the tissues ranged from Ni>Cu>Mn>Co. The levels of heavy metals varied among the various tissues in the fish species studied and the concentrations of the metals found in gill tissues were high.

KEYWORDS: fishes, nutritional benefits, contamination, dietary uptake, metal concentrations.

INTRODUCTION

The global consumption of fishes has gradually increased in the last few years as fish consumption possesses great therapeutic and nutritional benefits. Being on a robust demand for frozen shrimp and frozen fish in international markets, India had exported 11,34,948 MT of seafood with a worth of over US\$ 5.78 billion (Rs 37, 870.90 crore) in 2016-17. South East Asia remained the second largest destination of India's marine products, with a share of 29.91 per cent in dollar terms, followed by Japan, the Middle East, China and other countries. Vizag port exported 1,59,973 tons of marine cargo worth Rs 9,294.31 crore (1,401.94 million US dollars) in 2016-17 as compared to 1,28,718 tons worth Rs 7,161 crore (1,105.76 million dollars) in 2015-16. [16].

The demand for fish consumption is gradually increasing to facilitate the growing population which in turn is increasing the pressure over fish capturing sector. However, the aquatic life is under constant threat due to pollution caused by various anthropogenic sources. Heavy metal pollution occurs in sea due to industrial discharges, atmospheric depositions of pollutants and occasional accidental spills of toxic chemicals [15]; [25]. Through consumption of fish as a dietary intake, humans are prone to bioaccumulation of heavy metals due to continued intake of contaminated species through a long period of time. As fishes act as an indicator species [14] and are relatively situated at the top of the aquatic food chain, hence they have a tendency of accumulating heavy metals from food, water and sediments [32]; [33]; [12]. Various studies have been carried out on heavy metal exposure on fishes, which elucidated that histopathological alterations in the tissues, DNA damage, early death of sensitive fish species, decrease in fish population, disturbed body physiology and reproductive failure can take place due to the same. In addition to this, there have been several adverse effects of heavy metals on human health that have been reported over time [8]; [12]. Serious threats like renal failure, liver damage, cardiovascular diseases and even death has been reported till date [3]; [26]; [12]. Hence, several international monitoring programs have been established over a period of time in order to assess and evaluate the quality of fish for human consumption and to monitor the health of aquatic ecosystems [5]; [12].

Water quality of the coastal zones are deteriorating due to heavy metal pollution from sewage discharge, industrial effluent discharge, loading and unloading of cargo at shipyard, and agricultural run-off. The most important issue arises when heavy metals that are released in to the environment pose a threat to the aquatic life as well as their predators. The present study was carried out at Visakhapatnam which lies in the coastal belt of Andhra Pradesh that is being polluted due to fast growing urbanization. The city is also known to host a busy

Port and a number of small and large scale industries ranging from Pharmaceutical industry to Steel Plant. The present study investigated the concentrations of heavy metal trapped in Liver, Gills and Muscles in three different species of fishes namely Indian Mackerel (*Rastrelliger kanagurta*), Yellowtail Scad (*Atule mate*) and Pink Perch (*Nemipterus japonicas*) which are commonly consumed by the inhabitants of in and around Visakhapatnam due to their easy accessibility and seasonal availability throughout the year. As muscles of fishes are the chief edible part, hence it was necessary to determine if the metal content in the muscles were within the permissible limits and suitable for consumption.

MATERIALS AND METHODS

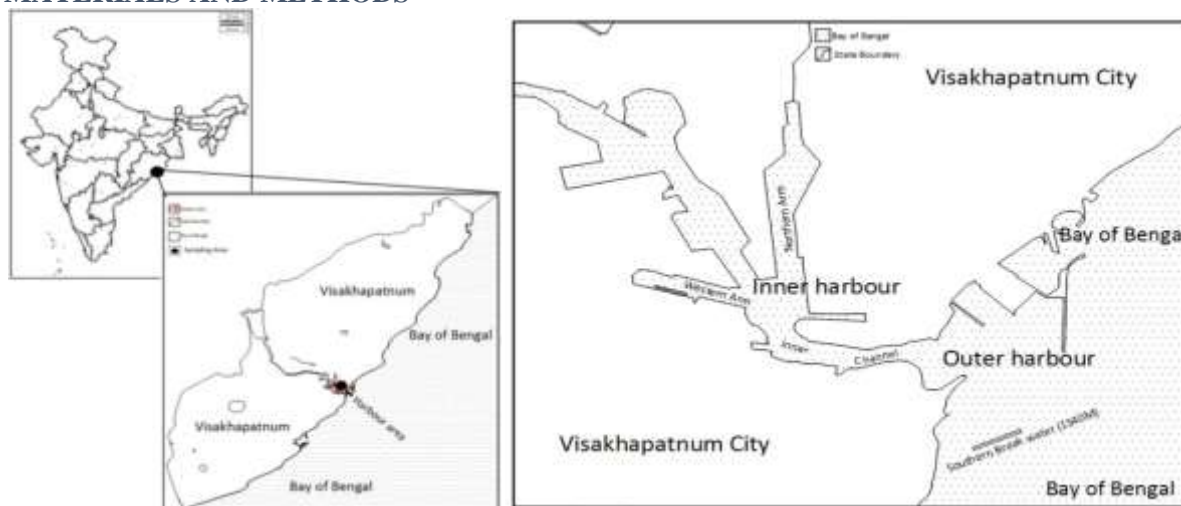


Figure 1: Map of Visakhapatnam showing the study area

Source: <https://www.google.co.in/maps/india> and AutoCAD 64bit

Study Area

Visakhapatnam has a coastline that stretches about 132 kms and has significant economic activity yielding out of the fisherman population. It also hosts a natural harbor and port which consists of local fish markets where the fishes caught from the coastal waters are regularly sold. In the present work, a total of twenty fish were collected from harbor fish market.

Sample Preparation

All fishes were dissected to obtain gills, muscle and liver following which they were dried by placing in hot air oven at 120°C for 8 hours. The samples were then finely powdered with the help of pre-acid washed mortar and pestle and each sample was weighed accurately by 1 gram. The samples were acid digested following standard techniques by using 10 mL HNO₃ (65% W/V Merck) followed by 2 mL Perchloric Acid (70% W/V Merck) and 5 mL H₂O₂ (50% W/V Merck) in order to get rid of organic content.

Analysis

In order to measure the concentration of the heavy metals Ni, Mn, Cu and Co, Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 400) was employed by using Air Acetylene gas. The results obtained in the present study were represented as mean ± standard error (SE).

RESULTS

Heavy metal concentrations of Mn, Cu, Ni and Co observed in species of Indian Mackerel (*Rastrelliger kanagurta*), Yellowtail Scad (*Atule mate*) and Pink Perch (*Nemipterus japonicas*) are represented in Table 1 and graphically represented with their concentrations in Figure 2. A comparison was made between the levels of heavy metals studied by different authors from in and around the same region. The results obtained were considered in dry weight as dry weight values are more reliable and consistent than wet weight values [1]; [27].

Organs	Mn			Ni			Cu			Co		
	Yellowtail Scad	Pink Perch	Mackerel	Yellowtail Scad	Pink Perch	Mackerel	Yellowtail Scad	Pink Perch	Mackerel	Yellowtail Scad	Pink Perch	Mackerel
Gills	0.16±0.11	0.14±0.02	0.16±0.2	0.27±0.01	0.49±0.1	1.63±0.08	0.31±0.03	0.31±0.1	0.21±0.01	BDL	0.21	BDL
Muscle	0.13±0.08	0.16±0.1	0.14±0.8	0.025±0.7	0.56±0.5	0.39±0.01	0.17±0.7	0.42±0.8	0.39±0.3	0.02	BDL	0.002
Liver	0.14±0.12	0.14±0.7	0.112±0.1	0.17±1.02	0.28±0.2	0.43±0.05	0.19±0.1	0.33±0.4	0.29±0.1	0.01	BDL	BDL

Table 1: Total heavy metal concentration (Ni,Cu,Mn,Co) in all three species of fish (expressed in µg/g)

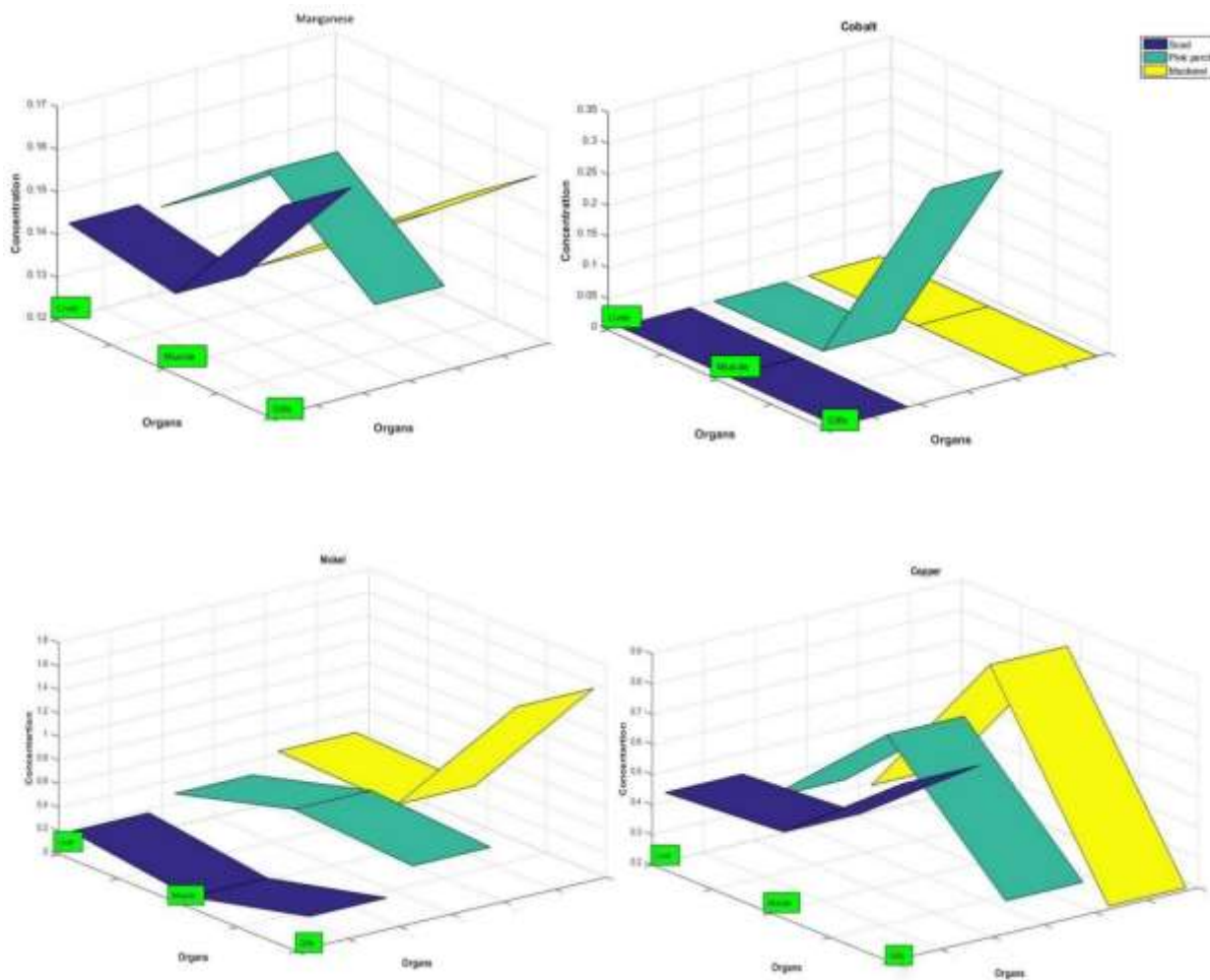


Figure 2: Graphical representation of Nickel (Ni),Copper (Cu),Manganese (Mn), Cobalt (Co) content in gills, muscle and liver in Indian Mackerel (*Rastrelliger kanagurta*), Yellowtail Scad (*Atule mate*) and Pink Perch (*Nemipterus japonicas*) expressed in µg/g (dry weight)

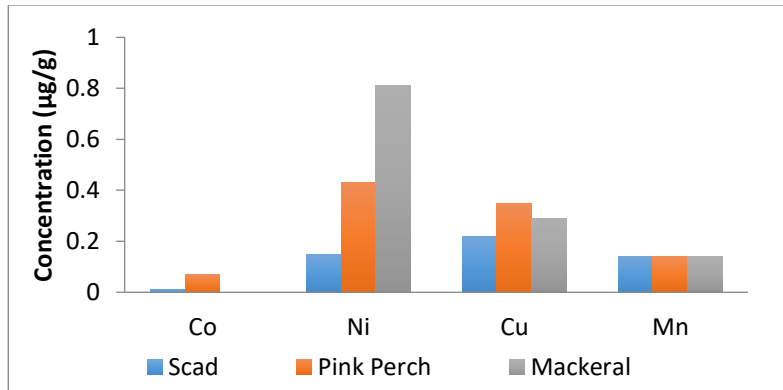


Figure 3: Species wise metal in three different species of fish Indian Mackerel (*Rastrelliger kanagurta*), Yellowtail Scad (*Atule mate*) and Pink Perch (*Nemipterus japonicas*)

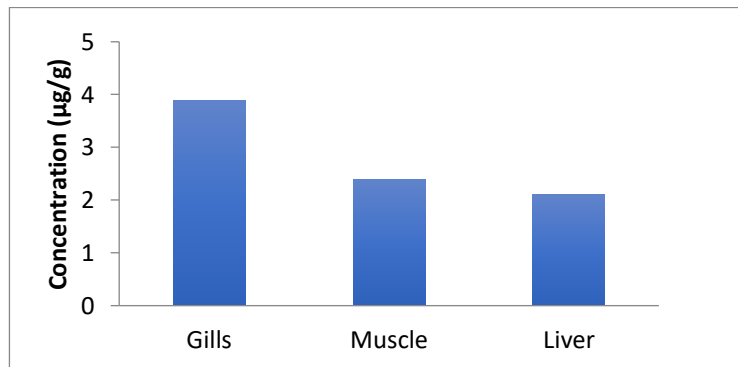


Figure 4: Organ wise metal concentrations in Gills, Liver and Muscle of Indian Mackerel (*Rastrelliger kanagurta*), Yellowtail Scad (*Atule mate*) and Pink Perch (*Nemipterus japonicas*)

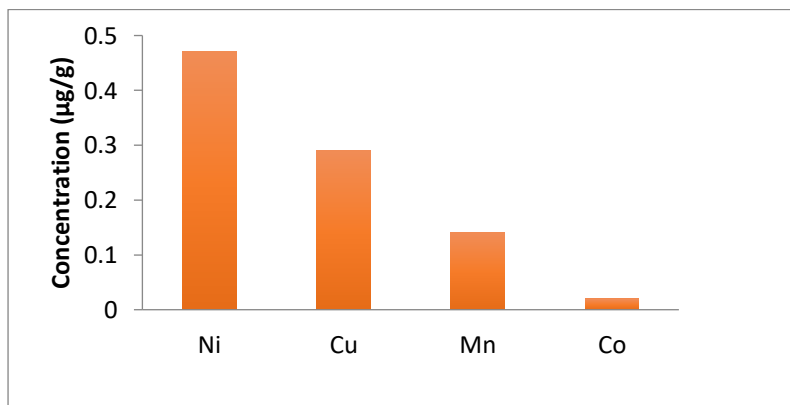


Figure 5: Mean Concentration of Nickel (Ni), Copper (Cu), Manganese (Mn) and Cobalt (Co) in pooled data of all samples of Indian Mackerel (*Rastrelliger kanagurta*), Yellowtail Scad (*Atule mate*) and Pink Perch (*Nemipterus japonicas*)

The results obtained showed the concentration of heavy metals in descending order of Ni>Cu>Mn>Co in pooled data of all the samples analyzed (Table 1). The organs reflected the accumulation pattern of metals mostly to be present in Gills followed by Muscle and then Liver (Figure 2). Among all the species of fishes studied which were Indian Mackerel (*Rastrelliger kanagurta*), Yellowtail Scad (*Atule mate*) and Pink Perch (*Nemipterus japonicas*), the highest amount of metal accumulation was reported in Indian Mackerel (*Rastrelliger kanagurta*).

Study done in Visakhapatnam Entrance Channel (VEC) by Kameswara Rao et al., 2015									
Metals	<i>Arioma indicum</i>	<i>Pentaprion longimanus</i>	<i>Rastrelliger kanagurta</i>	<i>Nemipterus Japonicus</i>	<i>Sphyraena obtusata</i>				
Cu	1.23±0.18	3.23±0.55	1.27±0.42	1.33± 0.16	1.01± 0.18				
Ni	0.019±0.008	0.18±0.02	0.04±0.008	0.19± 0.03	0.04±0.005				
Mn	1.379± 0.18	1.31±0.14	0.13± 0.02	1.08± 0.35	0.24±0.05				
Zn	4.37±2.18	7.21±2.38	5.20±0.57	3.53±0.63	4.21±1.39				
Pb	0.66±0.28	0.20±0.05	2.554± 0.53	0.87± 0.09	4.88±1.27				
Cr	1.23±0.23	1.09±0.19	0.27±0.07	0.38± 0.08	0.30±0.03				
Study done in Visakhapatnam and Bheemli Region by Byragi et al., 2015									
Metals	Seer (<i>Cybbium guttatam</i>)			Indian Mackerel (<i>Rastrelliger kanagurta</i>)			Silver Pomfret (<i>Pampus argenteus</i>)		
	Muscle	Liver	Gills	Muscle	Liver	Gills	Muscle	Liver	Gills
Cu	4.97± 0.16	7.08± 0.63	6.51± 0.73	3.46± 0.03	5.10± 0.41	4.82± 0.17	6.64± 0.05	8.62± 0.17	6.29± 0.36
Cd	0.04± 0.02	1.93± 0.16	1.67± 0.32	0.09± 0.05	3.27± 0.13	2.61± 0.21	0.12± 0.03	2.41± 0.12	1.57± 0.35
Zn	8.37± 0.09	12.49± 0.26	1.67± 0.32	15.64± 0.14	19.82± 0.18	17.05± 0.16	0.12± 0.03	2.41± 0.12	1.57± 0.35
As	0.37± 0.28	3.13± 0.16	2.90± 0.32	1.07± 0.03	3.02± 0.18	2.76± 0.07	0.16± 0.24	0.91± 0.04	1.63± 0.09
Pb	0.18± 0.02	2.41± 0.19	2.05± 0.21	0.15± 0.09	5.91± 0.27	3.85± 0.08	0.09± 0.04	4.14± 0.37	3.16± 0.16
Study done in Visakhapatnam and Bheemli Region by Chaitanya et al., 2017									
Metals	Silver Pomfret (<i>Pampus argenteus</i>)	Indian Mackerel (<i>Rastrelliger kanagurta</i>)	Yellow-striped Goatfish (<i>Upeneus vittatus</i>)						
Mn	5.18±1.06	3.46±0.33	1.93±0.45						
Co	1.71±0.21	1.16±0.09	0.31±0.02						
Cd	1.30±0.12	0.73±0.09	0.05±0.05						
Pb	0.49±0.06	0.51±0.08	0.41±0.13						
Cr	0.24±0.08	1.06±0.18	1.05±0.18						
Present Study done in Visakhapatnam Fishing Harbour region, 2017									
Metals	Indian Mackerel (<i>Rastrelliger kanagurta</i>)	Yellowtail Scad (<i>Atule mate</i>)	Pink Perch (<i>Nemipterus japonicas</i>)						
Cu	0.29±0.45	0.22±0.34	0.35±0.11						
Ni	0.81±0.12	0.15±0.87	0.43±0.21						
Mn	0.19±0.78	0.14±0.10	0.11±0.11						
Co	0.1±0.10	0.2±0.62	0.07±0.10						

Table 2: Comparison of concentration of heavy metals (in µg/g) in fish reported in previous studies.



The mean concentration of Nickel (Ni) which was accumulated highest compared to other metals was 0.47 $\mu\text{g/g}$ whereas Copper (Cu) was found to be 0.29 $\mu\text{g/g}$ followed by Manganese (0.14 $\mu\text{g/g}$). Cobalt (Co) was found to be 0.02 $\mu\text{g/g}$ in mean of all samples. Overall, the mean concentration of metals accumulated by organs showed that Gills had accumulated the most amount of heavy metals (3.89 $\mu\text{g/g}$) compared to Muscles (2.4 $\mu\text{g/g}$) and Liver (2.1 $\mu\text{g/g}$). Indian Mackerel (*Rastrelliger kanagurta*) reflected highest among the other species of fishes studied which were Yellowtail Scad (*Atule mate*) and Pink Perch (*Nemipterus japonicas*). The mean concentration of Nickel (Ni) accumulated in Indian Mackerel (*Rastrelliger kanagurta*) was found to be 0.81 $\mu\text{g/g}$ which were higher than other species with the distribution of 1.63 $\mu\text{g/g}$ in its Gills, 0.43 in Liver and 0.39 $\mu\text{g/g}$ was found in Muscle tissues. The mean concentration of Copper (Cu) accumulated by Indian Mackerel (*Rastrelliger kanagurta*) was 0.29 $\mu\text{g/g}$ with the distribution of 0.21 $\mu\text{g/g}$ in Gills, 0.39 $\mu\text{g/g}$ in Muscle and 0.29 $\mu\text{g/g}$ in Liver. Manganese (Mn) mean concentration in the same species was found to be 0.14 $\mu\text{g/g}$ with distribution of 0.16 $\mu\text{g/g}$ in Gills, 0.14 $\mu\text{g/g}$ in Muscles and 0.12 $\mu\text{g/g}$ in Liver. Cobalt (Co) was present in negligible concentrations. The mean concentration of Nickel (Ni) accumulated in Yellowtail Scad (*Atule mate*) was found to be 0.15 $\mu\text{g/g}$ with the distribution of 0.27 $\mu\text{g/g}$ in its Gills, 0.17 in Liver and 0.025 $\mu\text{g/g}$ was found in Muscle tissues. The mean concentration of Copper (Cu) accumulated by Yellowtail Scad (*Atule mate*) was 0.22 $\mu\text{g/g}$ with the distribution of 0.31 $\mu\text{g/g}$ in Gills, 0.17 $\mu\text{g/g}$ in Muscle and 0.19 $\mu\text{g/g}$ in Liver. Manganese (Mn) mean concentration in *Atule mate* was found to be 0.14 $\mu\text{g/g}$ with distribution of 0.16 $\mu\text{g/g}$ in Gills, 0.13 $\mu\text{g/g}$ in Muscles and 0.14 $\mu\text{g/g}$ in Liver. Cobalt (Co) was present in very scanty concentrations of 0.02 $\mu\text{g/g}$ in muscles and 0.01 $\mu\text{g/g}$ in liver. The mean concentration of Nickel (Ni) accumulated in Pink Perch (*Nemipterus japonicas*) was found to be 0.43 $\mu\text{g/g}$ with the distribution of 0.49 $\mu\text{g/g}$ in its Gills, 0.28 in Liver and 0.56 $\mu\text{g/g}$ was found in Muscle tissues. The mean concentration of Copper (Cu) accumulated by Pink Perch (*Nemipterus japonicas*) was 0.35 $\mu\text{g/g}$ with the distribution of 0.31 $\mu\text{g/g}$ in Gills, 0.42 $\mu\text{g/g}$ in Muscle and 0.33 $\mu\text{g/g}$ in Liver. Manganese (Mn) mean concentration in *Nemipterus japonicas* was found to be 0.14 $\mu\text{g/g}$ with distribution of 0.14 $\mu\text{g/g}$ in Gills, 0.16 $\mu\text{g/g}$ in Muscles and 0.14 $\mu\text{g/g}$ in Liver. Cobalt (Co) mean concentration was found to be 0.07 $\mu\text{g/g}$ with distribution of 0.21 $\mu\text{g/g}$ in Gills of Pink Perch (*Nemipterus japonicas*).

DISCUSSION

Aquatic pollution is one of the major rising problems globally and it also affects the total seafood demand from India. Fish is an important dietary source of protein but due to the increasing level of heavy metals in water, fishes tend to bioaccumulate and concentrate the smallest amount of heavy metal present in water. And in turn, this poses a greater risk to human beings due to our dependency on fish as a part of our diet. Ingesting of polluted marine fish and exposure to heavy metals causes health problems in people and animals which includes neurological and reproductive problems [28][17]. Also, fishes can be used as bioindicators for marine pollution [22]. Hence in order to take early precautions related to human health concerns, studying of marine pollutions and heavy metal accumulation is very important. The present study compared to other studies done by previous authors indicated lower levels of metals namely Cu, Mn and Co however Ni studies in the study area has not been reported previously. Also, there were species wise and area wise differences present in the total heavy metal content in contrast to other studies. Seasonal variability in accumulation levels and patterns has also been reported by many researchers worldwide. While a lot of work has been already carried out on this relevant topic around the world, in this region, research has begun recently and the amount of information available is scanty. Researchers have noticed that accumulation and concentration of heavy metals by fishes in their tissues depend on many factors such as concentration, duration of exposure, salinity, temperature, hardness of water and metabolic rate of organisms [4].

Nickel concentrations are comparatively elevated in aquatic plants and animals in the vicinity of nickel smelters, nickel-cadmium battery plants, electroplating plants, smelting, and petroleum industries stainless steel industries. It is also used in nickel alloys, jewelry, paint, spark plugs, catalysts, ceramics, disinfectants, magnets, batteries, ink, dyes, and vacuum tubes sewage outfalls, coal ash disposal basins, and found to be elevated in populated areas [26]. Food uptake as well as food processing is the major uptake of Ni for humans apart from other natural sources. The highest concentration of Ni (1.63 $\mu\text{g/g}$) was measured in the gill of Indian Mackerel (*Rastrelliger kanagurta*) which was higher than (0.95 $\mu\text{g/g}$) measured in the gill of *Oreochromis niloticus* reported by Akan *et al.*, [2], while the lowest detectable level of 0.02 $\mu\text{g/g}$ was detected in the muscle of Yellowtail Scad (*Atule mate*). In the present study, the mean concentration of Ni in Indian Mackerel (*Rastrelliger kanagurta*) was 0.81 $\mu\text{g/g}$ which was higher than 0.04 $\mu\text{g/g}$ reported by Kameswara Rao [18] during a study done in same region. Also, Ni concentration in Pink Perch (*Nemipterus japonicas*) in previous studies was found to be 0.19 $\mu\text{g/g}$ whereas the current study revealed a slight higher level of 0.43 $\mu\text{g/g}$. The

estimated maximum guideline for Ni is 70 - 80 $\mu\text{g/g}$ as per USFDA guidelines [31]. Thus the concentrations of Ni in all the samples were far below the stipulated limit however the metal showed an accumulating trend over time when compared to previous studies done in the two of the similar species in the same region.

The World Health Organization, WHO, has established as safety metal quantity limits from eating fishes a weekly maximum dosage, commonly known as Provisional Table Weekly Intake (PTWI), per kg of body weight. Copper (Cu) is an essential part of several enzymes and it is found as a dietary intake in Seafood and is also necessary for the synthesis of hemoglobin. However, if consumed excessively, Cu can be toxic. The content of Cu in seafood was limited (FAO, 1983) for fish and fishery products to 30 mg/kg. In the present study, the mean values of copper (Cu) was $0.29 \pm 0.01 \mu\text{g/g}$ which was relatively similar to $0.39 \mu\text{g/g}$ of Akan *et al.*, [2] but comparatively lesser than the values reported by Kameswara Rao *et al.*, [18] and B.Reddy *et al.*, [29]. However, the highest value obtained of $0.29 \mu\text{g/g}$ was below the FAO guideline of $30 \mu\text{g/g}$. Hence, the concentrations of Copper (Cu) in the analyzed fish samples were all below the FAO recommended guideline.

Manganese was determined in all fish samples. Manganese is a metal with low toxicity but has a significant biological influence and is an essential element for both animals and plants. Deficiencies of Mn result in severe skeletal and reproductive abnormalities in mammals. It has chances of increasing accumulation in any species with time. Mn is widely distributed throughout the body with little variation and does not accumulate with age [24]. The average concentration of manganese (Mn) in all samples analyzed was $0.14 \mu\text{g/g}$ which on par with earlier studies done in same region by Kameswara Rao *et al.*, [18] but lower than $5.3 \mu\text{g/g}$ analyzed in *H. nehereus* by Bhupander Kumar *et al.*, [6] and $3.45 \mu\text{g/g}$ which was detected in gill of *Tilapia zilli* by Akan *et al.*, [2]. However, the levels of Manganese (Mn) in all the fish samples exceeded the [WHO] limit of 0.01 ppm or $\mu\text{g/g}$ [31], which therefore poses a threat to the fishes with time.

Although the permissible limit of Co is not specified by BIS (1993) and WHO (2004) for drinking water, Cobalt (Co) exposure has been known to create complicated health problems. A concentration of $0.21 \mu\text{g/g}$ was recorded in the gills of Pink Perch (*Nemipterus japonicas*) which was in agreement with the findings ($0.039 - 1.44 \mu\text{g/g}$) of a study conducted in Jannapura Lake [10] but higher than that reported in *Rastrelliger kanagurta* ($0.0028 \mu\text{g/g}$) [10]. Hence, the accumulating trend of few of the metals which was reported in higher levels compared with previous studies done before can be considered as an alarming or warning situation regarding the entry of heavy metals into the environment due to rising urbanization and the same may enter the food chain through our feeding habits.

CONCLUSION

The study was carried out in order to focus on accumulation of certain heavy metal levels in liver, muscle, and gills of some common edible fishes of Visakhapatnam region. All results obtained were below the limits of fish suitable for human consumption proposed by International Standards of Fish. In the present study, the elevated levels of some heavy metals seized in muscle and gills throws some light on water pollution due to runoffs containing sewage, industrial and toxic pollutants. Moreover the fishes were collected from the port market which lies close to the Visakhapatnam Entrance Channel (VEC), which extends for several kilometers and is quite wide and deep enough to harbor major ships in it. The seaport activities including loading and unloading and the growing industrialization had altered the ecological integrity of the channel. Some fisherman still uses the same canal for fishing [18]. Hence, it is evident that due to subsequent industrialization, the entry of toxic effluents is increasing the risk of metal contamination in the environment which contributes to its further entry into human beings through food chains.

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