Bioconcentration Potential Studies of Heavy Metals in *Fenneropenaeus penicillatus* (Jaira or Red Tail Shrimp) along the Littoral States of Karachi City

Tuba Kamal[1,*], Muhammad Asad Khan Tanoli[1], Majid Mumtaz[1], Nida Ali[2] and Sara Ayub[1]

[1]*Department of Chemistry, Faculty of Science, University of Karachi, Karachi-75270, Pakistan*

[2]*Department of Environmental Science, Faculty of Science, Federal Urdu University of Arts, Science and Technology (Gulshan-e-Iqbal Campus), Karachi-75300, Pakistan*

Abstract: *Fenneropenaeus penicillatus* (commonly known as *Jaira* or *Red tail shrimp*) is one of the commercially important and abundant species in the coastal areas of Pakistan and export to more than 40 countries world wide. It is a good source of nutrients for human diet because of its highly rich composition of protein, calcium and vitamins. The littoral state of Pakistan is facing much environmental issues during the last many years because of increasing pollution and human induced environmental changes which have gradually declined the export of shrimps. Crustacean species are considered as the bio-indicators of toxic materials due to their high affinity to accumulate heavy metals than fishes. The study was undertaken to quantify the heavy metals like copper, zinc, cadmium and lead in the *Red tail shrimp*. For this purpose biosamples were collected in year 2011 to 2013 from the littoral states of Karachi city, Pakistan. Flame Atomic Absorption Spectroscopy (FAAS) technique was used to analyze the Cu and Zn while Graphite Atomic Absorption Spectroscopy (GAAS) technique was used to quantify the Cd and Pb. Results were compared with the WHO/FAO/FDA values. The concentrations of selected heavy metals were within the normal range in all analyzed samples except for cadmium. Bioconcentration of cadmium was found much higher than the recommended value which is an alarming condition. Analysis of variance (ANOVA) was applied to find out the concentration variation of heavy metals in three years research study at $p < 0.05$. The results suggested that there is no significant effect of year wise variation on accumulation level of heavy metals in *F. penicillatus.*

Keywords: *Fenneropenaeus penicillatus*, heavy metals, littoral states of Karachi city, FAAS, GAAS.

1. INTRODUCTION

Sea food products are considered worldwide as the good source of nutrients in human diet [1]. Especially, crustacean species reflects the highly rich composition of protein, calcium, vitamins and various extractable compounds [2]. On this basis, decapod crustacean represents an important economic source.

Fenneropenaeus penicillatus (old name *Penaeus penicillatus*) commonly known as Jaira or red tail shrimp, is a commercially important and abundant species of Pakistan Coast. Twenty five species of penaeid shrimps have been recorded in Pakistan. Out of these 25 species, only 12 species are commercially important [3-4]. Local fishermen divide the shrimps from commercial catch into three main categories. The division is based mainly on the size and color of the shrimp. These are categorized as Jaira (large), Kalri (medium) and Kiddi (small). *F. penicillatus*is suitable species to study the biochemical composition of shrimps in Pakistan due to its high temperature and salinity tolerance and its relative abundance and economic importance. Jaira or Red tail shrimps are dominantly composed of mainly two species *Penaeus penicillatus* followed by *Penaeus merguiensis*. *P. penicillatus* is seemed to be abundant specie in summer while *P. merguiensis* is found to be dominant existence in winter [5].

The average catch landing from the coastal and offshore areas of Pakistan is about to be 25,541 (± 4090 S.D.) metric tons annually. The annual shrimp catch during 1971-2007 ranged between 16,050 to 34,920 metric tons with an average value of 25,541 (± 4090 S.D.) metric tons. The rate of Kiddi shrimp catches increased from 20 % to 50-60 % of the total shrimp landings. This situation is an alarming condition which shows that the stocks of large-sized shrimps (Jaira and Kalri) have been overexploited [6].

Pakistan has geologically and ecologically diverse coastline which is dissected by harbors, estuaries, bays and creeks with diversified animals forms, exhibiting wide characteristics [7]. It is also economically very important to export seafood products. The shrimps are exported to about 40 countries worldwide. The United States of America and Japan are the major importers of frozen shrimps. The shrimp exported values have gradually declined during past five years [8], because the littoral state of Pakistan is facing many environmental issues due to increasing pollution and

*Address correspondence to this author at the Department of Chemistry, Faculty of Science, University of Karachi, Karachi-75270, Pakistan;
E-mail: lion.chemistry@hotmail.com

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human induced environmental changes. Fishing, coastal aquaculture, waste disposal, industrial activity, agriculture, domestic effluents, salt making and unplanned tourism etc, have contaminated the ecosystem, destroyed the live of aquatic animals, decreased the market value and increased bacterial diseases [9].

Aquatic animals mainly the phyla crustacean species are the bioindicators of toxic materials because invertebrates have more tendencies to accumulate contaminants as compare to fishes [10]. Hazardous material from the surrounding continuously enter in marine environment and deposit in biota from where it subsequently transferred in to human through the food chain and when the concentration of these substances reaches to a certain level, it become toxic [11].

Previous investigations have shown declined condition of aquatic environment, due to heavy metals contamination which is one of the most critical environmental issues worldwide. The increasing population in Asian countries also expanding their economic activities near the littoral zone [12]. Latest researches on the chemical composition and nutritional value of crustaceans and composition benefits to human health have been much promoted in the recent years but information is still rather dispersing [13].

Absence of such information about population and variation in species has been a great handicap for fishing industry in Pakistan on modern lines [7]. Therefore it is necessary to assess the quality of aquatic animals because of rapid increase in environmental pollution.

Figure 1: Image of Karachi city showing all sampling points.

The aim of the present paper is to highlight the accumulation level of heavy metals (Cu, Zn, Cd and Pb) in the muscles and exoskeleton of commercially important species of shrimp in Pakistan. Perkin Elmer precisely atomic absorption spectroscopy A.A analyst 700 technique were used to analyze the digested metals in biosamples and were interpreted statistically by using the statistical software i.e. **Minitab-14**.

2. EXPERIMENTAL

2.1. Materials and Methods

First, biosamples (red tail shrimps) were collected during the year 2011 to 2013 from the littoral states of Karachi city, Pakistan (Figure **1**). The samples were collected in the months of March and October of each year and the mean of these two different seasons were reported. The red tail shrimp species studied here is of commercial interest on local and national scales. This species has been exported to different countries, represented as an important food source. They were obtained directly from the fishing boats with a scoop net. After harvesting immediately packed in sealed plastic bags with particularly specified tags in ice box and were transported to the lab within 1 hr. Samples were weighed and washed under the tap water to remove the adhere sediments particles from the body of the organisms. These were then cleaned with distilled and deionized water [14]. Shrimps in premoult or that had recently moulted were not used. To minimize trace elements contamination, all lab wares were washed first with detergent and tap water and then soaked in 15% nitric acid for 24h, rinsed repeatedly in deionized water and then dried prior to use. Furthermore, to minimize the contamination of the samples, all chemicals used were of a HPLC grade.

2.2. Heavy Metals Analysis

The total length and weight of red tail shrimps were measured in cm and in gram respectively, before dissection. The average weights and total lengths of samples in each year were mentioned in Table **1**. For detail analysis, biotic samples were dissected and the shells were removed. The edible tissues and exoskeleton were dried to a constant weight in an oven at 70^0C. After drying, the weight was noted again for each sample that was the original weight of samples. After that samples were ground to a fine powder. 4g of each sample was taken and treated with a mixture of HNO_3, H_2SO_4 and $HClO_4$ in a ratio of 2:1:1 for the complete digestion. After digestion the solution was evaporated nearly to dryness on hot plate at 65–70^0C and the required volume was filtered with whatmann 542 filter paper and diluted up to 100ml with 1% HNO_3 and kept at 4^0C prior to further analysis. Finally, element contents in the samples were analyzed by using AAS against working standards solution of different concentrations [15, 16]. Working standard solutions were prepared by appropriate dilution of 1ppm stock standard solution, which was prepared by using the 1000 ppm standard solutions from Fisher Scientific of HPLC grade. All analysis was undertaken in triplicates on each sample and the mean values were calculated. As no certified reference material (CRM) of shellfish (red tail shrimp) was available, the accuracy of the adopted procedure was assessed by the analysis of aquaculture growing shrimp in Fish Harbor of Karachi city. Recoveries were consistently in the range of good agreement and were found certified and standard values. The achieved percent recoveries are delineated in Table **2**.

Table 1: Sample Characteristics i.e. Sampling Years, Average Wet Weights & Total Lengths of *Fenneropenaeus penicillatus*

		Length (cm) ± SD			Weight (g) ± SD		
Years		**2011**	**2012**	**2013**	**2011**	**2012**	**2013**
Sample	**n**						
Fenneropenaeus penicillatus	25	15.0±1.4	16.5±2.8	14.5±1.5	9.93±4.0	14.57±2.1	10.8 ±1.7

Table 2: Percent Recoveries of Heavy Metals in Aquaculture *Fenneropenaeus penicillatus*

Heavy Metals	% Recoveries	Heavy Metals	% Recoveries
Cu	99	Cd	91
Zn	95	Pb	98

Table 3: *Statistical* Analysis of Heavy Metals Concentration Profile in *Fenneropenaeus penicillatus* Samples

Metals	Samples of *Fenneropenaeus penicillatus*	years	n	Range (µg/g)		Mean (µg/g)	Standard Deviation
				Minimum	Maximum		
Copper	*Muscles*	2011	25	8.13	12.03	10.08	1.95
		2012	25	12.50	16.50	14.50	2.00
		2013	25	17.70	21.86	19.78	2.08
	Exoskeleton	2011	25	2.98	6.90	4.94	1.96
		2012	25	3.78	7.74	5.76	1.98
		2013	25	5.98	9.97	7.98	1.99
Zinc	*Muscles*	2011	25	21.75	25.90	23.83	2.08
		2012	25	18.90	22.90	20.90	2.00
		2013	25	20.03	24.02	22.03	1.99
	Exoskeleton	2011	25	5.15	8.95	7.05	1.90
		2012	25	5.60	10.00	7.80	2.20
		2013	25	4.70	8.96	6.83	2.13
Cadmium	*Muscles*	2011	25	1.47	5.67	3.57	2.10
		2012	25	3.66	7.86	5.76	2.10
		2013	25	3.70	7.51	5.61	1.90
	Exoskeleton	2011	25	6.13	9.91	8.02	1.89
		2012	25	11.78	15.98	13.88	2.10
		2013	25	9.69	11.89	10.79	1.10
Lead	*Muscles*	2011	25	0.04	0.08	0.06	0.02
		2012	25	0.02	0.07	0.05	0.02
		2013	25	0.04	0.09	0.07	0.02
	Exoskeleton	2011	25	ND	ND	-	-
		2012	25	ND	ND	-	-
		2013	25	ND	ND	-	-

*ND= Not detected.

2.3. Statistical Analysis

Bioconcentration potential of heavy metals in aquatic samples which were sampled in three different years were statistically analyzed by Minitab-14 software. Descriptive statistical analysis of concentration potential of heavy metals in shrimp samples were delineated in Table **3**. Analysis of variance (One way ANOVA) was performed to assess whether the heavy metals concentration varied significantly between the years. The criterion values of probabilities ($p < 0.05$) for correction significance were used.

3. RESULT AND DISCUSSION

Bioaccumulation potential of heavy metals in aquatic animals vary in various species. These variations depend upon number various of factors: biotic ones like body dimensions or mass, age, sex, diet, metabolism, position in trophic zones; and abiotic ones such as distribution of metals in its aquatic environment, salinity, temperature, pH of water, habitats and interaction with other metals [17]. In the present study, accumulation of the heavy metals has been focused in red tail shrimps along the littoral states of Karachi, Pakistan during the three years. Heavy metals are toxic if they exceeded from the certain level in the bio-system. Descriptive statistical analysis of concentration profile of heavy metals (Cu, Zn, Cd and Pb) in commercially important shrimp *Fenneropenaeus penicillatus* as µg/g dry weight in 2011 to 2013 years studied are reported in Table **3**.

In the current study, concentration of Zn and Cu were observed higher in the muscles as compare to the

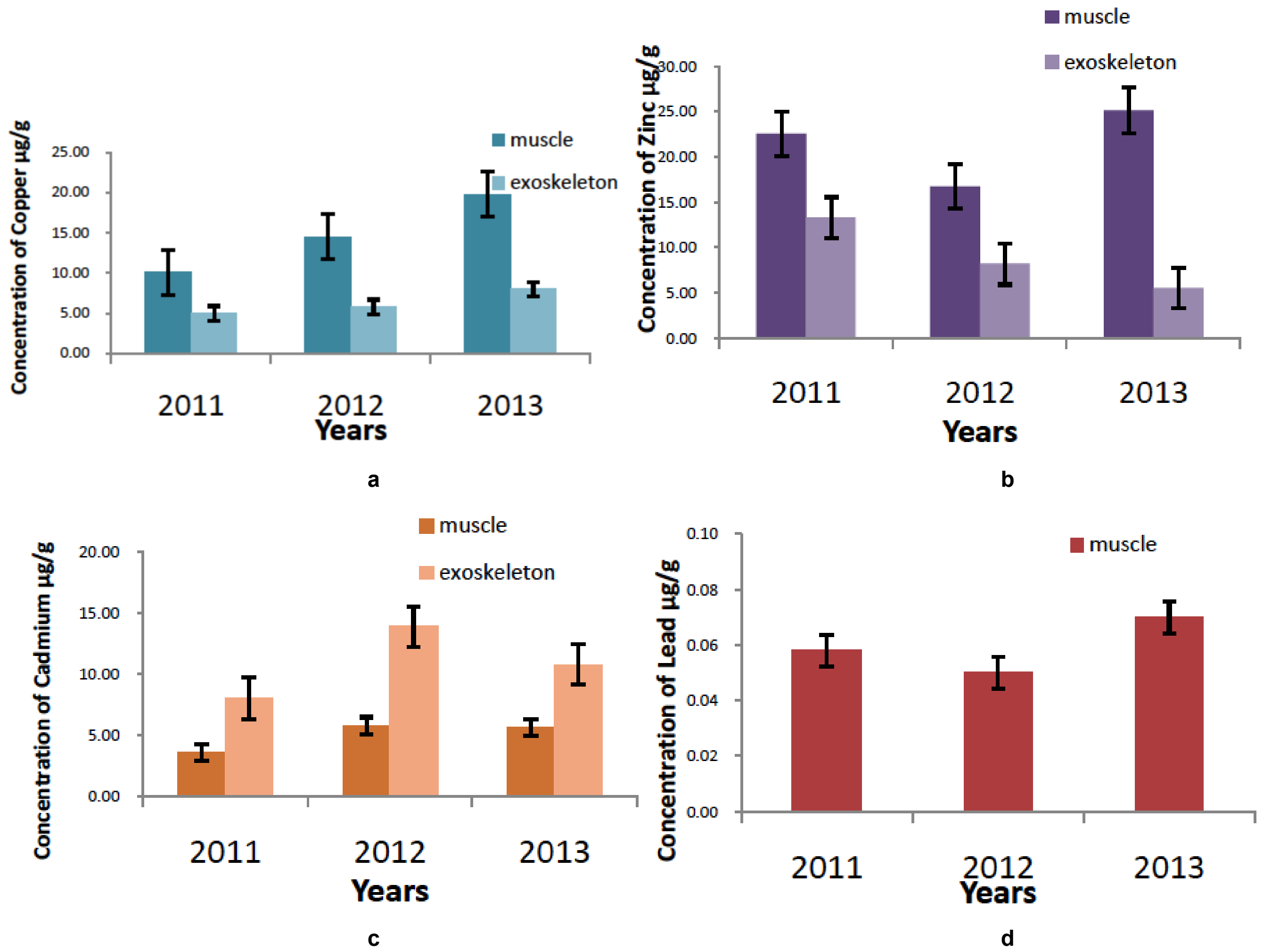
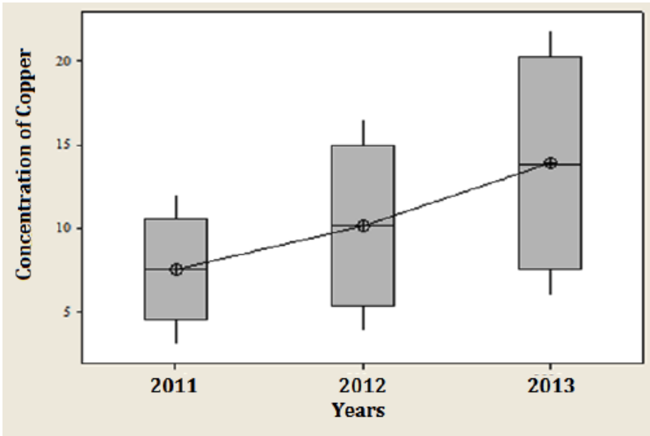


Figure 2: a. Concentration of **Cu** in *Fenneropenaeus penicillatus*. **b.** Concentration of **Zn** in *Fenneropenaeus penicillatus*. **c.** Concentration of **Cd** in *Fenneropenaeus penicillatus*. **d.** Concentration of **Pb** in *Fenneropenaeus penicillatus*.

exoskeleton of red tail shrimp in each year. The range of Zn varied from 20.90µg/g to 23.83µg/g in edible tissues and 6.83µg/g to 7.80µg/g in exoskeleton while the Cu concentrations were found from 10.08µg/g to 19.78µg/g in edible tissues of red tail shrimp and 4.94µg/g to 7.98µg/g in exoskeleton. Bioconcentration levels of Cu and Zn metals in muscles and exoskeleton of *Fenneropenaeus penicillatus*, determined in the sampling years i.e. 2011 to 2013 were also presented in Table **2** and Figure **1a** and **b**.

Cu concentrations in red tail shrimp were recorded far below the normal permissible range, i.e. 120 ppm [17, 18] and 30 ppm [19] as recommended for the crustacean tissue. The Food and Agricultural Organization (FAO) suggested limit for Cu is 30 mg/kg [20, 21]. The Turkish legislation establishes maximum levels for the Cu i.e. 20.0 mg/kg, above which human consumption is not permitted. The other food standards for Cu like Canadian Food Standard is 100µg/g, Hungarian standard is 60µg/g and the Malaysian Food Regulation limit is 120µg/g while the range of international standard is 10-100µg/g [21, 22].

According to the results obtained, the Zn levels in the muscle and exoskeleton were also found lower than the permissible level, i.e. 200-400 ppm in the crustacean tissue for human consumption [17]. However the values were much lower than the permissible limit for human consumption, which is 100 ppm for marine sea food [21, 23, 24], the Food and Agricultural Organization (FAO) suggested limit for Zn is 30 mg/kg. The Turkish legislation establishes maximum levels i.e. 50 mg/kg for the Zn, above which human consumption is not permitted. Other standards for like Canadian Food Standard is 100µg/g, Hungarian Standard is 80µg/g, Australian Standard is 10µg/g and Turkish Standards (TFC) it is 50mg/kg [21, 25, 26]. It is generally believed that crustacean can actively regulate Zn concentrations in tissues and therefore Zn tissues



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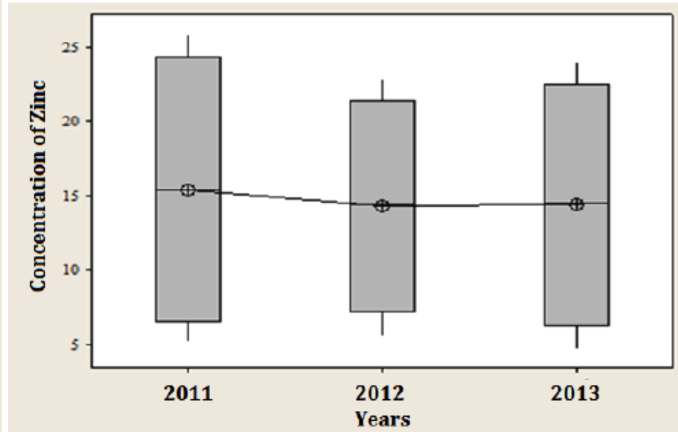

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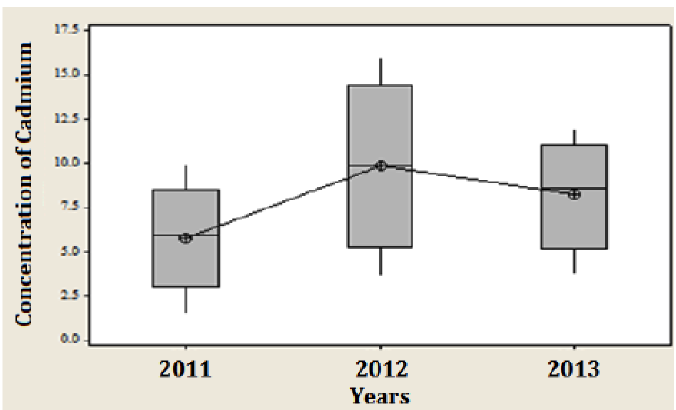

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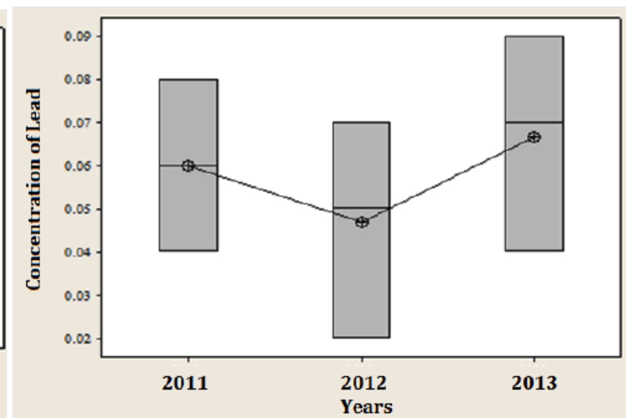

d

Figure 3: **a.** Box plot presents Concentration of Cu in *Fenneropenaeus penicillatus.* **b.** Box plot presents Concentration of Zn in *Fenneropenaeus penicillatus.* **c.** Box plot presents Concentration of Cd in *Fenneropenaeus penicillatus.* **d.** Box plot presents Concentration of Pb in *Fenneropenaeus penicillatus*.

level does not reflect the changes in Zn concentrations in the environment [17-27].

The main source of Cu and Zn metals in the present geographical location could be the effluents of Bin Qasim thermal power plants, sea port activities, industrial effluents of SITE (Sindh Industrial Trade Estate) through Layari River and unloading of raw materials for Pakistan steel mill; which is further fractionated into water, seaweed and sediments. Industrial effluents coming through Malir River, sewage water and oil refinery situated at the coastal region are the other sources of Cu and Zn contamination. Shrimps living in this environment meet the higher ionized metals in water which are absorbed by seaweed [18]. Cu and Zn are essential metals for normal growth, metabolism, enzymatic and respiratory processes of aquatic animals including crustaceans but at high level both are very toxic to aquatic life [15]. Major source of copper contamination in marine organisms is *via* food chain rather sea water. Thus increasing ambient pollution levels in water do not directly affects the marine life. Copper is considered highly toxic metal after mercury and silver for marine life because the existence of a number of detoxifying and storage systems for Cu [17].

Cadmium is mainly concerned pollutant because it is very much toxic metal to the aquatic organisms. Cadmium is absorbed in excess by human being through sea food and tends to accumulate mainly in liver and kidney. The main source of cadmium contamination along the coastal areas is electroplating and industrial waste because it is an important metal with industrial applications. Results obtained from the sampling years (2011 to 2013) shown that the bioaccumulation levels of Cd in exoskeleton of *Fenneropenaeus penicillatus*, was found higher as compare to the muscles tissues. Concentration of Cd was found higher in exoskeleton as compare to the muscles tissues in each year, range from 3.57µg/g to 5.76µg/g were observed in muscles and 8.02µg/g to13.88µg/g in exoskeleton, also reported in Table **2** and Figure **2c**. The presence of substantial concentrations of cadmium in the exoskeleton can be attributed to the involvement of this tissue in the excretion of these metals [28]. The results were also showed that Cd concentration was much higher as compare to the maximum allowable concentration of Cd in shrimp species for human consumption by WHO i.e. 0.2µg/g [24]. Reported concentration was also exceed form the other suggestible limits by European commission i.e. 0.5µg/g [29] and by FAO i.e. 3µg/g [30]. It is an alarming condition as exceeded concentration of Cd in red tail shrimps in Pakistan has declined the quality of seafood which is not suitable for human consumption.

Pb is a neurotoxin metal that causes many behavioral defects in biotic samples, as a result of which decrease in survival growth rates and metabolism occur. The main source of Pb in the present geographical locale could be the contamination from Ibrahim Hyderi coast. The discharge of industrial waste of Korangi industrial trading estate (KITE) and Gizri Creek causes the increase Pb concentration along the littoral states of Ibrahim Hyderi. In case of Pb, different results were recorded which are reported in Table **2** and Figure **2d**. Concentration of lead was found only in muscles tissues from 0.05µg/g to 0.07µg/g. In the present study, results obtained of Pb concentration were much lower than the recommended values of WHO i.e., 2ppm in prawns for human consumption [24]. These values were also found to be much lower than the suggested limits by international sea food standards i.e. 0.5ppm [31] and by FAO i.e. 1.5µg/g [30].

Analysis of variance (ANOVA) was performed to assess whether heavy metal concentrations varied significantly between the years. The results showed that the concentrations of copper, zinc, cadmium and lead in shrimp samples were not significantly affected during the three years study plan ($p<0.05$). Graphical representation of 1-factor ANOVA for Cu, Zn, Cd and Pb have been presented in the form of box plot given in Figure **3a**, **3b**, **3c** and **3d** respectively.

4. CONCLUSION

The findings of the present study suggest that metal concentrations in *Fenneropenaeus penicillatus* were in the following descending order: Zn>Cu>Cd>Pb in muscles and Cd>Zn>Cu in exoskeleton. The available evidence leads us to postulate that accumulation level of these metals in muscles and exoskeletons were varying in their physiological requirements. Results showed that these metals except Cd are still in their permissible limits set by WHO, FDA or FAO etc. The discharge of industrial waste specially from electroplating processes into the sea may be the reason of high level of Cd. This study will provide the data for future research work, to investigate the serious health risks associated with the *Fenneropenaeus penicillatus* due to metal contamination. It has been found that increasing industrialization and urbanization is producing adverse impact on the commercially important species of shrimp community day by day.

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