

HEAVY METALS CONCENTRATION AND POSSIBLE HEALTH RISKS FROM SHRIMP NURSERIES AT SOUTH-WEST REGION, BANGLADESH

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Abstract: Water pollution poses a threat of heavy metal accumulation in aquatic animals. This study was designed to determine the heavy metals contamination in shrimp PL (post larvae) nurseries of Khulna region and associated human health risks. Water (raw sea water, treated and outlet water) and PL samples of five shrimp nurseries of Khulna districts were analyzed to measure the concentrations of six heavy metals (Zn, Cr, Mn, Cu, Pb and Ni) by using Atomic Absorption Spectrometer (Model No: AA-7000, Shimadzu). The metal concentrations were higher in almost all PL samples of five nurseries except Mn, which value was below detection level (BDL). Concentrations of Zn and Cu were significantly higher ($P < 0.05$) in PL samples than inlet (raw sea water, treated water) and outlet water samples among five nurseries. The highest value for Cr was found in PL sample (2.95 ± 3.64) ppm but the lowest was in outlet water (0.004 ± 0.004) ppm. The highest value of Pb was found in PL samples (3.48 ± 7.78) ppm and the lowest was in treated water sample (0.54 ± 0.09). The Ni concentration was highest in PL samples (1.71 ± 1.98) ppm while the lowest in both raw seawater (0.01 ± 0.005) ppm and outlet water (0.01 ± 0.004) ppm. In some PL samples, the concentrations of Cr, Cu, Pb and Ni were higher than maximum allowable concentration. The estimated daily intake (EDI) of heavy metals in shrimp PL were followed the order $Zn > Cu > Pb > Cr > Ni > Mn$. The target hazard quotients (THQ) of PL samples were followed the rank $Pb > Cu > Zn > Ni > Cr > Mn$. Target cancer risk (TR) of Pb of nursery-2 PL for rural people was 1.1305×10^{-4} while the urban and average TR was 1.4757×10^{-4} and 1.2195×10^{-4} , respectively. The findings of the present study on heavy metal concentrations in PL nurseries indicate the necessities of quality water for prevention of accumulation heavy metals further in shrimp grown-up stages. Proper management strategies should be taken to avoid this risk.

Key words: Heavy metal, shrimp nurseries, inlet and outlet water, shrimp PL, health risks

INTRODUCTION

The major sources of heavy metals are agriculture and industrial activities which can cause pollution of the marine ecosystem and can invade the food chain through bioaccumulation process and thereby leading to human health problem (Jakimska *et al.* 2011). Therefore, heavy metals have been a serious problem to human health and ecosystem globally (Vrhovnik *et al.* 2013).

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Metals from natural and anthropogenic sources may pose a serious threat as a consequence of their toxicity, long persistence, bioaccumulation and biomagnification in the food chain of ecosystem (Papagiannis *et al.* 2004). Thus, residues of heavy metals in polluted habitats may accumulate in microorganisms, fishes, crustaceans another aquatic animals which may enter into the human food chain and cause health problems (Gupta *et al.* 2009). It has been reported that human health is under threat as the concentration of heavy metal in fish and crustaceans at the northern coastline of the Bay of Bengal has increased and exceeded the proposed health advisory levels (Borrell *et al.* 2016).

Shrimp is the second largest export item and because of its commercial high value tiger shrimp is known as 'white gold' of Bangladesh (Ahmed and Diana 2015). Mainly shrimp is cultured in the coastal districts of Bangladesh including Cox's Bazar, Chittagong, Khulna, Bagerhat, Satkhira and adjacent districts (Matin *et al.* 2016). Generally, crustaceans are more sensitive to heavy metals (Ahsanullah *et al.* 1981). Baki *et al.* (2018) analysed heavy metal contamination for Cr, Mn, Cu, Zn, As, Cd, Pb, Hg and Fe in six of the most consumed fish, a shrimp *P. sculptilis*, a lobster and three crabs collected from Saint Martin Island, Bangladesh. Sarker *et al.* (2016) assessed heavy metals contamination and health risk in shrimp (*Macrobrachium rosenbergii* and *Penaeus monodon*) collected from Khulna-Satkhira region in Bangladesh. Besides, bioavailability of heavy metals in marine invertebrates is influenced by extrinsic factors such as metal speciation, salinity and temperature variation etc. and by intrinsic or biological factors like size, age, diet, and sex of the organisms (Pourang and Amini 2001, Barrento *et al.* 2009). Very limited number of studies have been performed to estimate the heavy metal accumulation in tiger shrimp of Bangladesh. Still there is lack of information regarding heavy metal concentration assessment at different life cycle stages of tiger shrimp. Therefore, the aim of the study was to determine the heavy metal concentrations of the inlet and outlet water of shrimp PL rearing nurseries, and shrimp PL samples as well; and to undertake a health risk assessment of heavy metals consumption via shrimp.

MATERIAL AND METHODS

Study site: Khulna is one of the important shrimp farming area of south-west regions of Bangladesh. Many shrimp PL nurseries are located in this district to supply PL among nearby shrimp farmers. Therefore, five different nurseries situated in Dacope Upazila of Khulna were randomly selected to perform the present study (Fig. 1).

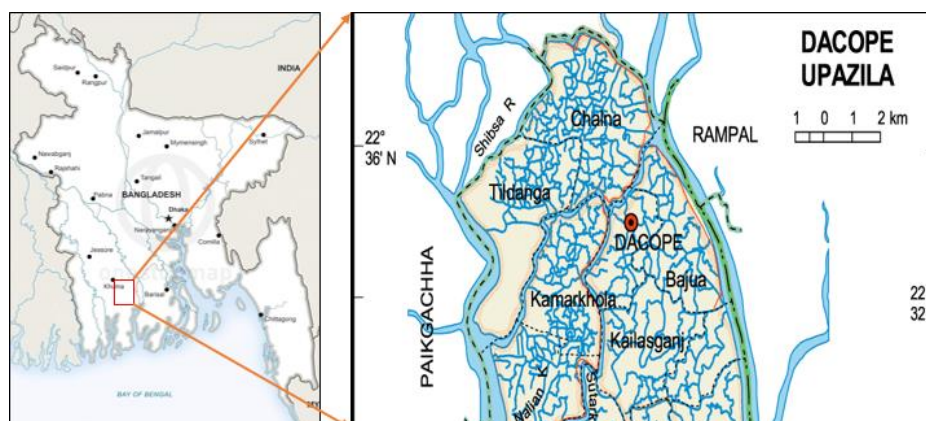


Fig. 1. Map showing the sample collection site of Dacope Upazila under Khulna district.

Usually, shrimp hatchery and nurseries get their containment water for PL production from the sea. After necessary disinfection, they used the water in hatchery and nursery operation. Therefore, four types of samples *viz.* shrimp PL and three kinds of water samples (raw sea water which had been collected from Bay of Bengal by the nursery, treated sea water and outlet water) were collected from each of five nurseries.

Collection of samples: Selected nurseries has been addressed in the study as nursery-1, nursery-2, nursery-3, nursery-4 and nursery-5. Water samples were collected in sterile plastic water bottles and kept at 4°C until further study. PL samples (about 1000 pieces of PL) were collected using scoop net and transferred to contamination free zipper bags. Then the samples were carried to the laboratory for further analysis. Authority of the nursery-2 did not allow to collect outlet water. Total 19 samples of water and shrimp PL were collected from five different nurseries.

Water sample preparation: In the laboratory, at first, water samples were filtered through Whatman 44 filter papers and then transferred to 100 ml plastic bottles. One ml nitric acid (HNO_3) was added to each plastic bottle and it was kept for 3 days to remove all the organic matter.

Shrimp PL sample preparation: Weighted PL samples were put into microwave oven for drying at 60°C for 48 hours. Dried samples were grinded in mortar pestle. Minimum 0.5g of samples were taken in 50 ml beakers. 10 ml HNO_3 was added in each beaker. A blank sample was prepared for calculation. Then the samples were put on hot plate at 60°C for digestion under fume hood. When the concentration became low, 5 ml H_2O_2 was added in each sample.

Finally, the mixture was placed under fume hood on hot plate at 120° C until it was clear. After that, the solution was diluted to 25 ml with deionized water. Then the solution was filtered and preserved into clean sterile plastic bottles for heavy metals determination in the samples.

Heavy metals determination: Six heavy metals (Zn, Cr, Mn, Cu, Pb and Ni) concentration were determined through Atomic Absorption Spectrometer (AAS) (Model No: AA-7000, Shimadzu) using following formula:

Concentration of heavy metals = (Reading – Blank reading) ×PDF×SDF

Where, Primary Dilution Factor (PDF) = $\frac{\text{Volume}}{\text{Weight of sample}}$

Secondary Dilution Factor (SDF) = $\frac{\text{Secondary Volume}}{\text{Secondary Weight of sample}}$

Potential human health risk assessment: Shrimp PL is not consumable product. However, the measured metal concentration in shrimp PL will not remain same in the farmed consumable shrimp rather increase during culture period. Therefore, it is logical to assess the human health risk associated with the consumption of shrimp containing harmful metal concentrations. Potential human health risks were assessed following Baki *et al.* (2018).

Estimated Daily Intake (EDI): Estimated Daily Intake (EDI) of metals in shrimp PL was predicted by using metal concentrations in shrimp PL, daily intake (rural, urban and total) and body weight. Following equation was utilized for EDI measurement.

Estimated daily intake (EDI) = $\frac{\text{FIR} \times \text{C}}{\text{BW}}$

Where, FIR = Fish/Shrimp intake rate (Rural: 45.8 g/person/day; Urban: 59.9 g/person/day; on average: 49.5 g/person/day), C = Heavy metal concentration in Shrimp (ppm), BW = Average body weight (60 Kg).

*Target Hazard Quotients (THQ):*In this investigation, the non-cancer-causing health threats related with the ingestion of shrimp species by the nearby occupants (low, medium and high fish consumers) were analyzed by Target Hazard Quotients (THQs) using the standard supposition for a coordinate USEPA risk examination.

Target Hazard Quotients (THQ) = $\frac{\text{EFr} \times \text{ED} \times \text{FIR} \times \text{C}}{\text{RfD} \times \text{BW} \times \text{AT}} \times 10^{-3}$

EFr = Exposure Frequency (365 days/year), ED = Exposure Duration (70 years), FIR = Fish/Shrimp Ingestion Rate (Rural: 45.8 g/person/day; Urban: 59.9 g/person/day; on average: 49.5 g/person/day), C = Heavy metal concentration in Shrimp (ppm), BW = Average Body Weight (60 Kg), AT = average exposure time for non-carcinogens (EF×ED) (365 days/year for 70 years), RfD = The oral

reference doses were based on 1.5, 0.02, 0.04, 0.0005, 0.3 and 0.0035 mg/kg/day for Cr, Ni, Cu, Cd, Zn and Pb, respectively.

Target cancer risk (TR): As per USEPA (1988), for cancer-causing agents, risks were evaluated as the steady likelihood of a person to create malignant growth of cancer over a lifetime introduction to that potential carcinogen. Target cancer risk (TR) was determined by utilizing following equation (Baki *et al.* 2018)

$$TR = \frac{EFr \times ED \times FIR \times C \times CSf_0}{BW \times AT} \times 10^{-3}$$

Where, CSf₀ is the oral carcinogenic factor. (CSf₀ of Pb = 8.5 × 10⁻³) (USEPA 2011).R of Pb was determined for PL samples and the samples were designated as YPL1, YPL2, YPL3, YPL4 and YPL5 representing five nurseries.

Statistical analysis: The data were analyzed using the statistical software, SPSS 20.0 and the graphs were made by using MS Excel 2013.

RESULTS AND DISCUSSION

Estimation of heavy metal concentrations: Concentrations of the six heavy metals (Zn, Cr, Mn, Cu, Pb and Ni) were estimated in all types of collected samples from shrimp nurseries. The concentration of Mn in all samples were below detection limit (BDL). The value of the other five heavy metals concentrations in each individual sample from five different nurseries are presented in following graphs (Fig. 2-6).

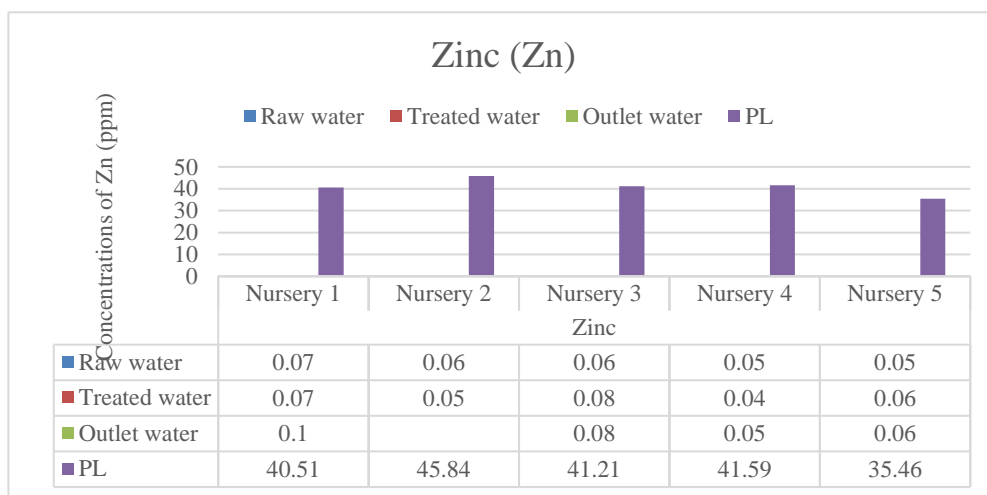


Fig. 2. Concentrations of Zn in four types of samples (raw seawater, treated water, outlet water and PL sample) collected from five different nurseries.

Highest Zn concentration was observed in PL sample of nursery-2 while the lowest concentration was observed in outlet water of nursery-4 (Fig. 2).

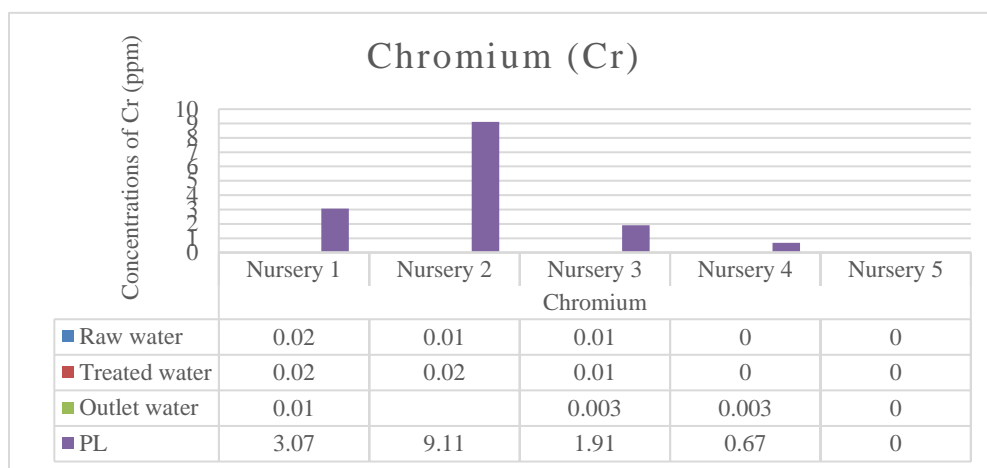


Fig. 3. Concentrations of Cr in four types of samples (raw seawater, treated water, outlet water and PL sample) collected from five different nurseries.

Highest Cr concentration was observed in PL sample of nursery-2 while the lowest concentration was observed in all samples of nursery-5. They were all below detection level (Fig. 3).

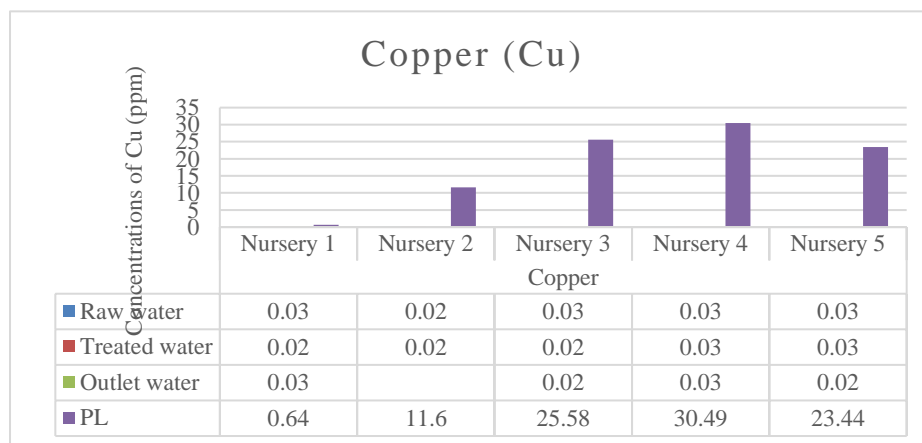


Fig. 4. Concentrations of Cu in four types of samples (raw seawater, treated water, outlet water and PL sample) collected from five different nurseries.

Highest Cu concentration was observed in PL sample of nursery-4 while the lowest reading was observed in raw water of nursery-2, treated water sample of nursery-1, 2 & 3 and outlet water sample of nursery-3& 5 (Fig. 4).

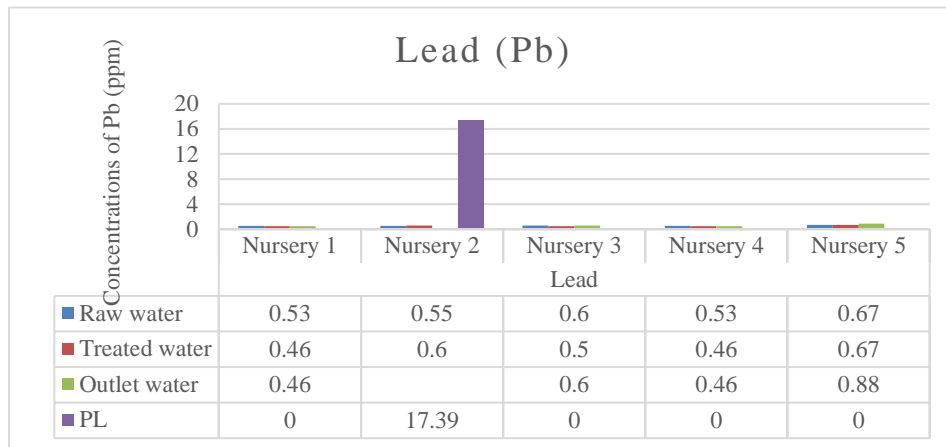


Fig. 5. Concentrations of Pb in four types of samples (raw seawater, treated water, outlet water and PL sample) collected from five different nurseries.

Highest Pb concentration was observed in PL sample of nursery-2 while the lowest one was observed in PL sample of other 4 nurseries (Fig. 5).

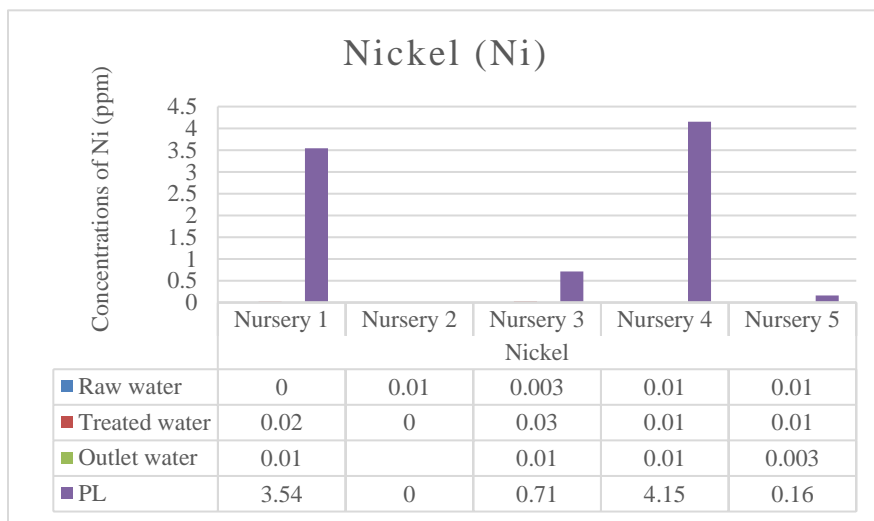


Fig. 6. Concentrations of Pb in four types of samples (raw seawater, treated water, outlet water and PL sample) collected from five different nurseries

Highest Ni concentration was determined in PL samples of nursery-4 while lowest concentration was found in raw water of nursery-1, treated water and PL sample of nursery-2. They were all below detection level (Fig. 6).

Table 1. Heavy metals concentrations (Mean \pm SD) in four types of samples among five nurseries of Khulna district

Heavy metals	Concentration of heavy metals (ppm)			
	Raw seawater	Treated water	Outlet water	PL of Shrimp
Zn	0.06 \pm 0.01 ^a	0.06 \pm 0.01 ^a	0.07 \pm 0.02 ^a	40.92 \pm 3.7 ^b
Cr	0.01 \pm 0.01 ^a	0.01 \pm 0.01 ^a	0.004 \pm 0.004 ^a	2.95 \pm 3.64 ^a
Mn	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Cu	0.30 \pm 0.004 ^a	0.024 \pm 0.005 ^a	0.025 \pm 0.005 ^a	18.35 \pm 12.09 ^b
Pb	0.56 \pm 0.05 ^a	0.54 \pm 0.09 ^a	0.60 \pm 0.20 ^a	3.48 \pm 7.78 ^a
Ni	0.01 \pm 0.005 ^a	0.014 \pm 0.01 ^a	0.01 \pm 0.004 ^a	1.71 \pm 1.98 ^a

(ANOVA, $P < 0.05$; *Different letters superscript (a, b) showed significant difference)

Table 1 shows the means and standard deviations of all six metals concentration of five nurseries of Khulna region. The highest value of Zn was observed in PL samples (40.92 \pm 3.7ppm) while the lowest was in outlet water (0.07 \pm 0.02ppm) after raw seawater (0.06 \pm 0.01ppm) and treated water (0.06 \pm 0.01ppm). There was significant difference between PL samples and water samples in Zn concentration. The highest value of Cr was found in PL sample (2.95 \pm 3.64ppm) but the lowest one was observed in outlet water (0.004 \pm 0.004ppm). The concentrations of Cr for both raw seawater and treated water were same (0.01 \pm 0.01ppm). For Cu, the lowest value was observed in treated water (0.024 \pm 0.004ppm) after outlet water (0.027 \pm 0.003ppm) and raw seawater (0.30 \pm 0.004ppm). The highest concentration of Cu was found in PL samples (18.35 \pm 12.09ppm) and there was significant difference between Cu concentration of PL samples and water samples. The highest value for Pb was observed in PL samples (3.48 \pm 7.78ppm) while the lowest was in treated water (0.54 \pm 0.09ppm) after raw seawater (0.56 \pm 0.05ppm) and outlet water (0.60 \pm 0.20ppm). There was no significant difference between PL samples and water samples. Lastly the highest value for Ni was observed in PL samples (1.71 \pm 1.98ppm) while the lowest was observed in both raw seawater (0.01 \pm 0.005ppm) and outlet water (0.01 \pm 0.004ppm) after treated water (0.014 \pm 0.01ppm). There was no significant difference in concentration of Ni between PL samples and water samples. The Zn concentrations of all samples did not exceed the reference value of EU/EC for Zn (50 ppm) according to EU (2006). On the other hand, the Cr concentrations of PL (nursery-1, nursery-2) was higher than the maximum allowable concentration (MAC)(FAO/WHO 2002).

The Cu concentration of PL samples (nursery-2, nursery-3, nursery-4 and nursery-5) exceeded the MAC of 4.5 ppm. For Pb, all raw water samples of five nurseries, treated water sample (nursery-2, nursery-3 and nursery-5), outlet water samples (nursery-3 and nursery-5) and PL sample of nursery-2 exceeded the MAC of 0.5 ppm (FAO/WHO 2002). Finally, the Ni concentrations of PL samples (nursery-1 and nursery-4) was far more than MAC of 0.8 ppm (FAO/WHO 2002).

Bioaccumulation of Zn, Pb, Cd and Cu was observed in the flesh, liver and gill of benthic and pelagic fishes collected from Lake Geriyo during two seasons (Bawuro *et al.* 2018). Rashid *et al.* (2015) observed the heavy metals in sea water, sediment and the toxic effects on sea shells, oysters along the east coast of the Bay of Bengal. The levels of heavy metals in sea sediments were ranged followed by Fe > Zn > Ni > Cr > Pb > Cd and the concentrations of Cu, Fe, Cd and Pb in sea water were higher than standard concentrations. The study also showed that Zn, Cu, Fe, Pb and Mg levels in sea shells and oysters were toxic and their growth were reduced. Cheung and Wong (2006) carried out a research in Mai Po Nature Reserve, Hong Kong and bioaccumulation of heavy metals in fish and shrimp including tilapia, grey mullet (*Mugil cephalus*), Gei wai shrimp, and caridean shrimp. They determined that the Zn, Cr, Cu, Ni and Cd levels were higher than the standard concentrations set by Food Regulations of Hong Kong. Sarkar *et al.* (2016) observed Pb concentrations (0.52-1.16 ppm) in all farmed *M. rosenbergii* and *P. monodon* samples of Khulna-Satkhira region were generally higher than recommended level.

Health risk assessment

Estimated Daily Intake (EDI): EDI was assessed for the heavy metal (Zn, Cr, Mn, Cu, Pb and Ni) concentrations of PL samples as they will be considered edible after certain time of culture period (Table 2).

The estimated daily intake (EDI) of shrimp post larvae were determined by using the mean concentration of each metal. For rural, urban and average EDI the ranking order was Zn>Cu>Pb>Cr>Ni>Mn (Table 2).

Target Hazard Quotients: Target hazard quotients of Zn, Cr, Mn, Cu, Pb and Ni were determined for the PL samples of 5 nurseries of Khulna district (Table 3).

Table 2. Estimated daily intake of PL samples for Zn, Cr, Mn, Cu, Pb and Ni.

Heavy metals	Estimated Daily Intake (mg/kg-body weight/day)		
	Rural	Urban	Average
Zinc	31.24	40.85	33.76
Chromium	2.25	2.95	2.43
Manganese	0	0	0
Copper	14.01	18.32	15.14
Lead	2.66	3.47	2.87
Nickel	1.31	1.71	1.41

Table 3. Target hazard quotients for Zn, Cr, Mn, Cu, Pb and Ni in PL samples

Heavy metals	Target Hazard Quotients		
	Rural	Urban	Average
Zn	0.104	0.136	0.113
Cr	0.002	0.002	0.002
Mn	0.000	0.000	0.000
Cu	0.350	0.458	0.378
Pb	0.759	0.993	0.820
Ni	0.065	0.085	0.071

The target hazard quotients (THQ) of PL heavy metal samples were determined with the mean concentrations of metals. The ranking order for rural, urban and average THQ was Pb>Cu>Zn>Ni>Cr>Mn (Table 3). Saha *et al.* (2016) investigated ten metals (Cd, As, Co, Zn, Cr, Mn, Pb, Se, Ni and Cu) from ten fish species from Bay of Bengal during four seasons. The highest THQ value was determined for Pb (0.993) but the minimum value was found for Mn (0) in that study. No value exceeded 1 so they considered each fish species was safe for human consumption. In this study, no value was greater than 1 so it can be said that there is no risk for considering the PL samples.

Target cancer risk: Carcinogenic risk for lead (Pb) was determined for all PL samples of five nurseries. They are given below (Table 4). Target cancer risk or TR was determined for Pb in PL sample of nursery-2 only. On the other hand, the target cancer risk of Pb was not measured for other PL samples as the concentration was below detection level. The rural TR of Pb of nursery-2 PL was 1.1305×10^{-4} while the urban and average TR was 1.4757×10^{-4} and 1.2195×10^{-4} ,

Table 4. Carcinogenic risk (TR) for Pb in PL sample of all five nurseries

FIR	Carcinogenic risk for Pb in PL samples of five nurseries				
	YPL1	YPL2	YPL3	YPL4	YPL5
Rural	0	1.1305×10^{-4}	0	0	0
Urban	0	1.4757×10^{-4}	0	0	0
Average	0	1.2195×10^{-4}	0	0	0

respectively. Arsenic (As) and lead (Pb) is known as carcinogenic metal according to USEPA. The target cancer risk (TR) were determined as these metals may cause both carcinogenic and non-carcinogenic effects based on their exposure dose. Mainly the risks lower than 10^{-6} are marked as safe for human consumption. But cancer risks greater than 10^{-4} are marked as unacceptable (USEPA 1988,2011) while the risks between 10^{-6} and 10^{-4} are considered acceptable generally. In this study, none of the value exceeded 10^{-4} and can be considered safe. Ezemonye *et al.* (2019) evaluate the health risk consequences of consumption of heavy metal-contaminated water, shrimp and fish from Benin River in Nigeria. They observed that Ni was the most dominant heavy metal in water, while Fe was the most dominant in shrimp and fish and concentration in water were below recommended limit set by World Health Organization (WHO) and Standard Organization of Nigeria (SON) except for cadmium (Cd), nickel (Ni) and lead (Pb). They also found that THQ for heavy metals in water (oral exposure) and consumption of fish were above threshold value of 1 indicating potential health risk.

In this study, health risks were assessed for shrimp PL although the PL are not consumable item but the PL shrimp will eventually be cultured and human will consume those. Therefore, the metal accumulation in shrimp PL are also harmful for human. After analysis of the heavy metal concentrations and health risk consequences, it can be said that the shrimp PL nurseries are at risk of heavy metal pollution. Future research and regular monitoring are required to evaluate the heavy metal pollution in shrimp hatcheries, nurseries and farms across the country.

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