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Assessment of Heavy Metals Concentration in the Tissue of Black Clam (*Villorita cyprinoides*) (Gray, 1825) from Aquaculture farms of Ezhikkara Village in Kerala, India

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Abstract

Upsurges of heavy metal pollution in aquatic ecosystem have affected the global aquaculture sector in different ways. The present study is focused on the assessment of heavy metals in Ezhikkara aquaculture farms in Ernakulam district, Kerala where Vembanadu Lake and the Periyar River are the major water sources. In Ezhikkara aquaculture farm the level of heavy metals were ranked as: sediment>black clam tissue>water. Biota sediment accumulation factor (BSAF) value and Bioaccumulation factor (BAF) values illustrate that water is the major source of accumulation of heavy metals in the clam tissue. Hg and Cd posed very high and moderate potential ecological risk (E^i_r) respectively, during the study time in Ezhikkara aquaculture farms. The recorded concentrations of As, Cd, Cr, Pb and Hg (0.08 ppm, 0.05 ppm, 4.37 ppm, 0.15 ppm and 0.06 ppm, respectively) in clam tissue were lesser than the permissible limits proposed by the EIC of India but were higher than the limits prescribed by WHO guidelines.

Keywords: Heavy metal, bioaccumulation, biomagnification

Introduction

The concentration of heavy metals proliferates in the environment due to anthropogenic activities, especially in aquatic ecosystems. Upsurges of heavy metal pollution in aquatic ecosystem is a dynamic problem worldwide (Malik et al., 2010). Heavy metals in the water, dumped without any treatments, polluted the water bodies and also accumulate in the different body parts of aquatic living organisms like fin fishes, shellfishes and seaweeds which form major part of human diet (Iyengar, 1991). Higher concentration of toxic heavy metals present in the diet such as fish and shell fish negatively affect human health with continued consumption (Castro et al., 2008; Reilly, 1991).

The aquaculture farms are exposed to many chemical, biological and other pollutants. Toxic effects of the heavy metals cause problems in aquaculture also. The concentration of metals in water positively correlates with the concentrations of metals in aquatic organisms (Castro et al., 2008).

The present study is focused on the heavy metal pollution in Ezhikkara aquaculture farms in Ernakulam district. Ezhikkara is one of the major aquaculture villages having a farming method based on the traditional polyculture of fishes, crustaceans and mollusc in the Pokkali fields. The area under study includes the prawn cultivating aquaculture farms in Ezhikkara, which are facing numerous problems, including flooding, salinity intrusion, water quality deterioration, weed infestation, diseases, water pollution etc. These farms are part of Vembanad wetland system, is lying below the mean sea level, characterized by a vast area of fertile agricultural and aquaculture spots. Water from Periyar also these farms through reaches different channels. Vembanadu Lake and the Periyar River are the two main water sources of Ezhikkara aquaculture farms and they are contaminated by industrial pollution.

Black clam (*Villorita cyprinoids*) is one of the most common food item of the local people of Ernakulam district and is a very good source of minerals such

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as calcium and other important nutrients. Black clams could be the bio-indicator species of heavy metal pollution in the farms of Ezhikkara since they are filter feeding organisms, feeding on detritus matter in sediment.

Materials and Methods

Ezhikkara (latitude10.14 N and longitude 76.23 E) is a village in Ernakulam district, Kerala, India. The village (1301 hectare) is surrounded by lagoons and pokkali farms. Ezhikkara is famous for its Pokkali rice cultivations and fresh water prawn farms.

Samples of live edible black clams (*Villorita cyprinoides*), sediments and water were collected bimonthly from selected random spots of aquaculture farms in Ezhikkara village during July, 2015 to December, 2015 in clean plastic bottles. Based on the method of APHA, (1980) and APHA, (2005) the pre-treatments of samples were carried out. Water sample was pre-treated using chloroform with the help of chelating agents (APDC and DDDC) in 1000 ml separating funnel. After the complete transferring of the metal ion to the organic layer from the water sample, shaken it with concentrated HNO_3 and milli Q water to separate polar layer using small separating funnel and collected in a 25 ml standard flask. It was sonicated to evaporate the chloroform layer and made up to the mark. Pre-concentrated samples were stored in plastic bottles.

Dried, pulverized and sieved sediment samples were taken in digestion tubes and digested with HNO_3 and HCl in the ratio 1:3 at 700°C in heating digestion furnace. The process was repeated up to the complete digestion of sediment particles. Evaporated samples were diluted using 2% HNO_3 in plastic bottles. Cleaned and macerated tissue samples were digested with concentrated HNO_3 and $HClO_4$ in the ratio 1:4 using a heating digestion furnace at 700^0C until all organic matter had been destroyed. After evaporation of HNO_3, the residual solution was diluted to 25 ml with milli Q water and stored in plastic bottles.

Metallic elements such as cadmium (Cd), chromium (Cr), copper (Cu), arsenic (As), mercury (Hg), lead (Pd) and zinc (Zn) in pre-treated samples were determined using flame atomic absorption spectroscopy (PerkinElmer, AAnalyst 200,version 8.0, 2013). The metallic elements such as As and Hg were determined using the mercuric hydride system-atomic absorption spectroscopy. All samples were analyzed in triplicate as per standard conditions. The operating parameters for working elements were set as recommended by manufacturer. The blank and calibration standard solution were also analyzed in the same way as for the samples.

Significant differences in the concentration of metals among the water, sediment and tissue samples were noticed (ANOVA test using the SAS software). According to Szefer et al. (1999) and Abdallah et al. (2008) bio-sediment accumulation factor (BSAF) of mollusc tissue was calculated as.

$$BSAF = C_x/C_s$$

where C_x is the mean concentration of heavy metals in organism and C_s is the mean concentration in sediment.

Rates of bioaccumulation factors of fish were calculated by means of the formula suggested by Gobas and Morrison, (2000) as shown below.

$BAF = C_x/C_w$, where C_w is the concentration of heavy metals in water

Ecological risk was assessed using risk indexing method proposed by Hakanson, (1980).

$$R_I=\Sigma E_r^i$$

$$\Sigma E_r^i=T_r^i C_f^i$$

$$C_f^i=C_o^i/C_n^i$$

where R_I is the calculated sum of all risk factors for heavy metals in sediment, E_r^i is the monomial potential risk factor and T_r^i is the toxic response factor for a given substance. C_f^i is the contamination factor, C_o^i is the concentration of metal in the sediment and C_n^i is a reference value for metals (Hakanson, 1980).

Results and Discussion

Results of the present investigation pertaining to the mean concentration of heavy metals in the water, sediment and the clam tissue samples are presented in Table 1 and calculated value of ecological risk index, BSAF value and BAF value are presented in Table 2.

In Ezhikkara aquaculture farms, the levels of heavy metals were ranked as: sediment> black clam tissue> water. The concentration of heavy metals except As in the sediment is higher than the heavy metals

Table 1. Mean concentration (ppm) of heavy metals present in water, sediment and clam tissue samples of Ezhikkara aquaculture farm

HEAVY METAL	Concentration of heavy metals in ppm (Mean ±SD)		
	Water	Sediment	Clam tissue
Arsenic	0.001±0.001	0.017±0.023	0.083±0.082
Cadmium	0.023±0.013	0.297±0.347	0.053±0.045
Copper	0.056±0.032	1.408±0.404	0.327±0.357
Chromium	0.447±0.104	6.290±1.865	4.372±1.197
Lead	0.072±0.013	1.170±0.613	0.155±0.111
Mercury	0.046±0.024	0.154±0.091	0.069±0.039
Zinc	0.256±0.044	4.053±0.251	3.119±0.241

present in the water. In water, concentrations of heavy metals were much lower than the sediment and clam tissue. Naminga & Wilhm (1976) have reported similar result that is the presence of heavy metal in water is very low and achieve significant changes in sediment and biota. According to the Yi et al. (2008), heavy metals in water do not degrade but are generally not found in high concentrations, primarily due to deposition in sediments and also because of uptake by plants and animals. The accumulation of metals in water and sediments affects various organisms in the environment, influencing their functions in several different ways (Regoli & Principato 1995). Intermediate levels of heavy metals were present in the tissue of the black clams collected from Ezhikkara farms. Gao, (2010) has reported that the heavy metals contained in the sediment are then absorbed and stored in the tissues of aquatic organisms. It is well known that molluscs and crustaceans accumulate organic and metallic pollutants at concentrations several orders of magnitude above those observed in the field environment (Bryan et al., 1983; Rao et al., 2016; Ashraf et al., 2007; Zynudheen & Ninan, 2007).

The concentration of heavy metals (As, Cd, Cu, Cr, Pb, Hg and Zn) present in Ezhikkara aquaculture farm is positively correlated to concentration of heavy metals in the sediment and the concentration of heavy metal in the clam tissue and there is significant difference between the concentration of each metal present in the sample of water, sediment and clam tissue.

The Bio-sediment Accumulation Factor (BSAF) was calculated to evaluate the efficiency of metal uptake by the clams and to describe the accumulation of the

Table 2. Ecological risk index, BSAF value and BAF value of the heavy metals present in Ezhikkara aquaculture farm.

Heavy metal	E^r_i	BSAF	BAF
Arsenic	0.011	4.917	56.885
Cadmium	17.833	0.178	2.265
Copper	0.235	0.233	5.885
Chromium	0.210	0.695	9.786
Lead	0.234	0.133	2.172
Mercury	30.88	0.449	1.525
Zinc	0.051	0.769	12.166

metals studied. From BSAF values, the metal concentrations in the tissues of clams were ranked in the order As>Zn>Cr> Hg >Cu>Cd >Pb. According to Dalliger (1993) BSAF value of metal more than 2 indicates that it is a macro-concentrator and BSAF value between 1 and 2 indicates that it is a micro-concentrator. BSAF value of arsenic (As) 4.91 indicates that clam tissue is a macro-concentrator of As. Higher concentration of arsenic in clam tissue than water and sediment is due to the presence of organic arsenic. Organic (arseno-sugars, arsenolipids, arsenobetaine, arsenocholine) and inorganic (arsenite and arsenate) compounds of arsenic have been identified in the tissues of bivalves and crustaceans (Francesconi et al., 1993 and Edmonds et al., 1997). According to Phillips et al. (1985) and Kaise et al. (1985) the organic forms of the element present in marine organisms are much less toxic. Chapman (1926) and Foa et al. (1984) reported that the arsenic ingested with seafood by man is rapidly cleared in the urine.

All the metals except arsenic have BSAF value less than 1 and this shows that clams in Ezhikkara farms are very poor accumulators of heavy metals (Zn, Hg, Cr, Cd, Cu and Pb). Thus the contamination levels of these metals in the farm do not exceed the clams' capacity to regulate them. The concentration of heavy metals such as Zn, Hg, Cr, Cd, Cu and Pb in the sediment is very high but bio availability of these metals is very little. According to Wang et al. (2002) the variations in the bioavailability of different metals are determined by the interactions between metal geochemistry and animal physiology. The high BASF value of arsenic indicates that the black clams in Ezhikkara farms were the macro accumulator of arsenic. Arsenic contamination levels in clams were found to be higher than the sediment, suggesting the higher rate of accumulation in black clams.

Heavy metals released into environment have potential to change and damage life activities by accumulating in the organisms (Hu, 2000; Gough et al., 1979) and these metals can be easily transferred to high trophic organisms through food chain (Dietz et al., 2000). In higher organisms, accumulated metals tend to biomagnify depending on the type of metal and the species (Hopkin, 1990; David et al., 2012). In clam tissue collected from Ezhikkara aquaculture farms the bioaccumulation rates were determined as As>Zn>Cr>Cu>Cd>Pb>Hg. All metals present in Ezhikkara farm showed very high BAF indicating that the black clams are highly capable of accumulating heavy metals from water. Bioaccumulation factor is different for different metals in different seasons. Bioaccumulation of heavy metals in the tissue of black clam depends on the type of heavy metals and season of farming and the farm conditions. Clam is a natural feed for many higher organisms in the aquatic systems. In river, fish are often at the top of the food chain and have the tendency to concentrate heavy metals from water (Yilmas et al., 2007; Zhao et al., 2012). The ecological needs, metabolism and feeding patterns of fish and also the season in which studies are carried out influence the metal accumulation (Mansour & Sidky, 2002; Wright & Welbourn, 2002; Ruelas-Inzunza & Paez-Osuna, 2008).

Mercury (Hg) posed the highest potential ecological risk (E^i_r and R_I) during the study in Ezhikkara aquaculture farms. Cadmium posed moderate ecological risk and other metals showed very less ecological risk. The potential ecological risk indices for single regulators (E^r_i) indicated that the harshness of pollution of the seven heavy metals decreased in the following sequence: Hg> Cd >Pb> Cu >Cr > Zn >As and R_I indicated that the Ezhikkara farms during the experimental period are at moderate ecological risk.

Heavy metals such as arsenic, cadmium, copper, chromium, lead, mercury and zinc were contained in the tissue of black clam collected from the Ezhikkara farm. To assess the public health risk of farm product of Ezhikkara, the concentration of heavy metals present in the biomonitor species, black clam tissue, were compared with the maximum permissible limit for human consumption (MPL) stipulated by Export Inspection Council of India (As- 75 ppm, Cd-3 ppm, Cr-12 ppm, Hg-1ppm, Pb- 1.5 ppm) and WHO (As- 0.01 ppm, Cd- 0.003 ppm, Cu-2.25 ppm, Cr-0.05 ppm, Pb-0.01 ppm, Hg-0.001 ppm and Zn- 5 ppm).

The concentrations of all tested heavy metals were found less than the maximum residual level agreed by the Export Inspection council of India. The concentration of biologically essential metals like Cu and Zn were found to be present below the permissible limits in the clam tissue during the experiment period as per the prescribed standards of WHO. The concentration of other analysed metals such as arsenic, cadmium, chromium, lead and mercury were higher than permissible limits of WHO but lower than the MPL prescribed by EIC.

The presence of As, Cr and the Hg concentration in the clam tissue at levels higher than the MPL suggested by WHO and FAO may cause dangerous health issues in human beings. Jack et al., (2003) reported that the arsenic is a carcinogen to both humans and animals. Arsenic has been associated with cancers of the skin, lung, and bladder. Clinical manifestations of chronic arsenic poisoning include non-cancer end point of hyper- and hypo-pigmentation, keratosis, hypertension, cardiovascular diseases and diabetes. It has been estimated that tens of millions of people are at risk of exposure to excessive levels of arsenic from both contaminated water and arsenic-bearing coal from natural sources. Excess dietary chromium intake may lead to serious health problems. Pellerin et al. (2000) reported that the Cr is a carcinogen and that it can cause other deleterious health effects including kidney and liver damage. Tchounwou et al. (2003) have reported that the consumption of mercury in food will cause diseases and mortality in human population. Countries such as Japan, Iraq, Ghana, the Seychelles, and the Faroe Islands have faced such epidemics. The unbearable effects of mercury poisoning include neurotoxicity particularly in children, central nervous system defects, erethism as well as arrythmias, cardiomyopathies, kidney damage and respiratory failure.

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