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EVALUATION OF HEAVY METAL CONTENTS AND ITS NOXIOUS EFFECTS ON BIOCHEMICAL CONSTITUENTS OF *PENAEUS MONODON* (FABRICIUS, 1798) OF ASHTAMUDI LAKE, KERALA, SOUTH INDIA

LEKSHMI PRIYA V [1]* AND E. SHERLY WILLIAMS[2]

[1, 2] *Environmental Sciences, Aquaculture and Fish Biotechnology Unit, Department of Zoology, Fatima Mata National College (Autonomous), Kollam, Kerala. Research Centre, University of Kerala.*

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ABSTRACT

***Penaeus monodons* were collected from three sites of Ashtamudi Lake for one year. Elemental analysis of Cadmium, Chromium, Copper, Lead and Zinc were done in the muscle samples of *Penaeus monodon* as described by APHA. Biochemical constituents such as carbohydrate, protein fat, moisture and ash contents were analyzed in tissues using standard methods. Significant differences between heavy metals concentrations and biochemical constituents of the candidate species from various sites, determined using One- Way analysis of variance (ANOVA) followed by Fisher's LSD post hoc test. The results revealed that samples collected from polluted sites have more heavy metal contents when compared with the reference site. Due to the noxious effects of heavy metals, significant reduction in the biochemical constituents has been analyzed in the samples collected from the polluted sites.**

KEY WORDS: Heavy metals, Pollution, *Penaeus monodon*, Muscle, Biochemical constituents.

INTRODUCTION

Contamination of aquatic environment especially due to heavy metal pollution has become a serious problem of recent years. Heavy metal pollution plays a striking role in decline in quality of water, sediments, and aquatic organisms, which in turn seriously affect the overall ecological status of the system. The second largest backwater lake in Kerala Ashtamudi Lake, is prone to several kinds of toxic aquatic pollutants especially heavy metals from various sources. Significant input of heavy metals into Ashtamudi Lake from the nearby industrial effluents and from other sources have been reported earlier (Razeena *et al.,* 2014; Sherly *et al.,* 2015; Suma *et al.,* 2012). Studies on the bioaccumulation of various pollutants in different organs of fishes of Ashtamudi Lake have been extensively studied by many. Some of the recent work includes those of Lekshmi and Sherly (2018); Sherly *et al.,* (2015) and Razeena *et al*., (2014).

A commercially important shell fishes of Ashtamudi lake, *Penaeus monodon* (Fabricius, 1948) were selected for the present study. *Penaeus monodon*, commonly known as the giant tiger prawn or Asian tiger shrimp, second most widely cultured prawn species in the world is widely reared for food. In 2010, Greenpeace added *Penaeus monodon* to its seafood red list – "a list of fish that are commonly sold in supermarkets around the world, and which has a very high risk of being sourced from unsustainable fisheries". Biochemical composition, such as protein, amino acids, lipid, fatty acids, carbohydrate, fiber and ash determines the nutritive values of crustaceans (Immaculate and Jamila, 2015). Many studies have proved that increase in the concentration of heavy metals significantly reduces the biochemical constituents of the tissues of *Penaeus monodon* (Azza *et al.,* 2014; Cicik and Engin, 2005; Snehalata *et al.,* 2001). The main aim of the present

study were to determine the status of selected heavy metals and thereby its correlating effects on the biochemical constituents of the muscles of *Penaeus monodon* from three sites of Ashtamudi Lake.

MATERIALS AND METHODS

Kureepuzha, Perumon and West Kallada regions of Ashtamudi Lake were selected as site 1, 2 and 3 respectively for the present study. The effluents from Parvathy Mills, Milma Dairy, KSRTC workshop, municipal waste dump site and many small scale industries are the major sources of heavy metals in this region (Lekshmi Priya and Sherly, 2018; Girish Kumar, 2016; Razeena *et al.,* 2014). The Aluminum Industries Ltd., Kerala Ceramics Ltd., Kerala Electrical and Allied/Engineering Company and Techno Park are the major industries discharging effluents at Site 2. (Lekshmi priya and Sherly, 2018; Girish Kumar, 2016). The region of West Kallada lake is not much disturbed with anthropogenic interferences and urbanization, is selected as the third and reference site (Lekshmi priya and Sherly, 2018).

Penaeus monodon of about about 5 to 10 cms long and 6 to 18 gm weight (120 numbers) were collected from each study site for one year. The analysis of the heavy metals Cadmium, Chromium, Copper, Lead and Zinc were done using an atomic absorption spectrophotometer (AAS, Pinnacle 900H) as described by APHA (1998). Metal concentrations were calculated in mg/kg. Total carbohydrate was estimated by the method of Dubois *et al.,* (1956). Total protein was analyzed by Folin – ciocalteau method using Bovine Serum Albumen (BSA) as standard (Lowry *et al.,* 1951) Total fat content was estimated by the method of Folch *et al.,* (1957). Moisture content was estimated by drying the pre weighed wet samples at 60 °C until a constant weight was obtained. The difference in weight was calculated and expressed as percentage moisture content of the sample. Total ash content was estimated by muffling the sample at 600 to 700 °c to dry ash.

Data obtained was generalized and the results were expressed as mean ± standard deviation. Statistical analysis of data was performed using SPSS statistical program (Package-22, registered). Significant differences between heavy metals concentrations and biochemical constituents of the candidate species from various sites, determined using One- Way analysis of variance (ANOVA) followed by Fisher's LSD post hoc test. The level of significance was $p < 0.05$.

RESULTS

The statistical results of the One way analysis of variance (ANOVA) showed that selected heavy metals for the present study such as Cadmium (F = 19.206), Chromium (F = 32.093), Copper (F = 82.354), Lead (F = 39.806) and Zinc (F = 96.600) were found to be different among each other with respect to the study sites. The level of significance was very higher at 1% ($p< 0.01$). The results of the Fisher's LSD (Least significant difference) Post hoc multiple comparisons further reveal that the three study sites significantly differ from each with respect to the accumulation of all the selected heavy metals such as Cadmium, Chromium, Copper, Lead and Zinc (Table 1). The accumulation of heavy metals in the muscle samples of *Penaeus monodon* of site 1 was in the decreasing order of Zn > Cu > Pb> Cr >Cd .With respect to site 2 the decreasing order was Zn > Cu >

Table 1. Analysis of variance (One-Way ANOVA) of heavy metals of the muscles of *Penaeus monodon* comparing study sites of the Ashtamudi Lake.

Heavy metals	Study sites				
	Site 1 (Mean ± SD)	Site 2 (Mean ± SD)	Site 3 (Mean ± SD)	F value comparing study sites	P Value
Cadmium	1.070 ± 0.586 [a]	0.675 ± 0.453 [b]	0.0±0.0 [c]	19.206	< 0.001*
Chromium	1.666 ± 0.743 [a]	0.825 ±0 .475 [b]	0.0± 0.0 [c]	32.093	< 0.001*
Copper	11.708±3.455 [a]	6.616 ± 0.719 [b]	0.889 ± 0.591 [c]	82.354	< 0.001*
Lead	7.183 ± 3.664 [a]	0.666 ±0.903 [b]	0.0± 0.0 [c]	39.806	< 0.001*
Zinc	63.091 ±18.569 [a]	13.179 ±2.613 [b]	7.041 ± 0.747 [c]	96.600	< 0.001*

* = $p < 0 .01$, The mean difference is significant at 1% level; SD – Standard deviation; [a, b, c] - Means within rows with differing subscripts are significantly different using Fisher's LSD post hoc test.

Cr > Cd > Pb. In the case of Site 3, the most abundant element was Zinc followed by Copper, and all other three heavy metals Cadmium, Chromium, and Lead were found to be below the detection limit. The decreasing order for heavy metal accumulation with respect to sites is site1 < site 2 < site 3.

Statistical Analysis of variance (One-Way ANOVA) of biochemical components of the muscles of *Penaeus monodon* (Table 2) showed that biochemical compositions such as carbohydrate (F= 6.349), protein (F =116.125), fat (F=15.148), moisture (F =9.530) and ash (F = 86.995) were found to be different in their values with respect to the sites and showed significance at 5% level ($p < 0.05$). The results of the Fisher's LSD (Least significant difference) Post hoc multiple comparisons further reveal that site 1 and 2 significantly differ from site 3 with respect to the biochemical constituents such as carbohydrate, fat, ash and moisture. For protein all the three sites were found to be significantly different from each other.

DISCUSSION

An assessment of heavy metal pollution loads of Ashtamudi Lake with respect to the mangrove crab, *Scylla serrata* done by Lekshmi *et al.,* (2018) reveals that the samples collected from the study sites Kureepuzha and Perumon were found to bioaccumulated with heavy metals when compared with the reference site, West Kallada. Razeena *et al.*, (2014) reported bioaccumulation status of heavy Metals in the muscles of the fish *Liza parsia* of Ashtamudi Lake. The order for heavy metal accumulation in the fishes collected from Perumon region is Zn (18.42 µg/g) > Cu (9.69 µg/g > Pb (1.45 µg/g) was, whereas Zn (26.35 µg/g) > Cu (16.50 µg/g) > Pb (1.58 µg/g) was the order of heavy metals accumulation in the fishes collected from Kureepuzha region. Morphological alterations caused by different kinds of aquatic pollutants, especially heavy metals on gills and fins of *Penaeus monodon* collected from Kureepuzha and Perumon region of Ashtamudi lake was done by Sherly *et al.,* 2015. In the present study also, the samples collected from the study sites Kureepuzha and Perumon were found to bioaccumulated with heavy metals when compared with the reference site.

Significant reduction in the biochemical profile with increasing concentrations of various heavy metals in fish tissues had been proved in numerous reviews (Azza *et al.,* 2014; Cicik and Engin, 2005; Snehalata *et al.,* 2001). Depletion of carbohydrate in the fish tissues after chronic exposure to heavy metals has been reported by Razeena Karim (2014), Cicik and Engin (2005) and many others. In their studies, they proved that in order to over the stress condition due to the toxic effect of heavy metals the fishes use carbohydrate as their energy source thereby results in the reduction of carbohydrate content. Similarly, in the present study a significant decrease in the carbohydrate composition has been noticed in the tissues from polluted site (site 1 and 2) when compared with the reference site.

The total proteins profile of adult brine shrimp *Artemia salina* tissue was analyzed after the treatment of Cadmium and Iron by Azza *et al.,* (2014). The results revealed that the total proteins intensity increased from all treatments of the beginning of the experiment then decreased gradually than the control by the end of exposure. In a study conducted by Snehalata *et al.,* (2001) protein contents indicated decline in the increase in

Table 2. Analysis of variance (One-Way ANOVA) of biochemical components of the muscles of Penaeus monodon comparing study sites of the Ashtamudi Lake.

Biochemical components	Study sites				
	Site 1 (Mean ± SD)	Site 2 (Mean ± SD)	Site 3 (Mean ± SD)	F value comparing study sites	P Value
Carbohydrate (%)	1.333 ± 0.313[a]	1.779 ± 0.949 [a]	3.098 ± 1.943 [b]	6.349	< 0.05*
Protein (%)	23.866 ± 10.697[a]	51.725 ± 7.496[b]	76.998 ± 6.951[c]	116.125	< 0.05*
Fat (%)	1.183 ± 0.523 [a]	1.320 ± 0.559 [a]	2.372 ± 0.647[b]	15.148	< 0.05*
Moisture (%)	63.654 ± 8.430[a]	67.685 ± 6.300 [a]	75.665 ± 5.513 [b]	9.530	< 0.05*
Ash (%)	1.575 ± 0.142 [a]	1.850 ± 0.355 [a]	3.276 ± 0.445 [b]	86.995	< 0.05*

* = $p < 0.05$, The mean difference is significant at 5% level; SD – Standard deviation; [a, b, c] - Means within rows with differing subscripts are significantly different using Fisher's LSD post hoc test.

time of the exposure to mercury in case of the liver of prawns *Penaeus indicus* and *P. monodon*. The recurring stress on the organisms with the increased concentration of the medium is attributed as the reason for this decline. Likewise in the present study also a reduction in the protein composition has been noticed in the tissues from polluted sites. A significant reduction in the fat, moisture and ash contents has been also noticed in the tissues of *Liza parsia* collected from the polluted of Ashtamudi Lake by Razeena Karim (2014). Similarly with regard to the above mentioned previous studies, in the present study also a significant reduction the biochemical constituents has been analysed in the samples collected from site 1 and 2 (polluted sites) than site 3 (reference site). A normal biochemical profile has been observed in the tissue of *Penaeus monodon* collected from the reference site. This agrees with the earlier reports on Glencross *et al.,* (2002) and Narasimhan *et al.,* (2013). The reduction in the biochemical profile in the samples of site 1 and 2 was due to the influence of heavy metals on them. Site 1 seems to be more polluted with heavy metal contamination than site 2 and hence the biochemical profile of the tissues from site 1 have significantly lower values than site 2 and 3. The recurring stress on the organisms with the increased concentration of heavy metals will result in the deterioration of the nutritional value of *Penaeus monodon*.

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