First report on significant alterations in biochemical constituents of Paphia malabarica due to heavy metal toxicity from Ashtamudi lake

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Abstract : Paphia malabarica 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 Paphia malabarica 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.

Index terms : Biochemical constituents, Heavy metals, Pollution, Paphia malabarica, Muscle.

1. INTRODUCTION

Studies on the biochemical constituent can be used an appropriate tool in order to monitor the level of deterioration in the tissues of organisms caused by aquatic pollutants. For the estimation of metal pollution, molluscs were considered the most significant indicators in freshwater systems [1]. The shortneck yellow clam Paphia malabarica is a dominant species of Ashtamudi Lake, which alone contribute 90% of India's clam meat export. Paphia malabarica is one of the nutritious and inexpensive food which has of greater demand in both national as well as international market [2]. The biochemical change that occurs to the tissues of organisms provides the first indication of the stress faced by the organisms. In order to counter act the stressful condition the organism needs sufficient energy such as glycogen, lipid and protein which is stored in the body as reserve materials. To meet the demand of stress, only stored glycogen is used if the stress is mild and the energy stored in lipid and protein may be used if the stress is strong. When comes in contact, heavy metals cause metabolic impairment and thereby toxic biochemical alterations in the tissues of organisms. The second largest backwater lake in Kerala Ashtamudi Lake, is prone to several kinds of toxic aquatic pollutants especially heavy metals from various sources.

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Significant input of heavy metals into Ashtamudi Lake from the nearby industrial effluents and from other sources have been reported earlier [3-5]. Studies on the bioaccumulation of various pollutants in different organs of fishes of Ashtamudi Lake have been extensively studied [3], [4], [6]. Very little is known about the changes in the biochemical constituents of aquatic organisms on exposure to toxic heavy metals especially from Ashtamudi Lake. Hence the present study were emphasized on the alterations in the biochemical components of the muscles Paphia malabarica of Ashtamudi lake with special reference to heavy metal toxicity in them.

2 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 [3], [6], [7]. 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, the Perumon region [6], [7]. The region of West Kallada lake is not much disturbed with anthropogenic interferences and urbanization, is selected as the third and reference site [6]. Paphia malabarica (180 numbers of about 20 to 30 mm width and 0.500 to 1.30 gm weigh) were collected from each study site for one year. The analysis of the heavy metals Cadmium, Chromium, Copper, Lead and Zinc were carried out using an atomic absorption spectrophotometer (AAS , Pinnacle 900H) as described by APHA [8]. Metal concentrations were calculated in mg/kg. Total carbohydrate was estimated by the method of Dubois et al. [9]. Total protein was analyzed by Folin – ciocalteau method using Bovine Serum Albumen (BSA) as standard [10]. Total fat content was estimated by the method of Folch et al. [11]. Moisture content was estimated by drying the pre weighed wet samples at 60^{o}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 ^{o}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 $p < 0.05$.

3 RESULTS

The results of the One way analysis of variance (ANOVA) showed that selected heavy metals for the present study such as Cadmium (F = 17.135) , Chromium (F = 37.337), Copper (F = 33.254), Lead (F = 55.698) and Zinc (F = 109.43) were found to be different among each other with respect to study sites. The mean level of significance was very higher at 1% ($p < 0.01$). The results of the Fisher's LSD Post hoc multiple comparisons further reveals that the site 1 and 2 significantly differ from site 3 with respect to the heavy metals Cadmium and Chromium. In the case of heavy metals Copper, Lead and Zinc site 1 significantly differ with site 2 and 3. Tabulation of the statistical results of ANOVA were shown in table 1.

Table 1: *Analysis of variance (One-Way ANOVA) of heavy metals of the muscle samples of Paphia malabarica comparing study sites of the Ashtamudi Lake.*

	Study sites				
Heavy metals	*Site 1 (Mean ± SD)*	*Site 2 (Mean ± SD)*	*Site 3 (Mean ± SD)*	*F value comparing study sites*	*P Value*
Cadmium	*0.508 ± 0.253* ^{a}	*0.375 ± 0.285* ^{a}	*0.0 ±0.0* ^{b}	*17.135*	*< 0.001**
Chromium	*4.008 ± 1.523* ^{a}	*3.266 ± 1.436* ^{a}	*0.0 ±0.0* ^{b}	*37.337*	*< 0.001**
Copper	*3.1583 ±1.066* ^{a}	*1.275 ± 0 .307* ^{b}	*0.894 ± 0.599* ^{b}	*33.254*	*< 0.001**
Lead	*8.416 ± 3.516* ^{a}	*1.150 ± 1.047* ^{b}	*0.0±0.0* ^{b}	*55.698*	*< 0.001**
Zinc	*74.283 ±20.697* ^{a}	*16.420 ± 2.775* ^{b}	*6.983 ± 0.833* ^{b}	*109.430*	*< 0.001**

7.718), protein (F =145.848), fat (F = 12.128), moisture (F = 9.638) and ash (F =60.491) were found to be different from their values with respect to the sites and showed significance of 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 , Protein, and moisture. For ash all the three sites were found to be significantly different from each other.

Table 12: *Analysis of variance (One-Way ANOVA) of biochemical components of the muscles of Paphia malabarica comparing study sites of the Ashtamudi Lake.*

	Study sites				
Biochemical components	*Site 1 (Mean ± SD)*	*Site 2 (Mean ± SD)*	*Site 3 (Mean ± SD)*	*F value comparing study sites*	*P Value*
Carbohydrate (%)	*8.840 ± 2.105* ^{a}	*12.500 ± 6.571* ^{a}	*16.920 ± 5.362* ^{b}	*7.718*	*< 0.05**
Protein (%)	*22.353 ± 3.722* ^{a}	*23.600 ± 4.397* ^{a}	*50.494 ± 5.403* ^{b}	*145.848*	*< 0.05**
Fat (%	*1.191 ± 0.239* ^{a}	*1.245 ± 0.285* ^{a}	*2.359 ± 1.072* ^{b}	*12.128*	*< 0.05**
Moisture (%)	*74.009 ±2.728* ^{a}	*75.046 ± 2.118* ^{a}	*80.013 ± 5.151* ^{b}	*9.638*	*< 0.05**
Ash (%)	*1.358 ± 0.197* ^{a}	*3.716 ± 0.899* ^{b}	*4.610 ± 0.911* ^{c}	*60.491*	*< 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

4 DISCUSSION

An assessment of heavy metal pollution loads of Ashtamudi Lake with respect to the mangrove crab, Scylla serrata done by Lekshmi and Sherly [6] 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 [3] 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 (18.42µg/g) > Cu (9.69 µg/g > Pb (1.45 µg/g), whereas Zn (26.35µg/g) > Cu (16.50µg/g) > Pb (1.58µg/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 [4]. In the present study also, the samples collected from the study sites Kureepuzha and Perumon were found to be 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 [12-14]. Razeena [3], Cicik et al. [13] and many others, has reported depletion of carbohydrate in the fish tissues after chronic exposure to heavy metals. In their studies, they proved that in order to recover 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 recorded range of the nutritional composition of Paphia malabarica from the West kallada region of Ashtamudi Lake was 9.84% to 27.14 %, 41.24% to 58.90%, 1.20% to 4.70%, 71.79% to 86.89%, 2.76% to 5.89% for carbohydrate, protein, fat, moisture, and ash respectively. Of the nutritional composition of Paphia malabarica studied by Nagvenkar et al. [15,] the lowest range of carbohydrate (4.29%), protein (15.64%), and fat (2.25 %) contents somewhat falls with the range as that of the present study; while the highest values of carbohydrate (72.73%), protein (86.79%), and fat (5.12 %) contents were much higher than the values of the present study. The proximate composition of carbohydrate (11.36%) and fat (4.22%) content as reported by Umesiet al. [16] in medium sized clam Senilia senilis and that of carbohydrate (24.54%) by Saritha et al. [17] in Green Mussel Perna Viridis agrees with the findings of the present study. Nutritional compositions as determined by Raghavan et al. [18] in the spawned eggs of paphia malabarica collected from Ashtamudi Lake were composed of 11.4% carbohydrate, 63.2% protein, 25.4% lipid, of which the value of carbohydrate is in congruent with the present study. The range of proximate composition such as carbohydrate (9.22 to 39.55%), protein (40.75 to 66.37%), fat (0.99 to 9.97%), moisture (79.4 to 86.83%) and ash (7.49 to 20.55%) studied by Appukuttan [19] in Paphia malabarica of Ashtamudi lake were consistent with the range of values recorded in clams in the present study. In the present study, when compared with the reference site – West Kallada , significant reduction in all the biochemical parameters has been observed the tissues of Paphia malabarica from the Perumon and Kureepuzha regions of Ashtamudi Lake. The level of significant reductions was more in the tissues samples collected from the Kureepuzha region. This reduction in the biochemical profile is in concordance with the pollution status of those regions. A consistent reduction in carbohydrate contents has been studied by Sandhya et al. [20] in the freshwater bivalve, Lamellidens due to the toxicity of mercuric chloride and cadmium chloride. When compared to the controlled fishes significant alterations in the glycogen contents were also observed in different tissues like gills, liver and kidney of freshwater fish, Channa gachua due to the chronic exposure of Zinc toxicity [21]. Sonia and Ali [22] observed a decline in the biochemical contents of glycogen, lipids, and proteins in fishes due to chromium toxicity. Venkatramreddy et al. [23] have also monitored similar trend of decline in the biochemical profile due to chromium. In the present investigation also the reported biochemical content of the muscles of the selected species collected from the Kureepuzha and Perumon region also showed a declining trends due to the impact on heavy metals as observed in previous reports.

5 CONCLUSION

The results of the present study reveal that the samples of Paphia malabarica collected from site 1- Kureepuzha and site 2 - Perumon are more contaminated with heavy metals, compared with reference site – West Kallada. Anthropogenic influences are seemed to the main sources of pollution of site 1 and 2. With respect to sites the decreasing order of heavy metal accumulation is site1< site2< site3 and those of biochemical parameters are site3 < site2 < site 1. A significant reduction in the biochemical parameters has been monitored in the samples of site 1 and 2, which is in concordance with the heavy metal pollution status of those regions. Accumulations of heavy metals in turn alter and impair the nutritional status of short neck yellow clams. If the bioaccumulation of heavy metals occurs in an increasing proportion in the tissues of targeted samples, it will further results in the deterioration of the organisms. Stringent and necessary measurements should be taken up by the responsible authorities to save edible molluscs such as Paphia malabarica and other organisms of the Lake from heavy metal pollution load.

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