Available online at www.elixirpublishers.com (Elixir International Journal)

Aquaculture

Elixir Aquaculture 41 (2011) 5855-5858

ISSN: 2229-712X

Bio chemical alterations due to the impact of Lead nitrate in sublethal levels on Muscle and Hepatopancreas tissues of an economically important Shrimp Tiger Prawn *Penaeus monodon*

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ARTICLE INFO

Article history:

Received: 24 November 2011;

Received in revised form:

20 November 2011;

Accepted: 9 December 2011;

Keywords

Biochemical,
Gill,
Muscle,
Hepatopancreas,
Lead nitrate,
P.monodon.

ABSTRACT

In this study the tiger shrimp *Penaeus monodon* subjected to lead nitrate toxicity under three sublethal concentrations (1.66,3.33 and 6.60 mg/L)for 24 hrs, 48 hrs 72 hrs and 96 hrs . The vital organs Muscle and Hepatopancreas were dissected from tiger shrimp and processed for biochemical assay .The results of Lead nitrate treated Shrimp shows a decrease in the level of Protein, Carbohydrate and Lipid comparing to the control Shrimp. The depletion results of Protein in Muscle from 65.22 to 60.45 percent ,Carbohydrate from 15.10 to12.10 percent and Lipid from 09.00 to 06.60 percent, similarly the hapatopancreas shows depletion of protein from57.48 to50.50 percent ,Carbohydrate from 18.57 to 14.42 percent and Lipid shows from 19.20 to 15.86 percent This shows depletion of three biochemical components due to the impact of Lead nitrate toxicity . Impact of Lead nitrate leads to active depletion of biochemical components of protein carbohydrate and Lipid resulting in accelerated metabolism.

Introduction

All heavy metals become toxic at some concentration (Bryan, 1971). Metal accumulation in the environment continuously increases owing to the anthropogenic activities and they tend to concentrate in all the aquatic matrices. The low-dose heavy metal exposure to aquatic organisms may result in various manifestations of biochemical, physiological, and histological alterations in primary tissues (Hinton et al. 1973; Ghate and Mulherkar 1979; White and Rainbow 1986; Kaliamurthy et al. 1994; Yamuna et al. 1996).Lead, one of the oldest known metals, is also one of the most widespread toxicants, and lead poisoning remains a health threat (Hernberg, 2000). The fish, as a bio indicator species, plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of fish indicates heavy pollution; the effects of exposure to sub lethal levels of pollutants can be measured in terms of biochemical, physiological or histological responses of the fish organism (Mondon *et al*., 2001).

According to Hashmi et al. (2002) continental sources (river runoff and atmospheric transport),oceanic sources (upwelling) and diagenetic exchanges at water-sediment interface have been identified as the factors that influence the heavy metals in aquaculture organisms. One of the greatest concerns for human health is caused by lead (Pb) contamination. Lead (Pb)occurs naturally in all soils, in concentrations ranging from 1 to 200 mg/ kg, with a mean of 15 mg/ kg (Chirenje et al., 2004). Moreover, anthropogenic atmospheric inputs, sewage sludge and fertilizers are often inferred to be significant because of important these metals input. Heavy metals are present in the atmosphere in ever increasing levels as a result of anthropogenic and natural emissions (Suzuki, 2006). Dural (2007) stated heavy metal pollution in estuaries and coastal area has been recognized as a serious environmental concern.

Materials and Methods

Experimental animals

The shrimps (Penaeous monodon)were collected from culture pond at Velankanni,Latitude:10.6833, Longitude: 79.8333, Lat (DMS): 10° 40' 60N, Long (DMS): 79° 49' 60E, Time zone (est) of Nagapattinam District, South India. The shrimps were acclimatized to laboratory condition by using in-door fiber tanks, each with 1.5 m in diameter and 1 m in height, containing 0.80 m water with adequate aeration, and 20% of water volume changed daily. The shrimps were acclimated under the light–dark cycle of 12:12 for two weeks before the experiment. The water temperature was maintained at 27–28 °C. Salinity, 40 ppt; total hardness, 255.0 mg/l; pH, 8.2; nitrate, 1.6 mg/l; chloride, 27.0 mg/l; ammonia, 0.058 mg/l; dissolved oxygen, 6.7 mg/l; BOD, 5.8 mg/l; COD, 14.7 mg/l; and total solid, 1.7 g/l). Commercial shrimp feed was provided daily at 3% of the body weight.

The biochemical analysis were carried out in both control animals and on lead nitrate treated animals. The tests were carried out in 3 different sublethal concentrations of 1.66 mg/L as SLC – 1,3.33 mg/l as SLC –II, 6,60 mg/L as SLC – III with 24 hrs, 48 hrs, 72 hrs and 96 hrs, the results were mentioned in four different tables (Table 19-22).

Biochemical assay

Biochemical assay was carried out for the above animal tissue using the method of Lowry et al.(1951) for protein, Folch et al.(1957) for Lipid and Roe(1955) for Carbohydrate.

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Results

The observations from the present study showes that, The Lead nitrate at sublethal and lethal concentrations altered the biochemical composition (protein, Carbohydrate and lipid) of the various organs of test animal.

Biochemical analysis of shrimp *Penaeus monodon*

The analyses were carried out on muscle and hepatopancreas of adult shrimp *Penaeus monodon.*

Control values of muscle and hepatopancreas tissues of shrimp *Penaeus monodon*

The control value for the shrimp *Penaeus monodon* protein in muscle tissue 66.95%, carbohydrate in muscle 15.05% and lipid muscle 9.87. Similarly the protein in hepatopancreas is 58.56%, carbohydrate in hepatopancreas is 18.66% and lipid in hepatopancreas is 20.45% respectively.

The table 19 presents biochemical parameters of protein, carbohydrate and lipid into the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* in three sublethal levels concentrations of 24 hrs. The results of table 15, the concentration of lead nitrate present in muscle tissue in three sublethal concentrations SLC-I, SLC-II and SLC-III are 65.22 to 62.25% this shows a decline and depletion of protein from the treated tissues similarly carbohydrates in muscle tissue shows a decline from 15.1 to 14.2%. The lipid concentration shows a decrease from 09.00 to 08.32%. The hepatopancreas tissue shows a decreasing trend in protein from 57.48 to 53.7, carbohydrate from 18.57% to 15.82% and Lipid 19.20 to 17.22, these observations are found (Table 19).

The table 20 presents biochemical parameters of protein, carbohydrate and lipid into the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* in three sublethal levels concentrations of 48 hrs. The results of table 16 shows a decrease in muscle protein the concentration of lead nitrate present in muscle tissue in three sublethal concentrations SLC – I, SLC – II and SLC –III are 65.0 to 61.56 for muscle protein 14.96 to 13.60 for muscle carbohydrate and 8.76 to 7.88 for muscle lipids all the three show a decrease in post exposure to lead nitrate similarly hepatopancreas protein shows a decreasing trend from 56.15 to 52.50, carbohydrate 18.10 to 15.22 and lipid 18.60 to 16.52 respectively (Table 20).

The table 21 presents biochemical parameters of protein, carbohydrate and lipid into the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* in three sublethal levels concentrations of 72 hrs. The concentration of lead nitrate present in muscle tissue in three sublethal concentrations SLC – I, SLC – II and SLC – III are 64.9 to 61.1% this shows a decline and depletion of protein from the treated tissues. Similarly carbohydrates in muscle tissue shows a decline from 14.22 to 13.16%. The lipid concentration shows a decrease from 08.45 to 06.90%. The hepatopancreas tissue shows a decreasing trend in protein from 55.9 to 51.22, carbohydrate from 17.98 to 15.05 and lipid 18.25 to 16.22, these observations are found (Table 21).

The table 22 presents biochemical parameters of protein, carbohydrate and lipid into the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* in three sublethal levels concentrations of 96 hrs. The observations found in table 18 shows a decrease in muscle protein the concentration of lead nitrate present in muscle tissue in three sub lethal concentrations SLC – I, SLC – II and SLC – III are 64.1 to 60.45 for muscle protein, 13.85 to 12.1 for muscle carbohydrate and 08.23 to 06.6 for muscle lipids all the three show a decrease in post exposure to lead nitrate similarly hepatopancreas protein shows a decreasing trend from 55.1 to 50.5 carbohydrate, 17.60 to 14.42 and lipids 18.00 to 15.86 respectively (Table 22).

Discussion

The P.monodon were exposed to a sublethal concentration of lead (1.44 ppm) for 30 days. The major biochemical constituents, including total carbohydrates, proteins, lipids and ninhydrin-positive substances (TNPS) were estimated using standard methods. Lead exposure resulted in retardation of growth with a significant decrease in length and weight occurring at day 10 and onwards. Of all the biochemical constituents, total protein showed the maximum decrease (79.3%) followed by total lipids (68.1%) and then by total carbohydrates (51.4%) in lead-exposed PL. The data suggest lead exposure causes reduced growth and the depletion of biochemical constituents. This may be due to metal interactions and inhibition of metabolic pathways responsible for synthesis of biochemical constituents or to greater utilization of these constituents under metal stress conditions (Chinni and Yallapragada, 2000). Similar observations were observed in shrimp *Penaeus monodon*. The protein, carbohydrate and lipid level in the body tissues of these animals treated with lead nitrate show a decreasing trend comparing to the control animals (prawn).

The biochemical components such as protein, lipid and carbohydrate of the liver of two important penaeid prawns were significantly reduced, following six days of exposure to 0.005 ppm and 0.01 ppm of mercuric chloride during various reproductive stages i.e., preparatory, prespawning, spawning and postspawning. Liver protein recorded highest in contrast to lipid and carbohydrate irrespective of the species, sex and medium depletion was at 0.01 ppm Hg medium. The effect of mercury was more in *Penaeus indicus* than that of *Penaeus monodon*, the female species and prespawning stage. Liver-lipid deleteriously affected the female *Penaeus indicus* during spawning while carbohydrate affected it prominently during preparatory stage. Hg concentration of 0.01 ppm had much damaging effect on liver. The change caused due to test solutions in the biochemical constituents of the liver of the prawns indicate that female had more affected than male. The protein content indicated decline with the increase of time period, the depletion of percentage also raised with the increase of time exposure for carbohydrates and lipids (Snehalata Das *et al.*, 2001). As in the above statement the present study enumerates the same, there was a decrease in protein, carbohydrate and lipid content in the tissues of P.monodon.

Endosulfan, a broad-spectrum non-systemic organochlorine (OC) pesticide is extensively used to control a wide variety of pests in agriculture, horticulture and public health programmes. Biochemical changes occurring in the metabolically active tissues of gills (GL), hepatopancrease (HP) and muscle (MU) of the penaeid shrimp, *Metapenaeus monoceros* (Fabricius) on exposure to two sublethal doses (40 and 60 ngl^{-1}) of endosulfan were studied for 23 days of exposure (DoE). Theresults of the study revealed that sublethal doses of endosulfan significantly alters the proximate composition of major tissues, particularly the TP levels in the MU tissues thereby reducing the nutritive value of this economically important panaeid shrimp. Since *M. monoceros* exhibits significant biochemical changes on exposure to endosulfan, this species could possibly be used as biosensor of coastal marine and estuarine pollution (Suryavanish *et al.*, 2009). Test results indicated that the brackish water juvenile

shrimp, *Palaemonetes africanus* were sensitive to the cadmium solution especially at concentration above 4.0 mg/l (Joel and Amajuoyi, 2009). Evaluation of toxic effect of lead on the edible lobster, *Thenus orientalis* for the LC_{50} value and effect of heavy metal lead on the nutritional status viz., protein, carbohydrate and lipid in ovary, spermatheca, hepatopancreas, muscle and haemolymph was made. The results assume greater interest as most water bodies are increasingly subjected to environmental pressure due to pollution (Kalyanaraman and Senthilkumar, 2009). As stated above similar observations found in the present biochemical study of the shrimp *Penaeus monodon*'s muscle and hepatopancreas tissues and the same exhibits similar kind of results in decreasing order in protein, carbohydrate and lipid depletion from the control animal.

Summary

The biochemical studies on estuarine fish and shrimp *P. monodon* show a maximum decrease in the protein, carbohydrate and lipid content in muscle, hepatopancreas of *P. monodon* due to accelerated metabolism enhanced by the presence of heavy metal Lead nitrate.

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Table 19 The results of biochemical parameters of protein, carbohydrates and lipid in to the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* at three sublethal level of concentrations of 24 hrs

Body organ	Treatment with lead nitrate	Exposure period (24 hrs)		
		Protein (%)	Carbohydrate (%)	Lipid (%)
Muscle	Control	66.95	15.05	09.87
	Conc.1.66 mg/l SLC-I	65.22	15.10	09.00
	Conc.3.33 mg/l SLC-II	64.90	14.80	08.78
	Conc.6.60 mg/l SLC-III	62.25	14.20	08.32
Hepatopancreas	Control	58.56	18.66	20.45
	Conc.1.66 mg/l SLC-I	57.48	18.57	19.20
	Conc.3.33 mg/l SLC-II	56.15	17.95	18.95
	Conc.6.60 mg/l SLC-III	53.7	15.82	17.22

Table 20 The results of biochemical parameters of protein, carbohydrates and lipid in to the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* at three sublethal level of concentration for 48 hrs

Body organ	Treatment with lead nitrate	Exposure period (48 hrs)		
		Protein (%)	Carbohydrate (%)	Lipid (%)
Muscle	Control	66.95	15.05	09.87
	Conc.1.66 mg/l SLC-I	65.0	14.96	08.76
	Conc.3.33 mg/l SLC-II	63.85	14.15	08.20
	Conc.6.60 mg/l SLC-III	61.65	13.60	07.88
Hepatopancreas	Control	58.56	18.6	20.45
	Conc.1.66 mg/l SLC-I	56.15	18.10	18.60
	Conc.3.33 mg/l SLC-II	55.80	17.12	18.15
	Conc.6.60 mg/l SLC-III	52.50	15.22	16.52

Table 21 The results of biochemical parameters of protein, carbohydrates and lipid in to the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* at three sublethal level of concentrations for 72 hrs

Body organ	Treatment with lead nitrate	Exposure period (72 hrs)		
		Protein (%)	Carbohydrate (%)	Lipid (%)
Muscle	Control	66.95	15.05	09.87
	Conc.1.66 mg/dl SLC-I	64.9	14.22	08.45
	Conc.3.33 mg/dl SLC-II	63.15	14.0	07.72
	Conc.6.60 mg/dl SLC-III	61.1	13.16	06.90
Hepatopancreas	Control	58.56	18.6	20.45
	Conc.1.66 mg/dl SLC-I	55.9	17.98	18.25
	Conc.3.33 mg/dl SLC-II	54.65	17.0	17.9
	Conc.6.60 mg/dl SLC-III	51.22	15.05	16.22

Table 22 The results of biochemical parameters of protein, carbohydrates and lipid into the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* and sublethal level of concentrations at 96 hrs

Body organ	Treatment with lead nitrate	Exposure period (48 hrs)		
		Protein (%)	Carbohydrate (%)	Lipid (%)
Muscle	Control	66.95	15.05	09.87
	Conc.1.66 mg/dl SLC-I	64.1	13.85	08.23
	Conc.3.33 mg/dl SLC-II	62.55	13.60	07.50
	Conc.6.60 mg/dl SLC-III	60.45	12.1	06.6
Hepatopancreas	Control	58.56	18.6	20.45
	Conc.1.66 mg/dl SLC-I	55.1	17.60	18.00
	Conc.3.33 mg/dl SLC-II	53.72	16.66	17.12
	Conc.6.60 mg/dl SLC-III	50.5	14.42	15.86