

BIOACCUMULATION AND METABOLIC EFFECTS OF ZINC ON MARINE ROCK OYSTER, *CRASSOSTREA CATTUCKENSIS*

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ABSTRACT

The Indian rock oysters, *Crassostrea cattuckensis* (80-90 mm shell length) were exposed to sub lethal levels of zinc for 15 days and 30 days for metal accumulation and next 15 days for metal depuration. The oysters, which served as experimental control after 15 days showed high amount of lipid (mg/100 mg) in hepatopancreas (3.32) followed by gills (3.20), mantle (3.08), gonad (2.90), adductor muscle (2.78) and siphon (2.11). During 15 days metal exposed oysters to (0.65) and (0.94) ppm concentrations there was changes observed in different body parts when compared to experimental control. The decreased rate upon 15 days in (0.65 ppm) concentrations was in mantle (2.96), than gill (2.22), hepatopancreas (1.72), gonad (1.59), siphon (1.51) and adductor muscle (1.50). While in (0.94 ppm) the protein was decreased in adductor muscle (2.51), gonad (1.61), siphon (1.44), gill (1.42) and it increased in mantle (3.90) and hepatopancreas (3.34) when it was compared to experimental control. Whereas upon 30 days exposure the lipid content increased among body parts in both concentration except the gills (1.35), gonads (1.97) and hepatopancreas (1.66) in higher concentration when compared with experimental control. The lipid increased in (0.65 ppm) from gonad (2.29), hepatopancreas (2.25), adductor muscle (2.22), gills (2.20), mantle (2.14) and siphon (1.44). While in (0.94 ppm) lipid increase trend was from mantle (4.27), adductor muscle (2.46) and siphon (1.42) and decreased from gonad (1.97), hepatopancreas (1.66) and gills (1.35) when compared with 30 days experimental control. During detoxification process the lipid content was increased in both concentrations except gill (2.11) in low and adductor muscle (0.99), siphon (0.74) in high concentrations when compared with respective concentrations of 15 days exposed oysters. The increase rate in low concentration was from mantle (4.32) than from adductor muscles (4.12), gonad (3.60), hepatopancreas (2.46) and siphon (1.05). In high concentration it was more in hepatopancreas (4.93) than gonad (4.42), gills (4.08) and mantle (4.01).

Figure : 00

References : 38

Table : 01

KEY WORDS : Accumulation, *C. cattuckensis*, Detoxification, Lipid, Zinc.**Introduction**

India has a very long coastline (7517 km) of which Maharashtra is 750 km. Maharashtra is the most industrialized state along the Indian coast. The coastal and offshore waters of the state are more used for sea transportation, exploration and exploitation of biological as well as geochemical resources as compared to other parts of the country. This part of the Arabian Sea is highly productive in terms of biological resources; the estuaries, creek

and sheltered coastal wet land along these state sustain, luxuriant mangrove forests, which ultimately support breeding and nursery grounds for a variety of shell and fin fishes of commercial importance. Amongst several marine living resources the shellfishes play a vital role in India's economy; bivalves such as oysters, mussels and clams are source of minerals, protein, glycogen and lipids for humans^{4,19,29}. Since last many decades, the coastal near shore waters of

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Maharashtra receives voluminous and increasing quantum of untreated or partially treated wastewater from industrial and domestic sectors^{8, 7}. Although, much of the more direct deleterious impacts due to environmental stress have been mitigated in cases of commercially important species especially, those which are not able to migrate have been threatened. Although, toxicological responses of organisms exposed to heavy metals and its bioaccumulation caused by heavy metal pollution have seriously threatened the security of ecosystems and food; investigations focused on the responses of certain organisms exposed to the long term and severe heavy metal contamination in specific environments are very scarce²¹. In unpolluted areas, zinc is found in water at nanomolar levels, reaching micromolar values in metal-contaminated environments³³. Zinc is an essential element for all organisms, playing a critical role in a variety of biochemical processes including regulatory, structural, and enzymatic functions. Although Zn is essential, it is toxic at high concentrations^{18,38}. When the external concentration gets too high, the organism's homeostatic capacity will fail and toxicity effects will occur³⁴. In addition, evidence shows that high intracellular free Zn promotes neuronal death by inhibiting cellular energy production¹³.

Lipids play an important role nutritionally and physiologically in marine bivalves by providing an efficient source of high energy content and essential fatty acids^{5,24,37}. The metabolism and transport of lipids play important role in reproduction and larval development in bivalves^{7,15,25}. Although have been carried out in bivalve molluscs to gain knowledge on the specific effect of zinc on lipids concentration and metabolism^{1,2,14,16,27,30,38} such effect is directly related with the degree of industrialization and the discharge not controlled of chemical effluents from anthropogenic activities. In addition, high levels of zinc in the sediment have been correlated with levels of this metal in tissues of bivalves²⁶. It has been proposed that soluble zinc and other metals are taken up mainly by gills and mantle of bivalves, while their particulate forms are taken up mainly by the digestive organs and stored in the digestive gland^{10, 32}.

Sub lethal toxicity provides a tool to assess the impact of contamination on the aquatic ecosystems and could be very useful for future toxicological studies. Hence, the main aim of the present study is to understand the effect of a wide range of concentration of zinc on lipid in different

body parts from the oysters, *Crassostrea cattuckensis* from Ratnagiri coast.

Materials and Methods

Ratnagiri is the southern district of Maharashtra state bearing important productive estuaries and creeks. In the present study the oyster species, *Crassostrea cattuckensis* has been selected and this species occurs fairly in large number in the estuary at Bhatye and adjacent interior areas of Kajvi River. The oysters were collected from Bhatye estuary in post winter (February). Soon after the fishing they were brought to the laboratory and the shells were brushed to clean the fouling biomass and mud. They were then stocked in continuous aerated filtered seawater pumped in the laboratory from the estuary for 24 to 48 h for depuration. Based on the acute toxicity tests the chronic test were performed on the oysters of (80-90 mm shell length) using sublethal concentrations, i.e. 1/10th of LC₀ and LC₅₀ values of zinc metal salts. The experiments were carried for 15 days and 30 days to metal exposure, and then transfer of oysters of 15 days metal exposed to normal seawater to next 15 days for detoxification. The control group in normal seawater was run simultaneously during each experiment. The oysters belonging to control and experimental were sacrificed separately to obtain soft body parts like mantle, gills, gonad, hepatopancreas, adductor muscles and siphons. These tissues were weighed and they were then kept in hot air oven at 92°C till constant weights were obtained. The dried product was ground to obtain fine powder. From the replicates of the three samples lipid was estimated⁶. The data obtained were statistically analyzed for confirmation of the results by ANOVA. The results were calculated using regression equations and expressed in mg/100mg dry weight.

Results

In the present study the oysters, which served as experimental control after 15 days, showed high amount of lipid in hepatopancreas followed by gills, siphon, mantle, gonad, and adductor muscle. Compared with experimental control group the content decreased from most of the body parts in both concentrations. In low concentration the decrease in the trend of the content was from hepatopancreas (48.38%; P<0.001), followed by adductor muscle (46.24%; P<0.001), siphon (45.59%; P<0.05), gonad (45.31%; P<0.001), gill (30.77%; P<0.05) and

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mantle (4.04%). In high concentration the level of the content was more decreased in gill (55.65%; $P<0.001$), followed by gonad (44.65%; $P<0.01$), siphon (31.96%; $P<0.001$) and adductor muscle (9.60%; $P<0.01$) but it increased from mantle (26.64%; $P<0.05$) and the level from hepatopancreas (0.69%) almost remained unchanged. The content when compared low concentration with high concentration showed increased trend, which was more from hepatopancreas (95.04%; $P<0.001$), followed by adductor muscle (68.15%; $P<0.001$) and mantle (31.96%; $P<0.05$), while gonad (1.21%) but the level decreased from gill (35.95%) and siphon (4.91%).

The oysters which served as experimental control after 30 days showed high level of the content in hepatopancreas followed by gill, gonad, adductor muscle, mantle and siphons. When compared with the oysters which served for 15 days as experimental control showed decreased in siphon (36.14%) followed by mantle (34.67%), hepatopancreas (33.33%), gill (32.74%), gonad (26.44%) and adductor muscle (26.11%) all at ($P<0.001$). Compared with the oysters of 30 days experimental control group shows low concentration, the lipid was more decreased in adductor muscle (7.96%) followed by gonad (7.38%), siphon (6.54%), mantle (6.43%) gill (2.05%) and hepatopancreas (1.72%). In high concentration when compared with the experimental control was decreased from gill (37.51%; $P<0.01$), hepatopancreas (25.06%) and gonad (7.43%) but it increased from mantle (112.3%; $P<0.01$), adductor muscle (19.67%; $P<0.01$) and siphon (5.20%). On the other hand, compared with low concentration in high concentration the content was decreased from gill (38.76%; $P<0.01$) followed by hepatopancreas (26.33%) and gonad (13.78%). However, it was increased in mantle (99.44%; $P<0.01$), adductor muscle (10.85%), siphon (1.26%). Further, the oysters exposed for 30 days was compared with the oysters exposed for 15 days it was found that it decreased in low concentration from mantle (27.55%), followed by siphon (4.91%) and gill (0.86%) (All at non-significant) but it increased from adductor muscle (48.36%; $P<0.05$), gonad (44.41%; $P<0.05$) and hepatopancreas (31.37%). Further in high concentration group similar comparison showed decreased trend from hepatopancreas (50.38%; $P<0.01$) followed by gill (5.23%), adductor muscle (2.20%) and siphon (1.26%) but increased

from gonad (22.98%) and mantle (9.51%).

In the detoxification process the content increased from the both the concentrations when it compared with those oysters exposed for 15 days metal accumulation. In the oysters from low concentration this increase was much from adductor muscle (176.06%; $P<0.001$) and gonad (126.85%; $P<0.001$), followed by mantle (46.36%; $P<0.001$), hepatopancreas (43.23%; $P<0.05$), siphon (30.62%) and gill (5.02%) non-significant. On the other hand, in the oysters from high concentration there was much increase in the content from gill (187.87%; $P<0.001$) and gonad (175.35%; $P<0.001$) followed by adductor muscle (60.43%; $P<0.001$), siphon (48.92%; $P<0.01$), hepatopancreas (47.62%; $P<0.001$) and mantle (2.86%; $P<0.001$). On the other hand, the content when compared with those exposed to low concentration, in high concentration there was increase in the trend of the content from hepatopancreas (101.20%; $P<0.001$), followed by gill (94.15%; $P<0.001$) and gonad (22.84%; $P<0.05$) but it decreased from the adductor muscle (99.76%; $P<0.001$), siphon (99.30%), mantle (7.28%).

Discussion

The oysters are used in the pollution assessment and monitoring studies in various ways which include changes in community structure, species diversity, species preference, bioaccumulation of toxicants. This community profile will hopefully constitute an educational source documented for all those interested in the ecological values of coastal water. The present study try to assess the alteration in lipid composition of *Crassostrea cattuckensis* after metal contamination to zinc and decontamination kinetics showed quite innovative long-term results. Upon 15 days exposure to the zinc our results indicated a different pattern of substrate utilization and gearing of metabolism in both the metal concentration groups of zinc. Heavy demand of energy exerted upon the oyster body parts was noticed especially by utilization of the lipid from gill; also in the hepatopancreas and adductor muscle lipid content decreased. Decrease in the lipid in most of the tissues shows its prime utilization in gearing of the metabolism. The regulation of biochemical shows that lipids in bivalves are multifunctional and in diverse species one or same of the functions during the maturation of gametes, drastic environmental conditions, starvation, population stress etc. can

TABLE-1: Changes in the lipid content from different body parts of *C. cattuickensis* after exposure to values for chronic test of zinc and detoxification process

Body parts	15 days exposure			30 days exposure			15 days detoxification		
	Exp. control	LC ₀	LC ₅₀	Exp. control	LC ₀	LC ₅₀	LC ₀	LC ₅₀	
Mantle	3.08 ±0.061	2.96 ±0.14 (4.05%)	3.90 ±0.34 (26.64%)* (31.96%) _{oo}	2.01 ±0.061 (34.67%) ΔΔΔ	2.14 ±0.36 (6.43%) (27.55%)	4.27 ±0.44 (112.3%)* (99.44%) _{ooo} (9.51%)	4.32 ±0.30 (46.36%) _{□□□}	4.01 ±0.18 (2.86%) _{□□□} (7.28%)	
Gills	3.20 ±0.061	2.22 ±0.59 (30.77%)*	1.42 ±0.26 (55.65%)* (35.95%)	2.15 ±0.012 (32.74%) ΔΔΔ	2.20 ±0.17 (2.05%) (0.86%)	1.35 ±0.18 (37.51%)* (38.76%) _{ooo} (5.23%)	2.11 ±0.12 (5.02)	4.08 ±0.18 (187.87%) _{□□□} (94.15%) _{ooo}	
Gonad	2.90 ±0.062	1.59 ±0.12 (45.31%)* (1.21%)	1.61 ±0.23 (44.65%)* (1.21%)	2.13 ±0.062 (26.44%) ΔΔΔ	2.29 ±0.24 (7.38%) (44.41%) _Δ	1.97 ±0.20 (7.43%) (13.78%) (22.98%)	3.60 ±0.20 (126.85%) _{□□□}	4.42 ±0.44 (175.35%) _{□□□} (22.84%) _o	
Hepato-pancreas	3.32 ±0.126	1.72 ±0.25 (48.38%)* (95.04%) _{ooo}	3.34 ±0.14 (0.69%)* (95.04%) _{ooo}	2.22 ±0.012 (33.33%) ΔΔΔ	2.25 ±0.29 (1.72%) (31.37%)	1.66 ±0.51 (25.06%) (26.33%) (50.38%) _{ΔΔ}	2.46 ±0.21 (43.23%) _□	4.93 ±0.25 (47.62%) _{□□□} (101.20%) _{ooo}	
Adductor Muscles	2.78 ±0.062	1.50 ±0.20 (46.24%)*	2.51 ±0.17 (9.60%)* (68.15%) _{ooo}	2.05 ±0.062 (26.11%) ΔΔΔ	2.22 ±0.30 (7.96%) (48.36%) _Δ	2.46 ±0.12 (19.67%)* (10.85%) (2.20%)	4.12 ±0.33 (176.06%) _{□□□}	0.99 ±0.12 (60.43%) _{□□□} (99.76%) _{ooo}	
Siphon	3.11 ±0.061	1.51 ±0.28 (45.59%)*	1.44 ±0.12 (31.96%)* (4.91%)	1.35 ±0.061 (36.14%) ΔΔΔ	1.44 ±0.25 (6.54%) (4.91%)	1.42 ±0.14 (5.20%) (1.26%) (1.26%)	1.05 ±0.15 (30.62%)	0.74 ±0.24 (48.92%) _{□□} (99.30%)	

(Bracket values represent percentage differences) (*, 0, Δ, □, - P<0.05; **, oo, ΔΔ, □□, - P<0.01; ***, ooo, ΔΔΔ, □□□, - P<0.001; * compared to experimental oysters, 0- compared to LC₀ oysters and Δ, □, - compared to 15 days exposed group of oysters)

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be more noticeable³⁴. However, upon 30 days exposure of zinc caused some how different trend as observed upon 15 days exposure, revealing different type of substrate utilization to meet the energy demand. During the study it was observed that lipid increased in the body parts of mantle, adductor muscle and siphon, which it could be correlated with a high synthesis of lipid in this body parts. In addition, in our study it was also observed that the lipid decreased in specific tissues like, gill, hepatopancreas and gonad, all these evidences suggest us the possibility of a pronounced lipolysis, which, could be related with the higher demand energy during the metal stress. According to our findings on brown mussel, *Perna perna* an increase in lipid peroxidation processes and peroxide levels after 7 and 21 days was observed, confirming the pro-oxidant action of zinc on this species³². Higher peroxide levels suggest an increase in ROS production, possibly causing macromolecular damage; although it is not clear by which mechanism zinc causes an increase in ROS production, it has been related with mitochondrial impairment; it is important to emphasize that it is not clear if the responsiveness of the antioxidant system in bivalves occurs after an acute or chronic stress³². It is still not clear which tissues are the primary targets for regulation viz. gills, digestive gland or others. Nevertheless, coordinated antioxidant response has been previously shown in other mollusks¹², which deserves special attention in previous studies³¹. The depletion of lipid from the different tissues of Indian rock oyster, *C. cattuckensis* when it was exposed to cadmium. However, they suggested that the possibility of its utilization to provide excess energy for cellular metabolic processes probably through lipolysis in anaerobic conditions due to non-essential metal cadmium toxicity²⁸. Previously, found considerable decrease in the total lipid in muscle of freshwater fish, *Tilapia mossambica*, which might be due to drastic decrease in glycogen content in the same tissue after long term exposure to pollutants. reported in blue oysters *Crassostrea hongkongensis* and green oysters, *Crassostrea angulata* from China coast the cellular debris was the main subcellular fraction binding the metals³⁵. With increasing Cu accumulation, its partitioning into the cytosolic proteins decreased. In contrast, metallothioneins-like proteins increased their importance in binding with Zn as tissue concentrations of Zn increased. In the most severely

contaminated oysters, only a negligible fraction of their Cu and Zn was bound with the metal-sensitive fraction. In addition to these, some reports are available on lipid alterations in different species of bivalves under stress conditions by different heavy metals³. Lipid content increased in the gill and muscle of green mussel, *Perna viridis* during exposure to cadmium and copper from Indian coast, however, a decreasing trend in the lipid level was observed in different body parts of freshwater bivalve *L. marginalis* during long term exposed to mercuric chloride²³. The effect of exposure time and concentration of mercury on body biochemical composition of *M. sallei* was studied in time-dependent and concentration-dependent experiments¹¹. In concentration-dependent experiments, there was also a decrease in glycogen/lipid ratio but the glycogen/protein ratio was almost constant at all exposure concentrations. They concluded the study with bivalve exhibited a differential preference in their utilization of biochemical constituents during time- and concentration-dependent stress of metals.

However, in the present study the oysters detoxified over a period of 15 days almost showed similar pattern of gearing of metabolism by utilizing the same substrate from most of the body parts. Further it was observed that that lipid content decreased from siphon but increased from mantle, gonad and hepatopancreas. It is evident that decrease in the protein and glycogen from gonad and hepatopancreas in the oysters depurated from both the concentration probably caused metabolism restricted to lypogenesis and maintenance by utilizing these two substrate. Whereas, lipid content increased in gill, gonad, hepatopancreas, adductor muscle and siphon from the oysters during detoxification process from the both metal concentrations. This showed utilization of protein and synthesis of lipid from all the body parts upon detoxification of the metal irrespective to its concentration in the outside medium. While, increase in the lipid content in the body parts of the oyster suggest us that an inhibition of lipase activity and lipid synthesis could be due to the impairment in carbohydrate metabolism and to the inhibition of enzyme activity in lipid metabolism. Similarly, the present results were correlated with reports from west coast of India²², the lipid content was depleted in different body organs viz. mantle, adductor muscles and gonads of green mussel, *P. viridis* when long term exposure (15 and 30 days) to zinc

metal salt from coast of Maharashtra. Further they observed during the study that lipid content was increased in some body parts like siphon, gill and hepatopancreas when an increased exposed time and even in detoxification process. The reports showed that the drastic changes in lipid content during the study from two estuarine clams, *Katelysia opima* and *Meretrix meretrix* when exposure to chronic concentrations of cadmium metal salt²⁰. On the other hand, the results on two marine bivalves, *Mytilus galloprovincialis* and *Callista chione*, using different concentrations of Cd, Ni, Fe and Zn for 20 days accumulation and 10 days depuration in a laboratory and were determined in their gills, mantle and other body

parts; amongst Fe and Zn levels were much more affected in *M. galloprovincialis*; in general, accumulation and distribution of heavy metal in the tissues showed a metal, species and time of exposure dependent⁹. Further the study revealed that the significant amounts of heavy metals remained in the tissues after 10 days depuration. Hence, our findings supports the idea that the lipid increase observed in some body parts it could be possibility with the fact that zinc was not depurated from those organs; wherever lipid increase or decrease might be due to zinc depurated more from those body parts along with some essential nutrients in process of metal accumulation and detoxification.

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