



Multi-biomarker Responses to Cadmium Toxicity in Indian Green Mussel *Perna viridis* (Linnaeus, 1758)

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Abstract

Bivalves are considered as good bioindicators for monitoring metal contamination in marine environment because of their ability to accumulate heavy metals in their soft tissues from sea water. Present study investigated biological response of Indian green mussel, *Perna viridis* (Linnaeus, 1758), to cadmium toxicity through antioxidant enzymes and overall accumulation during the period of study. The mussels were exposed to cadmium at different sublethal concentrations and the cadmium uptake and accumulation in the soft tissue were estimated after 21 days. Metallothionein formation in the cadmium exposed mussels were also evaluated. Results of the present study showed a definite linear increase in cadmium accumulation during the period of exposure. Mussels exposed to sub lethal concentrations of cadmium at $400 \mu\text{g L}^{-1}$, showed an overall gain of $1.466 \mu\text{g g}^{-1}$ and $1.981 \mu\text{g g}^{-1}$ on 15 and 21 days respectively. Cadmium exposure elicited a significant increase in metallothionein concentration in digestive gland of *P. viridis*, the concentration was found to increase with dose and period of exposure. Antioxidant enzyme levels were higher in hepatopancreas followed by gills on 15 and 21 days of exposure, except in the case of SOD which was higher in gills. Overall, the bioaccumulation of Cd^{2+} in *P. viridis* was found to display a concentration-dependent trend in antioxidant enzyme levels, with significant differences among different tissues exposed.

Keywords: Mussels, *Perna viridis*, cadmium toxicity, heavy metal, antioxidant enzyme, metallothionein

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Introduction

Marine invertebrates especially molluscs are known to accumulate trace metals in varying degrees in their body tissue and hence used widely for monitoring trace metal pollution (Phillips, 1977; 1980; Bryan, 1979; Bryan et al., 1980) and metal concentrations in whole or parts of them was taken as a measure of ambient concentration. Knowledge of accumulation and distribution of metals in the soft tissues may help us to understand the process involved in the uptake and excretion of metals by different parts of molluscs. Moreover, studies reveal that a simple linear relation exists between metal concentration in water and in marine organisms. Mussels are widely used in both, field and laboratory experiments as sensitive markers of trace metal or organic substance contamination (Krishnakumar et al., 1994; Regoli, 1998; Viarengo et al., 1997). Mussels of the genus *Perna* have tremendous potential to accumulate potentially toxic trace metals in their tissues far in excess of the environmental levels particularly in warm waters (Rainbow, 1995).

Exposure to metals is known to generate oxidative stress in living organisms, through the induction of antioxidant defenses, both enzymatic and non-enzymatic. The antioxidant defense system is being studied because of its potential utility to provide biochemical biomarkers that could be used in environmental monitoring system. Biomarkers in environmental monitoring confer significant advantages over traditional chemical measurements because these can be relevantly linked to environmental consequences so that environmental concerns can be directly addressed. The use of multiple biomarkers is more advantageous than the use of a single biomarker and offers an effective early warning system in biomonitoring of aquatic environment. Aim of this work was to evaluate alternations in the level of antioxidant enzymes viz., superoxide

dismutase, catalase, reduced glutathione and glutathione peroxidase along with no enzymatic biomarkers like metallothionein, which are physiologically related to both metal sequestration and defense against metal-induced oxidative stress, using *Perna viridis* as the model organism.

Materials and Methods

The green mussel, *P. viridis* both male and female (3 to 4 cm) were collected from rocky shores of Chellanam, Ernakulam district. The collected mussels were transported to lab in plastic containers in sea water of ambient salinity. On reaching the lab, they were cleaned to remove algae, mud or other fowlers and then washed in a jet of water. They were acclimatized in the laboratory conditions for 48 h in filtered sea water collected from the same station. Salinity of sea water ranged between 30-35‰ and p^H between 8.15 and 8.30.

For LC_{50} studies, ten mussels were randomly selected and exposed to Cadmium Chloride ($CdCl_2$) (Sigma-Aldrich) at a dose of 50, 100, 500, 1000 and 4000 $\mu g L^{-1}$ for a period of 96 h. Sea water was changed every day (2L/animal) and algal suspension (*Chlorella sp.*) as feed was given throughout the experiment. The number of mussels dead and alive was scored on each day for each concentration. The experiment was done in triplicate and the average mortality for each concentration was taken and the LC_{50} value was estimated by Probit analysis.

Sub lethal concentrations were selected on the basis of lethal toxicity studies of $CdCl_2$ on mussels. Ten mussels of shell length 3 to 4 cm were used for each sub lethal concentrations (10, 25, 50, 100, 200 and 400 $\mu g L^{-1}$) and were inspected every 12 h for 21 days. During the course of experiment, sea water was changed daily and the animals were given algal diet in the required quantities.

Mussels exposed to cadmium chloride was excised and whole tissue was kept in separate labeled petri dishes in oven at 65°C for 72 h. The dried tissues were finely ground using a mortar and pestle and a portion (1 g) of it was digested with nitric acid and 60% perchloric acid on a hotplate. Heating was continued until nitric acid evaporated followed by cooling. Heating was continued after adding 5 ml of nitric acid. The mixture was heated to white fumes and 5 ml of HCl and Milli Q water in 1:1 ratio was added. The digested samples were filtered and made up to appropriate dilutions. The obtained

solutions were filtered, analyzed using Air/ Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer) for estimating cadmium content.

The mussel after the exposure period were sacrificed and their gills, hepatopancreas and mantle tissue were dissected out. Enzymatic antioxidants were measured in each tissue samples by homogenizing (1:5 w/v) in 100 mM Tris-HCl buffer pH 8, 0.1 mM phenylmethylsulphonylfluoride (PMSF), 0.008 trypsin inhibitor units, 0.6% NaCl and differential centrifugation was carried out at 4°C to obtain cytosolic fractions. Super oxide dismutase was determined by the method of Mc Cord & Fridovich (1969). One unit of SOD is defined as the enzyme inhibiting 50% of NBT (Nitro blue tetrazolium) per minute mg^{-1} protein. Catalase activity was measured by the loss of absorbance due to consumption of H_2O_2 at 340 nm according to the method of Aebi (1984). Glutathione peroxidase was analysed by the method of Rotruck et al. (1973). Glutathione reductase was estimated by the method of Beutler et al. (1986).

Metallothionein (MT) content was evaluated according to the method of United Nations Environment Programme (UNEP)/RAMOGE (1999) modified from Viarengo et al. (1997). Pooled digestive gland tissues (1 g) were homogenized in 3 vols 0.5 M sucrose, 20 mM Tris-HCl buffer, pH 8.6, containing 0.006 mM leupeptine, 0.5 mM phenylmethylsulphonylfluoride (PMSF) and 0.01% b-mercaptoethanol. The homogenate was centrifuged at 30,000g for 20 min. To the 1 ml supernatant, 1.05 ml cold ethanol and 80 ml chloroform were added; the samples were then centrifuged at 6000 g for 10 min at 48°C. The collected supernatant was combined with 1 mg RNA and 40 ml 37% HCl and subsequently with 3 vols cold ethanol (to a final concentration of 87%). The sample was maintained at -20°C for 1 h, and then centrifuged at 6000 g for 10 min.

The MT containing pellet was washed with 87% ethanol/1% chloroform, centrifuged at 6000 g for 10 min, then dried under nitrogen gas stream. The pellet was resuspended in 150 ml 0.25 M NaCl and 150 ml 1 N HCl containing 4 mM ethylene diamine tetra acetic acid. A volume of 4.2 ml 2 M NaCl containing 0.43 mM DTNB (5,5-dithiobis-2-nitrobenzoic acid) buffered with 0.2 M sodium phosphate, pH 8, was added to the sample at room temperature. The sample was centrifuged at 3000 g

for 5 min, and the supernatant absorbance measured at 412 nm.

All data were represented as mean $\pm$ S.D. (n=6). Statistical analysis for biochemical parameters was carried out by two-way ANOVA using Sigmatat 3.5. ($p < 0.05$) was considered statistically significant.

Results and Discussion

The LC_{50} value (96 h of observation) for cadmium in *P. viridis* was found to be $500 \mu\text{g L}^{-1}$ by Probit analysis. After 15 days of exposure, Cd^{2+} concentration in mussel tissues ranged from 0.077 to $1.466 \mu\text{g g}^{-1}$ dry weight (Fig. 1). Significant ($p < 0.001$) difference in cadmium accumulation was found in 15 and 21 days of exposure (Sokolova et al., 2005; Gomes et al., 2014). Post treatment analysis of mussel exposed to sub lethal concentrations of cadmium, showed an overall gain of $1.466 \mu\text{g g}^{-1}$ and $1.981 \mu\text{g g}^{-1}$ for $400 \mu\text{g L}^{-1}$, for 15 and 21 days respectively. An abrupt increase in cadmium accumulation was reported at 200 and $400 \mu\text{g L}^{-1}$ on 15 and 21 days.

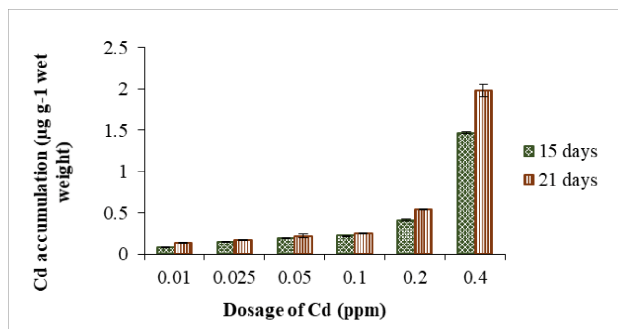


Fig. 1. Cadmium accumulation in *P. viridis* exposed to sub lethal concentrations on 15th and 21st day. Values are expressed as Mean $\pm$ S.D

Many authors have reported cadmium accumulation in the soft tissue of *P. viridis* (Kamaruzzaman et al., 2011; Ponnusamy et al., 2014; Budiawan et al., 2020). In the present study the rate of accumulation of cadmium showed an increasing trend from 15th to 21st day of exposure. Similar observations were also made by Chan (1988). According to Li et al (2006), bivalves do not regulate cadmium and hence they are incapable of regulating the metal in their body and tend to accumulate in tissues.

P. viridis exposed to cadmium showed increase in catalase activity with increase in concentration and time (Table 1). The activity was higher in hepatopancreas followed by gills and mantle ($p < 0.01$). In

bivalves increased catalase activity is associated with heavy metal accompanied tissue burden, which seem to induce CAT activity in bivalves.

CAT is the primary scavengers of H_2O_2 in the cell. Increased catalase activity in cadmium exposed *P. viridis*, further prove that stress caused by metal has elevated the formation of H_2O_2 . As referred by Martins et al. (2013), the mechanisms of CAT response have to be more rapid and efficient in gills than in other tissues. These results are in agreement with those reported by Rajkumar & John Milton (2011) who observed a significant induction of catalase in *Perna viridis* by Cd, Cu, Pb, and Zn under short toxicity test. Moreover, these results are comparable to the study by Pan et al., (2006) where it was shown that CAT activity increased in sites of metal contamination. Also, elevated activity of CAT was reported in *Mytilus galloprovincialis* in the Adriatic Sea (Borkovic' et al., 2005).

SOD activity significantly increased in a hepatic, mantle and branchial tissues with time of exposure and concentrations of Cd^{2+} ($p < 0.01$). Among the antioxidant enzymes, SOD is the first defensive enzyme to neutralize superoxide and helps to protect cells against destruction (Fridovich, 1975). Gills showed only slightly higher SOD activity than hepatopancreas and mantle on 21st day. Overall, SOD activity was higher for gills than other tissues except for $10 \mu\text{g L}^{-1}$ on 15th day of exposure. The elevated SOD levels in gills indicate the greater potential of O_2 production in gills during respiration. This is expected because gills are the prominent organ bioaccumulating metals. Similar observations were also made by Regoli in 1998, in his study on Mediterranean mussel *Mytilus galloprovincialis*. Enhanced levels of SOD activities would help to neutralize the toxic effect of Cd^{2+} and in turn protect the membrane lipids against oxidative stress.

The reduced glutathione decreased in all the three tissues after 21 days of Cd^{2+} exposure. The levels were higher in hepatopancreas than in gill tissue (Table 1). The lowest GSH level was observed for mantle compared to other tissues ($p < 0.01$). The metal detoxification process is directed through thiolate sulfur atom present in GSH, thus representing the first line of defense against heavy metal cytotoxicity, which may be responsible for reduced GSH content, observed in the study. As an initial response to Cd^{2+} stress, GSH might chelate with Cd^{2+} ions, as demonstrated by its decreased level

Table 1. Antioxidant response of *Perna viridis* to cadmium toxicity after 15th and 21st days of exposure

Cd Conc. ($\mu\text{g L}^{-1}$)	Biochemical Parameters							
	CAT ($\mu\text{moles mg}^{-1}$ protein)		SOD (U mg^{-1} protein)		GSH (nmoles 100 g^{-1})		GPx ($\mu\text{g mg}^{-1}$ protein)	
	15 th day	21 st day	15 th day	21 st day	15 th day	21 st day	15 th day	21 st day
Hepatopancreas								
10	11 $\pm$ 0.03	12.3 $\pm$ 0.09	4.27 $\pm$ 0.04	4.44 $\pm$ 0.05	13.82 $\pm$ 0.2	13.06 $\pm$ 0.1	5.2 $\pm$ 0.9	5.4 $\pm$ 1
25	11.4 $\pm$ 0.23	12.4 $\pm$ 0.23	4.31 $\pm$ 0.01	4.51 $\pm$ 0.2	13.75 $\pm$ 0.3	12.8 $\pm$ 0.5	5.29 $\pm$ 0.23	5.63 $\pm$ 0.9
50	11.6 $\pm$ 0.05	12.7 $\pm$ 0.4	4.39 $\pm$ 0.04	4.6 $\pm$ 0.6	13.5 $\pm$ 0.07	12.63 $\pm$ 0.07	5.35 $\pm$ 0.8	5.74 $\pm$ 1.1
100	11.7 $\pm$ 0.4	12.8 $\pm$ 0.4	4.4 $\pm$ 0.09	4.68 $\pm$ 0.26	13.36 $\pm$ 0.1	12.43 $\pm$ 0.1	5.48 $\pm$ 0.07	5.8 $\pm$ 0.3
200	12 $\pm$ 0.42	12.9 $\pm$ 0.04	4.52 $\pm$ 0.01	4.74 $\pm$ 0.01	13.01 $\pm$ 0.04	12.2 $\pm$ 0.5	5.71 $\pm$ 0.04	5.83 $\pm$ 0.7
400	12.3 $\pm$ 0.02	12.99 $\pm$ 0.02	4.6 $\pm$ 0.03	4.8 $\pm$ 0.03	12.6 $\pm$ 0.6	11.82 $\pm$ 0.01	5.86 $\pm$ 1	6.1 $\pm$ 0.84
Gills								
10	6.2 $\pm$ 0.04	6.7 $\pm$ 0.4	4.21 $\pm$ 0.03	4.55 $\pm$ 0.5	12 $\pm$ 0.09	11.64 $\pm$ 0.03	3.61 $\pm$ 0.04	3.86 $\pm$ 0.3
25	6.37 $\pm$ 0.2	6.8 $\pm$ 0.23	4.39 $\pm$ 0.23	4.61 $\pm$ 0.8	11.8 $\pm$ 0.43	11.55 $\pm$ 0.5	3.66 $\pm$ 0.7	3.9 $\pm$ 0.78
50	6.5 $\pm$ 0.04	6.91 $\pm$ 0.5	4.53 $\pm$ 0.05	4.69 $\pm$ 0.3	11.67 $\pm$ 0.5	11.34 $\pm$ 0.5	3.74 $\pm$ 0.5	4.02 $\pm$ 0.04
100	6.59 $\pm$ 0.09	7.23 $\pm$ 0.09	4.72 $\pm$ 0.4	4.73 $\pm$ 0.2	11.5 $\pm$ 0.09	11.29 $\pm$ 0.09	3.82 $\pm$ 0.1	4.11 $\pm$ 0.5
200	6.63 $\pm$ 0.31	7.5 $\pm$ 0.1	4.95 $\pm$ 0.04	4.8 $\pm$ 0.09	11.42 $\pm$ 0.31	11.1 $\pm$ 1	3.9 $\pm$ 0.04	4.2 $\pm$ 0.07
400	6.68 $\pm$ 0.03	7.79 $\pm$ 0.03	5.3 $\pm$ 0.02	4.91 $\pm$ 0.02	11.3 $\pm$ 0.03	11.02 $\pm$ 0.07	4.04 $\pm$ 0.03	4.35 $\pm$ 0.58
Mantle								
10	5.8 $\pm$ 0.1	6.0 $\pm$ 0.1	3.6 $\pm$ 0.1	4.01 $\pm$ 0.1	11.58 $\pm$ 0.08	11.55 $\pm$ 0.1	4.4 $\pm$ 0.2	4.11 $\pm$ 0.7
25	5.9 $\pm$ 0.02	6.23 $\pm$ 0.02	3.67 $\pm$ 0.02	4.15 $\pm$ 0.1	11.46 $\pm$ 0.1	11.41 $\pm$ 0.04	4.52 $\pm$ 0.05	4.2 $\pm$ 0.92
50	6.04 $\pm$ 0.03	6.54 $\pm$ 0.03	3.78 $\pm$ 0.03	4.24 $\pm$ 0.2	11.39 $\pm$ 0.24	11.2 $\pm$ 0.9	4.63 $\pm$ 0.06	4.23 $\pm$ 0.3
100	6.17 $\pm$ 0.5	6.67 $\pm$ 0.3	3.81 $\pm$ 0.3	4.36 $\pm$ 0.06	11.2 $\pm$ 0.11	10.53 $\pm$ 0.6	4.7 $\pm$ 0.3	4.5 $\pm$ 0.9
200	6.26 $\pm$ 0.3	7.1 $\pm$ 0.3	3.9 $\pm$ 0.3	4.42 $\pm$ 0.3	11.09 $\pm$ 0.4	10.15 $\pm$ 0.3	4.8 $\pm$ 0.3	4.63 $\pm$ 0.4
400	6.82 $\pm$ 0.2	7.01 $\pm$ 0.06	4.02 $\pm$ 0.2	4.56 $\pm$ 0.9	10.96 $\pm$ 0.04	9.5 $\pm$ 0.66	4.92 $\pm$ 0.2	4.72 $\pm$ 0.2

towards higher Cd^{2+} concentrations (Table 1). This may be due to the fact that, accumulation of Cd^{2+} can form a GSH-metal complex, resulting in the depletion of cellular GSH (Xia et al., 2016; Yao et al., 2020a). At higher Cd^{2+} concentration (400 $\mu\text{g L}^{-1}$), the significantly lower levels of GSH in the gill and mantle compared to hepatopancreas might indicate partial toxicity caused by Cd^{2+} (Yao et al., 2020b).

Pre-exposure to cadmium induced a significant dose dependent increase in gill GPx activity compared with the mantle and hepatopancreas ($p < 0.001$), suggesting that gill GPx activity was highly susceptible to cadmium exposure (Table 1). Present observations are in consistent with another study which concluded that inhibition of GPx was more

pronounced in the gill than in the hepatopancreas (Cossu et al., 1997). Induction of the enzymatic catalyst (GPX) indicates that the existing pool of glutathione is being utilized in defense against oxidative stress at a greater rate than usual.

The study suggests that Cd^{2+} exposure increases the formation of reactive oxygen species. It was reported in the Pacific oyster, *Crassostrea gigas*, that exposure to Cd^{2+} leads to increased H_2O_2 , SOD, CAT and GPX as compared with controls (Jo et al., 2008). These elevated levels may due to an increase in the defensive mechanisms of the antioxidant system as a result of ROS production exerted by Cd^{2+} . Funes et al. (2006) demonstrated that antioxidant enzyme activity is higher in metal polluted areas than in clean areas by comparing levels of SOD, CAT, and

GPX activity in Portuguese oysters, *Crassostrea angulata*, and Mediterranean mussels, *Mytilus galloprovincialis*. These increases may represent a protective mechanism against metal pollution, which induced the production of ROS. These results suggest that, to protect itself from ROS, the mussel intensify the antioxidant defense system with simultaneous increases in Cd^{2+} dose and exposure time. In addition, the results provide evidence that enzyme and non-enzyme biomarkers can be sensitive indicators of aquatic pollution caused by heavy metals.

Cadmium exposure elicited a significant increase in metallothionein concentration in digestive gland of *P. viridis* as it is a known MT inducer (Fig. 2). The concentration was found to increase with dose and period of exposure ($p < 0.05$), having an MT level of $786.2 \mu\text{g protein g}^{-1}$ wet weight for $400 \mu\text{g L}^{-1}$ on 21st day of exposure. The exposure of marine organisms to cadmium can result in some biochemical or physiological changes that can protect organisms from cadmium toxicity. Metallothioneins are non-enzymatic proteins which bind metals with their cysteine thiol groups ($-\text{SH}$), and this binding can detoxify toxic metals and regulate the metabolism of trace elements (Amiard et al., 2006; Zhu et al., 2018). Binding of Cd^{2+} to MT can lower the availability of free metal ions, thus controlling the intracellular levels of Cd^{2+} and reducing its toxicity (Lavradas et al., 2014; Chan & Wang, 2018).

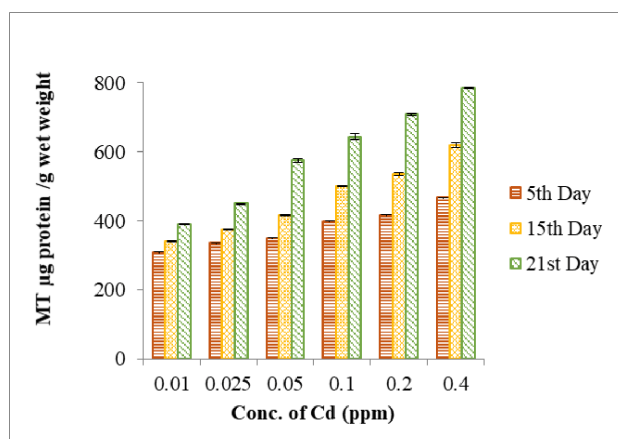


Fig. 2. Metallothionein activity in various tissues of *P. viridis* exposed to sublethal conc. of CdCl_2

The results suggested that in *P. Viridis*, response to the toxic effect of Cd^{2+} might occur through a combination of mechanisms, which involve both enhanced antioxidant enzyme activities and the

ability to bind and sequester Cd^{2+} via cysteine-rich molecules such as metallothionines, both of which would eventually lead to the reduction of heavy metal-induced oxidative stress. In conclusion, the results of this study show the suitability of enzyme and non-enzyme biomarker responses as indicators of heavy metal toxicity in mussels and *P. viridis* can be a suitable bioindicator of heavy metal pollution monitoring in aquatic environments.

References

- Aebi, H. (1984) Catalase. In: Methods in Enzymology (L. Packer Edn), pp121-126. Academic Press, Orlando
- Amiard, J. C., Amiard-Triquet, C., Barka, S., Pellerin, J. and Rainbow, P. S. (2006) Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. *Aquat. Toxicol.* 76: 160-202
- Beutler, E., Gelbart, T. and Pegelow, C. (1986) Erythrocyte glutathione synthetase deficiency leads not only to glutathione but also to glutathione-S-transferase deficiency. *J. Clin. Invest.* 1: 38-41
- Borkoviae, S.S., Saponjiae, J.S., Pavloviae, S.Z., Blagojeviae, D.P. et al. (2005) The activity of antioxidant defence enzymes in the mussel *Mytilus galloprovincialis* from the Adriatic Sea. *Comp. Biochem. Physiol. Toxicol. Pharmacol.* 141(4): 366-74
- Bryan, G. W., Langston, W. J., Hummerstone, L. G. (1980) The use of biological indicators of heavy metal contamination in estuaries. *Occ. Publ. mar. biol. Ass. U. K.* 1: 1-73
- Bryan, G.W. (1979) Bioaccumulation of marine pollutants. *Phil. Trans. R. Soc. Lond. (B)* 286: 483-505
- Budiawan, R. B., Aziz S. A and Suseno, H. (2020) Study of bioaccumulation and heavy metal depuration of cadmium in green mussel (*Perna viridis*). *IOP Conf. Ser.: Mater. Sci. Eng.* 902: 012057
- Chan, C. Y., and Wang, W. X. (2018) Seasonal and spatial variations of biomarker responses of rock oysters in a coastal environment influenced by large estuary input. *Environ. Pollut.* 242: 1253-1265
- Chan, H. M. (1988) Accumulation and tolerance to cadmium copper lead and zinc by the green mussel *Perna viridis*. *Mar. Ecol. Prog. Ser.* 48: 295-393
- Cossu, C., Doyotte, A., Jacquin, M. C, Babut, M, et al. (1997) Glutathione reductase, selenium- dependent glutathione peroxidase, glutathione levels and lipid peroxidation in freshwater bivalve, *Unio turmidus* as biomarkers of aquatic contamination in field studies. *Ecotox. Environ. Saf.* 38: 122-131
- Fridovich, I. (1975) Superoxide dismutases. *Ann. Rev. Biochem.* 44: 147-159

- Funes, V., Alhama, J., Navas, J.I., López-Barea, J., Peinado, J. (2006) Ecotoxicological effects of metal pollution in two mollusc species from the Spanish South Atlantic littoral. *Environ. Pollut.* 139(2): 214-223
- Gomes, T., Chora, S., Pereira, C.G., Cardoso, C. and Bebianno, M. J. (2014) Proteomic response of mussels *Mytilus galloprovincialis* exposed to CuO NPs and Cu²⁺: An exploratory biomarker discovery. *Aquat. Toxicol.* 155: 327-336
- Jo, P. G., Choi, Y. K., Choi, C. Y. (2008) Cloning and mRNA expression of antioxidant enzymes in the Pacific oyster, *Crassostrea gigas* in response to cadmium exposure. *Comp Biochem. Physiol. Toxicol. Pharmacol.* 47(4): 460-9
- Kamaruzzaman, B.Y., Zahir, M.M.S., John, B.A., Jalal, K.C.A., Shahbudin, S., Al-Bawarni, S.M. and Goddard, J.S. (2011) Bioaccumulation of some metals by green mussel *Perna viridis* (Linnaeus 1758) from Pekan, Pahang, Malaysia. *Int. J. Biol. Chem.* 5(1): 54-60
- Krishnakumar, P.K., Casillas, E. and Varanasi, U. (1994) Effects of environmental contaminants on the health of *Mytilus edulis* from Puget Sound, Washington, USA. *Mar. Ecol. Prog. Ser.* 106: 249-26
- Lavradas, R. T., Hauser-Davis, R. A., Lavandier, R. C., Rocha, R. C. C., Saint' Pierre, T. D., Seixas, T., et al. (2014) Metal, metallothionein and glutathione levels in blue crab (*Callinectes* sp.) specimens from south-eastern Brazil. *Ecotox. Environ. Saf.* 107, 55-60
- Li, Y., Yu, Z., Song, X., Mu, Q. (2006) Trace metal concentrations in suspended particles, sediments and clams (*Ruditapes philippinarum*) from Jiaozhou Bay of China. *Environ. Monit. Assess.* 121: 491-501
- Martins, M., Costa, P.M., Ferreira, A.M. and Costa, M.H. (2013) Comparative DNA damage and oxidative effects of carcinogenic and non-carcinogenic sediment-bound PAHs in the gills of a bivalve. *Aquat. Toxicol.* 142: 85-95
- Mc Cord, J.M., Fridovich, I. (1969) Superoxide dismutase. An enzymic function for erythrocyte (hemocytin). *J. Biol. Chem.* 244(22): 6049-55
- Pan, L.Q., Ren, J. and Liu, J. (2006) Responses of antioxidant systems and LPO level to benzo (a) pyrene and benzo (k) fluoranthene in the haemolymph of the scallop *Chlamys farreri*. *Environ. Pollut.* 141: 443-451
- Phillips, D. J. H. (1977) The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments - a review. *Environ. Pollut.* 13: 281-217
- Phillips, D. J. H. (1980) Quantitative aquatic biological indicators - their use to monitor trace metals and organochlorine pollution. *Appl. Sci. London.* 1-488
- Ponnusamy, K., Sivaperumal, P., Suresh, M., Arularasan S., Munilkumar S. and Pal. A.K. (2014) Heavy Metal Concentration from Biologically Important Edible Species of Bivalves (*Perna viridis* and *Modiolus metcalfei*) from Vellar Estuary, South East Coast of India. *J. Aquac. Res. Development.* 5(5): 1000258
- Rainbow, P. S. (1995) Biomonitoring of heavy metal availability in the marine environment. *Mar. Poll. Bull.* 31(4-12): 183-192
- Rajkumar, J.S.I., John Milton, M.C. (2011) Biochemical markers of oxidative stress in *Mugil cephalus* exposed to cadmium, copper, lead and zinc. *Int. J. Pharma. Bio. Sci.* 2: 41-50
- Regoli, F. (1998) Trace metals and antioxidant enzymes in gills and digestive gland of the Mediterranean mussel *Mytilus galloprovincialis*. *Arch. Environ. Contam. Toxicol.* 34: 48-63
- Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. (1973) Selenium: biochemical role as a component of glutathione peroxidase. *Science.* 179(4073): 588-90
- Sokolova, I.M., Sokolov, E.P. and Ponnappa, K. M. (2005) Cadmium exposure affects mitochondrial bioenergetics and gene expression of key mitochondrial proteins in the eastern oyster *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). *Aquat. Toxicol.* 73: 242-255
- UNEP/RAMOG. (1999) Manual on the biomarkers recommended for the MED POL biomonitoring programme. UNEP, Athens, Greece
- Viarengo, A., Ponzano, E., Dondero, F. and Fabbri, R. (1997) A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic molluscs. *Mar. Environ. Res.* 44 (1): 69-84
- Xia, L. P., Chen, S. H., Dahms, H. U., Ying, X. P. and Peng, X. (2016) Cadmium induced oxidative damage and apoptosis in the hepatopancreas of *Meretrix meretrix*. *Ecotoxicology.* 25: 959-969
- Yao, H., Hongchao, T., Jianyu, J., Meng, F., Alan, K. C and Xueping, Y. (2020 a) Effects of Waterborne Cadmium Exposure on Its Internal Distribution in *Meretrix meretrix* and Detoxification by Metallothionein and Antioxidant Enzymes. *Front. Mar. Sci.* 5: 502
- Yao, J., Yang, Z. G., Li, H. P., Qu, Y. B. X. and Qiu, B. (2020 b) Effects of waterborne exposure to cadmium on biochemical responses in the freshwater gastropod, *Bellamya aeruginosa*. *Ecotox. Environ. Saf.* 193: 110365
- Zhu, Q. H., Zhou, Z. K., Tu, D. D., Zhou, Y. L., Wang, C., Liu, Z. P. et al. (2018) Effect of cadmium exposure on hepatopancreas and gills of the estuary mud crab (*Scylla paramamosain*): histopathological changes and expression characterization of stress response genes. *Aquat. Toxicol.* 195: 1-7