Indian Journal of Experimental Biology
Vol. 58, November 2020, pp. 794-802

Environmental monitoring using biomarkers in relevance to heavy metal pollution in coastal areas of the Gulf of Mannar

N Nagarani*, M Anand & AK Kumaraguru

Department of Marine & Coastal Studies, School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai-625 021, Tamil Nadu, India

Received 22 June 2019; *revised 22 October 2020*

Marine organisms are vulnerable to harsh environment where fluctuation in physicochemical conditions including salinity and tides are common. These organisms over the period have evolved different mechanisms to maintain the physiological conditions of the body through metabolic activities. In the present work, we studied accumulation of about 10 heavy metals in about 20 marine organisms from the Gulf of Mannar, Southeast coast of India and also explored the passable relationship between distribution of these inorganic metals and biochemical response of these organisms. The effect on the accumulation of metals over the metabolism in the marine animals was evaluated by the level of antioxidant response. Concentration of metal in the body tissues was analyzed using ICP-AES. Interestingly, it was also observed that genotoxic potential pollutants were less or negligible in the selected sites. Tail DNA in the muscle cells was observed to be <5%. Our study concludes that fishes from the two areas are not heavily burdened with metals, but pollutants should be monitored periodically to avoid excessive intake of trace metals by humans due to bioaccumulation. The work concludes that a multiparameter analysis should be followed to study the ecological status of the aquatic environment.

Keywords: Antioxidant, Bioconcentration factor, Environmental impact assessment, Marine pollution

The Gulf of Mannar (GoM) is the first marine biosphere reserve area in Southeast Asia established to secure fundamental diversity of organisms while continuing to promote economic growth. However, Gulf of Mannar coastal waters and offshore have been contaminated by discharge from sewage, industrial outlets (organic carbon); fertilizer, chemical industries (chlorinated hydrocarbons and heavy metals); thermal power station (heated effluents and fly ash); fishing harbor, major port (oil spill) and shrimp farm effluents in that area[1,2]. Other anthropogenic activities such as destructive trawling, coral mining using cyanide to catch reef fishes, dredging of shipping channels produce large quantities of metal pollution, metals such as Al, Hg, Pb, etc., also enter the sea from the atmosphere as wet deposition (natural inputs)[3].

Most of the metals get dissolved in seawater or particulate in sediments. The metals in seawater enters the marine fish through gills, small quantity get absorbed by the marine organisms and utilized for their biological activities. Minerals such as Fe, Cu, Co, Mo, Zn, Mn and Ca play a vital role in cell metabolism. Metals such as Hg, Pb, Sn, Ni, Se, Cr and As that are generally not required for metabolic activity and are toxic to living organisms at even low concentrations[4].

Heavy metals accumulate in marine organisms to high levels in body tissue, which remains in the cytoplasm and induce oxidative stress via generation of free radicals or reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, hydroxyl radicals, etc.[5]. ROS leads to multiple distractions in cellular constituent and metabolism, which in turn results in lipid peroxidation, protein cleavage, DNA damages, etc[6].

The investigation on biomarkers for efficient environmental monitoring is essential for the researchers, environmentalist, government, etc.[7]. Choosing a suitable biological marker for the study of contaminant is a controversial issue, since two or more elements can either synchronize or compete with more adverse effect than by a single element[8]. Even very low exposure can cause dreadful effects. That too, when information on the mechanism of action of the contaminant is incomplete its effect is unpredictable with single biomarkers. Studies on bioindicator organism with respect to heavy metal contamination in coastal regions are not uncommon. A few of them are mollusc and seagrass[9], Crab[10], marine sponge *Haliclonatenuiramosa*, *Petrosia*

*Correspondence:
E-Mail: nagaranikannan@yahoo.co.in

testudinaria[11,12] , macroalgae[13], *Posidonia oceanica*[14], mussel *Perna viridis, M. galloprovincialis*[15,16], gastropod *Osilinusatrata*[17] and algae[18]. However, only limited investigations are available for ecotoxicological studies in a natural environment from southeast coastal regions of India. In the present study, we made an attempt to establish a baseline report on the Gulf of Mannar Biosphere Reserve (GoMBR) on the impacts of heavy metal pollutants and assess the biomarkers to predict the environmental changes. Further, the information on the biomarkers assessments in marine organisms can be used for site specific risk assessments and in establishing water quality guidelines or criteria for the Indian coastal regions.

Materials and Methods

Sample collection and site descriptions

The study area Gulf of Mannar (8° 47’ to 9° 15’ N Latitude and 78° 12’ to 79° 14’ E Longitude) is the first marine biosphere reserve in South and Southeast Asia located in the Indo-Pacific region. It is known for its richness of biological wealth with ecological uniqueness, scientific research and global significance. Healthy specimens of fish and invertebrate were procured and collected during January to December 2014 from Mandapam (Latitude 9°16’50’’N and Longitude 79°10’35’’E) and Chinnamuttom fishing harbor (Latitude 8°5'45"N and Longitude 77°33'47"E) of the Gulf of Mannar, Southeast coast of India (Fig. 1). Seawater samples from each region were collected using Teflon coated Niskin samplers (to avoid metal contamination) from 10 m depth. Collected samples were immediately wrapped in a sterile bag and placed in an isolated container and transported to the University Research Laboratory located at Pudumadam. Temperature of 4°C is maintained while transport. The samples were stored at –80°C for further analysis.

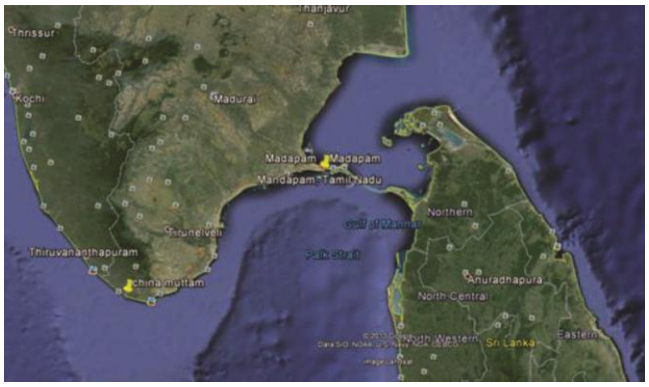

Fig. 1 — Map showing the sampling site

Water quality analysis

Water quality parameters such as pH, dissolved oxygen (DO), salinity and temperature were measured in situ using water and soil analysis kit model 1160-E.and Winkler method[19].

Heavy metal extraction and BCF analysis

To minimize contamination, all the materials used in the experiments were previously washed in $KMNO_4$ followed by ultra pure water, and a stainless steel knife was used to cut the tissues. The concentration of heavy metals (Lead, Manganese, Nickel, Mercury, Magnesium, Arsenic, Iron, Copper, Cadmium, Molybdenum and Zinc) was determined according to the standard double acid digestion methods[20] and analyzed using ICP-AES (Model: ARCOS from M/s. Spectro, Germany, SAIF- IIT Bombay). Standards were made using certified solutions (Merck, UK) acidified with HNO_3 to the same pH as the samples. Results are expressed as the means ± S.E. of three replicate samples. The percentage of bio-concentration factor (BCF) was calculated according to the equation

$$BCF = \frac{Cb}{Cw}\ x\ 100$$

Where, Cb is the concentration of the heavy metals in the fish and Cw is the concentration of the heavy metals in the water[21].

Biochemical enzyme analysis

The muscle tissue (1.0 g) were homogenized in trichloro acidic acid (10 mL; 5%) and ice cold phosphate buffered saline separately for analyzing lipid peroxidation and enzymes, respectively. The extract was centrifuged at 8000 rpm for 10 min at 4ºC. MDA level was determined to indicate the level of lipid peroxidation in the collected species as described by Buege & Aust[22]. The antioxidant enzyme response was measured using Double beam UV spectrophotometer (Model 2201; Systronics) following the methods viz., catalase[23], superoxide dismutase[24], glutathione peroxidase[25], reduced glutathione[26] and ascorbate peroxidase[27].

Nuclear damage studies

Micronucleus (MN)

The whole blood and hemolymph were collected from the healthy fish (by caudal vein puncture techniques) and invertebrates respectively using a heparinised syringe.The slides were prepared by

smearing one drop of collected liquid on clean microscopic slides, fixed in methanol for 10 min and air dried at room temperature. The slides were stained with 6% giemsa in phosphate buffered saline for 5-10 min. Five microscopic slides were prepared for each fish. Micronucleus test was preceded based on Bahari *et al*.[28]. Slides were scored by a single observer using blind review, in the light microscope, lens using 100X oil immersion magnification. For the MN scoring purpose, only non refractive small nuclei (>1/3rd of the diameter of the main nucleus) located close to the main oval nucleus of round erythrocytes with intact cytoplasm were considered. Mean MN frequencies and standard deviation were expressed as the number of MN per 5000 erythrocytes (500 cells/slide), for each concentration for five fish (mean of 10 slides).

Comet assay or alkaline single cell gel electrophoresis

The healthy or freshly dead samples with >75% (for reliability) of active cell were used for the comet assay. The viability of the cell was tested using the trypan blue method[29]. The comet assay was performed as per the methodology of Tice *et al*.[30] with some modifications. Hydrogen peroxide was used as a positive control. All preparation steps were performed under dim light and ice cold conditions to prevent additional DNA damage and to inactivate photoactive DNA repair process. The slides were analyzed using a Carl Zeiss HB50 fluorescence microscope (magnification 40X) with an excitation filter of 510–560 nm. Images of 100 randomly selected cells (50 counts on each duplicate slide) were analyzed for each sample. Mean score and standard deviation were calculated for the comet classes.

While scoring there was a close relation between the subjective visual score. A number of objective measurements were used such as percentage DNA in tail, tail length (measured from the leading edge of the comet head) and tail moment. Tail moment was calculated as follows:

$$Tail\ moment = \frac{Tail\ length \times \%\ Tail\ DNA}{100}$$

In 90% of the cells, the percentage of DNA in the tail region categories into the following non-overlapping ranges: class 0 (no damage), <5%; class 1 (low damage), 5–25%; class 2 (medium damage), 25–45%; class 3 (high damage), 45–70%; class 4 (very high damage), >70%.

Statistical analysis

DNA damage was analysed using Comet version 1.1 software. Data were analyzed through ANOVA followed by post-hoc mean comparison test (Tukey's test). Significance level adopted was 95%. Results were expressed as mean ± standard error. All assays were repeated thrice for setting concordant values. All data presented in the paper are the means of at least three replicates.

Results and Discussion

Physicochemical characteristics of sampling sites

The physicochemical parameters have been analyzed in order to characterize the Gulf of Mannar coastal waters and to find possible correlations between metal concentrations and some of these parameters were depicted in Table 1. The surface water temperature varied from 30 to 32^{o}C during the sample collection. Generally, surface water temperature is subjective to the natural parameters like, the intensity of solar radiation, freshwater inflow, etc.[31]. The average dissolved oxygen level for polluted waters in the Gulf of Mannar as previously reported as 3.79 mL/L[31]. Phosphate and nitrate concentrations were less and nitrite was higher when compared to the previous report[32]. Nitrite when enters the biological systems it can oxidize the antioxidants, that are significantly more toxic to aquatic life than nitrate[33].

Heavy metal analysis

Heavy metal analysis in biological samples usually involves membrane obliteration by double acid digestion and preventing the localization of potentially toxic elements in situ. The concentrations (µg L^{-1}) of heavy metals in the study sites ranged within the permissible limits (30 µg L^{-1} for Cu, 100 µg L^{-1} for Zn and Fe; 1 µg L^{-1} for Mn and Cd), whereas concentrations of Hg, Cd, Pb, MO and As, were very low or below the detectable limits (BDL) (Table 1).

The distribution of metal contents in the marine fish is shown in Table 2. The pattern of metal accumulation is species specific and significant with P <0.5 for Mandapam and Chinnamuttom region (18; P = 0.431058 and 10; P = 0.4521, respectively). It seems that the tendency of fish to accumulate the metals was exactly the same order in all the species, Mg > Fe > Zn >Mn> Ni > Cu. In the present study bioaccumulation of Mercury, Arsenic, Lead, Molybdenum and Cadmium in the marine fish were negligible or below the detectable range (0.01 01 µg. g^{-1}). At the same time the Pb and As are found to bioaccumulate in marine fish from the Chinnamuttom region and found to be in the order of

Table 1 — Water Quality Parameters

Physicochemical parameters	Mandapam	Chinnamuttom
Temperature (°C)	30.2±1.0	29.1±1.0
pH	8.1±0.1	8.31±0.1
Salinity (‰)	32.0±1.5	34.4±0.1
Dissolved oxygen (mg.L^{-1})	5.5±0.3	8.2±0.1
BOD (mg.L^{-1})	119.3±5.4	3.6±0.01
COD (mg.L^{-1})	3.50±0.2	108±0.01
Total organic carbon %	0.25±0.040	0.24±0.01
Ammonia (mg.L^{-1})	0.01±0.001	6.98±0.11
Nitrite (mg.L^{-1})	0.014±0.01	0.08±0.001
Nitrate (mg.L^{-1})	0.043±0.01	0.44±0.02
Phosphate (mg.L^{-1})	0.014±0.008	0.34±0.01
Silicate (mg.L^{-1})	0.03±0.008	0.149±0.01
Zn (ppb)	12±0.01	1.9±0.3
Cu (ppb)	0.83±0.002	0.59±0.05
Pb (ppb)	15.09±0.1	38±0.05
Cd (ppb)	0.45±0.001	0.8±0.01
Cr (ppb)	1±0.001	2±0.1
Ni (ppb)	2.0±0.1	3±0.01
Mn (ppb)	BDL	0.67±0.001
Fe (ppb)	67.03±0.5	74.5±0.05
Hg	BDL	BDL

Mg > Cu > Fe > Zn > Ni >Mn > Pb > AS. The species collected from Chinnamuttom region show no significant variation among all species in the accumulation of Zn, Fe, Cd, MN, Hg and Mo metals. Some species showed higher values for specific metals such as *Mugil cephalus* (Cu; 90%); *Carangoides malabaricus* (Ni) and *Scarus ghobban*, *Anchoviella commersoni* and *Chanos chanos* (Mg; 41.5, 38.8 and 38.7%, respectively) (Table 2). The present study reveals that the accumulation of Zn and Fe (83%) were closely related than Ni (51.99%) in fishes from Mandapam region (Fig. 2A).

The calculated bio-concentration factor (BCF) for the different metals in water and tissues of Chinnamuttom generally showed the highest enrichment of heavy metals that of Mandapam collected samples (10 to 50 times in waters for Zn, Fe and Cd), which are strongly influenced by the anthropogenic introduction of heavy metal. The BCF values of estimated metals in the Mandapam shore

Table 2 — Heavy metal accumulation in marine organisms of Mandapam and Chinnamuttom region

Species	Heavy metal concentration (µg.g^{-1})							
Mandapam region	Cu	Zn	Fe	Mn	Ni	Mg		
Megalaspis cordyla	0.13±0.01	3.035±0.42	3.957±0.01	0.215±0.01	0.162±0.1	87.032±0.45		
Chanos chanos	0.115±0.03	1.667±0.01	3.311±0.01	0.159±0.01	0.031±0.2	44.59±4.32		
Scarus ghobban	0.078±0.1	2.275±0.02	1.692±0.12	0.399±0.2	0.389±0.2	57.49±0.66		
Thunnus albacares	0.11±0.02	0.848±0.02	3.869±0.0	0.167±0.1	0.162±0.01	46.73±0.2		
Anchoviella commersoni	0.068±0.04	0.775±0.01	8.92±0.02	1.395±0.1	0.053±0.2	67.55±0.0		
Tetradon immaculatus	0.4±0.01	0.55±0.05	1.25±0.05	0.271±0.1	0.4±0.01	80.01±0.01		
Scarus russelii	0.112±0.05	0.87±0.6	0.9±0.06	0.192±0.1	0.127±0.1	66.91±0.2		
Thunnus obesus	0.04±0.03	0.62±0.01	1.22±0.089	0.66±0.2	0.291±0.1	71.92±0.3		
Terapon jarbua	0.22±0.1	0.59±0.001	2.1±0.01	0.322±0.01	0.071±0.1	67.22±0.4		
Sardinella brachysoma	0.04±0.05	1.2±0.02	2.3±0.001	0.2±0.1	0.089±0.01	42.8±0.5		
Rhincobatus djeddensis	0.012±0.01	1.76±0.01	3.2±0.02	0.34±0.02	0.22±0.1	49.2±0.7		
Rastrelliger kanagurta	0.8±0.04	0.98±0.1	1.4±0.01	0.42±0.03	0.176±0.1	49±0.6		
Epinephelus malabaricus	0.78±0.01	0.78±0.02	2.5±0.02	0.32±0.05	0.091±0.1	45.21±0.01		
Exocoetus volitans	0.02±0.01	0.55±0.0	1.9±0.01	0.24±0.06	0.22±0.1	50±0.01		
Lactoria cornuta	0.12±0.01	0.9±0.01	3.4±0.1	0.9±0.07	0.311±0.22	49.91±0.2		
Lutjanus fulviflamus	0.3±0.01	0.82±0.05	2.9±0.1	0.3±0.01	0.299±0.011	50.1±0.1		
Lutjanus sanguineus	0.1±0.001	0.71±0.11	2.7±0.1	0.26±0.02	0.301±0.1	46±0.2		
Selaroide sleptolepis	0.24±0.02	0.89±0.01	1.9±0.1	0.23±0.3	0.278±0.1	74.01±0.3		
Tetrosomus gibbosus	0.5±0.02	0.8±0.01	3.01±0.2	0.312±0.01	0.201±0.1	69.01±0.4		
Crassostrea madrasensis	1.01±0.4	5.011±0.1	9.521±0.3	0.399±0.03	0.154±0.2	90.1±0.5		
Donax faba	0.9±0.01	3.9±0.1	10.9±0.1	0.321±0.04	0.116±0.6	89.2±0.66		
Chinnamuttom region								
s	Cu	Zn	Fe	Mn	Ni	Mg	Pb	As
Lutjanus fulviflamus	0.099±0.01	0.172±0.01	0.353±0.1	0.081±0.1	0 .11±0.1	33.24±0.1	0.013±0.1	0.047±0.1
Seriolina nigrofasciata	0.109±0.02	0.159±0.66	0.279±0.1	0.132±0.1	0.19±0.1	27.69±0.2	0.013±0.2	0.094±0.1
Anchoviella commersoni	0.09±0.01	0.19±0.4	0.12±0.2	0.091±0.2	0.09±0.2	39.01±0.2	ND	ND
Scarus ghobban	0.078±0.1	0.12±0.01	0.12±0.1	0.145±0.1	0.089±0.2	40.9±0.1	0.01±0.1	0.01±0.1
Carangoides malabaricus	0.523±0.6	0.32±0.04	0.11±0.4	0.101±0.2	43.98±0.9	ND	ND	ND
Chanos chanos	0.115±0.01	0.2±0.5	0.298±0.5	0.121±0.1	0.16±0.1	39.1±0.3	0.012±0.1	ND
Rastrelliger kanagurta	0.8±0.2	0.178±0.01	0.2±0.01	0.09±0.0	0.15±0.2	29.87±0.2	0.01±0.1	ND
Mugil cephalus	0.98±0.11	0.192±0.01	0.35±0.01	0.082±0.2	0.17±0.2	27.8±0.1	ND	ND
Lutjanus spp	0.1±0.01	0.231±0.01	0.25±0.01	0.13±0.02	0.09±0.1	27.27±0.2	ND	ND
Parastromateus niger	0.109±0.02	0.134±0.02	0.305±0.2	0.109±0.01	0.1260.1	23.910.1	ND	ND

[Note: Cd; Pb; Hg; As; Mo were in non-detectable range (<0.01 µg.g^{-1})]

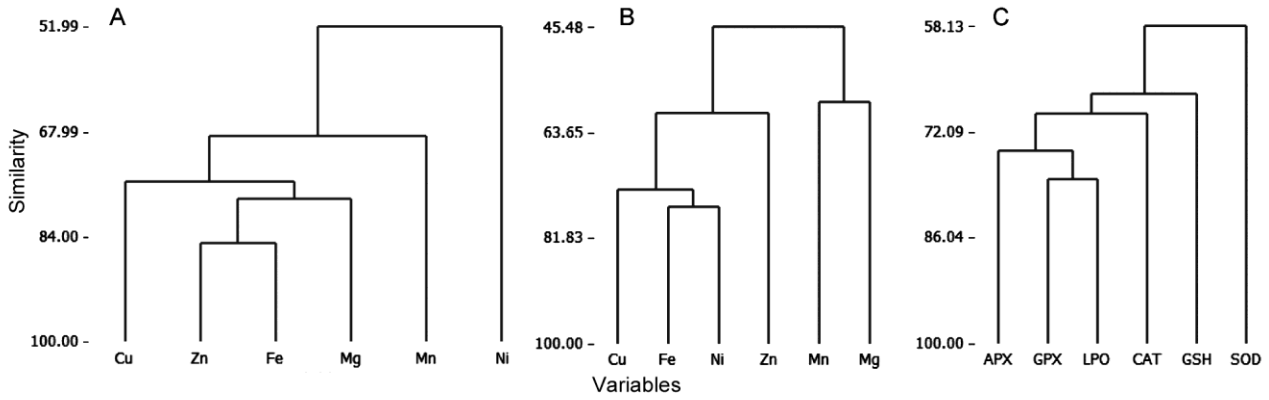

Fig. 2 — Clustral analysis of heavy metals accumulation and biochemical response in marine animals to the environmental stress. (A) Mandapam region; (B) Chinnamutam; and (C) Biochemical response

Table 3 — Mean bio-concentration factor of heavy metals in tissues

Heavy metals	Mean Bio-concentration factor in tissue	
	Mandapam	Chinnamuttom
As	0	0.27
Cu	0.079	0.163
Cd	0	0
Fe	0.039	0.045
Mn	0.19	0.136
Mg	29.63	0.049
Mo	0	0
Ni	0.127	0.154
Pb	0.005	0.005
Hg	0	0
Zn	0.106	0.193

decrease in the following order: Mg > Mn > Ni > Zn > Cu > Pb, which has been slightly altered based on the accumulation in the Chinnamuttom region as As > Zn > Cu > Ni >Mn > Mg > Pb, respectively (Table 3).

The clustral analysis of heavy metal analysis was depicted in Fig. 2A and B. Cu and Mg pattern of accumulation was found to be closely similar with Zn and Fe. In contrast to this the fish from Chinnamuttom showed two similar patterns of accumulation firstly with Fe, Ni (>70%); secondly between Mn and Mg (nearly 60%; Fig. 2B).

On a continuous exposure to metals, the marine organisms can absorb the available metals directly from the environment via gills and skin, or through food chain. Metals are then transported by the bloodstream, which brings it into contact with the various organs and tissues. Fish can regulate metal concentrations to a certain extent which are directly related to the amount of the metal accumulated in the specific tissue. Furthermore, physiological differences in species also affect the bioaccumulation of a particular metal. Variations in bioaccumulation of metals were observed in the present study when compared to other report for different marine fish collected from the Northeast coast of India, which may be due to various environmental factors including physicochemical parameters[34].

Metal distribution and accumulation in different tissues of fish varies depending on the sources, uptake, diet and or waterborne exposure. Similar to the observations of Ragupathi Raja Kannan *et al*.[35] and Ural *et al*.[36] the levels of essential elements like Fe, Mg, Mn, Cu and Zn were higher than the level of non-essential elements like Pb and Hg in our present study. Uptake and elimination processes are the most important factors in metal metabolism and metal toxicity studies. Generally, elimination rates of metals in fish are through the liver, gill, bile, urine and mucus. Metal accumulation is more rapid than metal elimination because of the presence of metal-binding proteins in tissue[37].

Most of the metals play a vital role in the biological metabolism, such as Fe (hemoglobin, SOD and CAT), Cu (respiratory pigments), Zn (SOD), Co (Vitamin B12), Mo and Mn (enzyme cofactor)[38]. Magnesium is essential for skeletal tissue metabolism and neuromuscular transmission[4]. Since iron (Fe) is an indispensable element in the functioning of organs and tissues of higher animals, including fish, as the most important micronutrients in terms of it's vital role in oxygen transport, defense and cellular respiration. In the present study distribution of copper were recorded as less when compared to other essential metals, it may be due to the increase in Zn- Mn- SOD. Zn has a tendency to get accumulated in the fatty tissues (liver) of aquatic organisms and it plays important roles in piscine growth, reproduction, development, vision and immune function.

Biochemical enzyme analysis

Usually fish encounter the oxidative stress upon exposure to pollutant or heavy metals, which disturbs their cellular ionic homeostasis through their oxidative defense mechanisms such as enzymes, chelation, etc[38]. The metabolic activity of marine fish and invertebrate in their native sites Mandapam and Chinnamuttom are depicted in Table 5. No significant variation was found in APX between the two selected areas (Table 5). Statistical comparisons were made between each metal and biochemical parameters. Irrespective of the sites the species showed some significant differences (P <0.5) towards the biochemical analysis (Tables 5). Among the species of Mandapam region the LPO and GPx was found to be higher in *Tetradon immaculate* (93.6 and 56.28 %), *Scarus russelii* (96.08 and 48.54%), *Thunnus obesus* (93.5 and 59.98%) and *Exocoetus volitans* (96.08 and 53.14%). The maximum level of GSH and SOD was observed in *Scarus ghobban*, *Thunnus albacores* and *Anchoviella commersoni*. *Anchoviella commersoni* produces higher levels of GSH, SOD and CAT. The level of GSH was found to be higher among the invertebrate *Crassostream adrasensis* and *Donax faba* than marine fish. Our findings reveal the increased level of GSH and SOD among the Chinnamuttom species, but APX and LPO were observed to be normal. MDA an intermediate product for expression of lipid peroxidation was found to be significant in the species of the Chinnamuttom region (Table 5).

Cluster analysis (CA) was used to detect the similarity between the biochemical enzymes. It yielded a dendrogram, grouping 6 enzymes 5 groups, LPO and GPx are closely related and grouped under one cladogram (Fig. 2C). Since we used hierarchical agglomerative CA, the number of clusters was also decided by the practicality of the results as there is ample information (e.g., stress response) on the biological system. Results indicate that the CA

Table 5 — Analysis if stress biomarkers in marine organisms of Mandapam and Chinnamuttom region

Species	LPO (µM)	GSH (mM)	CAT (U/mL)	SOD (U/mL)	GPx (U/mL)	APX (U/mL)
Mandapam region						
Megalaspis cordyla	0.77±0.1	126.15±0.5	29.38±0.1	791.52±0.5	90.2±0.1	0.198±0.1
Chanos chanos	1.2±0.01	5.594±0.1	15.29±0.1	890.21±0.5	132.4±6.9	0.406±0.05
Scarus ghobban	0.5±0.4	171.76±2.3	53.4±0.5	1341±0.1	80.2±0.7	0.085±0.1
Thunnus albacares	0.22±0.2	166.8±1.06	15.66±0.7	1068.7±0.005	77.27±9.34	0.289±0.5
Anchoviella commersoni	0.122±0.1	125.74±0.5	108.87±0.5	69.196±0.01	110.3±23.4	0.446±0.1
Tetradon immaculatus	1.41±0.002	190.94±0.5	60.41±0.25	62.32±0.05	129.7±4.49	0.479±0.22
Scarus russelii	2.3±0.02	33.64±0.51	47.47±0.7	224.931±2.3	110.2±0.1	0.423±0.12
Thunnus obesus	1.39±0.66	137.63±0.1	101.22±11.5	878.31±23.05	141.71±0.05	0.232±0.005
Terapon jarbua	0.66±0.87	150.1±0.01	56.76±0.5	313.67±0.05	96.73±0.5	0.373±0.7
Sardinella brachysoma	0.9±0.7	9.820±0.1	33.41±0.05	324.54±0.06	107.02±0.05	0.333±0.05
Rhincobatus djeddensis	0.34±0.1	182.9±0.5	151.87±0.5	98.01±0.005	76.03±0.11	0.414±0.04
Rastrelliger kanagurta	0.09±0.5	276.6±0.11	90.34±0.22	131.78±9.3	56.7±0.22	0.372±0.1
Epinephelus malabaricus	0.61±0.5	28.2±0.5	40.23±0.5	1338.73±1.5	88.99±2.3	0.429±0.22
Exocoetus volitans	2.3±0.1	44.74±0.5	44.52±0.4	8.90±0.3	121.04±0.7	0.449±0.05
Lactoria cornuta	1.6±0.5	17.63±0.5	57.48±0.8	25.28±0.05	125.4±13.3	0.378±0.1
Lutjanus fulviflamus	0.78±0.5	6.74±0.115	60.6±0.5	160.1±2.3	150.87±2.3	0.412±0.22
Lutjanus sanguineus	0.65±0.05	63.36±0.05	71.5±0.05	165.11±0.8	81.08±9.3	0.068±0.22
Selaroide sleptolepis	0.41±0.5	56.8±0.001	45.98±0.9	189±2.34	99.1±0.22	0.34±0.05
Tetrosomus gibbosus	0.52±0.5	49.98±0.5	52.76±0.72	122.9±9.3	101±0.77	0.23±0.22
Crassostrea madrasensis	0.321±0.7	320.7±0.7	35.686±0.05	23.9±4.35	2.941±0.1	0.54±0.05
Donax faba	0.301±0.05	210.8±0.5	7.83±0.5	10.9±0.1	1.118±0.07	0.078±0.05
Chinnamuttom region						
Lutjanus fulviflamus	0.290±0.01	212.497±0.660	42.339±0.33	121.6±0.01	2.61±0.9	0.523±0.06
Seriolina nigrofasciata	0.363±0.02	180.37±0.01	23.165±0.024	129.8±0.02	4.328±0.1	0.45±0.24
Anchoviella commersoni	0.230±0.33	225.74±0.012	92.87±0.001	81.23±0.005	20.64±0.02	0.523±0.2
Scarus ghobban	0.450±0.01	201.76±0.06	39.4±0.2	341±0.01	39.01±0.01	0.16±0.1
Carangoides malabaricus	0.235±0.01	165.8±0.005	45.89±0.03	445.91±0.11	11.02±0.1	0.234±0.04
Chanos chanos	0.198±0.04	93.760±0.024	34.29±0.06	890.21±0.02	89.34±0.33	0.506±0.8
Rastrelliger kanagurta	0.099±0.02	290.9±0.01	79.74±0.011	225.310±0.01	69.01±0.1	0.394±0.02
Mugil cephalus	0.451±0.001	45.89±1.2	44.2±0.01	169.6±0.02	67.81±0.2	0.198±0.01
Lutjanus spp.	0.660±0.02	67.8±0.9	60.6±0.09	162.9±0.9	45.32±0.78	0.465±0.45
Parastromateus niger	0.227±0.01	57.3±0.01	56.63±0.5	729.2±0.01	58.29±0.5	0.251±0.066

technique is useful in offering reliable classification of coastal waters in the selected region and will make it possible to design a future spatial sampling strategy in an optimal manner, which can reduce the number of sampling stations and associated costs.

The increase in the reduced glutathione level in certain species in the present study may be due to the synthesis of metal chelator-phytochelations[39]. The GSH (reduced glutathione) was found to be higher in *Terapon jarbua*; CAT (Catalase was higher in *Tetradon immaculatus*, low level was found in *C. chanos* at the same time APX (Ascorbate peroxidase) was observed to be higher in the ray, *Scarus ghobban* and low in *S. brachysome*. The date of enzyme analysis in species indicates the impact of the environment in the regulation of metabolisms in which depends on individual species. The normal LPO level in the fish shows the non occurrence of lipophilic toxicants on both sites. Factorial ANOVA results in Table 6, indicate a highly significant effect of metal and enzymes in the tissue (P <0.0001). Comparison of F values indicates that metal accumulation and enzyme activities had the strongest effects on marine fish. The paired interactions between the different factors of evaluation also had a significant effect on tissue. There was a significant interaction effect between metal concentration and enzyme.

Table 6 — Factorial ANOVA summary for the metal accumulation on the muscle tissue of marine organisms and biochemical response

Effect	P value	R square	Effect	P value	R^2
Metal	<0.0001	0.9241	Fe/SOD/LPO	<0.0001	0.3428
Enzymes	<0.0001	0.3424	Zn/CAT/APX	<0.0001	0.6478
Cu-Enzy	<0.0001	0.3632	Zn/GSH/GPx	<0.0001	0.4297
Fe-Enzy	<0.0001	0.3621	Zn/SOD/LPO	=0.0008	0.344
Zn- Enzy	<0.0001	0.3628	Mn/CAT/APX	<0.0001	0.6522
Mn- Enzy	<0.0001	0.3632	Mn/GSH/GPx	<0.0001	0.4345
Mg-Enzy	<0.0001	0.3462	Mn/SOD/LPO	<0.0001	0.3446
Ni- Enzy	<0.0001	0.3633	Mg/CAT/APX	<0.0001	0.6302
Cu/CAT/APX	<0.0001	0.6525	Mg/GSH/GPx	=0.0198	0.1225
Cu/GSH/GPx	<0.0001	0.4349	Mg/SOD/LPO	<0.0001	0.3131
Cu/SOD/LPO	<0.0001	0.3447	Ni/CAT/APX	<0.0001	0.6529
Fe/CAT/APX	<0.0001	0.6384	Ni/GSH/GPx	<0.0001	0.4354
Fe/GSH/GPx	<0.0001	0.4198	Ni/SOD/LPO	<0.0001	0.3447

Micronucleus and Comet assay

Apart from morphological and antioxidant enzyme activity (degrade free radical) the toxicants can directly interact with the genetic material and alters the gene function causing genotoxicity[40,41]. Mean Frequency of molecular damage as assessed by Micronucleus and Comet assay in marine fish are given in Figs 3 and 4. The frequency (number) of micronuclei was 1.00/1000 erythrocytes in the marine fish. The frequency of micronuclei (MN) was observed as 0.66 MN/1000 (mean erythrocytes). The DNA damage was measured as percentage of DNA in the tail portion. Our study reveals the absence of genotoxic potential toxicants in the selected sites. Since only class 1 type of DNA damage (0-5% of Tail DNA) was observed in all species[29].

Anthropogenic activities, particularly industrial (domestic and urban) effluent discharge, disturb natural cycling processes of metals. Fisheries industries at Mandapam, Ramanad discharge the wastes into the sea. Further, overexploitation of resources threatens the marine biodiversity of the Gulf

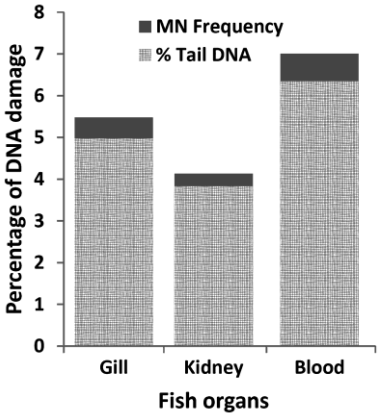

Fig. 4 — Mean DNA Damage observed in marine fish

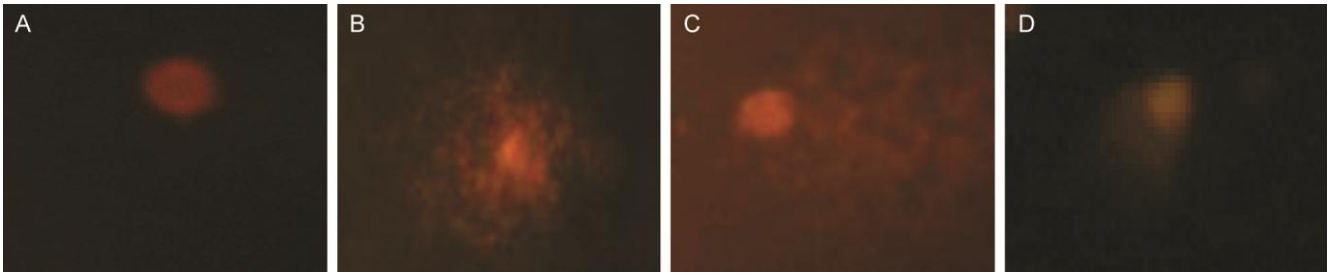

Fig. 3 — DNA damage studies using Single Gel Cell Electrophoresis or comet assy. (A) Control: showing zero damage with intact DNA in head; (B) Necrosis induced by H_2O_2 Positive control; (C) class 4 DNA damage showing the percentage of DNA in tail region; and (D) DNA damage in hemolymph of invertebrate

of Mannar[41]. Variations in the heavy metal concentrations between the fish species reflect the differences in uptake capabilities and their further translocation to the parts of the animals. While the concentration of heavy metal in the tissues of different species showed significant difference by the magnitude of their accumulation levels, the pattern is generally similar to each other. Concentration of other metals measured in the muscle of the species studied generally lower than the levels issued by WHO/FAO[42].

Conclusion

The results of this study revealed that consuming fish from the Gulf of Mannar coast may not have harmful effects because the concentration of bioaccumulated heavy metals is below the permissible limits prescribed by WHO and FAO (30 µg L^{-1} for Cu, 100 µg L^{-1} for Zn and Fe; 1 µg L^{-1} for Mn and Cd). The analysis of environmental matrices such as water provides a picture of the total contaminant load rather than of that fraction of direct ecotoxicological relevance. Thus, the use of biomarkers eliminates the need for complex studies on the chemical speciation (and hence presumptive bioavailability) of aquatic contaminants. Though the concentration of heavy metals in coastal water of Gulf of Mannar is low, continuous contamination over a period may put consumers at health risk. The present study concludes that a multiparameter analysis and regular monitoring of the ecological status of Gulf of Mannar is necessary.

Acknowledgment

The authors are grateful to Council for Scientific and Industrial Research, New Delhi for the financial support.

Conflict of interest

The authors declare no conflict of interests.

References

1 Savitha S, Srinivasalu S, Suresh S & Jayamoorthy K, Distribution of Heavy metals in the Marine Sediments of various sites in Karaichalli Island, Tutcorn, Gulf of Mannar, India. *Silicon*, 10 (2018) 1419.

2 Srikanth K & Venkateswara Rao J, Spatial and seasonal variation of potential toxic elements in *Adocia pigmentifera*, seawater and sediment from Rameswaram, southeast coast of India. *Environ Earth Sci*, 72 (2014) 2905

3 Fang GC & Yang HC, Comparison of heavy metals in marine sediments from coast areas in East and Southeast Asian countries during the years 2000–2010. *Toxicol Ind Health*, 27(8) (2011) 754

4 Pal M & Ghosh M, Assay of Biochemical Compositions of Two Indian Fresh Water Eel with special emphasis on accumulation of toxic heavy metals. *J Aquat Food Prod Technol*, 22 (2013) 27.

5 Nagarani N & Kumaraguru AK, Influence of marine alga against mercury-induced biochemical changes, structural destabilization, and genotoxicity in marine tiger fish, *Therapon jarbua*. *Toxicol Environ Chem*, 94 (2012) 1383.

6 Kandziora-Ciupa M, Ciepał R, Nadgórska-Socha A & Barczyk G, A comparative study of heavy metal accumulation and antioxidant responses in *Vaccinium myrtillus* L. leaves in polluted and non-polluted areas. *Environ Sci Pollut Res*, 20 (2013) 4920.

7 Qunfang Zhou, Jianbin Zhang, Jianjie Fu, Jianbo Shi & Guibin Jiang, Review: Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. *Analytica Chimica Acta*, 606(2008) 135.

8 Jonny Beye, Karina Petersen, You Song, Anders Ruus, Merete Grung, Torgeir Bakke & Knut Erik Tollefsen, Environmental risk assessment of combined effects in aquatic ecotoxicology: A discussion paper. *Marine Environ Res*, 96 (2014) 81.

9 Conti ME, Bocca B, Iacobucci M, Finoia MG, Mecozzi M, Pino A & Alimonti A, Baseline trace metals in seagrass, algae and mollusks in a southern Tryyhenian ecosystem (Linosa island, Sicily). *Archives Environ Conta Toxicol*, 58 (2010) 79.

10 Schuwerack PM, Lewis JW & Jone P, The Potential Use of the South African River Crab, *Potamonautes warreni*, as a Bioindicator Species for Heavy Metal Contamination. *Ecotoxicol*, 10 (2001) 159.

11 Venkateswara Rao J, Kavitha P, Chakra Reddy N & Gnaneshwar Rao T, *Petrosia testudinaria* as a biomarker for metal contamination at Gulf of Mannar, southeast coast of India. *Chemosphere*, 65(4) (2006) 634.

12 Venkateswara Rao J, Srikanth J, RamjeePallela T & Gnaneshwar Rao, The use of marine sponge, *Haliclonatenuiramosa* as bioindicator to monitor heavy metal pollution in the coasts of Gulf of Mannar, India. *Environ Monit Assess*, 156 (2009) 451.

13 Akcali I & Kucuksezgin F, A Biomonitoring study: heavy metals in macroalgae from eastern Aegean coastal areas. *Mar Pollut Bull*, 62 (2011) 637.

14 Pergent-Martini C, Leon, V, Pasqualini V, Ardizzone GD, Balestri E, Bedini R, Belluscio A , Belsher T, Borg J, Boudouresque CF , Boumaza S, Bouquegneau JM, Buia MC, Calvo S, Cebrian J, Charbonnel E, Cinellic F, Cossu A, Di Maidak G, Dural B, Francour P, Goberti S, Lepointi G, Meinesz A, Molenaar H, Mansour HM, Panayotidis P, Peirano A, Pergenta G, Piazzi L, Pirrottak M, Relini G, Romero J, Sanchez-Lizaso JL, Semroud R, Shembri P, Shili A, Tomasello A & Velimiro B, Descriptors of *Posidonia oceanica* meadows: use and applications. *Ecol Indic*, 5 (2005) 213.

15 Ali AM, Shoaib N, Naaaqvi GZ & Siddiqui Pirzada JA. Genotoxic effect of pesticide on gill tissues of green-lipped mussel *Perna viridis* (L.). *Indian J Expl Biol*, 56 (2018) 611.

16 Vlahogianni T, Dassenakis M, Scoullos MJ & Valavanidis A, Integrated use of biomarkers (superoxide dismutase, catalase

and lipid peroxidation) in mussels *Mytilus galloprovincialis* for assessing heavy metals' pollution in coastal areas from the Saronikos Gulf of Greece. *Mar Pollut Bull*, 54 (2007) 1361.

17 Ramírez R, The gastropod *Osilinusatrata* as a bioindicator of Cd, Cu, Pb and Zn contamination in the coastal waters of the Canary Islands. *Chem Ecol*, 2 (2013) 208 .

18 Bibak M, Sattari M, Tahmasebi S, Ali A, & Javid IN, Marine macro-algae as a bio-indicator of heavy metal pollution in the marine environments, Persian Gulf. *Indian J Geo-Mar Sci*, 49 (2020) 357.

19 Grasshoff, Ehrhardt, M., Kremling, K; *Methods of Seawater Analysis*, 2nd Edn. (VerlagChemie, Weinheins, Germany), 1983, 419.

20 Kremling K, Determination of the major constituents. In: *Methods of Seawater Analysis*, 3rd Edn. (Eds. Grasshoff K, Ehrhardt M & Kremling K; VCH Publishers, Weinheins, Germany), 1999, 600.

21 Mackay D & Fraser A, Bioaccumulation of Persistent Organic Chemicals: Mechanisms and Models. *Environ Pollut*, 110 (2000) 375.

22 Buege JA & Aust SD, Microsomal Lipid Peroxidation: *Methods in Enzymology*, 52 (1978) 302.

23 Matsumura T, Tabayashi N, Kamagata Y, Soum C & Saruyama H, Wheat catalase expressed in transgenic rice can improve tolerance against low temperature stress. *Physiol. Plant*, 116 (2002) 317-327. http://dx.doi.org/10.1034/j.1399.2002.1160306.x

24 Calatayud A, Ramirez JW, Iglesias DJ & Barreno E, Effects of ozone on photosynthetic CO2 exchange, chlorophyll *a* fluorescence and antioxidant system in lettuce leaves. *Physiol. Plant*, 116 (2002) 308.

25 Boyne AF & Ellman GL, A methodology for analysis of tissue sulfhydryl components. *Anal. Biochem*, 46 (1972) 639.

26 Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG & Hoekstra WG, Selenium: Biochemical role as a component of glutathione peroxidase. *Sci*, 179 (1973) 588.

27 Nakano Y& Asada K, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*, 22 (1981) 867.

28 Bahari I, Noor F & Daud NM, Micronucleated erythrocytes as an assay to assess actions by physical and chemical genotoxic agents in *Clarias gariepinus*. *Mutat Res*, 313 (1994) 1.

29 Cortes-Gutierrez EI, Fernandez JL, Davila-Rodriguez MI, Lopez-Fernandez C & Gosalvez J, Two-Tailed Comet Assay (2T-Comet): Simultaneous Detection of DNA Single and Double Strand Breaks. In: Histochemistry of Single Molecules, Methods and Protocols, Methods in Molecular Biology, vol. 1560, (Eds. Pellicciari C & Biggiogera M; Humana Press, New York), 2017, 285. NY. https://doi.org/10.1007/978-1-4939-6788-9_22.

30 Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC & Sasaki YF, Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. *Environ Mol Mutagen*, 35 (2000) 206.

31 Bindu Sulochanan PK, Krishnakumar D, Prema P, Kaladharan KK, Valsala Bhat GS & Muniyandi K, Trace metal contamination of the marine environment in Palk Bay and Gulf of Mannar. *J Mar Biol Assoc India*, 49 (2007) 12.

32 Thangaradjou T & Kannan L, Nutrient characteristics and sediment texture of the seagrass beds of the Gulf of Mannar. *J Environ Biol*, 28 (2007) 29.

33 WHO, Nitrate and Nitrite in Drinking-water WHO Guidelines for Drinking-water Quality. (WHO Press, Switzerland), 2011, 21. WHO/FWC/WSH/16.52

34 Kumar B, Sajwan K S & Mukherjee DP, Distribution of Heavy Metals in Valuable Coastal Fishes from North East Coast of India**.** *Turkish J Fish Aquat Sci*, 12 (2012) 81.

35 Ragupathi Raja Kannan R, Arumugam R & Anantharaman P, Chemometric Studies of Multielemental Composition of Few Seagrasses from Gulf of Mannar, India. *Biol Trace Elem Res*, 143 (2011) 1149.

36 Ural M, Arca S, Örnekçi GN, Demirol F, Yüce S, Uysal K, Çiçek A, Köse E & Koçer MAT, Metal accumulation in sediment, water, and freshwater fish in a Dam Lake. *Toxicol Environ Chem*, 94 (2012) 49.

37 Hennig HF, Metal-binding proteins as metal pollution indicators. *Environ Health Perspect*, 65 (1986) 175.

38 Nagajyoti PC, Lee KD & Sreekanth TVM, Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett*, 8 (2010) 199.

39 Srikanth K, Pereira E, Duarte AC & Ahmad I, Glutathione and its dependent enzymes' modulatory responses to toxic metals and metalloids in fish--a review. *Environ Sci Pollut Res Int*, 20 (2013) 2133.

40 Mundhe AY, Hari Pandit B & Sangeeta V, Genotoxicity and oxidative stress as biomarkers in fresh water mussel, *Lamellidens marginalis* (Lam.) exposed to monocrotophos. *Indian J Exp Biol*, 54 (2016) 822.

41 Karunanidhi K, Rajaram R, Dhinesh P, Arumugam G, First report on distribution of heavy metals and proximate analysis in marine edible puffer fishes collected from Gulf of Mannar Marine Biosphere Reserve, South India. *Toxicol. Rep*, 4 (2017) 319327.

42 Gazette of Eritrean Laws, vol. 12/2003, No. 4 of 30, (Eds**.** Ahmcd Haj Ali; FAO, Eritrea, Eastern Africa), 2003, 34.