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RESEARCH

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Biomarkers as ecological indices in monitoring the status of market fish

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Abstract

Background Environmental contamination has become a major concern over the past few decades, drawing the attention of numerous researchers from both developed and developing nations. The aquatic system serves as the primary sink for the disposal of garbage, which has a negative impact on the aquatic environment and biota. The reality is that heavy metals cannot be totally removed from the ecosystem because they can bioaccumulate and grow in strength as they move up the food chain. Particularly heavy metals can build up in the tissues of aquatic animals, and as a result, tissue concentrations of heavy metals may be harmful to both human and animal health. Our study aimed to elucidate the possible use of biomarkers in monitoring and assessing the heavy metals contaminants among fresh water fish.

Results From the present study, we conclude that glutathione peroxidase can be used as the bioindicator for nickel and iron contamination. Ultimately, these studies focus on measuring levels of pollution that may induce irreversible ecological changes to aquatic ecosystems. Till now the level of toxicity was moderate, and it was progressing toward the danger. Efforts can be made to control the activities that release pollutants unnaturally into the environment from both public and government so that the clean and clear environment can be maintained.

Conclusions The work concludes that a multiparameter analysis is needed to assess and monitor the ecological status of the aquatic environment.

Keywords Antioxidant enzymes, Bioindicator, Environmental management, Heavy metal, Pollution

Background

Over the last three decades there has been increasing global concern over environmental awareness over water pollution. Water forms a basis for transfer of nutrients in all ecosystem, which ultimately threatens aquatic life and ends in human via the food chain (Afshan et al., 2014; Garg et al., 2009; Nagarani et al., 2020). About a quarter of the diseases facing humankind today occur due to prolonged exposure to environmental pollution (Prüss-Ustün et al., 2011). It was reported that the two biggest crises all over the earth are contamination and massive disposal of waste in aquatic environment (Anh et al., 2010; Arkoosh et al., 2010).

Environmental pollutants represent a risk factor for human and animals in all areas of occurrence in the form of gas, solid and liquid forms as a single or synergistic action (Kovacik, 2017). The normal prevalence of heavy metals is not harmful to the environment, but their presence at higher concentrations becomes toxic, and pollution in relation to their toxicity to aquatic organisms affects the ultimate well-being of humans. Such occurrence of high level of pollutants, i.e., heavy metals, is known to inhibit biochemical and physiological mechanisms vital for fish metabolism (Nagarani et al., 2020; Shuhaimi-Othman et al., 2013). Bioaccumulation

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of any metal above its threshold level results in irreversible physiological conditions (Zhang et al., 2010). Despite the large scale of natural source for heavy metals, previous report reveals that the higher contribution toward heavy metals pollution is by anthropogenic source, especially for Pb, Hg, Cd, Zn and Cu than natural release, via., anthropogenic and industrial effluents into fresh water and marine resources (Bhattacharyya et al., 2021).

Heavy metals inhibit the functions of structural proteins, enzymes and nucleic acids by forming metal complexes (Jaishankar et al., 2014). In addition it also induces structural or morphological alterations, chromosomal aberrations, and ultimately results in impairment in the immune system (Coen et al., 2012). The nature of heavy metal toxicity in fish primarily depends on various physico-chemical parameters including its solubility, hardness, pH and ecosystem complexity via gills, food and skin (Tao et al., 2001). Fishes are one of the main nutritional components consumed by humans. Besides, this fish was at apex in aquatic food chain; hence, they can be a best bioindicators for aquatic pollution. Fish have the ability to uptake and concentrate metals directly from the surrounding water or indirectly from other organisms such as small fish, invertebrates and aquatic vegetation. Fish accumulate pollutants preferentially in their fatty tissues like liver the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic or acute diseases (AL-Yousuf et al., 2000).

Heavy metals are known to induce oxidative stress and/or carcinogenesis by mediating free radicals/reactive oxygen species (Javed et al., 2015). Redox active metals (Fe, Cu, Cr, Hg, Pb, Cd, Ni) produced ROS through redox cycling which disturbs the thiol groups containing antioxidants and enzymes (Jomova et al., 2010; Kurtuas, 2015; Stohs & Bagchi, 1995) and damage the fish at various level including DNA, gills, membrane lipids and proteins. Each oxidative stress parameter is specific for some of the heavy metals. Based on this information, selection of specific oxidative biomarkers has to be done for accurate analysis. With the backdrop of this information the present study was conducted to test the environmental risk assessment in market fish.

Methods

Sample collection and preservation

The healthy fresh fish were procured during early morning at 5.30 am from the local fish market at Narimedu (9.9372° N, 78.1258° E), Madurai, Tamilnadu, India. The study was conducted during the period of January–April 2019**.** The samples used in the study were selected by physical observation based on the criteria such as grown fish (fingerlings were avoided), fresh without rotten smell, red colored gill, muscle smooth without mucus (indicate the presence of microbes or pathogens) and chemical smell free. Collected samples were immediately stored in ice-cold conditions (4 °C). For each analysis three individuals per species were used. Fish were washed thoroughly with sterilized distilled water and oven-dried for metal analysis. The concentrations of metals were determined according to the standard double acid digestion methods analyzed using an atomic absorption spectrometer. Standards were made using certified solutions (Merck, UK) acidified with HNO_3 to the same pH as the samples. Fresh samples were stored at − 80 °C for further enzymatic studies. The whole samples were homogenized in trichloro acidic acid (TCA) for lipid peroxidation analysis and in phosphate buffer (pH 7) for reduced glutathione analyses.

Determination of non-enzymatic biomarkers

Lipid peroxidation in fish liver, gill, microsomes (pooled sample mixture) was evaluated by the thiobarbituric acid (TBA) method (Buege and Aust, 1978). The reaction mixture, 8.1% sodium dodecyl sulfate (0.2 ml) and thiobarbituric acid 20% in trichloroacidic acid (2 ml) were heated for 1 h in boiling water bath. After cooling, n-butanol: pyridine (15:1) mixture solution was added and centrifuged to obtain n-butanol: pyridine layer. The absorbance of the sample was estimated at 532 nm. The level of lipid peroxide was expressed as malondialdehyde (MDA) nmol/mg protein using the extinction coefficient for the MDA $\left(\sum = 1.55\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}\right)$.

The concentration, c, from the equation

$= \frac{A}{\varepsilon x l}$, the light path, l, is 1 cm.

Reduced glutathione (GSH) was assayed by the method of Boyne and Ellman (Boyne & Ellman, 1942). Briefly, One milliliter of homogenate (PBS, pH 7.4) was centrifugation at 8000 rpm for 15 min at 4 °C. The assay mixture contained 0.1 ml filtered aliquot and 2.7 ml phosphate buffer (0.1 M, pH 7.4) in a total volume of 3.0 ml. After centrifugation, 2.0 ml of the protein-free supernatant was mixed with 0.2 ml of 0.4 M Na_2HPO4 and 1.0 ml of DTNB (5,5- dithio-bis-(2-nitrobenzoic acid)) reagent (40 mg DTNB in 100 ml of aqueous 1% trisodium citrate). The yellow color developed was read immediately at 412 nm in a spectrophotometer. GSH concentration was expressed as nmol/mg.

Post-mitochondrial supernatant preparation (PMS)

Tissues were perfused with ice-cold saline (0.9% sodium chloride) and homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 3000 rpm for 5 min at 4 °C to separate the

nuclear debris. The supernatant so obtained was centrifuged at 10,000 rpm for 20 min at 4 °C to get the post-mitochondrial supernatant which was used to assay biomarker enzymes. The antioxidant enzyme response was measured using a double-beam UV spectrometer (Model 2201; Systronics).

One unit of enzymes is equal to 50% inhibition.

$$\text{Inhibition}(\%) = \frac{\text{Blank} - \text{Sample}}{\text{Blank}} \times 100$$

Biomarker enzyme analysis

The enzyme biomarkers were quantified by standard protocol. Superoxide dismutase (SOD) activity was assayed by the method of Kono et al., 2000; catalase activity (Matsumura et al., 2002); acetyl choline esterase enzyme (AChE) activity was measured by using spectrophotometer based on Ellman's method (1961), and glutathione peroxidase (GPx) activity in the homogenate was evaluated by the NADPH (nicotinamide adenine dinucleotide phosphate) method with minor modification (Rotruck et al., 1973).

One unit of enzymes is equal to 50% inhibition.

$$\text{Inhibition}(\%) = \frac{\text{Blank} - \text{Sample}}{\text{Blank}} \times 100$$

Statistical analysis

All the experiments were performed thrice to get the concordant values. All statistical tests are performed using GraphPad Prism (version 8). Data are reported as Mean ± SD, and statistical difference will be accepted at $P < 0.05$.

Results

The work was carried out to assess the environmental stress in the marine fish at local market. This study also measures the bioaccumulation of pollutants and its effect during transport. The list of fish samples collected from the local market is given in Table 1.

The malondialdehyde (MDA), an intermediate of the oxidation of polyunsaturated fatty acids, is considered as a useful index of general lipid peroxidation. Malondialdehyde (MDA) forms an adduct with thiobarbituric acid which can be quantified by spectrophotometer at 532 nm. In practice, TBARS is expressed in terms of malondialdehyde (MDA) equivalent which is depicted in Fig. 1. The TBARS in the sample is 2 to 200 µM MDA. Among the order Periciformes *Alectis indica* showed lower MDA formation. The non-antioxidant compounds glutathione, a part of glutathione peroxidase, and glutathione reductase enzymes were measured in the reduced form. The glutathione content was found to be varying from 5 to 39 µM in the species under study, as shown in Fig. 2.

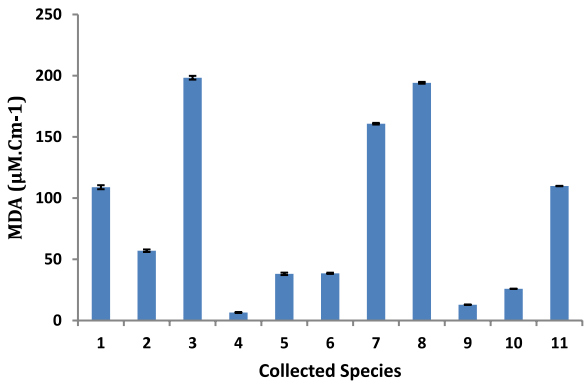

Fig. 1 Level of MDA formation in the collected fish

Table 1 List of fish collated to study the environmental risk assessment

S. no	Vernacular name	Scientific name	Class	Order
1	Emperor Long Face emperor bream	*Lethrinus olivaceus*	Actinopterygii	Perciformes
2	Indian goat fish (Nagarai Meen)	*Parupeneus indicus*	Actinopterygii	Perciformes
3	Malabar Trevally/Jack Fish—Paarai Meen	*Carangoides malabaricus*	Actinopterygii	Perciformes
4	Blue Fin Travelly	*Alectis indica*	Actinopterygii	Perciformes
5	Silver Pomfret	*Pampus argenteus*	Actinopterygii	Scombriformes
6	Chaalai or Sardine	*Sardinella longiceps*	Actinopterygii	Clupeiformes
7	Ailai/Dolphin	*Coryphaena hippurus* (Linnaeus, 1758)	Actinopterygii	Perciformes
8	Mural, Needle fish, Viraal, Gar fish	*Hemiramphus far* (Forsskal, 1775)	Actinopterygii	Beloniformes
9	Barracuda (ooli)	*Sphyraena forsteri* (Cuvier, 1829)	Actinopterygii	Scombriformes
10	Kilanga, Lady Fish	*Elops machnata* (Forskal, 1775)	Actinopterygii	Elopiformes
11	Crab	*Portunus pelagicus*	Malacostraca	Decapoda

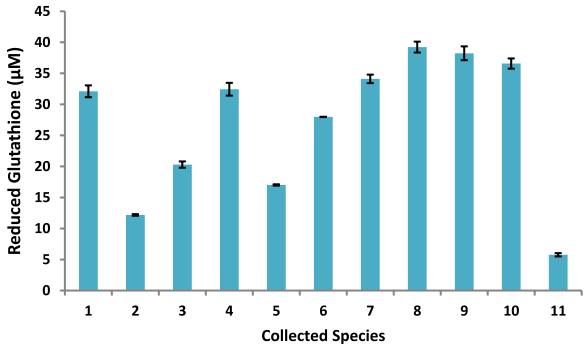

Fig. 2 Level of reduced glutathione in the collected fish

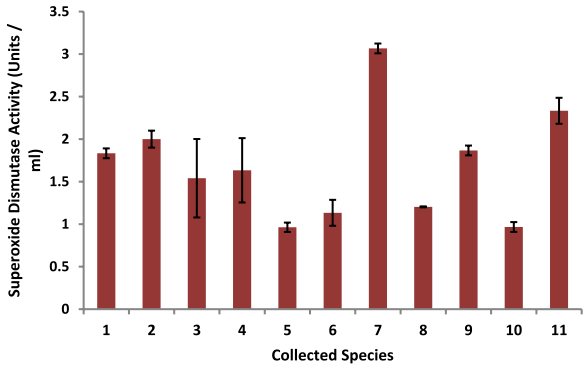

Fig. 3 Superoxide dismutase activity

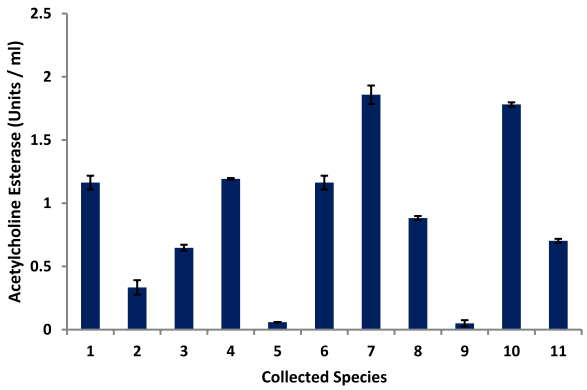

Fig. 4 Acetylcholine esterase activity

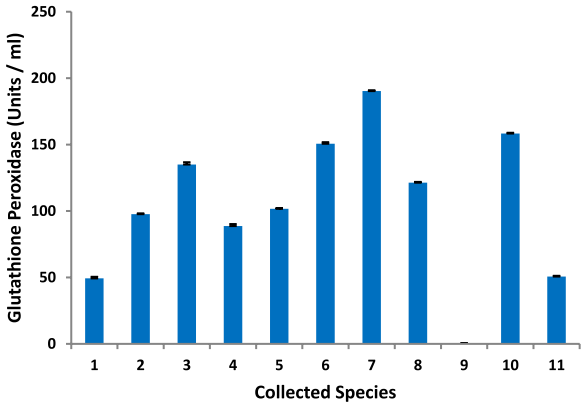

Fig. 5 Glutathione peroxidase activity

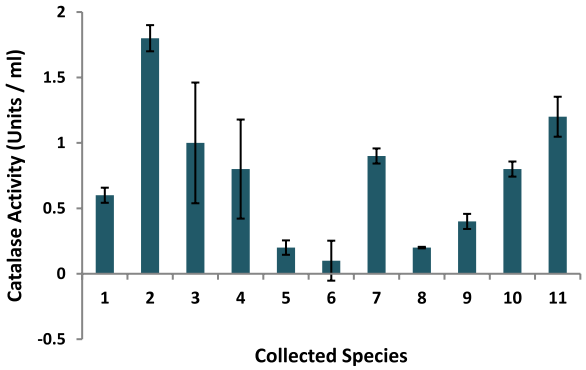

Fig. 6 Catalase activity

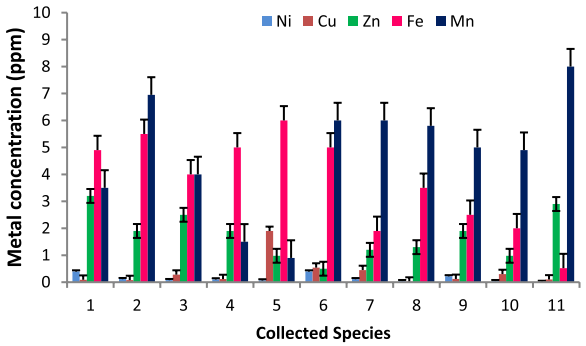

Fig. 7 Heavy metal studies in the collected fish samples

Enzymes play an important role during the metal toxicity in eliminating and converting the free radical into stable molecule and thus prevent cellular damage. The biochemical markers of environmental stress are depicted Figs. 3, 4, 5 and 6. *Coryphaena hippurus* was observed to have higher concentration of superoxide dismutase enzymes than other species. Silver Pomfret and *Sphyraena forsteri* of order Sconmrifoemes were observed to have higher concentration of acetyl choline esterase enzyme activity than other species (Fig. 4). *Coryphaena hippurus* was observed to have higher concentration of GPx (Fig. 5). The catalase activity was noted to be moderate among all fish species and ranges between 0.1 and 1.2 units and is depicted in Fig. 6.

The concentration of metals in the muscle tissues is depicted in Fig. 7. The level of metals was below the permissible limit; hence, the less or no environmental risk was found in the collected species (FAO, 1984). The order of accumulation of metals in the fish was Fe > Mn > Zn > Cu > Ni irrespectively to the species. Fe was low in crab when compared with fish due to the role of iron in hemoglobin formation, followed by manganese, zinc and copper which participate as cofactors in SOD formation; on the other hand, Ni was found to be low since Ni was one of the non-essential metals.

Discussion

Compared to the order Perciformes the other two order Beloniformes and Scombriformes have higher levels of reduced glutathione. Usually animals encounter oxidative stress upon exposure to pollutants or heavy metals. This disturbs their cellular ionic homeostasis through their oxidative defense mechanisms such as enzyme, chelation (Nagarani et al., 2009). The reduced glutathione (GSH) was found to increase since GSH has a vital role in protein metabolism. The increase in the reduced glutathione level in the present study may also be due to the synthesis of metal chelator (Nagarani et al., 2012). The increase in the levels of MDA may be due to external physiological stress. Fish exhibit many of the same defenses against oxidative stress as do mammals. These defenses include both low molecular weight free radical scavengers such as GSH and ascorbic acid, as well as enzymatic defense such as SOD, Ach E, CAT and GPx. The high concentration of SOD with reference to the level of Cu indicates the role of Cu as cofactor ions in the formation Cu-SOD in fish.

Copper are bonded with many cytoplasmic and membrane proteins like ferritin, which in turn would release and increase the metal ions in the tissues. These free ions were able to catalyze the breakdown of hydrogen peroxide into water molecules through the Fenton reaction. The level of CAT and SOD activity in animals usually reflects the face of environmental pollutants (Dautremepuits et al., 2004), since SOD-CAT was the first line of defense against oxidative stress. The CAT activity was noted to decrease; this may be due to the flux of superoxide ion formation which in turn decreases the formation of hydrogen peroxide and inhibit CAT activity (Pandey et al., 2003). The low level of CAT also confirms that the sample is pathogen free.

The variation in the antioxidant enzyme activities among the species indicates that there exists a species response pattern such as sensitivity to toxicants, nature of toxicants, bioaccumulation and detoxification processes (Abhijith et al., 2016; Balk et al., 2011). Tracing a suitable biomarker in natural fish populations to the biomarker responses in fish from highly polluted areas close to a point source is quite challenging since response to toxicants may also differ between areas and fish species (Balk et al., 2011).

Conclusions

There is a growing concern that the elements through the natural cycling process are being disturbed by anthropogenic activities, especially the growth of industrial, domestic and urban discharge of its effluents. From the present study, we conclude that glutathione peroxidize can be used as the biomarkers for Ni, Fe contamination. Ultimately, these studies must focus on measuring levels of pollution that may induce irreversible ecological changes to aquatic ecosystems. Till now the levels of toxicity were moderate, and it was progressing toward the danger. Efforts can be made to maintain and control the activities that release pollutants unnaturally into the environment from both public and government so that the clean and clear environment can be maintained.

Abbreviations

AChE	Acetyl choline esterase enzyme
CAT	Catalase
DTNB-5,5	Dithio-bis-(2-nitrobenzoic acid)
GPx	Glutathione peroxidase
GSH	Reduced glutathione
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
PMS	Post-mitochondrial suspension
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloro acidic acid

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Author contributions

All authors were involved for sampling, field work, laboratory activities, data collection and statistical analysis. The authors GK, DD, KGM, MAJ, PVM and RS involved in data collection and laboratory works. The manuscript was prepared by GK, while edited by NN. All authors have read and approved the manuscript.

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Availability of data and materials

We declare that the data generated from this study are readily available as well as the materials used.

Declarations

Ethics approval and consent to participate

The ethical conditions concerning Animal Research outside the Laboratory as stated by Nisbet and Paul (2004) were strictly observed in this research.

Consent for publication

Not applicable.

Competing interests
Authors declare that there is no conflict of interest among authors.

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