

**Original Research**

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**Biochemical properties and heavy metal analysis of a commercial fish *Opisthopterus tardoore***

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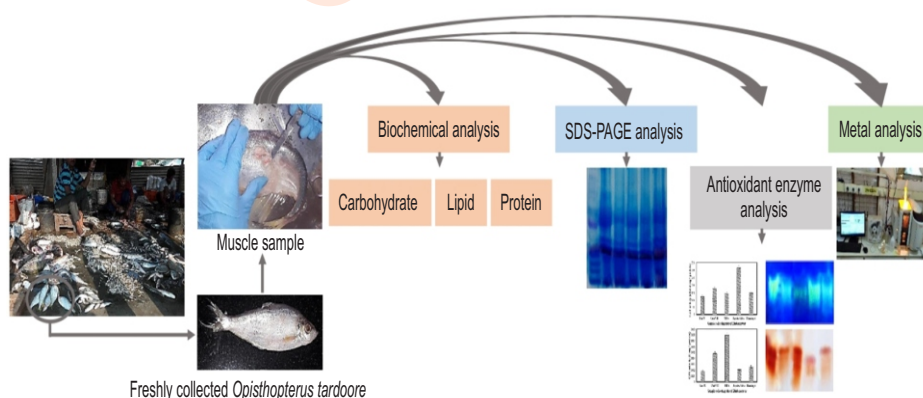
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**Abstract**

**Aim:** The study was designed to evaluate various biochemical parameters, protein expression, antioxidant enzymes and heavy metals by testing an edible fish *Opisthopterus tardoore*.

**Methodology:** The experimental fish were collected from five major fish markets of Bhubaneswar, India and distribution of carbohydrate, lipid and protein content were assessed in muscle tissues. To enhance the understanding of molecular mechanism, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of crude proteins was conducted. Catalase (CAT) and guaiacol peroxidase (GPX) activities were determined both spectrophotometrically and by native gel electrophoresis. For heavy metal analysis, three toxic elements viz., chromium, cadmium and lead were examined by atomic absorption spectrophotometer.

**Results:** The muscle tissues showed variation in the concentrations of carbohydrate ( $0.17 \pm 0.02$  to  $0.28 \pm 0.04$  % f.wt.), lipid ( $3.48 \pm 0.27$  to  $6.62 \pm 0.44$  % d. wt.) and protein ( $1.56 \pm 0.11$  to  $2.92 \pm 0.16$  % f. wt.) with respect to the markets from where fish samples were collected. The protein profiling study revealed the disappearance as well as



*de novo* synthesis of some proteins. The activities of CAT and GPX were determined for establishing a possible bioindicator of metal toxicity in fish. A rapid disturbance in their enzymatic actions was noticed which was also confirmed through native polyacrylamide gel electrophoresis with respect to fish samples. The heavy metals analysis manifested a varying level of accumulation of Cr whereas Pb and Cd were found below the detection limits.

**Interpretation:** The biochemical disturbances and accumulation of Cr in muscle tissues of *O. tardoore* reflect the pollution status of nearby coastal areas of the Bay of Bengal, Odisha.

**Key words:** Biochemical analysis, Chromium toxicity, Guaiacol peroxidase, *Opisthopterus tardoore*, Protein expression

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## Introduction

Fish are one of the most widely distributed creatures in the aquatic environment and are considered as one of the vital dietary sources for human beings (Taweel, 2012; FAO, 2020). Fish is consumed widely as they are rich source of various kinds of quality animal proteins with abundant quantities of essential and non-essential amino acids (Mohanty *et al.*, 2014). Most fish species are also a good source of healthy unsaturated and polyunsaturated fatty acids, especially omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, which have great medicinal benefits (Taşbozan and Gökçe, 2017). They are highly recommended in diet as they help to reduce blood pressure, improve cardiovascular risk factors, lower the rate of depression and mental illness (Torpy *et al.*, 2006; Fawole *et al.*, 2013). Anthropogenic activities such as discharge of industrial wastes, urban and agricultural runoff, littering of municipal wastes, stormwater discharge, oil and hydrocarbon wastes along with fishing and shipping activities have severely polluted the marine ecosystem often resulting in bioaccumulation of toxic heavy metals (Azaman *et al.*, 2015; Bashir *et al.*, 2020).

The presence of elevated concentrations of heavy metals like arsenic, cadmium, chromium, copper, iron, manganese, lead, nickel and zinc are well documented in marine habitat (Khan *et al.*, 2017; Ahmed *et al.*, 2019; Shah, 2021; Hao *et al.*, 2022). Sometime, these toxic metals are accumulated by different body parts of fish either in epidermal skin surfaces or gills or food tracts and/or combinations of all parts but it differs from species to species and also the nature of bioavailability (Dohaish, 2018; Feng *et al.*, 2020; Han *et al.*, 2021; Tabrez *et al.*, 2021). Fish are abundantly available in the ocean and are being consumed by other levels of consumers including humans, therefore, it is obvious that humans are at great risk of deposition of heavy metals in their tissues. The fish population also face various detrimental effects on the growth rate, mortality, biomass production and biochemical activities when exposed to heavy metal pollution (Fatima *et al.*, 2015; Zuluaga Rodríguez *et al.*, 2015; Sonone *et al.*, 2021), however, biochemical parameters in particular enzymatic antioxidants such as CAT and GPX are considered as the most sensitive bioindicators to assess metal causing toxicity in fish (Rahimikia, 2017).

*Opisthopterus tardoore* is an important marine fish species, commonly found in marine habitat of the Indo-West Pacific region, including coastal belt of the Bay of Bengal. It is considered as one of most commercialized fish types in Odisha, which belong to family Pristigasteridae. The exact health benefits of this fish is nowhere reported; however, the herring fish groups significantly contribute reducing the problem of arthritis and anti-obesity, improve heart health and eye vision, balance cholesterol metabolism, increase red blood cell production and optimizing nerve function (Reames, 2012; Pradhan *et al.*, 2020). The selected fish species are commonly found in markets throughout Odisha which is originally collected from one marine source that is the Bay of Bengal. On the other hand, several reports suggest the

deposition of various heavy metals in water as well as sediment samples of different location of Bay of Bengal, which is mostly due to direct atmospheric deposition, geological location, weather condition, agricultural runoff, and discharge of industrial, mining, municipal and domestic wastes (Satapathy and Panda, 2015; Hasan *et al.*, 2016; Khan *et al.*, 2017; Perumal *et al.*, 2021; Khadanga *et al.*, 2022). Moreover, several studies also demonstrate the bioavailability of various toxic metals in fish of different location of Bay of Bengal (Kumar *et al.*, 2012; Saha *et al.*, 2016; Reksten *et al.*, 2021), however, metal content of fish from Odisha coastal belt has not been reported. Therefore in the present study, *O. tardoore* was selected as a representative fish to examine the load of toxic heavy metals and subsequent impact through analyzing various biochemical parameters which may potentially act as a possible bioindicator of heavy metal pollution.

## Materials and Methods

**Study area and sampling location:** Five fish markets (Unit IV, Unit VIII, BDA, Jayadev Vihar, Khandagiri) of Bhubaneswar were selected as sites for sample collection. The sources of original catching were coastal fishing points of Odisha like Gopalpur, Puri, Paradeep and Balasore.

**Experimental fish:** Marine fish species *O. tardoore* were purchased (six numbers from each market having about  $156.51 \pm 10.43$  g weight and  $25.50 \pm 2.57$  cm length) in poly bags and transported in an insulated icebox to the laboratory immediately, stored under  $-20^{\circ}\text{C}$  for further analysis.

## Biochemical analyses

**Extraction and estimation of carbohydrate:** To estimate the crude carbohydrate content, 100 mg of muscle sample was homogenised in 10 ml 80% ethanol with prechilled motor and pestle followed by centrifugation at 5000 rpm for 5 min. One ml of supernatant was mixed with 5 ml of anthrone-sulphuric acid solution and the contents were kept in a hot water bath at  $20^{\circ}\text{C}$  for about 15 min and the absorbance was read at 630 nm using a UV-Vis Spectrophotometer. The concentration of carbohydrate was calculated by taking glucose as a standard and was expressed as the percentage of fresh weight of sample (Dubois *et al.*, 1956).

**Extraction and estimation of lipid:** Total lipid content was extracted and estimated following the procedure of Folch *et al.* (1957). To 500 mg of dried muscle sample, 3 ml of chloroform methanol (2: 1; v: v) mixture was added and homogenised. After 24 hrs of incubation, the mixture was filtered into a beaker and allowed to dry in a hot air oven at  $60^{\circ}\text{C}$ . The total lipid content of the sample was calculated by taking the final (with lipids) and initial (without anything) weight of the beaker and the concentration was expressed as the percentage of dry weight of sample.

**Extraction and estimation of protein:** To extract soluble protein, 1 g of fresh muscle tissue was homogenised with phosphate buffer saline (pH 7.4) and 50 mM EDTA in mortar and

pestle under ice cold condition. The homogenates were centrifuged at 15000 rpm for 10 min at 4°C followed by protein estimation (Lowry *et al.*, 1951). Here, bovine serum albumin was taken as standard and the protein quantity was expressed as the percentage of fresh weight of sample. The collected supernatant was kept at -20°C for protein profiling and enzymatic analysis.

**Analysis of protein profile by SDS-PAGE:** After estimation, 100 µg of protein aliquot was pooled and resolved by SDS PAGE by applying 5 % and 10 % stacking and resolving gels, respectively (Laemmli, 1970). The Coomassie Brilliant Blue staining method was applied for visualization of separated polypeptides (Blakesley and Boezi, 1977) and the captured images were analyzed using a gel documentation system with quantity one software from Bio-Rad (Bio-Rad, Italy). The appeared protein bands were identified by comparing with a precision plus protein molecular weight marker (Bio-Rad, CA).

**Assay of antioxidant enzymes (CAT and GPX):** CAT activity of the collected supernatant of muscle tissue samples was estimated by the method of Aebi (1974). The enzyme activity was measured followed by decrease in absorbance of H<sub>2</sub>O<sub>2</sub> at 240 nm and was expressed as nKatal mg<sup>-1</sup> protein. The reaction was initiated with a mixture of 2.0 ml of 100 mM phosphate buffer (pH 6.8), 0.5 ml of 10 mM H<sub>2</sub>O<sub>2</sub> and 0.5 ml of enzyme extract (50 µg of protein). GPX activity was assayed as per the method of Bergmeyer (1974) and the activity was expressed in units mg<sup>-1</sup> of protein by observing the oxidation of guaiacol in the presence of H<sub>2</sub>O<sub>2</sub> at 436 nm. The buffer substrate mixture was prepared by taking 2.8 ml of 100 mM phosphate buffer (pH 7.0), 50 µl of 10 mM H<sub>2</sub>O<sub>2</sub> and 50 µl of 0.018 M guaiacol and 100 µl of crude extract (50 µg protein).

**Activity staining of CAT and GPX:** The isoenzymes of CAT and GPX were studied using non-denaturing and non-reducing polyacrylamide gel electrophoresis with 10% acrylamide gels (Laemmli, 1970). For CAT isoforms, the electrophoresed gels were incubated in 0.003 % H<sub>2</sub>O<sub>2</sub> and followed by development in 2 % ferric chloride and 2 % potassium ferricyanide for 10 min (Woodbury *et al.*, 1971). The separated isoenzymes were clearly visualized as colorless bands on a blue background on the gel. GPX isozymes were separated according to the staining procedure of Hamill and Brewbaker (1969). The gels were soaked in 0.018 M guaiacol for 30 min at room temperature, followed by incubation in

a developing solution of 0.015 % H<sub>2</sub>O<sub>2</sub> in 1 % acetic acid until it turned to reddish brown colour indicating GPX bands.

**Sample preparation and heavy metal analysis:** Muscle tissues of collected fish samples were dissected according to the method of Dybem (1983). Samples were completely dried to achieve a constant weight at 80°C. Five gram of each sample was digested with 65% of nitric acid and 35% of hydrogen peroxide in 3:1 ratio. A blank digest was prepared with a similar treatment. After digestion, the sample was filtered and diluted to 25ml with Milli-Q water. In order to assess the accuracy of developed method, the certified reference standard materials of Cd, Cr and Pb (with product no 51994, 02733 and 16595, respectively) were obtained from Sigma-Aldrich, Switzerland. The concentration of specific heavy metals was assessed by applying a flame atomic absorption spectrophotometer (iCE3000 FL AA system, Thermo Scientific, UK) and the results are expressed in mg kg<sup>-1</sup> d.wt.

**Statistical analyses:** All obtained results are mean of three independent experimental replicates and data are presented as mean ± standard error. The values were statistically analyzed by ANOVA and validated for least significance differences by Duncan's New Multiple Range Test at P ≤ 0.05.

## Results and Discussion

Among all sampling locations, the carbohydrate content in fish samples of Khandagiri was found to be in higher concentration (0.28 ± 0.04 % f.wt.) as compared to other fish samples collected from other sites. A significant reduction in carbohydrate content was noticed in the fish samples which were collected from Jaydev Vihar and BDA (0.17 ± 0.02 and 0.19 ± 0.01% f.wt., respectively). Similarly, changes in the amount of lipids and proteins were observed with respect to sampling locations. The values of lipid content varied between 3.48 ± 0.27 to 6.62 ± 0.44 % d.wt. The maximum amount (6.62 ± 0.44 % d.wt.) of lipid was detected from samples of Unit VIII and the minimum (3.48 ± 0.27 % d.wt.) resulted from fish collected from BDA market. In case of protein, the maximum (2.92 ± 0.16 % f. wt.) protein was attributed in Unit IV sample whereas 1.56 ± 0.11 % f.wt. was yielded from the samples of Unit VIII, which was two times less than that of the Unit IV (Table 1). Changes in primary metabolites like carbohydrate, lipid and protein in fish tissue samples are the key symbol of imbalances at various levels of

**Table 1:** Values of biochemical parameters in muscle tissues of marine fish, *Opisthopterus tardoore* collected from local market in Bhubaneswar

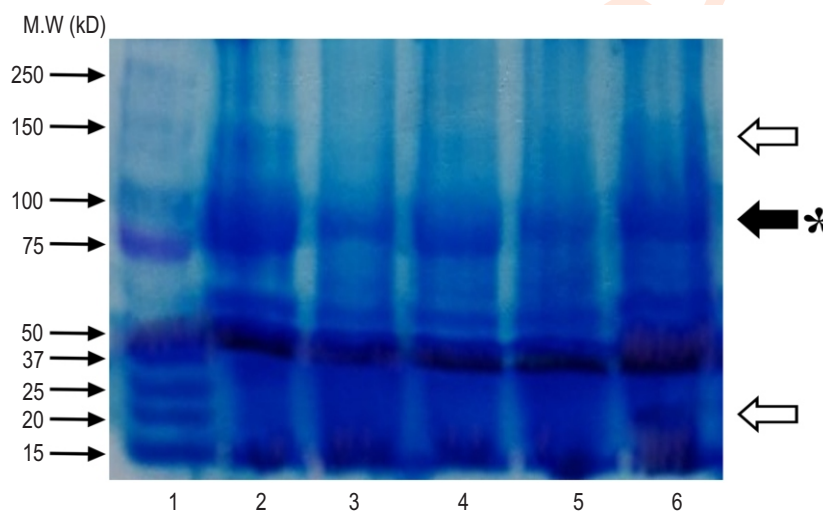
| Parameters                 | Samples collected sites of Bhubaneswar, Odisha, India |                          |                          |                          |                          |
|----------------------------|---|--------------------------|--------------------------|--------------------------|--------------------------|
|                            | Unit IV   | Unit VIII                | BDA                      | Jayadev Vihar            | Khandagiri               |
| Carbohydrate (% fresh wt.) | 0.25 ± 0.03 <sup>b</sup>                              | 0.24 ± 0.03 <sup>b</sup> | 0.19 ± 0.01 <sup>c</sup> | 0.17 ± 0.02 <sup>c</sup> | 0.28 ± 0.04 <sup>a</sup> |
| Lipid (% dry wt.)          | 5.15 ± 0.22 <sup>b</sup>                              | 6.62 ± 0.44 <sup>a</sup> | 3.48 ± 0.27 <sup>d</sup> | 4.68 ± 0.37 <sup>c</sup> | 5.61 ± 0.39 <sup>b</sup> |
| Protein (% fresh wt.)      | 2.92 ± 0.16 <sup>a</sup>                              | 1.56 ± 0.11 <sup>b</sup> | 1.99 ± 0.12 <sup>b</sup> | 1.66 ± 0.11 <sup>b</sup> | 2.36 ± 0.12 <sup>a</sup> |

Data values are mean ± SE of three replicates. Values in the same row carrying different letters are significantly different at P ≤ 0.05.

**Table 2:** Values of heavy metals in muscle tissues of marine fish, *Opisthopterus tardoore* collected from local market in Bhubaneswar

| Heavy metals<br>( $\mu\text{g g}^{-1}$ ) | Samples collected sites of Bhubaneswar, Odisha, India |                               |                                |                                |                               | MAL (FEPA,<br>2003; WHO,<br>1985) |
|--|---|-------------------------------|--------------------------------|--------------------------------|-------------------------------|-----------------------------------|
|  | Unit IV   | Unit VIII                     | BDA                            | Jayadev Vihar                  | Khandagiri                    |                                   |
| Chromium                                 | 17.39 $\pm$ 0.06 <sup>ab</sup>                        | 18.84 $\pm$ 0.08 <sup>a</sup> | 17.37 $\pm$ 0.06 <sup>ab</sup> | 17.42 $\pm$ 0.05 <sup>ab</sup> | 16.44 $\pm$ 0.07 <sup>b</sup> | 0.15 mg kg <sup>-1</sup>          |
| Cadmium                                  | BDL   | BDL                           | BDL                            | BDL                            | BDL                           | 2.0mg kg <sup>-1</sup>            |
| Lead                                     | BDL   | BDL                           | BDL                            | BDL                            | BDL                           | 2.0mg kg <sup>-1</sup>            |

Data values are mean  $\pm$  SE of three replicates. Values in the same row carrying different letters are significantly different at  $P \leq 0.05$ . MAL: Maximum allowable limit; BDL: Below detection limit



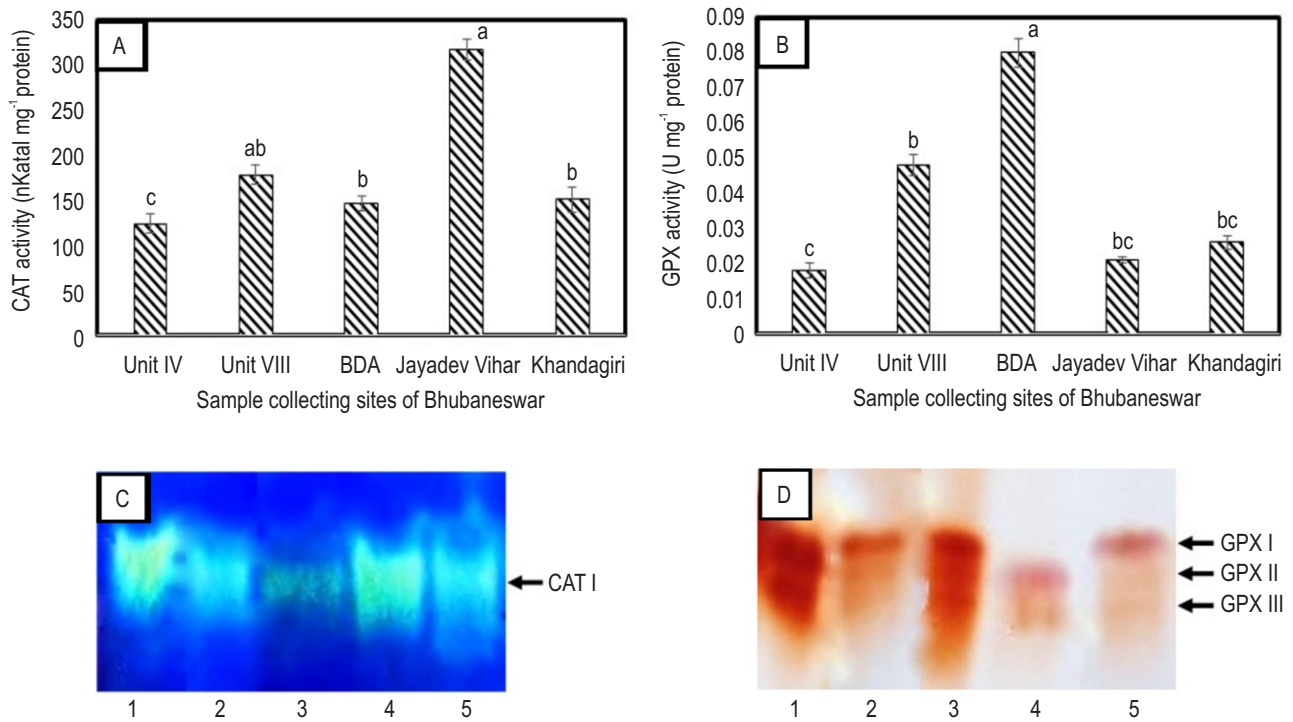
**Fig. 1:** Protein profile of muscle samples of *Opisthopterus tardoore*. Lane 1: Molecular weight marker; Lane 2: Unit IV; Lane 3: Unit VIII; Lane 4: BDA; Lane 5: Jayadev Vihar; Lane 6: Khandagiri. The white and black arrow with asterisk indicate newly synthesized and increased expression of polypeptides, respectively.

metabolic activity which generally occur due to involvement of various unhealthy environments (Javed and Usmani, 2015). Heavy metals causing abnormality is one of them, that interact at molecular level and cause serious injuries in the cellular compartment. Depletion and/ or augmentation of carbohydrates may be due to its rapid requirement/ loss to combat the situation under toxic manifestation (Bhilave *et al.*, 2008).

Lipids are another significant constituent of cellular structure which are also considered as stress indicator due to conversion into carbohydrates at the time of cellular crisis (Ilavazhahan *et al.*, 2012). Interaction of heavy metals also significantly impact the degree of damages of protein molecules. Variation in protein concentrations is a prime factor of cellular toxicity, as it involves various kinds of major physiological, biochemical and molecular activities (Raja and Puvaneswari, 2017; Ullah *et al.*, 2021). Similar patterns of biochemical changes have been studied on different fish samples however the experiments were conducted by supplementing the increasing concentrations of various heavy metals. Fe induced biochemical toxicity was investigated by Karanjkar and Deshpande (2016) on

*Gonoproktopterus kolus* and found that the lethal dose of Fe caused depletion in biochemical parameters in tissue samples of gill, liver, muscle, kidney and intestine. Specifically, the reduction of protein content may be due to stress induced proteolysis, hydrolysis and oxidation to meet the increased demand for energy metabolism. Moreover, supply of necessary keto acids through transamination, and deamination is considered as key changes during heavy metal stress in fish which acts as precursors for maintaining carbohydrate metabolism. Raja and Puvaneswari (2017) also studied Pb toxicity on *Labeo rohita* and found significant changes of carbohydrate, lipid and protein in liver and muscle tissues.

The alterations in carbohydrate, lipid and protein play a significant role in body construction and energy production, which directly involves physiological changes in fishes. The Coomassie Brilliant Blue stained one dimensional banding pattern of proteins (Fig. 1) indicate significant disturbances of protein signals. Among all protein bands, the major polypeptides like 50.12, 58.44 and 81.72 kDa were separated and found common in each sample, however, 81.72 kDa of peptide was expressed more in Unit IV.



**Fig. 2:** The specific assay (A, B) and activity staining (C, D) of CAT (A, C) and GPX (B, D) from muscle samples of *Opisthopterus tardoore*. For activity staining, Lane 1: Unit IV; Lane 2: Unit VIII; Lane 3: BDA; Lane 4: Jayadev Vihar; Lane 5: Khandagiri. For the enzymatic assay, the data plotted represent mean  $\pm$  SE of Six replicates. Symbol carrying different letters are significantly different between treatments and control at  $P \leq 0.05$ .

The protein band with a molecular weight of 64.84 kDa appeared in each case but was not noticed in muscle tissues of Unit IV. Two differentially expressed new proteins having molecular weights of 138.14kDa and 21.23 kDa were detected in Unit IV and Khandagiri, respectively (Fig. 1).

Protein metabolism is being treated as a vital mechanism underlying the biological strategy of toxicity. Hence, major changes in terms of either disappearance/ *de novo* synthesis/ more expression of proteins or polypeptides explain adjustment mechanism to cope with the stress caused within the cellular environment (Ko *et al.*, 2019; Rout *et al.*, 2019). Paruruckumani *et al.* (2015) characterized differentially regulated proteins in *Lates calcarifer* under Cu stress in which, the fish samples were exposed up to 28 days. Similarly, *Periphalmodon schlosseri* revealed a diversified range of proteins ranging from 33-184 kDa under heavy metals stress (Hidayaturrahmah *et al.*, 2019). A significant change in CAT and GPX activities were observed in the muscle tissues of *O. tardoore*, which were analysed by both spectrophotometrically and by native gel electrophoresis.

The maximum CAT activity was observed (317.18 n Katal mg<sup>-1</sup> protein) in samples collected from Jaydev Vihar whereas other samples showed mild variation ranging from 124.13  $\pm$  09.61 to 178.70  $\pm$  11.22 nKatal mg<sup>-1</sup> protein (Fig. 2A). The isoenzyme

activity of CAT showed single isoformic band (CAT I) in all samples, however, the intensity of activity varied (Fig. 2C). A similar pattern was also noticed in case of GPX, where the maximum activity (0.081  $\pm$  0.004 U mg<sup>-1</sup> protein) was found in tissue samples collected from BDA, followed by Unit VIII (0.048  $\pm$  0.003 U mg<sup>-1</sup> protein) [Fig. 2B]. The specific staining activity of GPX was carried out in non-denaturing PAGE where three isoforms (GPX I, GPX II and GPX III) appeared in all muscle samples, except for samples from Jayadev Vihar (GPX II and GPX III). However, the maximum intensity was observed in the tissue samples collected from Unit IV and BDA (Fig. 2D).

An inconsistent expression of antioxidant enzymes is considered as an early warning of toxicological symptoms at various levels and metal induced pollution is one of them. As most repair enzymes are capable of changing their enzymatic activity due to the interaction of various metals ions, therefore it is a sensitive process to identify metal causing pollutants (Ullah *et al.*, 2017; Kerry *et al.*, 2018; Kumar *et al.*, 2021). Changes in antioxidant enzymes in fishes depend on various factors like age, size, body weight, nature of the habitat, gender and developmental stages (Kurhaluk, 2019). In this study, the fish samples had constant factors, except for the source of marketing places (Unit IV, Unit VIII, BDA, Jayadev Vihar, Khandagiri of Bhubaneswar). However, they were harvested from a common

catching origin that is Bay of Bengal. The study revealed stimulation of key antioxidant enzymes like CAT and GPX may regulate the overproduction of AOS under Cr toxicity. Similar responses to Cr toxicity have been reported in *Channa marulius* and *Wallago attu* (Batool *et al.*, 2014), *Labeo rohita* (Kumari *et al.*, 2014), *Clarias batrachus* (Madhavan and Elumalai, 2016) and *Anabas testudineus* (Kumar *et al.*, 2022).

Elevation of antioxidant enzymes in the current study attributed to the protective mechanism of efficient regulation of AOS formation under stressful condition. This reimbursing response of antioxidant enzymes prevents the production of free radicals by producing less harmful products like H<sub>2</sub>O and O<sub>2</sub> in fish under metal stress (Rahimikia, 2017; Aliko *et al.*, 2018; Ullah *et al.*, 2021). The accumulating concentration of Pb and Cd were found below detection limit whereas Cr was detected in fluctuating concentrations in tissue samples. The maximum concentration ( $18.84 \pm 0.08 \mu\text{g g}^{-1}$ ) of Cr was recorded in the samples of Unit VIII followed by Jayadev Vihar ( $17.42 \pm 0.05 \mu\text{g g}^{-1}$ ), Unit IV ( $17.39 \pm 0.06 \mu\text{g g}^{-1}$ ), BDA ( $17.37 \pm 0.06 \mu\text{g g}^{-1}$ ), Khandagiri ( $16.44 \pm 0.07 \mu\text{g g}^{-1}$ ), respectively (Table 2).

The Cr concentration among fish samples ranged from  $16.44 \pm 0.07$  to  $18.84 \pm 0.08 \mu\text{g g}^{-1}$ , which was far above the maximum permissible limit of Federal Environmental Protection Agency (FEPA, 2003) and World Health Organization (WHO, 1985). Further, rise in anthropogenic activities and economic development have significantly contributed to the maximum load of various kinds of heavy metals in marine ecosystems. Industrial and agricultural runoff, boating and fishing activities, urban and municipality waste and mining activities are the chief sources of Cr contamination in the coastal belt of Bay of Bengal (Khan *et al.*, 2017; Ali *et al.*, 2019). Non-essential elements affect the metabolic activities of fish, additionally their bioaccumulation has further proved to be toxic for human through food chain even at low concentrations (Rajesh Kumar and Li, 2018). The current findings indicate that fish samples containing higher levels of Cr may be a threat indicator for human health. High Cr concentrations have been previously been reported in different fishes like *Chanos chanos*, *Lutjanus fulviflamma* and *Terapon jarbua* (Lakshmanasenthil *et al.*, 2013), *Scomberomorus guttatus* (Thomas and Mohaideen, 2015), *Arius cruciger* (Saha *et al.*, 2016), *Hypophthalmichthys molitrix* and *Pangasius pangasius* (Ullah *et al.*, 2017) and *Pampus chinensis* (Ahmed *et al.*, 2019).

The present findings point out significant disturbances in the concentration of carbohydrate, lipid and protein in muscle tissues of *O. tardoore*. In addition, changes in protein patterns and antioxidant enzymes indicated possible heavy metal bioaccumulation and its detoxifying mechanism against AOS. The accumulation of high concentration of Cr was observed in tissues which is the most edible part of this fish, indicates the bioavailability of Cr in the coastal areas of Bay of Bengal. Hence, it is not only a threat to marine fish population but also to local people as consumption of such fish may lead to serious public health hazard. This necessitates further investigation of toxicants

at sub-cellular and molecular levels of marine fish samples.

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### Add-on Information

**Authors' contribution:** J.R. Rout: Supervised and wrote the manuscript; Y. Panda: executed the laboratory work. All authors reviewed and approved the final manuscript.

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**Ethical approval:** Not applicable.

**Conflict of interest:** The authors declare that they have no conflict of interest.

**Data from other sources:** Not applicable.

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