Fuzzy Systems and Soft Computing
ISSN : 1819-4362

SEASONAL CHANGES INFLUENCES ON OXIDATIVE STRESS AND ANTIOXIDANTS RESPONSE IN THE MARINE BIVALVE DONAX INCARNATUS FROM THE TWO SOUTH INDIAN COASTAL REGIONS

Eswari S Research Scholar Postgraduate and Research Department of Zoology, Pachaiyappa's College for Men, Chennai-600 030 India.
Parthiban. R Research Scholar Postgraduate and Research Department of Zoology, Pachaiyappa's College for Men, Chennai-600 030 India.
P.C Sathyanarayanan, Assistant Professor Postgraduate and Research Department of Zoology, Pachaiyappa's College for Men, Chennai-600 030 India.

Abstract:
As filter feeders that contribute to the cycling of nutrients in water quality, bivalves serve a critical role in aquatic ecosystems. They are also frequently employed as sentinel species in environmental toxicology. Because it is rich in biological substances and has a high nutritional value, it makes up the majority of fisheries resources. The bivalve Donax incarnatus, is found in the seas of the Indo-Pacific area. Oxidative stress is brought on by certain environmental variables that produce an excess of reactive oxygen species or a decrease in the antioxidant defense system's functionality. In this work, the edible clam Donax incarnatus, which was collected from the Muttukadu-kovalam estuary and Pullicat Lake over several seasons from May 2021 to April 2022, is studied to determine the effects of seasonality on oxidative damage and antioxidant response. The alterations were evaluated for oxidative stress and the antioxidant defense mechanism. The information obtained suggests that antioxidant biomarkers and lipid peroxidation levels vary seasonally. The biochemical components of Donax tissue are regulated by seasonal variations, as demonstrated by this finding.

KeyWords:
Donax incarnates, bivalves, biological substances, Oxidative stress

1.Introduction

Estuarine systems are coastal bodies of brackishwaterthat are partially enclosed,have one or more rivers or creeks feeding into them and have a free access to the open sea.Estuary are extremely productive ecosystem and plays important role in many different ecological and biological process.each estuary is differs in structural,climate,tidal,intrusion,chemical process all estuary sustain a variety of vegetation and wildlife [1] Due to the significant degradation of the marine eco-system, trace metal induced marine pollution is a serious concern.Marine molluscs are an inexpensive source of high biological value protein as well as essential minerals and vitamins. Due to their extensive distribution, ability to adapt to changing environmental circumstances, and .frequently utilised as tools for the biomonitoring of chemical pollution Biomarkers are a broad category of molecular, cellular, or physiological alterations that can be detected in organisms in response to toxins or other environmental stressors [2].

There hasn't been much investigation into the Donax sp value.People living around the coast consume bivalve muscles, there is no much evidence that *Donax incarnatus* is edible in India. The current work was estimate *D. incarnatus's* biochemical components such as oxidative stress and antioxidant status are regulated by seasonal variations.

2. Related work

Bivalves are crucial in aquatic ecosystems due to their role as filter feeders, which significantly contribute to nutrient cycling and water quality. As filter feeders, they help maintain ecosystem health by removing particulate matter from the water and enhancing nutrient dynamics. Their ecological role makes them valuable indicators for assessing environmental health and changes in water quality [3]. Due to their sensitivity to environmental changes and their role in nutrient cycling, bivalves are frequently used as sentinel species in environmental toxicology. Their tissues accumulate pollutants from their surroundings, making them effective biomarkers for monitoring contamination levels and

assessing the impacts of environmental stressors [4]. Studies have shown that bivalves can reflect the quality of their environment and provide insights into the effects of pollution on aquatic ecosystems.
Oxidative stress in bivalves is influenced by environmental factors that either increase the production of reactive oxygen species (ROS) or impair the functionality of antioxidant defense systems. Reactive oxygen species can cause damage to cellular components, leading to oxidative stress and lipid peroxidation. Antioxidant defense mechanisms, including enzymes like catalase and molecules like reduced glutathione, play a crucial role in mitigating this oxidative damage [5]. However, exposure to pollutants such as heavy metals can disrupt these defense mechanisms, leading to increased oxidative stress and damage.
Seasonal changes can significantly impact the oxidative stress and antioxidant response in bivalves. For instance, variations in temperature, salinity, and pollutant levels throughout the year can affect the biochemical and physiological processes in these organisms. Studies have shown that oxidative stress markers and antioxidant enzyme activities in bivalves can fluctuate with seasonal changes, influencing their overall health and the integrity of their tissues [6]. The edible clam *Donax incarnatus* is commonly found in the Indo-Pacific region and is a valuable species for studying environmental impacts due to its biological richness and nutritional value. Despite its significance, research on *D. incarnatus* has been limited. The current study investigates the effects of seasonality on oxidative damage and antioxidant response in *D. incarnatus* collected from the Muttukadu-Kovalam estuary and Pulicat Lake over several seasons. The study aims to determine how seasonal variations influence oxidative stress and antioxidant defenses in this bivalve species.

3. Materials and Methods

This section of research work discuss about the material and method used in research. This methods and material include sample collection, tissue preparation and the statistical analysis.

Study Area and Sample Collection: In this study, samples were collected from two coastal regions near the mouth of the Muttukadu Backwater and Pulicat Lake, both situated along the eastern coast of India. The geographical coordinates for the Muttukadu Backwater are approximately Latitude 12° 48’01" N and Longitude 80° 14.54' E, while Pulicat Lake is located at Latitude 13° 27’25" N and Longitude 80° 18′35" E. These sites were chosen due to their ecological significance and proximity to each other.

Sampling was conducted during different seasons to capture a comprehensive understanding of the variations in the environmental conditions and their potential impact on the clams. At each site, 30 individual clams were selected for collection. These clams were carefully chosen to have similar weight, length, and breadth to ensure consistency in the study and to minimize variability in the results due to size differences.

The clams were collected from areas exposed during low tide, which are typically rich in benthic organisms and provide an ideal environment for clam habitation. Once collected, the clams were transported to the laboratory within 3 hours to ensure they remained in good condition for analysis. This prompt transportation was crucial to maintain the integrity of the biological samples and to prevent any changes in their physiological state that could occur due to prolonged exposure to ambient conditions.

Upon reaching the laboratory, the clams were likely subjected to various analyses to assess their health, growth patterns, or response to environmental factors, though specific procedures were not detailed in the initial description. The careful selection, rapid transportation, and controlled laboratory conditions suggest a well-planned study aimed at obtaining reliable and accurate data on clam populations in these coastal regions.

Tissuse Preparations

In the study, the process of sample preparation for biochemical analysis involved several meticulous steps to ensure the integrity and reliability [7]of the data obtained from the clams. Here's a detailed explanation of the procedure.

Sample Dissection and Storage

Dissection: The mantle, gills, and heptopancreas of each clam were carefully extracted [8]. These tissues are crucial for understanding various physiological and biochemical parameters, as they are involved in essential functions like respiration, digestion, and excretion.

Storage: The extracted tissues were placed in sterilized tubes and stored at -20°C [9]. This low temperature preserves the biological samples and prevents degradation or alteration of biochemical substances within the tissues until further analysis.

Homogenization

Preparation: The tissue samples were homogenized using a 100 mM sodium phosphate buffer with a pH of 7.0. This buffer helps maintain a stable pH environment that is essential for enzyme activity and biochemical reactions [10]. The buffer also contained 0.5 mM EDTA (ethylenediaminetetraacetic acid), which chelates metal ions that could otherwise catalyze unwanted oxidative reactions.

Protease Inhibition: A few crystals of phenylmethylsulfonyl fluoride (PMSF) were added to the buffer. PMSF is a protease inhibitor that prevents the degradation of proteins by inhibiting proteolytic enzymes, ensuring [11] that the proteins of interest remain intact during homogenization.

Centrifugation

Process: The homogenized tissue samples were subjected to centrifugation at 4°C for 30 minutes at 12,000 RPM (revolutions per minute) [12]. Centrifugation separates cellular components based on their size and density. At this speed and temperature, larger particles like cell debris and intact cells are sedimented at the bottom of the centrifuge tube, while the soluble proteins and other small molecules remain in the supernatant.

Supernatant Collection: The supernatant, which contains the soluble proteins and other biochemical substances, was carefully collected. This fraction is used for further analysis.

Aliquoting and Storage

Aliquoting: The collected supernatant was divided into different aliquots [13], which were placed in separate tubes. This step ensures that each sample can be analyzed independently, reducing the risk of contamination or sample degradation.

Storage: The aliquots were stored at -20°C [14] to maintain their stability until required for oxidative and antioxidant studies. This temperature ensures that biochemical reactions remain stable and that the integrity of the samples is preserved for accurate analysis.

By following these procedures, the study ensures that the tissue samples are well-preserved, homogenized consistently, and free from enzymatic degradation, allowing for reliable assessment of oxidative and antioxidant activities in the clams.

Assay for Oxidative Stress And Antioxidant

In the work, several biochemical assays were employed to assess oxidative stress and antioxidant activity in bivalve tissues. Here's an elaborate explanation of the methods used for these assays.

Lipid Peroxidation (LPO) Measurement

Lipid Peroxidation (LPO) is a process where free radicals attack lipids in cell membranes, leading to the formation of malondialdehyde (MDA), a by-product of lipid peroxidation. MDA is commonly used as a biomarker to assess oxidative stress.

Method Reference: Muthukumaran. [15]

Procedure Overview:

Tissue Preparation: The tissue samples are homogenized and centrifuged to obtain a clear supernatant.

Reaction Setup: An aliquot of the supernatant is mixed with specific reagents to induce a reaction that produces MDA.

Detection: The level of MDA is typically determined using thiobarbituric acid (TBA) which forms a colored complex with MDA. This color is measured using a spectrophotometer at a specific wavelength (usually 532 nm).

Quantification: The MDA content is quantified based on the absorbance of the colored complex, and results are expressed as nmol of MDA per gram of tissue.

Catalase (CAT) Activity Measurement

Catalase (CAT) is an enzyme that decomposes hydrogen peroxide (H_2O_2) into water and oxygen, playing a crucial role in protecting cells from oxidative damage.

Method Reference: Sinha (1972) [16]

Procedure Overview:

Reaction Setup: A known amount of tissue homogenate is added to a reaction mixture containing hydrogen peroxide.

Catalase Reaction: Catalase in the sample breaks down hydrogen peroxide, and the rate of this reaction is measured.
Measurement: The activity of catalase is typically assessed by measuring the decrease in hydrogen peroxide concentration, often using a colorimetric or spectrophotometric assay where the remaining hydrogen peroxide reacts with specific reagents to produce a measurable signal.
Quantification: The activity is usually expressed as micromoles of hydrogen peroxide decomposed per minute per milligram of protein.

Superoxide Dismutase (SOD) Activity Measurement
Superoxide Dismutase (SOD) is an enzyme that catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide, playing a crucial role in the defense against oxidative stress.
Method Reference: Marklund and Marklund (1974). [17]
Procedure Overview:
Reaction Setup: The tissue homogenate is added to a reaction mixture containing pyrogallol, which auto-oxidizes in an alkaline environment to form a color.
SOD Inhibition: SOD inhibits the auto-oxidation of pyrogallol. The degree of inhibition is proportional to the SOD activity in the sample.
Measurement: The extent of pyrogallol auto-oxidation is measured spectrophotometrically by monitoring the change in absorbance at a specific wavelength (typically 430 nm). The inhibition of this auto-oxidation is used to infer SOD activity.
Quantification: The activity is quantified based on the degree of inhibition of pyrogallol auto-oxidation, expressed in units per milligram of protein.

In summary the Lipid Peroxidation (MDA Content): Measures the extent of oxidative damage to lipids by quantifying MDA. Catalase (CAT) Activity: Assesses the enzyme's ability to break down hydrogen peroxide, reflecting antioxidant defense. Superoxide Dismutase (SOD) Activity: Evaluates the enzyme's capacity to neutralize superoxide radicals, another important indicator of oxidative stress. These methods collectively provide a comprehensive assessment of oxidative stress and antioxidant capacity in the bivalve tissues.

BIOACCUMULATION HEAVY METAL ANALYSIS

To analyze heavy metals in the soft tissues of bivalves, a series of methodical steps were followed to ensure accurate and reliable results. Here's a detailed explanation of the process:

Sample Preparation
Thawing and Rinsing: Thawing: The stored frozen samples were thawed at room temperature to facilitate handling.
Rinsing: Each sample was rinsed with deionized water to remove any surface contaminants and salts.

Drying and Grinding
Drying: The samples were dried to a constant weight in an oven set at 95°C. This step ensures that all moisture is removed from the samples, which is crucial for accurate weight measurements and subsequent analysis.
Grinding: After drying, the samples were ground into a fine powder using a suitable grinder. The resulting powder was sieved through a 100 μm mesh to achieve uniform particle size, which is essential for consistent digestion and accurate analysis.

Digestion of Samples
Digestion Process - Digestion Mixture: The powdered samples were subjected to acid digestion using a mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a ratio of 2:1. This acidic mixture is highly effective in breaking down organic matter and dissolving metals.
Hot Block Digester: The digestion was carried out in a hot block digester, a device that allows precise control of temperature.

Temperature Protocol: Initial Phase: The samples were first digested at a low temperature of 40°C for one hour. This initial phase helps to gradually decompose the organic material without excessive loss of metals.
Final Phase: The samples were then subjected to a higher temperature of 180°C for at least 3 hours. This high temperature ensures complete digestion of the samples, breaking down any remaining organic material and solubilizing the heavy metals.

Dilution and Filtration
Dilution: After digestion, the samples were diluted to a specific volume with double distilled water. This step ensures that the concentration of metals is within the detectable range of the analytical equipment.
Filtration: The digested samples were filtered to remove any particulate matter that might interfere with the analysis.

Heavy Metal Analysis
Spectroscopy - Instrument: The filtered samples were analyzed for heavy metals using a Spectra AA-Varian atomic absorption spectrophotometer (AAS). AAS is a highly sensitive technique used to measure the concentration of metals in liquid samples.
Procedure: The AAS measures the absorption of light by metal atoms in the sample. Each metal absorbs light at a specific wavelength, allowing for the identification and quantification of individual metals.

Quality Control
Quality Control Measures: Digestion Blanks: Blanks were prepared and digested alongside the samples to account for any contamination that might occur during the digestion process.
Reference Materials: Certified reference materials (IAEA shrimp MA-A-3/TM and IAEA simulated freshwater W-4) were used to validate the accuracy of the metal analysis. These reference materials have known concentrations of metals and are used to ensure that the analytical results are accurate and reliable.
Deviation Check - Certified Values: The measured values obtained from the analysis were compared with the certified values of the reference materials. A deviation of less than 10% from the certified values was considered acceptable, indicating that the analysis was performed correctly and the results are reliable.

In summary, the heavy metal analysis of bivalve tissues involved careful preparation, digestion, and analysis using advanced spectroscopic techniques. The process included rigorous quality control measures to ensure the accuracy and reliability of the results. The detailed steps provided ensure that the heavy metal concentrations in the bivalve tissues are accurately quantified, contributing to a comprehensive understanding of environmental contamination and its impact on aquatic organisms.

STATISTICAL ANALYSIS
Analysis for obtained results was carried out with the aid of the SPSS computer software programme. Data for antioxidant variables were expressed as mean ± S.E. To evaluate seasonal variations in antioxidant response in *Donax incarnatus* throughout a study year, analysis of variance (One-way ANOVA test, $p < 0.05$) was performed. The differences in body composition between the seasons were also evaluated by comparing the data obtained using Student 't'test

4. Results
The result of oxidative stress and antioxidant response tissue is discussed in this section.

4.1oxidative Stress Biomarker In Tissues Of *Donax Incarnatus*

Oxidative stress biomarkers in the tissues of *Donax incarnatus* are critical indicators of cellular damage and oxidative stress. These biomarkers, including lipid peroxidation (MDA content), catalase (CAT) activity, and superoxide dismutase (SOD) activity, help assess the impact of environmental stressors on the bivalves.

Lipid Peroxidation Of *Donax Incarnatus*

GILLS : The bivalve collected during pre-monsoon period, monsoon and post monsoon from pulicat coastal region showed higher MDA levels in gills and such elevated MDA levels was also observed to be significantly different from summer period. Sample collected from Muttukadu coastal region shows that the MDA levels was greater during post monsoon period and lowest MDA levels during summer season.

MANTLES: The bivalve collected during pre-monsoon period and monsoon periods from pulicat coastal region showed higher MDA levels in mantle and such elevated MDA levels was not significantly differ from summer and post monsoon period. the sample collected from muttukadu coastal region shows that the MDA levels was greater during pre-monsoon and lowest MDA levels in post monsoon season.

HEPATOPANCREAS: The bivalve collected during monsoon period and pre-monsoon periods from pulicat region showed higher MDA levels in hepatopancreas and such elevated MDA levels was significantly differ from summer and post monsoon period. The sample collected from muttukadu coastal region shows that the MDA levels was greater during monsoon and post monsoon and lowest MDA levels during pre-monsoon season.

4.2 ANTIOXIDANT RESPONSE TISSUE IN *Donax incarnatus*

Catalase activity of *Donax incarnatus*

Gills: The gills of bivalve collected from pulicat coastal region shows greater catalase activity during summer and Pre-monsoon sampling period and lowest catalase activity during post monsoon sampling period. The gills of bivalve *Donax* collected from Muttukadu backwater and pulicat, shows much similar catalyse activity.

Mantles: The mantle of bivalve collected from pulicat coastal region shows greater catalase activity during summer and pre-monsoon sampling period and lowest catalase activity during post monsoon period.the mantles of bivalve donax collected from muttukadu backwater and pulicat shows similar activity.

Hepatopaancreas: The hepatopancreas of bivalve collected from pulicat coastal region shows greater catalase activity during monsoon and Pre-monsoon sampling period and lowest catalase activity during post monsoon period. The hepatopancreas of bivalve *Donax* collected from Muttukadu backwater and pulicat, shows much similar catalyse activity.

Superoxide dismutase activity in gills of *Donax incarnatus*

Gills: The gills of bivalve collected from pulicat coastal region shows greater SOD activity during pre-monsoon and Post-monsoon sampling period and lowest catalase activity during monsoon period. The gills of bivalve *Donax* collected from Muttukadu backwater and pulicat, shows much similar SOD activity.

Mantle: The mantle of bivalve collected from pulicat coastal region shows greater SOD activity during summer and Pre-monsoon sampling period and lowest SOD activity during post monsoon period. The mantle of bivalve *Donax* collected from Muttukadu backwater and pulicat, shows much similar catalyse activity.

Hepatopanctreas: The hepatopancreas of bivalve collected from pulicat coastal region shows greater SOD activity during monsoon and Pre-monsoon sampling period and lowest SOD activity during post monsoon period. The hepatopancreas of bivalve *Donax* collected from Muttukadu backwater and pulicat, shows much similar SOD activity.

Bioaccumlation of Heavy metals in *Donax incarnatus*

The bivalve collected from Muttukadu backwater during different seasons. As indicated the levels of metals in both the tissues were generally high during monsoon and post monsoon when compared to premonsoon and summer. Donax collected from Pulicat coastal region during different seasons. the levels of metals in were generally high during pre-monsoon and monsoon when compared to post monsoon and summer. On an average the levels of metals accumulated in bivalve collected from pulicat were comparatively lesser than the sample collected from muttu kadu. Among the metals iron, copper, cadmium and cobalt are higher in both the station.

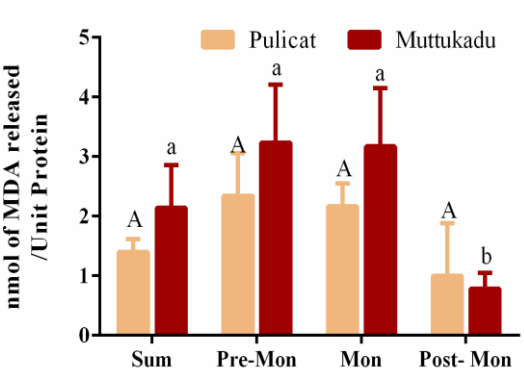

Fig.1: Lipid Peroxidation level different tissues of bivalves

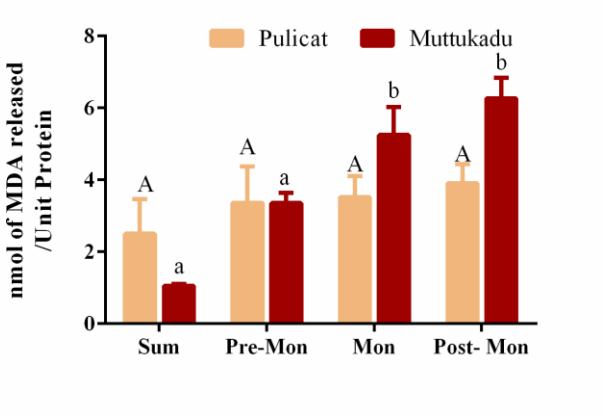

Fig 2: Gills

Fig 1 is the sampl collected from Pulicat and Muttukadu coastal regions. Each bar represents mean± standard error of six determinations using samples from different preparations. Two-way analysis of variance followed by Tukey's post hoc test was used. The same letters (a, b, c,d) indicate no significant difference between the exposure groups whereas different letters indicate statistically significant differences ($p < 0.05$) between different group**.** Fig.2 Catalae acitivities different tissues of bivalves collected from Pulicat and Muttukadu coastal regions. Each bar represents mean± standard error of six determinations using samples from different preparations.

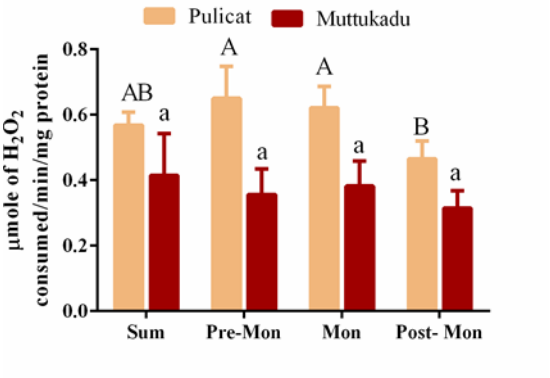

Fig 3: **MANTLES**

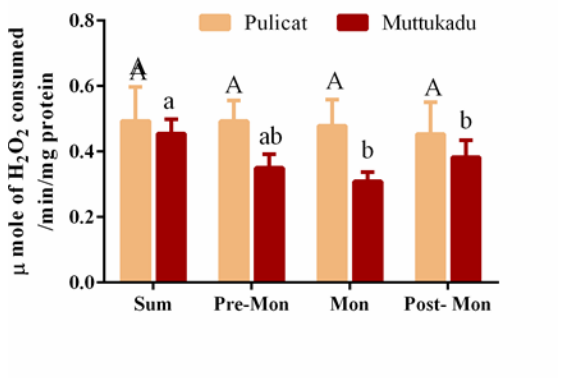

Fig 4: **HEPATOPANCREAS**

Fig 3 and 4 Two-way analysis of variance followed by Tukey's post hoc test was used. The same letters (a, b, c,d) indicate no significant difference between the exposure groups whereas different letters indicate statistically significant differences ($p < 0.05$) between different group.

Fig.3 Superoxide dismutase activities in different tissues of bivalves collected from Pulicat and Muttukadu coastal regions. Each bar represents mean± standard error of six determinations using samples from different preparations. Two-way analysis of variance followed by Tukey's post hoc test was used. The same letters (a, b, c,d) indicate no significant difference between the exposure groups whereas different letters indicate statistically significant differences ($p < 0.05$) between different groups.

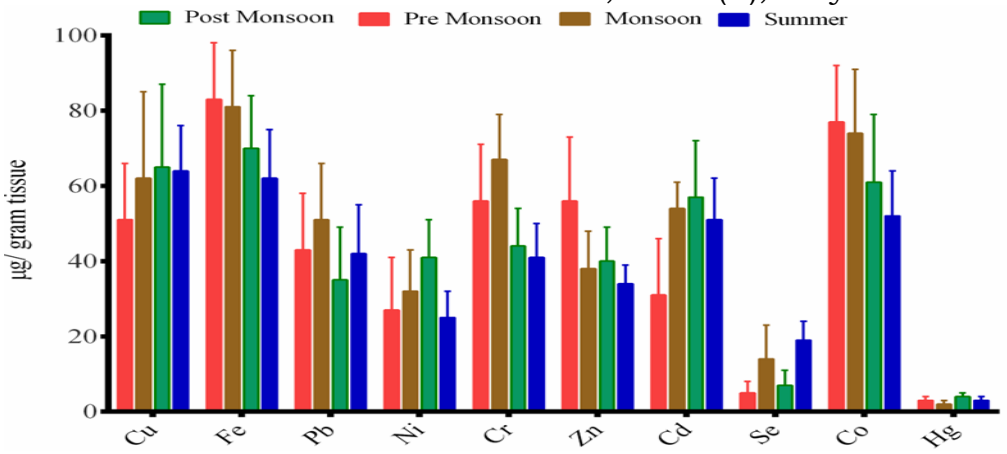

Fig. 5: Bioaccumulation of heavy metals in bivalve collected from Pulicat coastal region during different seasons

The study you are referencing suggests that high levels of lipid peroxidation (LPO) in the gill, mantle, and hepatopancreatic tissues of bivalves from Muttu Kadu indicate significant oxidative stress. Here's a detailed elaboration on the observations and their implications:

Oxidative Stress and Lipid Peroxidation

Oxidative Stress: Oxidative stress arises when there is an imbalance between the production of reactive oxygen species (ROS) and the biological system's ability to detoxify these reactive intermediates. This imbalance can lead to cellular damage and is often indicated by elevated levels of lipid peroxidation.

Lipid Peroxidation (LPO): LPO is a process where free radicals attack lipids in cell membranes, resulting in the formation of lipid peroxides. This damage can compromise cell integrity and function. High LPO levels, as observed in the study, are a marker of increased oxidative stress and are indicative of damage to cellular membranes and tissues.

Comparison Between Sampling Stations

Muttu Kadu vs. Pulicat: The study compares bivalves from two different locations: Muttu Kadu and Pulicat. The significantly higher LPO levels in the tissues from Muttu Kadu suggest that these bivalves are experiencing more severe oxidative stress. This is attributed to higher levels of metal accumulation in these tissues compared to those from Pulicat.

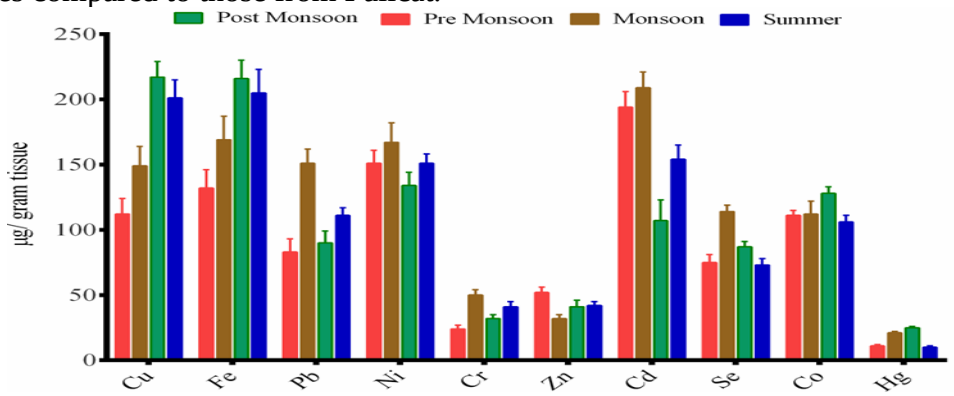

Fig. 6. Bioaccumulation of heavy metals in bivalve collected from Pulicat coastal region during different seasons

Metal Accumulation and Oxidative Stress: Heavy metals are known to contribute to oxidative stress by generating ROS and disrupting antioxidant defenses. Metals such as lead, cadmium, and mercury can catalyze the formation of ROS, which further damages lipids, proteins, and DNA. The elevated metal levels in Muttu Kadu bivalves likely exacerbate oxidative stress and LPO.

Antioxidant Defense Mechanisms

Role of Antioxidant Enzymes: Organisms have evolved antioxidant defense mechanisms to mitigate oxidative stress. Key enzymes include:

- **Catalase:** This enzyme catalyzes the decomposition of hydrogen peroxide (a common ROS) into water and oxygen. It is crucial in protecting cells from oxidative damage.
- **Glutathione:** Reduced glutathione acts as a potent antioxidant, neutralizing free radicals and protecting cells from damage by reactive intermediates and electrophiles.

Inhibition by Heavy Metals: Heavy metals can interfere with the functioning of these antioxidant defenses. For example:

- **Catalase Inhibition:** Heavy metals may bind to catalase, reducing its activity and impeding its ability to neutralize hydrogen peroxide.
- **Glutathione Disruption:** Metals can also deplete glutathione levels or alter its functionality, diminishing the cell's capacity to counteract oxidative stress.

Implications

The observed high LPO levels in bivalves from Muttu Kadu, coupled with the higher metal accumulation, suggest that these organisms are under significant oxidative stress. The study highlights the potential disruption of antioxidant defense mechanisms due to heavy metal exposure. This impaired defense, in turn, exacerbates lipid peroxidation, leading to further cellular damage. Overall, the findings emphasize the need for monitoring and managing metal pollution to protect aquatic organisms and maintain ecological balance. The study also underlines the importance of understanding the interplay between environmental contaminants and biological responses to better assess and mitigate the impacts of pollution.

5. CONCLUSION

The study revealed that bivalves from Muttu Kadu exhibited significantly higher levels of lipid peroxidation (LPO) in their gill, mantle, and hepatopancreatic tissues compared to those from Pulicat. This increase in LPO indicates elevated oxidative stress in bivalves from Muttu Kadu, which is likely due to higher metal accumulation in these tissues. The presence of heavy metals can lead to the generation of reactive oxygen species (ROS), which in turn exacerbates oxidative damage. The findings suggest that prolonged exposure to metal contaminants can overwhelm the antioxidant defenses of bivalves. Antioxidant enzymes, such as catalase and reduced glutathione, which normally help mitigate oxidative stress, appear to be compromised by the presence of heavy metals. This disruption in antioxidant activity results in increased lipid peroxidation and cellular damage. In conclusion, the study underscores the significant impact of metal pollution on oxidative stress in aquatic organisms. It highlights the need for effective monitoring and management of metal contamination to protect aquatic life and maintain environmental health. Additionally, the results emphasize the importance of further research into the mechanisms of metal-induced oxidative stress and its implications for aquatic ecosystems.

Reference

[1] Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., and K..N.(2014)., "Toxicity, mechanism and health effects of some heavy metals.", *Interdisciplinarytoxicology*, Issue7, Vol.2, pp. 60–72. https://doi.org/10.2478/intox-2014-0009.

[2] Devasagayam, T. P., and Tarachand, U. Decreased lipid peroxidation in the rat kidney during gestation. Biochemical and biophysical research communications, Vol. 145, Issue 1, pp. 134–138. https://doi.org/10.1016/0006- 291x(87)91297-6.

[3] Joshy, Aswathy, SR Krupesha Sharma, K. G. Mini, Suja Gangadharan, and P. Pranav., "Histopathological evaluation of bivalves from the southwest coast of India as an indicator of environmental quality." *Aquatic Toxicology* Issue 243, pp. 106076, 2022.

[4] Chahouri, Abir, Bouchra Yacoubi, Abdellatif Moukrim, and Ali Banaoui., "Bivalve molluscs as bioindicators of multiple stressors in the marine environment: recent advances." *Continental Shelf Research* , issue264 , pp. 105056, 2023.

[5] Pisoschi, Aurelia Magdalena, Aneta Pop, Florin Iordache, Loredana Stanca, Gabriel Predoi, and Andreea Iren Serban. "Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status." *European Journal of Medicinal Chemistry*, issue 209 , pp. 112891, 2021.

[6] Zhang, Chuanxu, Yizhou Sun, Jiahua Wen, Boya Xu, Wenlu Zhu, Hongkuan Zhang, Xiaodong Liu, Lingshan LiChu, and Huaiping Zheng. "Effects of chronic cold stress on tissue structure, antioxidant response, and key gene expression in the warm-water bivalve Chlamys nobilis." *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, Issue.* 50 , pp. 101225, 2024.
[7] Molnar, Agnes, Tamas Lakat, Adam Hosszu, Beata Szebeni, Anna Balogh, Laszlo Orfi, Attila J. Szabo, Andrea Fekete, and Judit Hodrea., "Lyophilization and homogenization of biological samples improves reproducibility and reduces standard deviation in molecular biology techniques." *Amino Acids*, Issue53, no. 6 , pp. 917-928, 2021.
[8] Joshy, Aswathy, SR Krupesha Sharma, K. G. Mini, Suja Gangadharan, and P. Pranav., "Histopathological evaluation of bivalves from the southwest coast of India as an indicator of environmental quality." *Aquatic Toxicology, Issue.* 243, pp. 106076, 2023.
[9] Liu, Meimei, Sishao Fan, Zhichao Rong, Hao Qiu, Susu Yan, Hongwei Ni, and Zhiguo Dong. "Exposure to polychlorinated biphenyls (PCBs) affects the histology and antioxidant capability of the clam Cyclina sinensis." *Frontiers in Marine Science, Vol.* 10 pp. 1076870, 2023.
[10] Zhang, Huan, Gaigai Sun, Zhihua Lin, Hanhan Yao, and Yinghui Dong. "The razor clam Sinonovacula constricta uses the strategy of conversion of toxic ammonia to glutamine in response to high environmental ammonia exposure." *Molecular Biology Reports, Vol.* 47, pp. 9579-9593, 2023.
[11] Garcia, Miguel. "Large Scale Purification of Glutathione S-transferase from the Marine Gastropod Tritonia Diomedea." PhD diss., California State University, East Bay, 2021.
[12] Le Vern, Lise. "Biomonitoring of two marine mollusks, the Mediterranean mussel Mytilus galloprovincialis and the limpet Patella sp. in three North Corsican ports and inter-annual comparisons." (2022).
[13] Sıkdokur, Ercan, Murat Belivermiş, Narin Sezer, Murat Pekmez, Ömür Karabulut Bulan, and Önder Kılıç. "Effects of microplastics and mercury on manila clam Ruditapes philippinarum: feeding rate, immunomodulation, histopathology and oxidative stress." *Environmental Pollution, Issue* 262 ,pp. 114247, 2020.
[14] Gnatyshyna, Lesya, Halina Falfushynska, Oksana Stoliar, and Reinhard Dallinger. "Preliminary study of multiple stress response reactions in the pond snail Lymnaea stagnalis exposed to trace metals and a thiocarbamate fungicide at environmentally relevant concentrations." *Archives of environmental contamination and toxicology, Issue* 79, no. 1 , pp. 89-100, 2020.
[15] Muthuraman, Arunachalam, Narahari Rishitha, Nallupillai Paramakrishnan, Bhaskaran Mahendran, and Muthusamy Ramesh. "Role of lipid peroxidation process in neurodegenerative disorders." *Lipid Peroxidation Research* , issue1, pp. 13, 2020.
[16] Singh, R., Gautam, N., Mishra, A., and Gupta, R. (2011)., "Heavy metals and living systems: An overview. *Indian journal of pharmacology"*, Vol. *43*, No 3,pp. 246–253. https://doi.org/10.4103/0253-7613.81505.
[17] Marklund, S., and Marklund, G, " Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase", European journal of biochemistry, Vol. 47, No.3,pp. 469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x