TOXICITY ASSESSMENT IN GREEN CHROMIDE (*Etroplus suratensis*) COLLECTED FROM SLUICE GATE HARVEST SITES

BY

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UNDER THE SUPERVISION OF

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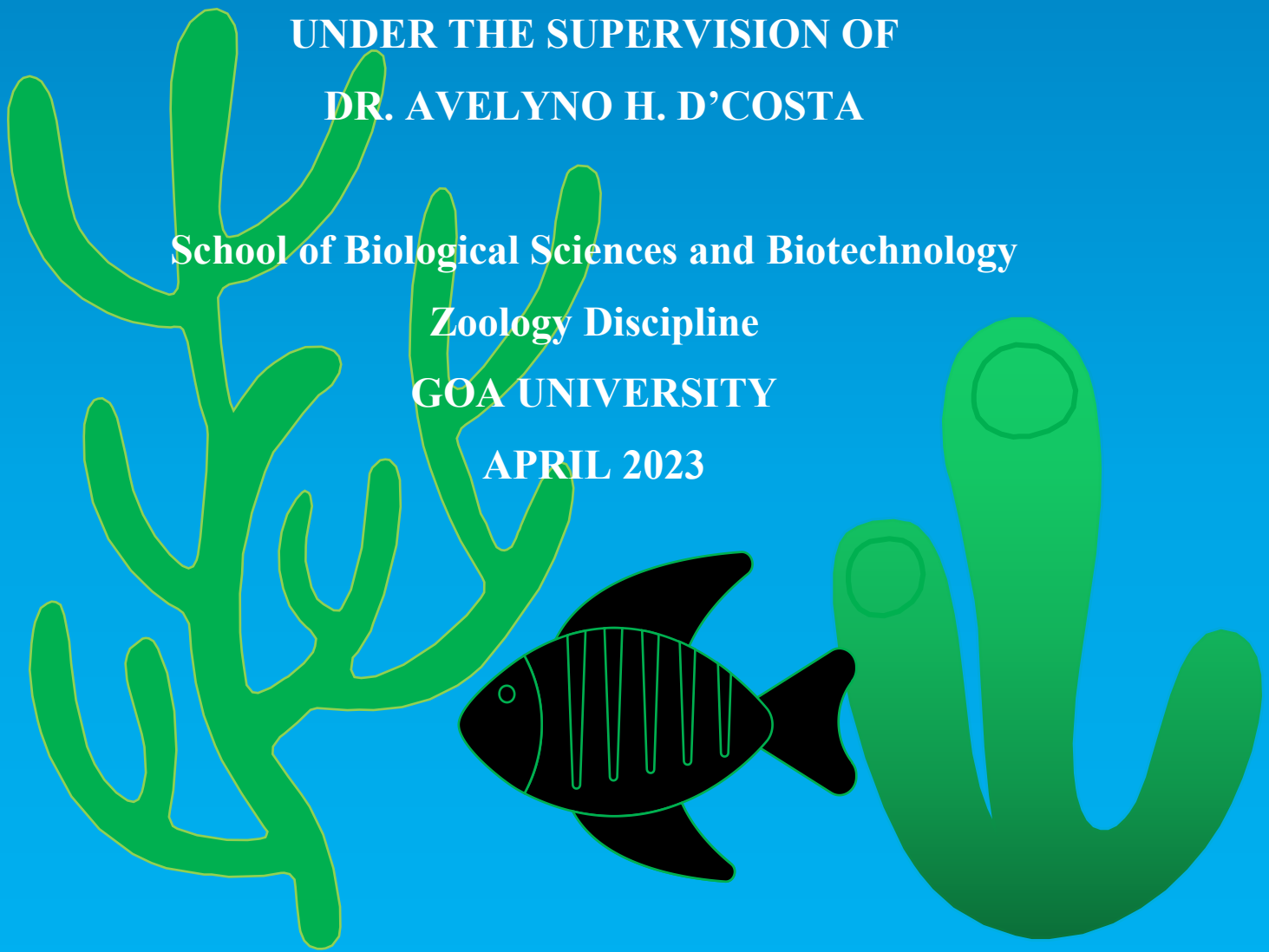

Toxicity Assessment in Green chromide (*Etroplus suratensis*) collected from Sluice gate harvest sites

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Submitted in the partial fulfilment for the Master's degree in Zoology

by

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April 2023

Examined by:

Seal of the School

COMPLETION CERTIFICATE

This is to certify that the dissertation **"Toxicity Assessment in Green chromide (*Etroplus suratensis*) collected from Sluice gate harvest sites"** is a bonafide work carried out by **Ms. Mugdha Deepak Chodankar** under my supervision/mentorship in partial fulfilment of the requirements for the award of the degree of **Master of Science in Zoology** in the Zoology Discipline at the School of Biological Sciences and Biotechnology, Goa University.

Dr. Avelyno H. D'costa
Assistant Professor
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Date: 24/4/2023 .

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation entitled, **"Toxicity Assessment in Green chromide (*Etroplus suratensis*) collected from Sluice gate harvest sites"** is based on the results of investigations carried out by me in the Zoology Discipline at the School of Biological Sciences and Biotechnology, Goa University under the Supervision/Mentorship of **Dr. Avelyno D'costa** and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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INTRODUCTION

INTRODUCTION

Goa is the smallest state of India which is located on the Western coast along the Arabian Sea (Rubinoff, 1999). With a coastline of about 104 kms and 250 kms of inland waterways, the fishery sector contributes significantly to the economy of the state. The presence of major rivers like Mandovi, Zuari, Sal, Talpona, Chapora, Galgibagh etc provide excellent sources of fish as well as a good shelter for fishing crafts (Kamat and Faria, 2016). The use of mechanised fishing and improved fishing technologies have expanded the Goan fish markets. The livelihood of most people of Goa depend on fisheries. There is an increase in demand for supply of fish as the staple food of Goans consists of fish and rice and also due to the development of Tourism industry in Goa that attracts visitors because of its beautiful beaches and adorable destinations (Rubinoff, 1999).

Fish are the major part of human diet (Turkmen et al., 2005) and are considered as healthy food because it is rich in essential nutrients like proteins, vitamins, polyunsaturated fatty acids (PUFA) such as ω-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Mohanty et al., 2019). Macrominerals such as Calcium, Phosphorous, Sodium, Potassium, Chloride along with some trace elements like Iron, Manganese, Zinc, Iodine that are also found in fish (Lall and Kaushik, 2021). Fish can be processed into various products that have varying market value. Not only the fish flesh but other parts like head, viscera, skin are used as raw materials for production of value added products (Benjakul et al., 2014). Thus, fish are a major contributor to human health due to its richness in all the essential nutrients that are necessary to provide a balanced nutrition (Mohanty et al., 2019).

Proteins of high biological values are found in fish. About 140 gm of fish can provide 50 - 60% of daily protein that is required by humans. These proteins have immunoglobulins which act as defence mechanisms against bacterial and viral infections and they also prevent protein calorie malnutrition (Balami et al., 2019). Nutritional value of protein depends on factors like structure of proteins, protein susceptibility to enzymatic digestion, changes in protein during processing, content and composition of amino acids in proteins. A high amount of essential amino acids like Lysine and Leucine are found in high quality proteins. Non essential amino acids like Glutamic acid, Alanine, Aspartic acid are also found in very high amounts (Dale et al., 2019).

Fish contains long chains of poly unsaturated fatty acids namely EPA and DHA. These fatty acids mostly occur in liquid form and flow in the blood vessels freely which make them different from other fats and oils. These are considered important for human health and in prevention of diseases (Balami et al., 2019).

Cholesterol is also found in fish and is a type of lipid molecule that plays an important role in humans as it is a key regulator in transporting membrane fluidity, helps in fat transportation and is precursor of steroids (Mohanty et al., 2019). However, high levels of cholesterol in the body can lead to blocking of blood vessels and causing cardiovascular problems (Carey, 2018). Some fish may have high levels of cholesterol which can be harmful to people with high cholesterol levels. Fish that are rich in omega - 3 fatty acids maintain cholesterol levels by lowering them. (Fatima, 2023).

Carbohydrates are present in minute quantities in fish (Balami et al., 2019). Carbohydrates are macromolecules that are required by the human body as they

act as an energy source, control blood glucose levels and insulin metabolism and they also participate in triglyceride and cholesterol metabolism (Holesh et al., 2022).

Fish is also considered as an important source of micronutrients which are not available in other sources of diet. Fish have a good amount of Calcium which is required by humans for increasing bone density. Fish also have minerals like iodine, selenium etc and are rich sources of Vitamins A and D that prevent problems of poor eyesight, help in formation of bones and avert various diseases (Balami et al., 2019).

But, in the last few decades, the aquatic ecosystem has been polluted and this has emerged as a major environmental issue (Maurya and Malik, 2019). Pollution is the major challenge faced by modern human society. Swift development in agricultural as well as industrial sectors has increased the contamination of water bodies (Ali and Khan, 2019).

The population of the world is increasing and so there is an increase in the production and usage of plastic materials (Saha et al., 2021). Plastics have been used indiscriminately (Nikki et al., 2021) and the management of plastic waste is very limited which posses serious societal and health problems. Plastics are synthetic polymers which are made of non-renewable resources, some of these materials are chemically inert (Saha et al., 2021). Plastics have become an integral part of daily life due to its highly versatile nature. Plastics are categorised into various types like Macroplastics (>25 mm), Mesoplastics (5-25 mm), Microplastics (< 5mm), Nanoplastics (< 0.1 μm) (Nikki et al., 2021).

Microplastics are basically small pieces of plastic which are less than 5mm in size. They can be formed by fragmentation of larger objects. Due to the persistence, ubiquity and toxicity of microplastics, they have been recognised as an emerging threat to the environment. Microplastics have been classified into primary and secondary types depending on their chemical composition as well as on their usage (Saha et al., 2021). Primary microplastics are produced intentionally to be used in consumer products like exfoliants in cosmetics, in abrasive activities like air blasting etc (Prata et al., 2019). The primary microplastics reach coastal and marine habitats by the release of intermediate plastic feedstock like pellets or micro-beads (Saha et al., 2021).

The natural waters are also polluted with heavy metals that originate either from natural or anthropogenic sources (Ali and Khan, 2019). Pollution due to heavy metals is recognized as a serious environmental concern as they accumulate in the organisms residing there (Dural et al., 2007). Heavy metals have high atomic numbers (Z) and specific density greater than 5 g cm−3 (Ali and Khan, 2019). These substances can get accumulated in the tissues of aquatic organisms and be biomagnified to higher trophic levels. They can become toxic for humans after reaching a substantially high level (Dural et al., 2007). Heavy metals accumulation in humans can lead to reduced or damaged mental and central nervous function, it can lower energy levels and damage blood composition, vital organs like lungs, kidneys, liver can also be harmed leading to various life threatening diseases (Zeitoun and Mehana, 2014).

Heavy metals such as Mercury, Cadmium, Lead etc are considered toxic, even in trace amounts (Turkmen et al., 2005). These metals have altering physiological

effects in fish tissues and so fish are used as indicators of heavy metal contamination in the ecosystem (Yardi et al., 2012). Cadmium is present in fertilisers, industrial emissions. It is used as stabilisers in PVC products, alloys and colour pigments and is most commonly used in nickel - cadmium batteries (Zeitoun and Mehana, 2014). Cadmium causes bone demineralization directly through bone damage or indirectly through renal dysfunction. It is also nephrotoxic to proximal tubular cells (Ali and Khan, 2019). Mercury is used in the chlor - alkali industry, it is also used as an electrode in the electrochemical process of manufacturing chlorine. Organic mercury exists as methylmercury which accumulates in the food chain. Mercury poisoning during nervous system development causes neural impairment in infants (Yardi et al., 2012). Lead occurs in mines and smelters, is used in paints as well as battery plants (Zeitoun and Mehana, 2014). Lead poisoning causes disturbance in haemoglobin synthesis, decreases intelligence capacity in children and damages most organs of human beings (Kinuthia et al., 2020).

SIGNIFICANCE OF THE STUDY

This study helps to understand the presence of toxic substances such as Microplastics and Heavy metals in the tissues of Green chromide. It also helps to understand if there is effect of toxic substances at the genetic level in the fish. It also helps us understand the nutritional constituents of the fish species studied. No such work has been previously carried out in Green chromide found in Sluice gate harvest sites of Goa.

LITERATURE REVIEW

LITERATURE REVIEW

A study was carried out by Saha et al., (2021) in Sal estuary, Goa to determine microplastics in three different matrices i.e water, sediments and biota (shellfishes and finfishes). Microplastics were recorded in all matrices and the predominant ones were polyacetylene, polyvinylchloride, polyacrylamide and polyamide. The microplastics found in biota closely resemble those found in the water and sediments.

The amount of microplastics were evaluated in the water column as well as in some important bottom feeding fishes and shellfishes of Vembanad lake like *Arius maculatus, Etroplus suratensis, E. maculatus* and *Villorita sp.* (Nikki et al., 2021). The microplastics analysis showed a higher value for the water column than the finfishes and shellfishes. The most abundant microplastic found was fibre.

Selvam et al., (2021) examined the microplastics and their trace metal composition in the muscle and intestine of five commercially important fish species like *Sufflamen fraenatus, Heniochus acuminatus, Atropus atropos, Pseudotriacanthus* and *Leiognathus brevirostris* from Thoothukudi, Gulf of Mannar coast in South India. Polyethylene, polypropylene, polyamide and fiber were present in muscles as well as in the intestine. Higher number of microplastics were found in *Pseudotriacanthus.*

In 2019, a study was carried out by Pozo et.al to identify and characterise microplastics in six commercially important fish species from oceanic and

coastal habitats of central Chile. The species were *Trachurus murphyi*, *Strangomera bentincki*, *Merluccius gayi*, *Eleginops maclovinus*, *Aplodactylus punctatus*, *Basilichthys australis*. The results showed that the coastal fish species had higher microplastics as compared to the oceanic species and constituted mainly of red microfibers, polyester, polyethylene terephthalate and polyethylene.

Microplastics were analysed in eight common freshwater fish species from Chi river, Thailand (Kasamesiri and Thaimuangphol, 2020). 72.9% of the fish showed the presence of microplastics. *Puntioplites proctozysron* had ingested the highest percentage of microplastics as compared to others and most of the microplastics were blue coloured and fiber shaped.

Heavy metals such as lead (Pb), cadmium (Cd) and mercury (Hg) were detected in muscle and intestine of seven fish species (*E. suratensis, L. equulus, T. nigroviridis, L. tade, G. giuris, C. macrolepidotus, A. commersoni)* and one prawn species (*M. malcolmsonni*) from Savitri river, Raigad district of Maharashtra (Yardi et al., 2012). The results showed that concentration of these metals were higher in fish organs compared to water.

Another study was carried out by Maurya and Malik (2019) to measure the concentration of heavy metals like Cd, Cr, Pb, Cu and Zn in muscle, gill and liver tissues of 18 fish species from Ganga river. The fish species were *C. gachua, C. marulius, C. punctatus, C. nama, C. ranga, H. fossilis, C. batrachus, P. ticto, P. phutunio, L. rohita, L. calbasu, L. gonius, T. putitora, T. tor, R. rita, G.*

chapra, H. ilisa, and N. botia. Higher concentrations of metals were found in gills and liver tissues of the fish than in their muscle.

Turkmen et al., (2005) detected the concentrations of metals such as cadmium, lead, copper, chromium, cobalt, aluminium, iron, zinc, manganese, nickel in the edible parts of three commercially valuable fish species like *Saurida undosquamis*, *Sparus aurata*, *Mullus barbatus*, from Iskenderun Bay, Turkey in August 2003. The concentration of metals in the parts of investigated fish species were in the permissible safety levels for human consumption.

A study was carried out by Dalman et al., (2006) in sediments as well as in a frequently consumed fish species i.e *Dicentrarchus labrax* from Bay of Gulluk in the Southeastern Aegean Sea, Turkey. The results showed the presence of Pb, Zn, Cu, Cd in the sediments and in the fish. The concentrations of these metals were lower than their maximum level and so were considered fit for human consumption.

Indrajith et al., (2008) assessed the levels of eight metals namely Pb, Cd, Cu, Ni, Cr, Mn, Zn and Hg in two fish species from Negombo estuary located in Sri Lanka. Metals were found in body tissues of both fish species and positively correlated with their body weight and length. The edible muscle was found to be safe for consumption by human beings.

OBJECTIVES

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The Objectives in this Dissertation titled "Toxicity Assessment in Green chromide (*Etroplus suratensis*) collected from Sluice gate harvest sites" includes:

- Detection of Microplastics and Heavy metals in tissues of Green chromide collected from two sluice gates located in Goa.

- Detection of micronuclei in the peripheral blood of fish collected from these sites.

- The quantify the biomolecules of nutritional relevance in the tissues of Green chromide.

MATERIALS AND METHODS

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SPECIES OF FISH STUDIED

Etroplus suratensis (Marcus Elieser Bloch, 1790)

Classification

Kingdom : Animalia

Phylum : Chordata

Class : Actinopterygii

Order : Cichliformes

Family : Cichliidae

Genus : *Etroplus*

Species : *E. suratensis*

Green chromide

It is also known as Green chromide or Pearl spot. In Goa, it is locally known as Kalundar. It is mostly found in fresh and brackish water bodies. The body of Green chromide is oval in shape and has greyish green colour with dark bands. A peculiar dark spot is present at the base of the pectoral fin. It mainly feeds on aquatic plants such as filamentous algae, diatoms and occasionally on mollusks and other animal matter.

SITE SELECTION

Two sites were selected for the purpose of study.

Site A: Sluice gate located at Nevagi nagar, Panaji - Goa

Site B: Sluice gate located at Gandaulim, Goa

Map of Site A and Site B

Green chromide (dead) was procured from local fishermen from both the sites for a period of five months (October - February). The fishes were brought to the laboratory and dissected inorder to obtain the desired tissue samples for testing various parameters such as heavy metals, microplastics, carbohydrates, cholesterol etc.

CHEMICALS AND LABORATORY WARES

The chemicals used for this Project work were Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium chloride, Potassium hydroxide, Hydrogen peroxide, Perchloric acid, Nitric acid, Chloroform, Methanol, Sodium hydroxide, Anthrone, Concentrated Sulphuric acid, Ferric chloride, Orthophosphoric acid, Acetic acid, Lowry's reagent, Folin Cicalteis reagent and Giemsa stain.

The glassware used were beakers, Conical flask, Test tubes, Funnel, Glass rod, Petri plates, Measuring cylinder, Reagent bottles, Amber coloured bottles, Centrifuge tubes, Micro pipettes, Mortar and pestle.

The instruments used were Weighing machine, Vacuum filtration unit, Hot plate, Vortex, Water bath, pH meter, Visible spectrophotometer, Atomic absorption spectrophotometer, OLYMPUS Fluorescence microscope, LEICA EZ4D Stereomicroscope.

METHODS

A. Estimation of Microplastics (Saha et al., 2021)

Reagents:

a. Saline:

Phosphate buffered saline was prepared using 1.065g of Disodium hydrogen phosphate in 50ml distilled water and 1.71g of Sodium dihydrogen phosphate in 50ml distilled water.

The working buffer solution was prepared using 41ml of Disodium hydrogen phosphate and 9ml of Sodium dihydrogen phosphate.

Normal saline was prepared by dissolving 0.9g of Sodium chloride in 100ml of distilled water.

The saline was prepared using 50ml of Working buffer solution and 50ml of Normal saline.

b. 10% Potassium hydroxide

10g of Potassium hydroxide was dissolved in 100ml of distilled water.

Processing of fish tissue:

1g of gastrointestinal tract of fish was homogenised in 5ml of Saline using mortar and pestle. The homogenate was collected in a beaker and to it, 100ml of 10% Potassium hydroxide and 20ml of Hydrogen peroxide was added. The solution was incubated at 60°C until digestion. The solution was then filtered using vacuum filter on 5µm membrane filter paper. The filter paper was kept in petri plate and dried in the oven at 42°C for 24hrs.

Estimation:

The dried filter paper was observed under LEICA EZ4D Stereomicroscope to observe the presence of microplastics.

B. Estimation of Heavy metals (Ali and Khan, 2019)

Reagents:

a. Perchloric Acid

b. Nitric Acid

Processing of fish tissue:

1g of muscle tissue was taken in a beaker. To it, 7.5ml of Nitric acid and 2.5ml of Perchloric acid was added. The sample was heated on a hot plate at 80 °C until yellow solution was obtained. The solution was cooled at room temperature and then filtered through Whatman filter paper. The filtrate obtained was diluted to 50ml with distilled water.

Estimation:

The sample was then detected for the presence of heavy metals using Atomic Absorption Spectroscopy.

C. Biochemical estimations

Reagents:

a. Phosphate buffer

Phosphate buffer was prepared using 0.077g of Disodium hydrogen phosphate and 0.029g of Sodium dihydrogen phosphate in 50ml distilled water.

b. Perchloric acid

c. Chloroform

d. Methanol

e. Sodium hydroxide

Extraction of sample:

5g of muscle tissue was taken. 10ml of Phosphate buffer was added to it and homogenised using mortar and pestle. The homogenate was transferred in 15ml centrifuge tubes and centrifuged at 4000 rpm for 10 minutes. The supernatant was used for estimation of carbohydrates. To the remaining residue, 4ml of Perchloric acid was added and centrifuged at 4000 rpm for 10 minutes. The supernatant was discarded. To the residue, 4ml of chloroform and 2ml of methanol was added and centrifuged at 4000 rpm for 10 minutes. The supernatant is used for estimation of cholesterol. To the remaining residue, 5ml of Perchloric acid was added and was centrifuged again at 4000 rpm for 10 minutes. The supernatant was discarded. 2ml 1N Sodium hydroxide was added to the centrifuge tube, shaken thoroughly and was transferred in a test tube. The test tube was then kept in a boiling water bath for 30 minutes to get a clear solution. This solution was used for estimation of proteins.

1. Carbohydrate test (Hedge and Hofreiter, 1962)

Reagents:

a. Glucose stock solution

0.005g of Glucose is added in 50ml of distilled water.

b. Anthrone reagent

0.125g of Anthrone is added to 50ml concentrated Sulphuric acid to make Anthrone reagent.

Estimation:

The standard solution (Glucose) was added in increasing concentrations in six test tubes (0 - 1ml) and was diluted with distilled water to make the volume upto 1ml. 5ml of Anthrone reagent was added to it and was incubated at 100 °C for 20 minutes. The absorbance was taken at 620nm using Visible spectrophotometer. To test the unknown sample, 1ml of extracted fish tissue sample was taken and 5ml of Anthrone reagent was added. Distilled water was not added. The concentration of carbohydrates was estimated from the unknown sample with the help of a standard curve.

2. Cholesterol test (Zak and Ressler, 1955)

Reagents:

a. Cholesterol stock solution

0.010g of Cholesterol was added to 20ml Acetic acid to prepare a stock solution.

b. Ferric chloride solution

0.0125g of Ferric chloride was added to 5ml Orthophosphoric acid. This solution was then mixed with 50ml concentrated Sulphuric acid.

Estimation:

The standard solution (Cholesterol) was added in increasing concentrations in seven test tubes (0 - 0.6ml). Acetic acid was added in appropriate amounts to make the volume up to 3ml. 2ml of Ferric chloride solution was added to it and it was kept for 10 minutes at room temperature. The absorbance was taken at 550nm using Visible spectrophotometer. To test the unknown sample, 0.1ml of

extracted fish tissue sample was taken. 2.9ml of Acetic acid was added to it followed by 2ml of Ferric chloride solution. It was kept at room temperature for 10 minutes and absorbance was taken at 550nm. The concentration of cholesterol was estimated from the unknown sample with the help of a standard curve.

3. Protein test (Lowry et al., 1951)

Reagents:

a. BSA stock solution

0.005 g of BSA is added to 20ml 1N Sodium hydroxide. The solution is warmed slightly to dissolve the BSA.

b. Lowry's reagent

Solution i: 4g Sodium carbonate was dissolved in 100ml distilled water.

Solution ii: 0.1g of Copper sulphate was dissolved in 5ml distilled water.

Solution iii: 0.2 g of sodium potassium tartarate was dissolved in 5ml of distilled water.

98ml of Solution i + 1ml of Solution ii + 1ml of Solution iii were mixed to obtain Lowry's reagent.

c. Folin Cicalteis reagent

10ml of Folins was mixed with 10ml distilled water to make 1:1 Folin's reagent.

Estimation:

The standard solution (BSA) was added in increasing concentrations in six test tubes (0 - 1ml) and was diluted with distilled water to make the volume up to 1ml. 5ml of Lowry's reagent was added to it. It was kept for 10 minutes at room temperature. Then, 0.5ml of Folin Cicalteis reagent was added and it was kept again at room temperature for 10 minutes. The absorbance was taken at 660nm

using Visible spectrophotometer. To test the unknown sample, 1ml of extracted fish tissue sample was taken. All the reagents were added similarly except for distilled water which was not added at all. The concentration of total proteins was estimated from the unknown sample with the help of a standard curve.

D. Micronucleus test (Andreikenaite et al., 2007)

Reagents:

a. Giemsa stain

b. Methanol

Estimation:

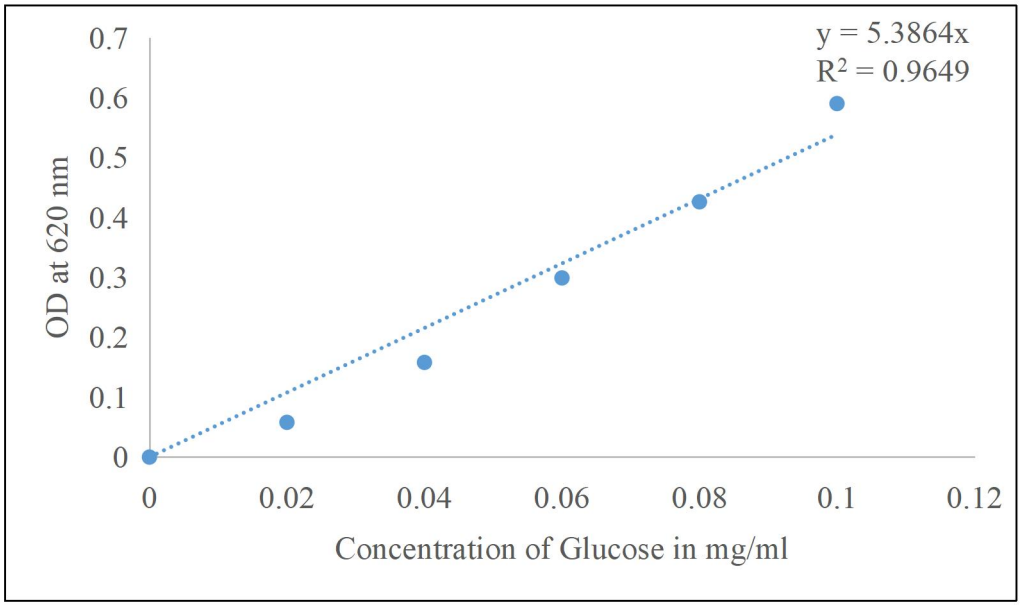
Blood was extracted from the fish's caudal region using a syringe. A smear was made on the slide. It was air dried and then kept in methanol for 2 minutes. It was air dried again and kept in Giemsa stain for 20 minutes. The slide was air dried again and observed under OLYMPUS Fluorescence microscope to detect the presence of micronucleus if any.

Statistical analysis

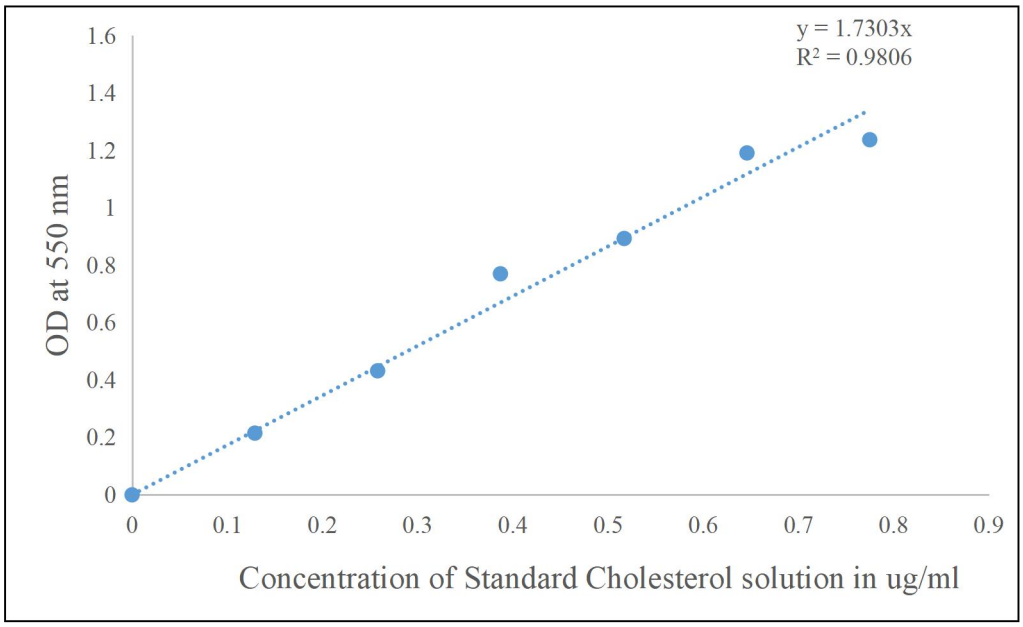
The data was analyzed using Graph Pad Prism App. Student's t-test was used to compare the differences between the two sites. Correlation test was used to analyse the variance in different parameters.

RESULTS

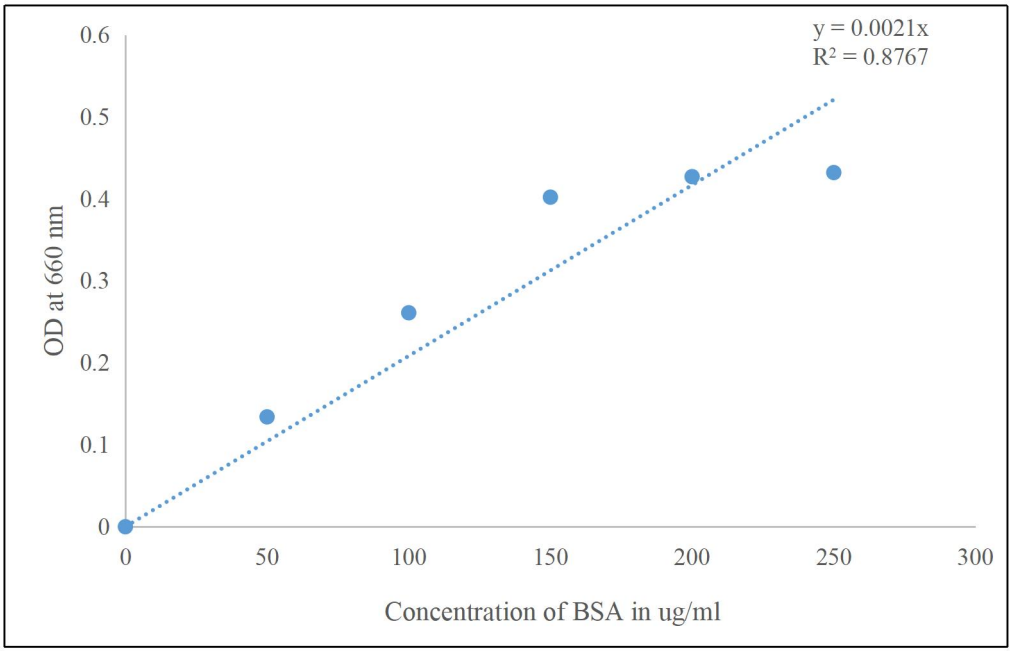
STANDARD CURVES OF BIOCHEMICAL ESTIMATIONS



Standard curve for Carbohydrates



Standard curve for Cholesterol



Standard curve for Proteins

PLATES

PLATES

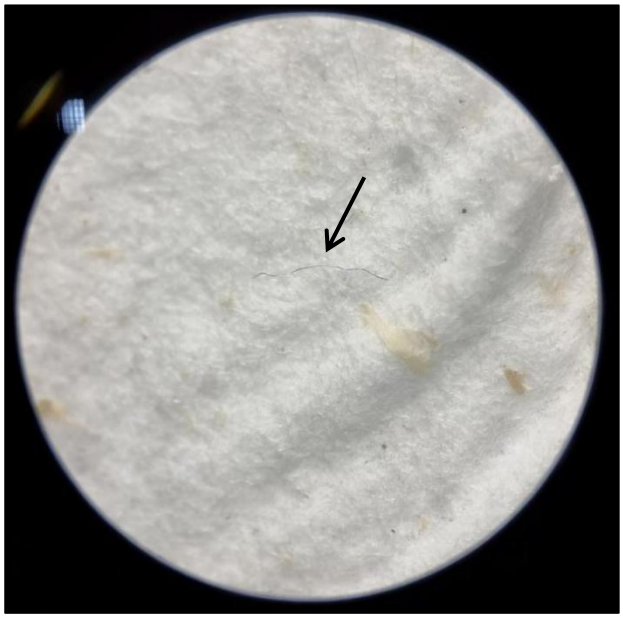


Plate 1: Microplastic - Blue fiber observed

at 25x magnification

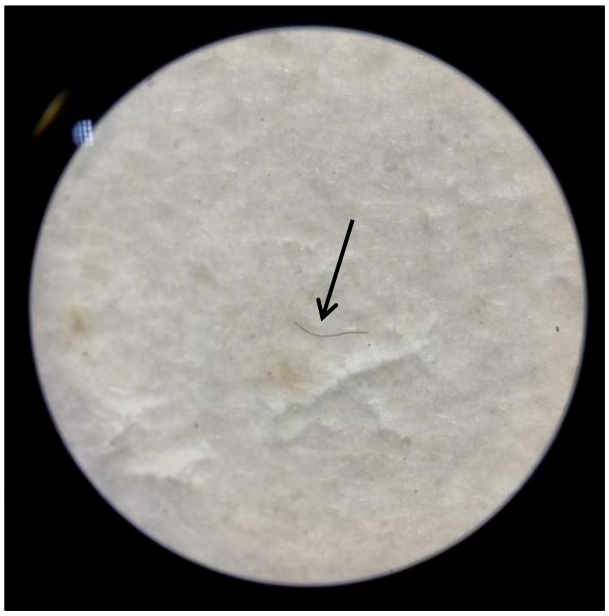


Plate 2 : Microplastic - Black fiber observed

at 25x magnification

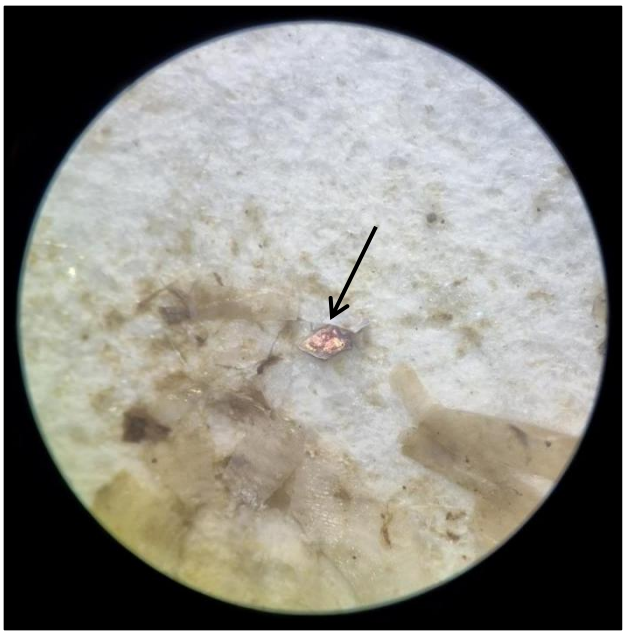


Plate 3 : Microplastic - Multi coloured fragment observed at 25x magnification

Plate 4 : Standard test for Carbohydrates

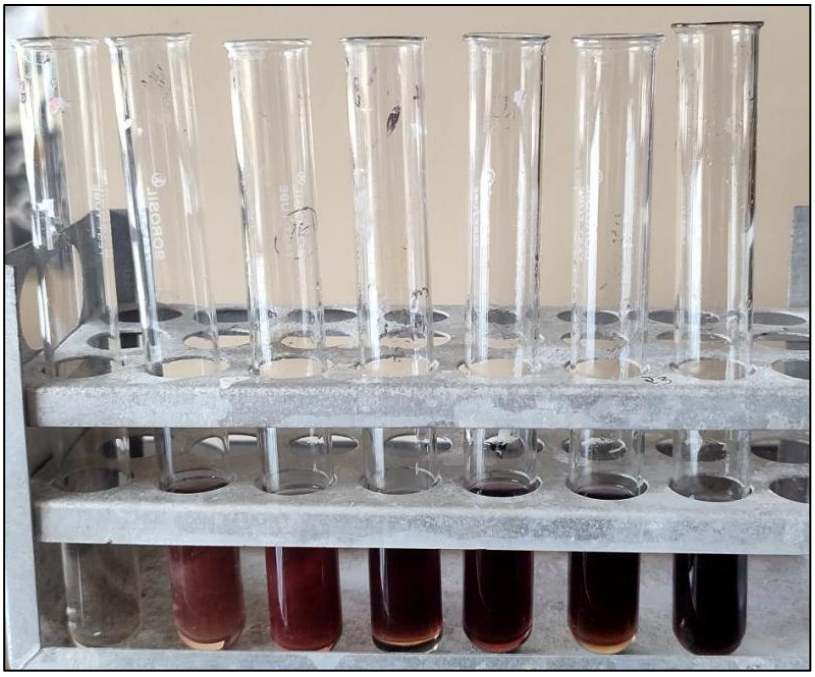


Plate 5 : Standard test for Cholesterol

Plate 6 : Standard test for Proteins

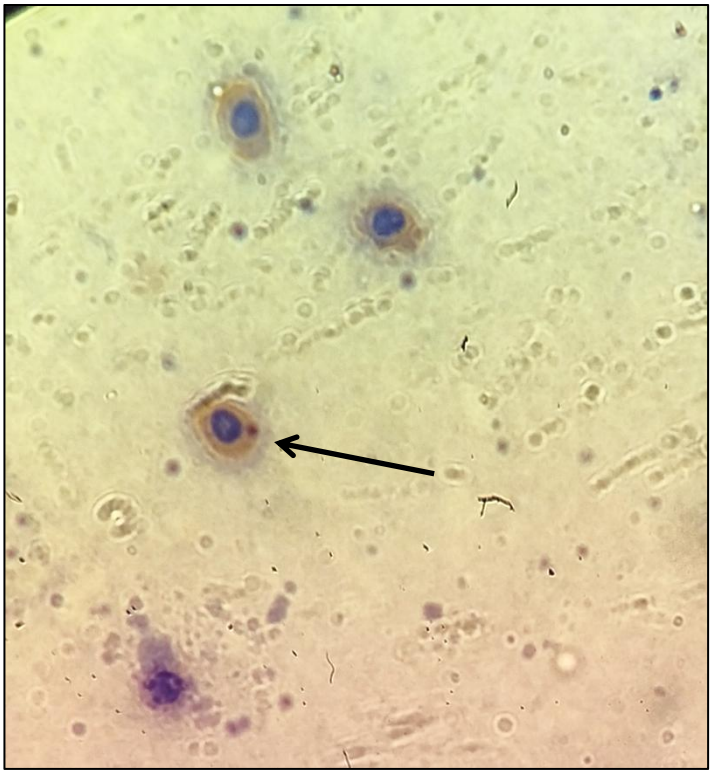


Plate 7 : Presence of Micronucleus in blood smear

TABLES

TABLES

Test for Microplastics		
Months	Site A	Site B
October	1	1
November	1	2
December	1	2
January	5	1
February	2	3

Table 1 : Microplastics from gut of *E. suratensis*

collected from Site A and Site B

Heavy metal analysis in ppm				
	Cadmium		Lead	
Months	Site A	Site B	Site A	Site B
October	0.02	0.02	0.11	0.05
November	0.02	0.02	0.06	0.05

Table 2 : Concentration of Heavy metals such as Cadmium and Lead

in muscle tissue of *E. suratensis* collected from Site A and Site B

Carbohydrate estimation in µg/ml		
Months	Site A	Site B
October	457.2 ± 65.99	326.6 ± 49.21
November	322.2 ± 58.39	353.3 ± 75.03
December	315.5 ± 61.15	512.6 ± 9.29
January	495.7 ± 19.58	314.6 ± 5.68
February	500.5 ± 14.27	299.3 ± 15.82

Table 3 : Concentration of Carbohydrate in muscle tissue of *E. suratensis* collected from Site A and Site B

Cholesterol estimation in µg/ml		
Months	Site A	Site B
October	0.014 ± 0.012	0.008 ± 0.004
November	0.008 ± 0.005	0.034 ± 0.003
December	0.017 ± 0.006	0.010 ± 0.001
January	0.009 ± 0.003	0.033 ± 0.003
February	0.026 ± 0.006	0.011 ± 0.004

Table 4 : Concentration of Cholesterol in muscle tissue of *E. suratensis* collected from Site A and Site B

Protein estimation in µg/ml		
Months	Site A	Site B
October	461.54 ± 130.69	814.44 ± 382.59
November	505.13 ± 216.81	773.96 ± 95.90
December	372.13 ± 82.18	576.98 ± 166.27
January	582.01 ± 69.43	231.42 ± 32.89
February	570.59 ± 96.01	300.15 ± 13.31

Table 5 : Concentration of Protein in muscle tissue of *E. suratensis* collected from Site A and Site B

Micronucleus Test in Percent		
Months	Site A	Site B
October	1.6	1.9
November	3.0	3.6
December	2.5	2.1
January	1.9	3.2
February	2.1	1.7

Table 6 : Micronucleus test per 1000 cells in caudal blood smear of *E. suratensis* collected from Site A and Site B

Pearson's correlation test for Site A				
	Carbohydrates	Cholesterol	Protein	Micronucleus test
Carbohydrates	-	0.1293	0.4297	-0.9001 *
Cholesterol	0.1293	-	0.04199	-0.07033
Protein	0.4297	0.04199	-	-0.8783
Micronucleus test	-0.9001 *	-0.07033	-0.8783	-

Table 7 : Pearson's correlation test for *E. suratensis* collected from Site A

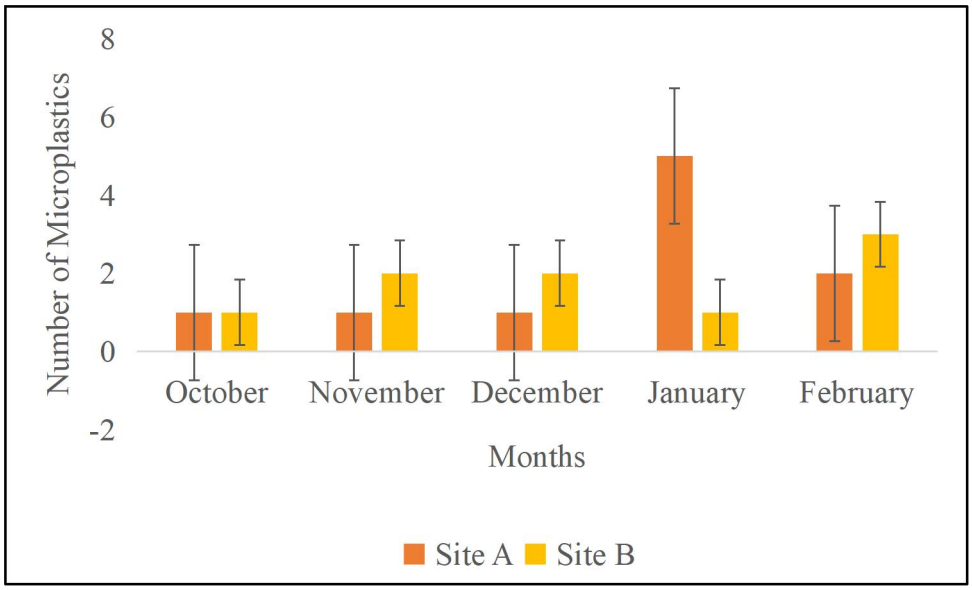
p <0.05, p <0.01**, p <0.001****

Pearson's correlation test for Site B				
	Carbohydrates	Cholesterol	Protein	Micronucleus test
Carbohydrates	-	-0.2462	0.1826	-0.1573
Cholesterol	-0.2462	-	-0.09858	0.9175 *
Protein	0.1826	-0.09858	-	-0.1798
Micronucleus test	-0.1573	0.9175 *	-0.1798	-

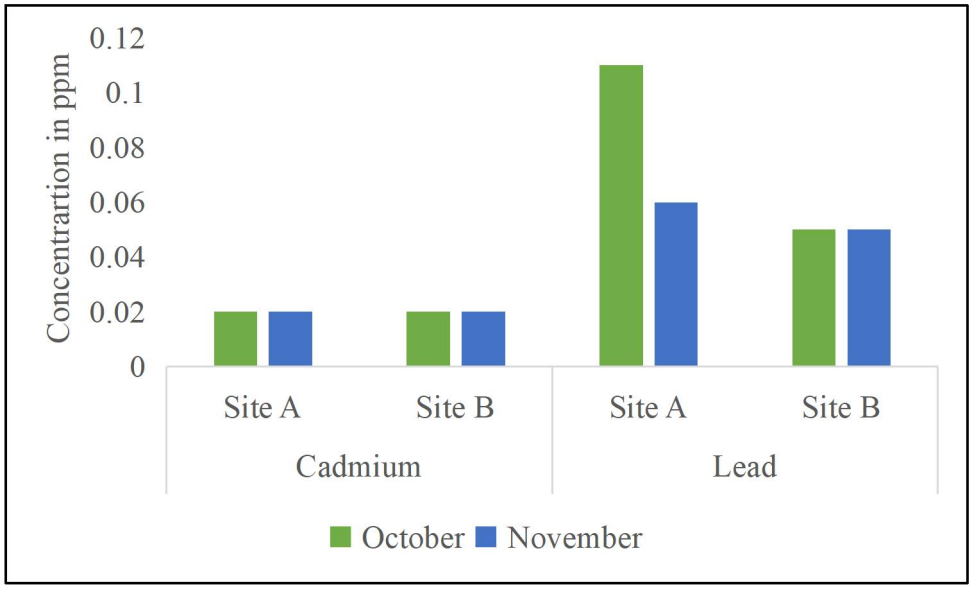
Table 8 : Pearson's correlation test for *E. suratensis* collected from Site B

p <0.05, p <0.01**, p <0.001****

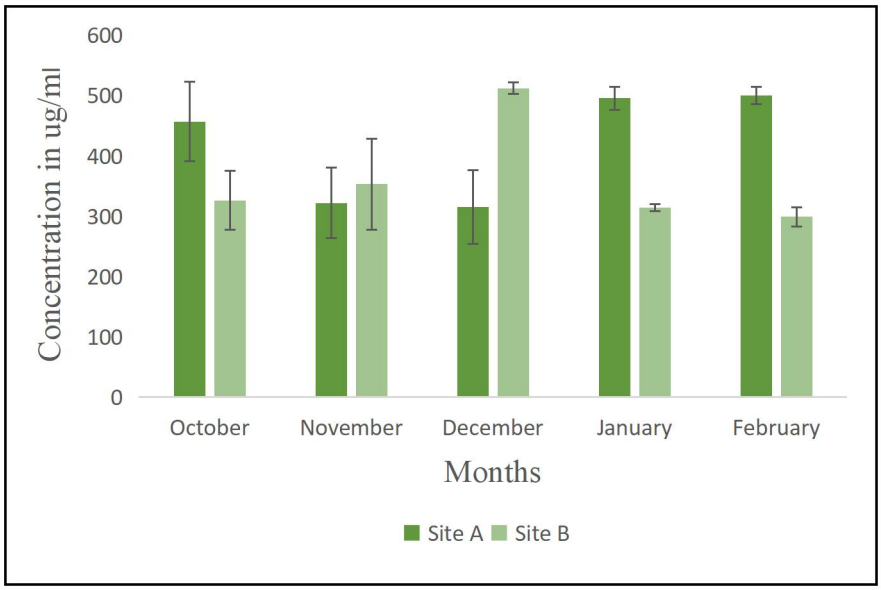
GRAPHS



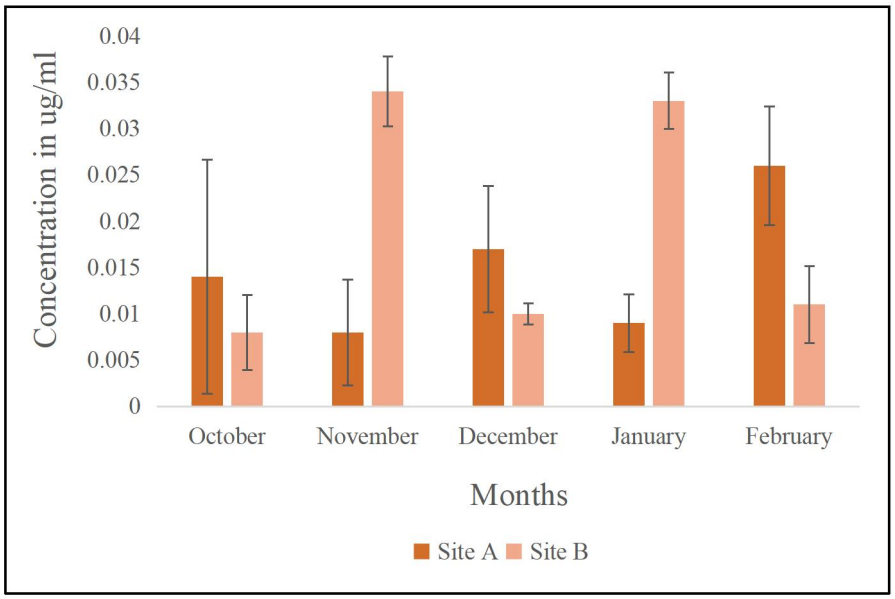
Graph 1: Number of microplastics in the gut of *E. suratensis* collected from Site A and Site B during October 2022 to February 2023



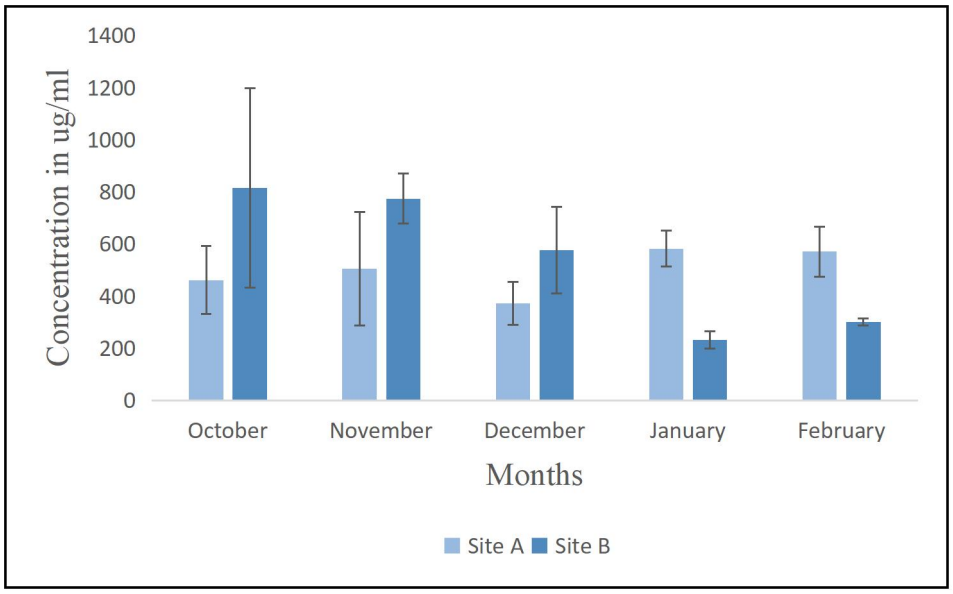
Graph 2 : Concentration of Cadmium and Lead in the muscle tissue of *E. suratensis* collected from Site A and Site B during October 2022 and November 2022



Graph 3 : Variation in Carbohydrate content in muscle tissue of *E. suratensis* from Site A and Site B

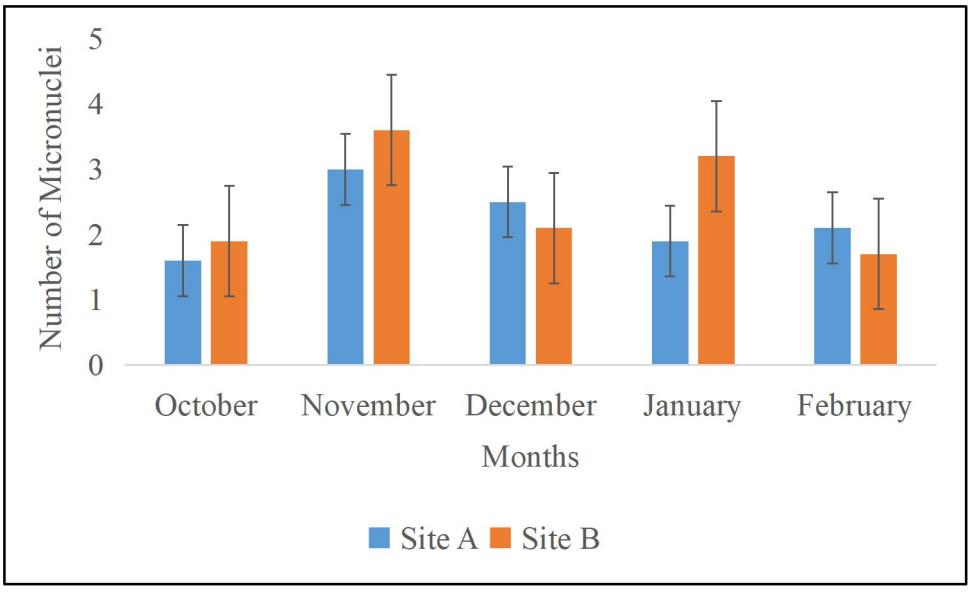


Graph 4 : Variation in Cholesterol content in muscle tissue of *E. suratensis* from Site A and Site B



Graph 5 : Variation in Protein content in muscle tissue

of *E. suratensis* from Site A and Site B



Graph 6 : Micronuclei found in caudal blood smear

of *E. suratensis* from Site A and Site B

RESULTS

Microplastics

Microplastics were found in all the fish samples analyzed from both the sites using LEICA EZ4D Stereomicroscope (Table 1). Site A had higher number of microplastics as compared to Site B. The microplastics found at both the sites consisted of fragments and fibers. Microplastics were also classified according to their colour and it was found that black colour was dominant among them. The difference between the two sites was found to be insignificant as per Student's t-test ($p = 0.8220$).

Heavy metals

The fish muscle sample from both the sites were analyzed using Atomic Absorption Spectrophotometer for the presence of heavy metals such as Cadmium and Lead during the months of October and November (Table 2). The concentration of Cadmium was found to be same for both the months at both the sites while the concentration of Lead at Site A was highest during the month of October and its concentration was the same for Site B during both the months.

Carbohydrates

The Carbohydrate content of fish muscle sample was analyzed (Table 3). For Site A, the Carbohydrate content was found to be lowest during the month of December while it was highest during the month of February. For Site B, the Carbohydrate content was found to be lowest during the month of February and highest during the month of December. Student's t-test was used for statistical

analysis and p value was found to be insignificant between Site A and Site B ($p = 0.3447$).

Cholesterol

The Cholesterol content of fish muscle sample from both the sites was analyzed (Table 4). For Site A, the Cholesterol content was found to be lowest during the month of November while it was highest during the month of February. For Site B, the Cholesterol content was found to be lowest during the month of October and highest during the month of November. Student's t-test was used for statistical analysis and p value was found to be insignificant between Site A and Site B ($p = 0.7932$).

Proteins

The Protein content of fish muscle was analyzed (Table 5). For Site A, the Protein content was found to be lowest during the month of December while it was highest during the month of January. For Site B, the Protein content was found to be lowest during the month of January and highest during the month of October. Student's t-test was used for statistical analysis and p value was found to be insignificant between Site A and Site B ($p = 0.7512$).

Micronucleus

Micronucleui were found in caudal blood smear of fish from both the sites (Table 6). Higher number of micronuclei were found in fish collected from Site B. The difference between the two sites was found to be insignificant as per Student's t-test ($p = 0.5510$).

Pearson’s correlation test

Pearson’s correlation test was used for data analysis.

For Site A, concentration of Carbohydrates was negatively correlated with Micronucleus test ($r = -0.9001$, $p = 0.0374$) (Table 7) while for Site B, Cholesterol concentrations were positively correlated with Micronucleus test ($r = 0.9175$, $p = 0.0281$) (Table 8).

DISCUSSION

DISCUSSION

Pollution due to microplastics is a global problem (Atamanalp et al., 2021). These microplastics are generated through fragmentation and are usually consumed by fish mistaking them for natural prey (Selvam et al., 2021). In the present study, the number of microplastics in the gut of Green chromide was found to be high at Site A which is a urbanized area. Fiber was the most dominant type of microplastic found in both the Sites. Based on the colour, black microplastics were found to be abundant. This result finds similarity with the studies of Nikki et al., (2021) and Selvam et al., (2021) who reported microplastics in fishes and shellfishes of the heavily urbanized Vembanad lake and Gulf of Mannar coast, India respectively.

Toxic pollutants such as heavy metals have severe impact on health of aquatic ecosystem (Maurya and Malik, 2019). Heavy metals can become toxic when the required limit is exceeded (Indrajith et al., 2008). In this study, concentration of Cadmium and Lead were tested in muscle tissue of Green chromide for the months of October and November. The results showed that the muscle tissue of Green chromide collected from both the sites during the months of October and November was contaminated with Cadmium and Lead but were overall fit for consumption as the levels of these metals were low. This results can be correlated with the studies of Turkmen et al., (2005) and Indrajith et al., (2008) who studied concentration of heavy metals in frequently consumed fish species from Iskenderun Bay, Turkey and Negombo estuary, Sri Lanka respectively. Their results showed that concentrations of these metals were lower than their maximum level and were considered fit for human consumption.

A higher number of micronuclei were found in the blood smear of Green chromide procured from Site B as compared to Site A. This was correlated with the study of Ali et al., (2008) who collected fish from different locations to display environmental stresses caused to them. This damage can be due to the contaminants present in the aquatic ecosystem. Similarly, in this study, since the fish collected from Site B had high micronuclei in them, it indicates that the water body is polluted with genotoxic compounds.

The Carbohydrate, Cholesterol and Protein content of muscle tissue of Green chromide was found to vary at both the sites during different months. It was found that Carbohydrate concentration increased during the months of December and February, Cholesterol concentration increased during the months of November and February while the Protein concentration increased during the months of October and January. This can be correlated with the study carried out by Shamsan and Ansari, (2010) in an economically important fish *Sillago sihama* from Zuari Estuary, Goa which indicates that Carbohydrates do not contribute notably to the total reserves in the body. It also states that during the maturity and spawning phase of the fish, there is rise in Protein content. Another study carried out by Mathew et al., (1999) found differences in cholesterol content of different fish analyzed and reported that this variation suggested a relationship between metabolism and biosynthesis of sterols. Thus, as the spawning phase of *E. suratensis* is from November to February, there is rise in Protein content a month prior for maturation of gonads to occur while the Carbohydrate levels keep on altering as it is utilized for muscle movement and other metabolic activities. The rise in Cholesterol levels is due to the production of hormones. But the

concentration of these biomolecules is said to decrease due to rise in concentration of Cadmium as investigated by Rani et al., (2015) in some freshwater fish species. A study carried out by Habib and Samah., (2013) in Catfish states that heavy metals can disturb the biological activity of the fish and in turn cause decrease in protein biosynthesis of muscle tissue. The study carried out by Banaee et al., (2019) states that the presence of microplastics in fish alter cellular homeostasis as well as change the biochemical parameters of blood which results in health hazards of fish. These results can be correlated with this study as, changes in biomolecules were observed in the muscle tissue of Green chromide which may be caused by toxic pollutants present in water.

CONCLUSION

CONCLUSION

In this study of "Toxicity Assessment in Green chromide (*Etroplus suratensis*) collected from Sluice gate harvest sites**",** the fish was collected from two different Sluice gates located in Goa : Site A - Panaji and Site B - Gandaulim. The fish procured was analyzed for the presence of microplastics, heavy metals and micronuclei. Biomolecules such as carbohydrate, cholesterol and proteins were also estimated in the muscle tissue of fish. The tests were performed from October 2022 to February 2023. Higher number of microplastics were found in gut of Green chromide procured from Site A while higher number of micronuclei were found in blood smear of Green chromide procured from Site B. The analysis of heavy metals such as Cadmium and Lead was carried out for both the sites during the month of October and November. The results showed low concentrations of these metals in muscle tissue of fish and were found to be fit for consumption. The amount of biomolecules kept on varying between the months which might be due to the presence of various toxic pollutants in the water body.

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