Effect Of Coal Tar on mud clam *Polymesoda erosa*

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In partial fulfillment of the degree of

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By

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Examined by: Seal of the School

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation entitled, **"Effect of Coal Tar on mud clam *Polymesoda erosa*"** 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 **Dr. Shamshad Bi M. Shaikh** 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 not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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CERTIFICATE

This is to certify that the dissertation report titled "**Effect of Coal Tar on mud clam *Polymesoda erosa***" is a bonafide work carried out by Miss Feazel Richea Dias under the supervision in partial fulfilment of the requirements for the award of the degree of Masters of Science in the discipline Zoology at the School of Biological Sciences and Biotechnology, Goa University, 2022 -2023

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Feazel Richea Dias

PREFACE

This thesis is submitted in fulfilment of the requirement for the degree of Masters in Zoology and comprises research work carried out by the author under the guidance of Dr. Shamshad Bi M. Shaikh Assistance Professor of Zoology, Goa University from 2022to 2023. Coal tars contain a wide range of both organic and inorganic elements. The majority of the detected organics are polynuclear aromatic hydrocarbons (PAHs), heterocyclic PAHs, phenolic compounds (cresols, xylenols, etc.), and monocyclic aromatic hydrocarbons like benzene, toluene, and xylene (EPRI, 1993). Most of these chemical compounds are toxic and may be cancer-causing. Depending on the kind and source of the tar, they account for around 40% of its total mass. The remainder is made up of other heavy residual materials as well as pitch, free carbon, metals, and other minerals (EEI, 1984; Beck, 1950; EPRI, 1993). NAPL is frequently accumulated underground as a result of unintentional spills and ongoing leaks of fuel oils or other hydrocarbon-rich materials (such as coal tar) properties. Groundwater contamination may result from NAPL when it is hydrologically related to an aquifer (Sauer et al., 2003). There are very few studies, undertaken to analyze the toxicity of Coal Tar. There are almost no reports on their toxicity in animals as it is a newly rising issue. This thesis is contributing to the knowledge investigating their toxicity in anatomy, gills, and mantle functioning of *Polymesoda erosa.* The thesis is divided into four main chapters. The first

chapter's introduction gives the current status of Coal Tar, their applications, and their exposure to humans. The 2nd chapter includes a survey of literature and the aims and objectives of the work. Chapter 3 gives the material and methods used for the study. The exposure techniques along with different biochemical estimations.

Chapter 4 represents the results embodying observations of anatomy, gills, and mantle functioning of *Polymesoda erosa*. Chapter 5 gives elaborate discussions. The reasons and the effects of changes occurring in the bivalve as a result of Coal Tar toxicity are discussed. Conclusion with a summary, future work references, and contributions from the thesis follows chapter 5.

LIST OF FIGURES

ABBREVITIONS

μm – micrometer

μmole- micromole

ANOVA- Analysis of variance

CAT- Catalase activity

Fig- Figure

GSH- Reduced Glutathione

H2O- Water

H2SO4- Sulphuric Acid

HCL- Hydrochloric Acid

IU- unit

Kg- Kilogram

M- Molarity

Mg- milligram

Min- minutes

NaoH- Sodium Hydroxide

NS- Non significant

INTRODUCTION

1. INTRODUCTION

PAHs are chemical compounds with at least two fused aromatic rings that are lipophilic in nature. They are ubiquitous and can be created by natural processes like organic matter diagenesis and forest fires (Wolska et al., 2012). Pyrogenic and petrogenic PAHs are two common forms of PAHs, and human activities are a significant source that can increase their concentrations. Incomplete combustion of fossil fuels (coal, diesel, and petroleum) and other organic materials, such as wood, produces pyrogenic PAHs, which can reach marine waters via indirect pathways by sorbing to air particles that land on the water. Petrogenic PAHs are released directly into the water through fuel discharge and oil spills. A high relative abundance of parent chemicals and a low relative abundance of alkylated compounds are characteristics of PAH assemblies from pyrogenic sources. Contrarily, PAHs with petrogenic ancestry typically contain larger levels of alkylated molecules. (Lima et al., 2005). Particularly concerning is the rise in the possibility of oil and other hydrocarbon spills. The contaminants that are most concerning after a spill are polycyclic aromatic hydrocarbons (PAHs), which have the potential to have both carcinogenic and non-carcinogenic impacts on the general populace and especially on vulnerable groups including children, pregnant women, and subsistence farmer seafood eaters (Yender et al., 2002). Concerns about harmful health effects linked to seafood pollution is brought on by rising industrial development and marine shipping activity near coastal communities (Chan et al.,2011). Individual PAHs; physiochemical characteristics influence their fate, behaviour, and mobility in the environment. Based on their chemical

characteristics and environmental factors (Such as temperature), PAHs will divide between environmental matrices in very different ways. As a result, when PAHs travel to the environment, their concentration varies from one location to the next (Chen et al., 2020). The PAHs bioavailability in the soil will be impacted by other environmental factors and processes, including soil aggregation, wetness, aeration, dryness, volatilization, biodegradation, transformation, leaching, and uptake by biota. As a result, PAHs with higher bioavailability and mobility have more harmful effects all around. Due to their structure, non-polar PAHs are more likely than polar PAHs to have long-term negative impacts on health (Mackay and Callcott, 1998). Polycyclic aromatic hydrocarbons (PAHs) are the primary contaminants in coal tar (lee et al., 2001). Human exposure to polycyclic aromatic hydrocarbons (PAH) occurs through complex mixtures such as coal tar (Marston et al., 2001).

1.1. COAL TAR

A Non-aqueous phase liquid (NAPL) that is denser than water and frequently quite viscous is coal tar. As a result of the careless disposal of process residuals at previously manufactured gas plant (MGP) sites, subsurface contamination with coal tar occurs today. Due to the extensive usage of natural gas and the exploitation of petroleum, the manufactured gas industry came to an end in the 1950s. Because the constituent chemicals from subterranean coal tar slowly and continuously dissolve, groundwater pollution at MGP sites continues decades later. The fact that coal tar NAPLs are combinations of hundreds of molecules, predominantly polycyclic aromatic hydrocarbons (PAHs), is the main research

obstacle. By measuring and documenting the partitioning of each component, it would be impossible to fully characterize the equilibrium-phase compositions of coal tar/solvent/water mixtures (Peters et al., 1993). Depending on where they were manufactured, different types of coal tar have different chemical compositions. Coal tars contain a wide range of both organic and inorganic elements. The majority of the detected organics are polynuclear aromatic hydrocarbons (PAHs), heterocyclic PAHs, phenolic compounds (cresols, xylenols, etc.), and monocyclic aromatic hydrocarbons like benzene, toluene, and xylene (EPRI,1993). Most of these chemical compounds are toxic and may be cancer-causing. Depending on the kind and source of the tar, they account for around 40% of its total mass. The remainder is made up of other heavy residual materials as well as pitch, free carbon, metals, and other minerals (EEI, 1984; Beck, 1950; EPRI, 1993). NAPL is frequently accumulated underground as a result of unintentional spills and ongoing leaks of fuel oils or other hydrocarbon-rich materials (such as coal tar) properties. Groundwater contamination may result from NAPL when it is hydrologically related to an aquifer (Sauer et al., 2003). For instance, when spilled on the ground, coal tar gradually migrates deeper since it is a dense non-aqueous phase liquid. However, due to factors like its viscosity and capillary effects, some of the phases are still trapped in the porous medium, leaving the medium with a residual saturation. This section might not even budge even when subjected to a water low (Illangasekare et al., 1995). As trapped pools, residual saturation in soil macropores or soil micropores, coal tar can get immobilized in the subsoil. Immobilized coal tar may eventually pollute soil and groundwater because of how slowly PAH compounds and other solutes dissolve in this material (Ghoshal et al., 1996).

Numerous oil spill accidents have occurred globally during the past few decades, releasing significant amounts of crude oil into the aquatic environment (Honda et al.,2020). Depending on the location and amount, oil spills over the surface caused by oil-tanker accidents or illegal bilge disposal of tankers can seriously harm the environment. Deliberate oil spills are frequently brought on by ships improperly disposing of oily debris while cleaning. The most frequent oil leak, though, is the spread of car oil on our highways. Everyday vehicles like automobiles and trucks often drip or leak oil, which when it rains flushes into storm drains and eventually contaminates the water (Mdakane et al., 2020). The composition and chemical characteristics of the crude oil are profoundly altered by the weathering processes of the crude emulsions. A crucial step in the breakdown of oil residue is the emulsification and dispersion of thespilled petroleum. Tar balls and oil-mineral aggregates are also created as a result of these processes. (OMA) (Kiruri et al., 2023).

1.2. TOXICITY OF COAL TAR

Chronic skin conditions including psoriasis and eczema can be effectively treated with coal tar (JHJ et al., 2005). It has significant quantities of more than 10,000 substances, including polycyclic aromatic hydrocarbons (PAHs). Benzo(a)pyrene is one PAH that has been identified as a human carcinogen. Concerns have been raised pertaining to the possibility of cancer following coal tar therapy in patients with skin problems due to the carcinogenic potential of PAHs. Following dermatological exposure to coal tar, the skin represents a significant route of absorption. Therefore, a number of research have looked into the possibility of developing skin cancer following exposure to coal tar, but except for one by Stern et al. [2003], the majority of

studies did not find an elevated risk. Coal tar is absorbed and metabolized by the skin and body after application. Several PAH metabolites are eliminated in the urine after metabolism. Therefore, using coal tar topically may raise your risk of developing cancers other than skin cancer, including bladder cancer. Only a small number of researchers examined the incidence of internal malignancies in patients receiving coal tar treatment, and the majority of these studies found no elevated risk. According to a study, coal tar stimulates the AHR signalling pathway, which in turn causes filaggrin levels to rise and the IL-4/STAT6 signalling pathway to be inhibited. These effects improve epidermal differentiation. These findings point to a critical role for the AHR signalling pathway in the molecular mechanism by which coal tar, the oldest known drug in dermatology corrects epidermal abnormalities in a common skin disease called atopic dermatitis, and shows that coal tar enhances skin barrier function and dampens keratinocyte response to the major cytokines involved in AD (Bogaard et al., 2013).Additionally, a study found that workers had a higher incidence of skin cancer following long-term occupational exposure to coal tar products (typically 20–30 years). However, several case reports show that skin tumours have appeared in patients who have used substantial doses of coal tar repeatedly over long periods of time (often years) (Bickers et al., 1978). Hence its clearly evident that many PAHs are mutagenic, carcinogenic, teratogenic, and immunotoxic to living organisms, including microorganisms, animals, and humans (Burchiel and Gao,2014; Rangarajan et al., 2015). PAHs bioaccumulate in the liver of aquatic animals, especially fishes. The reports suggested that fishes contaminated by PAHs have involved benthic or

bottom-feeding habitats (Honda et al., 2020). Specifically, the PAHs have ecotoxic effects on aquatic life and birds (Abdel-Shafy and Mansour, 2016). The majority of the contaminated material entering the marine environment via rivers and direct discharges settles out on the bottom of estuaries and coastal seas (MclNTYRE et al., 1992). Therefore, Polymesoda erosa found in brackish waters also known as mud clams as they are found buried inside the mud and also show tolerance to brackish and freshwater and shows the positive correlation between pollutant concentration (Azis et al., 2021)

1.3. Polymesoda erosa as MODEL ORGANISM

Classification

Kingdom: Animalia

Phylum: Mollusca

Class: Bivalvia

Order: Venerida

Family: Cyrenidae

Genus: Polymesoda

Species: erosa

The Bottom-feeding bivalves commonly come into contact with soil and water that contain hydrophobic contaminants (Wang et al., 2020). Filter feeders are capable of capturing significant amounts of suspended particles (Nakamura et al., 1988). Bivalves are particularly vulnerable to exposure and the negative impacts of aquatic contaminants due to their unique feeding and breathing habits, as well as their close association with sediments (Goldberg et al., 1980). When present in concentrations that are frequently found in water bodies, many trace metals are dangerous to some ecological receptors (W. Boening et al., 1997).

1.3.1. MORPHOLOGY;

a. THE SHELL

Polymesoda erosa, a mangrove clam with a large shell and no predators, lives on the landward edge of the dense mangrove forest. Polymesoda erosa has a huge, thick, outwardly dark shell that is white on the inside. The clamshell is globular and fat, with large, hefty valves. The majority of clams live with severely eroded umbonal beaks. It has an aragonitic shell. All corbiculids have a similar shell, according to Taylor and Brand (1975). Younger, smaller clams have more rounded shells. The left and right valves on the shell are symmetrical, and their weights are comparable. There are three cardinals, two anterior teeth, and one posterior tooth in the right valve. Strong hinge teeth tightly lock the shell when the valves are joined. There is no pallial sinus, but the shell has a pallial line. Near the anterior end, the umbones are noticeable. The variations in umbones' height appear to account for the majority of shape variety. The inside of the shell is white. Younger shells are dark green, but as they get older, the colour fades and they become stained black.

b. THE SIPHONS

Both the inhalant and exhalant siphons of Polymesoda erosa are relatively brief. 20–30 tentacles make up the crown of the inhalant siphon. At the base of the siphon, there is an outer circlet of tiny papillae that encircles the tentacles. The exhalant siphon is considerably smaller than the inhalant siphon and has a conical shape. The papillae that encircle this siphon instead of the tentacular crown

run in two parallel rows, one on each mantle lobe. The papillae gradually get shorter and eventually stop. Since Polymesoda erosa siphons and mantle margins are heavily coloured, it is challenging to see the siphons in their full open form. With the exception of when they are submerged in water and held between the borders of the valve.

c. THE PEDAL GAPE

From the inhalant siphon to the anterior adductor muscle, the pedal gape extends ventrally. The huge, muscular foot in cream-white colour might stick out through the gape. The shell valves gape a little when they are not submerged in water, and they close tightly when the animal is taken out of its burrow. A jet of water is frequently fired from the pedal gape when they close, which typically occurs if disturbed.

1.3.2. MORPHOMETRIC RELATIONSHIPS

It has been observed that Polymesoda erosa clams with fully grown shells are heavier, either as a result of increased shell mass or as a result of more water or mantle fluid being present inside the shell. The volume of mantle fluid may change seasonally based on Polymesoda erosa's reproductive status and may be related to the species; survival strategy. The mangroves; exceptionally harsh environments are ideal for habitation thanks to their enormous, thick shells.The high zones of the mangrove forest, which is submerged for extended periods of time, are where Polymesoda erosa can be found. Since these clams are filter feeders, they could be at risk for malnutrition, desiccation, and a variety of salinities. They require large, securely closing valves to protect the body from predators

and unfavourable climatic conditions. Bivalves living in occasionally dry zones have been reported to require heavier and thicker shells than usual (Seed, 1968).

1.3.3. ECOLOGICAL SIGNIFICANCE OF BIVALVES

The role of bottom-feeding bivalves in estuarine and marine ecosystems has been extensively documented through research in ecology, physiology, biogeochemistry, mariculture, interdisciplinary marine science, and fisheries science. Bottom-feeding bivalve molluscs consume at the lowest trophic level, feeding largely as herbivores (Duarte et al., 2008). On average an oyster filters 15-55 liters of water per day and releases dissolved ammonia and other nutrients either directly or through microbial decomposition of their pseudofaeces. Bivalves sequester nitrogen and produce protein in muscle tissues, and sequester carbon as CaCO3 in their shells. They are considered keystone species controlling phytoplankton density by grazing and also nutrient removal through biodeposits. Considering their significance, bivalve aquaculture for edible species and non-edible species like pearl oysters for bioremediation of polluted sites have been explored (Gallardi, 2014). Hence the introduction of different pollutants in the niche of Polymesoda erosa would ultimately deteriorate their physiological functions, affecting their chemical cycling, and biomonitoring characteristics thus also paving way for the pollutants like coal tar to enter the food chain leading hazards to life.

LITERATURE REVIEW

2. LITERATURE REVIEW

Bivalves are one of the typical nearshore animals having economic importance and ecological relevance among microbenthic animals. They are often used as sentinel species or environmental indicators owing to their ability to accumulate chemical contaminants, lack of mobility, and wide distribution throughout the coastal waters of the world.

2.1 *Toxicity Studies in Bivalves*

Among the multitude of toxicity studies, Waykar et al., (2011) reported that Three types of freshwater bivalves, *Lamellidens marginalis, Lamellidens corrianus* and *Indonaia caeruleus,*were exposed to chronic concentrations of heavy metals whose results showed that *Lamellidens corrianus* had the highest concentrations of lead and arsenic, *Lamellidens marginalis* had the highest concentrations of zinc, and Indonaia caeruleus had the highest concentrations of cadmium, copper and mercury. Subsequently, two freshwater bivalves, *Hyridella depressa* and *Velesunio* ambiguous, from an area with minor pollution had comparable Manganese, Cobalt, Copper, Zinc, Lead, Cadmium, and Uranium whole tissue contents, resulting in equal mean concentrations in both bivalves (Markish et al 2000).Sukumaran et al., (2005) also documented that Cadmium chloride acute toxicity in *Anadara rhombea* exhibited a relatively higher toxic response and was suggested as a candidate species to monitor the toxicity of Cadmium which would be very useful to provide a future understanding of the ecological impact. Additionally, the widely widespread clams *Corbicula fluminea* were exposed to stabilized gold nanoparticles, that were retained through the digestive tract (Hull et al., 2011).

2.2 Toxicity of PAH in Bivalves

The incidence of PAHs that occur naturally in coal, crude oil, and gasoline has boosted in the environment due to anthropogenic activities. The inherent properties of PAHs such as heterocyclic aromatic ring structures, hydrophobicity, and thermostability have made them recalcitrant and highly persistent in the environment. PAH pollutants have been determined to be highly toxic, mutagenic, carcinogenic, teratogenic, and immunotoxicogenic to various life forms (Patel et al., 2020). According to a comparative study by Murphy et al. (2019). different bivalve species; biodeposits have different mineralization rates depending on how bioavailable they are to the microbial community. The mussel *Geukensia demissa*, the oyster Crassostrea Virginian, and the clam *Mercenaria mercenaria* were shown to be the most bioactive. The presence of 16 PAHs was simultaneously checked in the adductor, gills, gonads, hepatopancreas, and mantles of the pearl oyster *Pinctada martensii* and mussel *Perna viridis* collected from the coastal environment wherein the PAH were accumulated in decreasing order of mantles; hepatopancreas; gonads; gills; adductor tissue. Mantle accumulated PAHs in higher concentrations due to their direct contact with the ambient environment (Wang et al., 2020). Al-Hashem et al., (2017) suggested that oysters exposed to PAHs resulted in Histopathological changes with necrosis and edemas of branchial lamellae. Along with that complete degeneration of gill filaments, loss of inter-filament junctions, loss of regular shape, hemolysis, and inflammation was accompanied by degeneration. Subsequently, Salgueiro et al., (2009) tested for PAHs in canned pickles, mussels, and clams wherein the bivalves with the highest and lowest total PAH levels were in direct relationship to their lipid content. Thuy et al., (2018)

represented a comparative study on the PAHs content between mussels and clams. Since mussels like *Mytilus galloprovincialis* usually grew on rocks or breakwaters at the air-water interface, they filter large quantities of water. Therefore, mussels are mainly exposed to LPAHs, which are preferentially soluble in marine water. On the other hand, clams *Donax trunculus* located in shallow waters of sandy beaches in close contact with the sediment, were more exposed to heavier and particle-associated compounds (HPAHs). Similarly, Varanasi et al. (1985) studied the rate of PAHs bioaccumulation by clams from a contaminated estuary in the USA. They found that HPAHs are clearly accumulated by clams and amphipods. Sun et al., (2021) also showed that exposure to realistic-relevant doses of MPs and a mixture of PAHs had significant toxic effects on the haematic parameters of blood clams by disrupting complex physiological and molecular processes. Further, Oros et al., (2005) reported from an estuary segment that minor amounts of the PAH in bivalves are derived from biomass and coal combustion, with even lesser amounts derived from unburned petroleum such as motor lubricating oil and crude oil. Vehicular traffic is likely the major source of PAH from petroleum combustion, which could be transported into the estuary primarily by atmospheric deposition and paved surface runoff. Unburned petroleum was most prominent in clams that altered their physiological functioning.

2.3 Toxicity Studies of Coal Tar

Coal tar, one of the core constituents of PAHs is turning out as a bane to the environment due to its increasing emissions. Chiovatto et al., (2021) reported the use of coal tar-based paints to contaminate water with metal and PAH, which eventually bio-accumulated in oyster tissues showing 9 trace elements (As, Cr,

Cu, Fe, Mg, Mn, Pb, Ni, and Sn) along with 17 PAH that integrated its composition. Organisms exposed to structures covered with coal tar-based paints underwent threats, such as damage to the integrity of the lysosomal membranes of haemocytes, which lead to irreparable physiological damages. In a study by Bryer et al., (2010) at the highest exposure level of coal tar sealant pavement a clear decrease in community health in terms of both abundance and diversity was observed in the macro-invertebrates.

2.4 Toxicity studies in Polymesoda species.

Polymesoda expansa and *Polymesoda erosa* are well-known as the mangrove clams that live in association with the mangrove forests. Studies reported by Yusoff et al., (2021) explain that heavy metals were found to be accumulated in the tissues of marsh clams *Polymesoda expansa* hence suggesting that the metals transferred through aquatic food webs to marsh clams and humans are of environmental and human health concern. In addition, the concentrations of Zn, Pb and Cd in the marsh clams exceeded the maximum permissible levels as recommended by WHO. Similarly, to this another study by Yaakub et al., (2019) mentioned the highest concentration of Cd and Ni in the tissue of *Polymesoda expansa* was from the wet season compared to the dry season, indicating that *Polymesoda expansa* accumulated more metals during monsoon seasons than dry seasons. They were influenced by environmental changes such as temperature, pH, changes in salinity, occurrences of obnoxious blooms of phytoplankton, and rainfall. According to Cruz et al., (2020) the accumulation of metals by edible bivalve P. erosa was reported above the permissible levels prescribed by WHO and other referred standards which was an indication of metal toxicity to the bivalve and human health. It also indicated a higher

accumulation of Zn than other metals and classified edible bivalve P. erosa as a macro-concentrator. A study by Azis et al., (2021) reported that micro pollutants in the sediments and in the clam P. erosa contains hydrocarbon and wax, triacylglycerol, free fatty acids, sterol, polar lipid, and monoalkyl diacylglycerol also 16 PAHs were found thus banning the consumption of mud clams. Gawade et al., (2013) studied the variations in heavy metal concentration in oysters, clams, and fish among which the metals Fe showed the highest percentage followed by Zn and Mn which suggested and indicated that bivalves accumulate higher metal concentration than fishes hence elevating to bioaccumulation of heavy metals that eventually enter the food chain giving rise to detrimental disorders. According to Daud et al., (2021) the microplastics found in clams P. erosa, namely line, fibre, fragments, and pellets can form knots or clots and can be dangerous because the fiber can block the digestive tract and block food entry. Thus, the health impacts that can arise in humans after ingestion of these clams like intestinal, and stomach disorders (irritation), and plastics' chemicals eventually trigger cancerous growth.

2.5 Lacunae

This literature summary evidently states that the majority of the contaminated material entering the marine environment is dispersed in the estuaries and it settles to the bottom and hence bottom feeding bivalves Polymesoda erosa commonly come in contact with the contaminated waters. This study also supports the toxicity of PAHs but completely no studies are been reported with respect to the effect of Coal tar on Polymesoda erosa bivalves which is one of the main sources of economy for the locals. Hence this research study will curtail

this gap by documenting data on the “toxic effects of coal tar on *Polymesoda erosa*”.

2.6 Hypothesis

The study hypotheses that coal tar can cause adverse toxic effects on the anatomy and physiology of gill and mantle tissue of bivalves functioning and it will in turn affect the normal functioning of the body as a whole.

2.7 Objectives of the study

 To analyze the chronic effects of coal tar on the anatomy of *Polymesoda erosa.*

 To evaluate the chronic effects of coal tar on the gill and its functions in *Polymesoda erosa.*

 To evaluate the chronic effects of coal tar on the mantle and its functioning in *Polymesoda erosa.*

 To determine the chronic effects of coal tar on the physiological functioning such as respiratory rate, excretory rate and osmoregulation of *Polymesoda erosa.*

MATERIALS AND METHODS

3.MATERIALS AND METHODS

3.1 Collection of materials

CHEMICALS

The chemicals used were of Analytical Grade from Sigma-Aldrich, Thermo Fisher, Supelco. Etc

GLASSWARES

Glasswares and lab wares including beakers, conical flasks, burette stand, test tubes, test tube stands, pipettes, pipette canisters, measuring cylinders, eppendorf tubes, centrifuge tubes, droppers, coupling jars, Petri plates, motor and pestle and funnels of high quality were used.All glassware was first soaked in 4% chromic acid and kept overnight and cleansed thoroughly with detergent and water. These clean Glasswares were rinsed with distilled water and dried in the oven before use.

INSTRUMENTATION

Spectrophotometer (BI/CI/SP/SDB-S-04), centrifugation machine, water bath, vortex (REMI

CM 101), incinerator, cold centrifuge, hot air oven (MIC 165), fluorescence microscope, and

other instruments were used.

BIOLOGICAL MATERIALS

The animal chosen for the conduct of research work was mud clam Polymesoda erosa. The

uniform body weights of the bivalves i.e., 50-55g were collected from Chorao, Tiswadi Goa.

Necessary approval was taken for carrying out experimentation on animals with prior

clearance from the Animal ethic committee of Goa University

(Ref. No. GU/ZOO/2021/Q/35)

3.2 MAINTAINANCE OF *Polymesoda erosa*

24 bivalves (Polymesoda erosa) weighing approximately 50-55 grams were collected from the authorized supplier and maintained in the animal house of the Zoology Department as per the guidelines provided by CPCSEA (Committee for control and supervision on experiments on animals). They were acclimatized for 2 weeks and exposed to 12 hours day/night cycle before the exposure period. A group of 6 bivalves were maintained in each of aquarium tanks along with the provision of adequate water along with mud, aerators, microalgae, constant temperature, and controlled light. The water was changed after alternate days along with cleaning of the aerators.

3.3 EXPERIMENTAL SETUP

The bivalves were acclimatized for 2 weeks before exposure studies. The bivalves were divided into 4 groups after the period of acclimatization. Each group comprising of 6 individuals. 3 groups were part of the experimental set and one group was a controlled setting. (Chiovatto et al., 2021). The experimental set ups were suspended with three cylindrical pipes, each of different surface area coated with coal tar pain for 28 days exposure period. The

surface area of cylindrical pipes coated with paint used in the experimental groups is mention in the table No 1

Table No 1 The surface area of the cylindrical pipes coated with coal tar paint

	Control	Experimental 1	Experimental 2	Experimental 3
No. of bivalves	6	6	6	6
Surface area of the pipe	-	123.15m²	153.94m²	197.92m²
Paint coated on the pipe	-	3.8	6.2	8.9

3.4 BEHAVIOUR

The Clams were observed routinely every day for any minute changes in their behaviour like burrowing inside the mud, swimming with the help of foot or any other distinct behavioural change during the 28 days exposure period.

3.5 MORPHOLOGY

The clams were also observed routinely for any morphological differences after getting exposed to Coal Tar. The different morphological characteristics of the

animals like the removal of the siphon, foot movements were observed and evaluated during the exposure period.

3.6 EUTHANASIA

After 28 days of exposure, all the clams were placed in ice cold water and further dissected for isolation of tissues.

3.7 HISTOLOGY

After sacrificing the clams, the mantle and gills were isolated, removed aseptically, and washed in physiological saline. They were weighed and immediately stored in 10% formalin. They were transferred to 70% ethyl alcohol and stored until processed. The tissue specimens were processed, embedded in paraffin, sectioned at 0.1 μm, and stained with hematoxylin and eosin by the Ashwini Pathology lab. They were further analyzed under a light microscope at 20x and light micrographs were captured.

3.8 EXTRACTION AND ESTIMATIONS OF BIOMOLECULES

Mantle and gill samples were used for biochemical estimations.

A. Estimation of Metabolites

Extraction

10% tissue homogenate (mantle, gill) was prepared in ice-cold water. By adding equal parts of 0.3 N barium hydroxide and 5% zinc sulphate to the homogenate. The supernatant was stored after centrifugation at 800 x g for 15 minutes to estimate the amount of total carbs and free sugars. (Roy et al., 1991).

1. *Total carbohydrate*

Carbohydrates are dehydrated by conc. H2SO4 to yield furfural, which condenses with anthrone to create a blue-colored complex that can be calorimetrically detected at 620 nm.

• ***Reagents***

Anthrone reagent: 0.2gm of anthrone was dissolved in 100m1 of concentrated sulphuric acid.

Protocol:

0.1m1 of the deproteinized aliquot was diluted with distilled water to make 1.0m1, to which 4m1 of the anthrone reagent was added, and the mixture was then incubated for 10 minutes in a boiling water bath. At 620 nm, the intensity of the color generated was assessed in comparison to a suitable blank (Carroll, 1956). Using a total carbohydrate standard curve, the amount of total carbohydrate was determined. (100ug of glucose/ml).

2. ***Free sugars***

When sugars undergo combustion with alkaline copper reagent, cuprous oxide is formed, which when combined with arsenomolybdate reagent results in a compound with a blue colour whose intensity may be detected at 540 nm.

Reagents:

Alkaline copper reagent-A) In 125 ml of distilled water, 6.0 g of sodium potassium tartrate and 12.0 g of anhydrous sodium carbonate were dissolved. In 25 ml of distilled water, 2.0g of copper sulphate was dissolved (Solution b). To create solution A, 8.0 g of sodium bicarbonate were added while agitating the mixture of solutions a and b.

B) In 250 ml of distilled water, 90.0g of anhydrous sodium sulphate was dissolved. Solution B is prepared by boiling it to remove the air and letting it cool to room temperature. Now, the solutions A and B were combined, and 500 cc of distilled water was added to the mixture. Arsenomolybdate colour reagent: 450 ml of distilled water were used to dissolve 25.0 g of ammonium molybdate, and while the mixture was being stirred, 21 ml of concentrated sulfuric acid was added. 3.0 g of disodium hydrogen arsenate, which had previously been dissolved in 25 mL of water, was then added, stirred, and kept at 37°C for 48 hours in the amber-colored bottle.

Protocol:

Alkaline copper reagent was added to 1.0 ml of the deproteinized sample and the mixture was incubated in a boiling water bath for 20 minutes. Arsenic-molybdate colour reagent was added after the liquid had cooled to room temperature and before 7 ml of distilled water was added to dilute it. At 540 nm,

the color's intensity was measured against a suitable blank (Nelson, 1944). With the aid of a glucose standard curve that was created using 100 g/ml of standard solution glucose, the quantification of tissue-free sugar concentration was evaluated.

3. Total Protein

Protein and copper from the alkaline copper reagent combine to create a protein complex. A blue colour results from a reaction between the complex's amino acids and the tungstic acid in the Folin Cio-Calteau reagent. At a wavelength of 690 nm, the intensity of the blue colour is precisely proportional to the amount of tyrosine and tryptophan present.

- ***Reagents***

Lowry's reagent-To 98.0m1 of 4% sodium carbonate, l ml of each 2% copper sulfate and 4% sodium —potassium tartrate was added to make the volume up to 100 ml.

Protocol:

The tissue extract for protein was diluted with distilled water to a volume of 0.5 ml from 0.1 m1, then 5 ml of Lowry's reagent was added, and the mixture was let to sit for 15 minutes at room temperature. Following that, 0.5m1 of Folin Cio-Calteau reagent (1:2 dilution) was applied and held for a further 30 minutes of incubation. At 690 nm, the intensity of the blue-coloured complex was measured in comparison to a suitable blank (Lowry et al., 1951). The amount of protein in the sample was quantified using a bovine serum albumin standard curve (100 g/ml BSA in 1N NaOH).

4. ***Reduced Glutathione***

GSH and 5, 5'-dithiobis, 2-nitrobenzoic acid combine to form a yellow molecule. At 412nm, the color's intensity can be determined spectrophotometrically.

Extraction

Tissue homogenate was prepared using 2 ml of 5% TCA and centrifuged at 500 x g for 5 minutes to remove the precipitate.

Protocol:

2, 5'-dithiobis, 2-nitrobenzoic acid (DTNB) reagent was added to 1.0 ml of diluted tissue extract resulting in a final volume of 3.0 ml (Moron et al., 1979). At an appropriate blank, absorbance was measured at 412 nm. With the aid of a reduced glutathione standard curve (0.2 moles/m1 in 5% TCA), the samples' reduced glutathione contents were measured.

3.8.1 Estimation of Enzyme Activity

Extraction

Mantle and Gills were thawed and homogenized in PBS (0.01M phosphate buffer, pH (7.0) for catalase assay.

1. ***Catalase***

Hydrogen peroxide is reduced into water and oxygen molecules in catalase's process.

2 H202 ----------> 2 H20 + 02

The enzyme unable to break down any remaining H202 in the samples. gives out a blue precipitate of perchromic acid when it reacts with dichromate. After being heated, this unstable precipitate is broken down to produce a stable compound that is green in colour. At 620 nm, the green color's intensity can be observed.

• ***Reagents***

Dichromate acetic acid reagent- 5% potassium dichromate and glacial acetic acid were taken in a 1: 3 ratio to prepare this reagent.

Protocol

0.4 ml of the substrate (0.2 M H202) and 1.5 ml of phosphate buffer (0.01 M, pH 7.0) were combined, and they were incubated at 37°C for 5 minutes. To produce the enzyme blank, 0.1 ml of phosphate buffer was added, then 0.1 ml of serum or tissue homogenate was added to measure the enzyme activity. For 15 minutes, this reaction mixture was incubated at 37°C. By adding 2.0 m1 of dichromate acetic acid reagent, the reaction was stopped. The colour intensity was then measured in comparison to a reference blank at 620 nm after the solution had been boiled in a boiling water bath for 10 minutes. As mentioned earlier, an estimate of the enzyme's protein content was calculated. Using, the enzyme activity was measured. The enzyme activity was quantified with the help of a reference curve of hydrogen peroxide (2 μmole/ ml) and expressed as moles of H202 consumed/min/mg protein.

3.8.2 Ion transporting enzymes (ATPases*):*

The ATPases (Na+, K+, Ca++, and Mg++-ATPases) are essential for transforming ATP's metabolic energy into the movement of ions across cell membranes like Na+, K+, Ca++, and Mg++. As a result, the hydrolysis of ATP and the activity of ATPases are strongly connected. After ATP is hydrolysed, the released inorganic phosphate is quantified using the ammonium molybdate reagent to produce phosphomolybdate acid, which is then reduced by ascorbic acid to produce a blue colour.

Reagents

- *Ammonium molybdate reagent: 0.42% of ammonium molybdate solution in 1(N) sulphuric acid and 10% aqueous ascorbic acid solution were combined in a ratio of 6:1.*

Protocol

To 1 ml of reaction mixture, 0.1 ml of sample suspension were added. The mixture was incubated for 10 min at 37°C. The reaction was initiated with addition of substrate and the mixture was incubated at 37° C for 10 minutes. After 10 minutes of incubation 0.2 ml of 10% TCA was added to the mixture and centrifuged at 1000 x g for 5 minutes and supernatant was collected for estimation of released inorganic phosphates. This gives the total activities of Na+-K+-ATPase. In another set, in the reaction mixture 0.1 ml of 0.1 mM ouabain was added and the same procedure was followed in order to determine the activity of Mg++-ATPase only. The quantification of released phosphorus was done with the help of standard curve of phosphorus (12 µmole of Phosphate/ml).

3.8.3 Estimation of Respiratory and excretory rate

3.8.3.1 Estimation of aquatic respiration:

The Manganous sulphate becomes Manganous hydroxide in water. The oxygen in the water sample oxidizes Manganous hydroxide to manganic hydroxide. The acidified manganic hydroxide liberates iodine from potassium iodide. The liberated iodine is titrated against sodium thiosulphate. Thus one molecule of oxygen can liberate 4 atoms of iodine which is titrated with 4 molecules of thiosulphate.

Reagents

- Winkler's Solution A- 20g of manganous sulphate was dissolved in 100ml of pre-boiled distilled water.
- Winkler's Solution B- l00 g of Potassium hydroxide and 20g of Potassium iodide was dissolved in 200 ml of pre-boiled distilled water.

Protocol:

To estimate the dissolved oxygen: 250 ml of water sample was taken into an amber-coloured bottle from the aquarium tanks; 1 control and 3 experimental groups (Welsh and Smith, 1960). The collected water was immediately fixed by adding 2 ml of 20% Manganous sulphate and 2 ml of 10% Potassium iodide solution. The brown precipitate formed was dissolved with 5 ml of concentrated Sulphuric acid. 20 ml of resulting solution was titrated against 0.025 (N) Sodium thiosulphate solution using 1% starch as indicator till the colour changes from blue black to colourless. Then with the help of the formula the dissolved oxygen was calculated.

Calculations:

$$\text{Dissolved Oxygen (DO)} = 8 \times 100 \times \frac{N}{V} \times v$$

V = Volume of sample taken

v = Volume of used titrant

N = Normality of the titrant.

Oxygen Consumption = Blank - experimental

3.8.3.2 Estimation of excretory rates

The amine or imine in the presence of sodium hypochlorite reacts with phenol to give p-nitroso phenol which gives green coloured substance. The 410 intensity of colour depends upon the ammonia present, which can be measured at 650nm.

Reagents:

Phenol colour reagent- 27 mg of Phenol and 125 mg of Sodium nitroprusside were dissolved

in 100 ml distilled water.

Sodium Hypochlorite solution

Protocol:

Samples aliquot was made up to 4ml with distilled water. To this 1 ml of sodium hypochlorite solution and 1ml of Phenol reagent was added. It was incubated in water bath at 40°C for 15 minutes and then cooled to room temperature. The intensity of colour was measured at 650 nm against a suitable

blank (Chaney and Marbach, 1962). The amount of ammonia present in samples was calculated by using standard curve of ammonia (l0 g/ml).

3.9. Statistical Analysis

Statistical analyses were done using GraphPad Prism 9 software. Results were expressed as mean ± standard deviation. One-way ANOVA analysis of variance was carried out to compare the differences of means among multi-group data calculating P and F values. The student-T test was used to compare the differences between the experimental groups and the control group. Statistical significance for all tests was set at $P < 0.05$ as significant and $P < 0.001$ as highly significant.

RESULTS

4.RESULTS

Effects of Coal Tar on the anatomy of major organs of *Polymesoda erosa.*

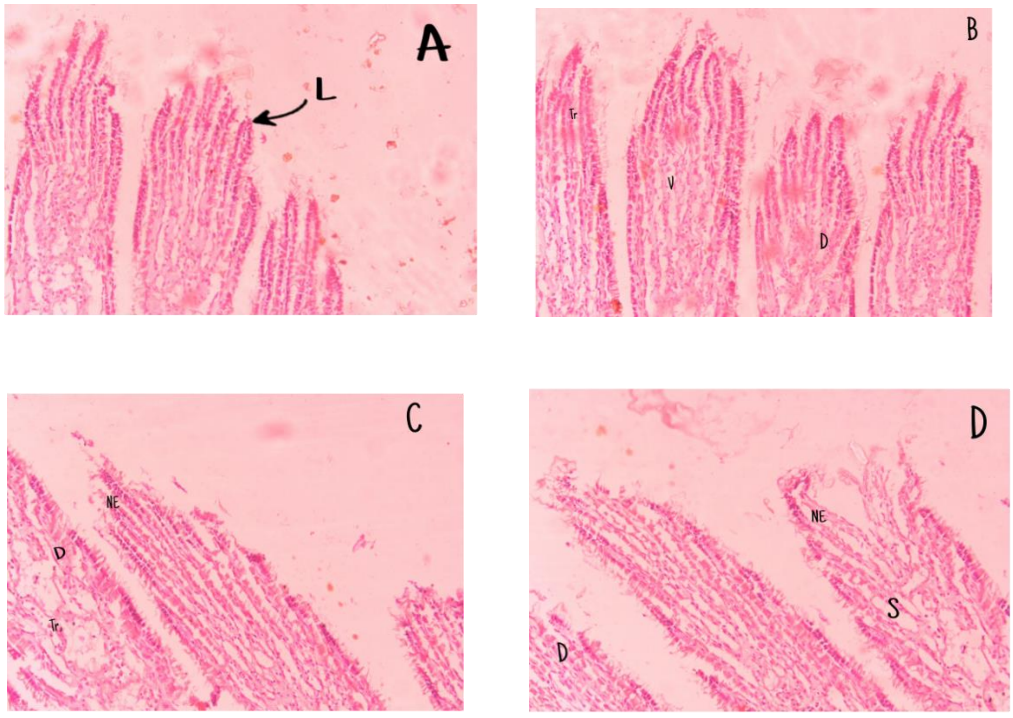
Gills

The light microscope of the control group gills was observed with normal undamaged basic features of the primary and secondary lamellae. In experimental group 1 the bivalves exposed to Coal Tar showed vacuolated regions on undergoing stress along with tissue damage and distortion. Experimental group 2 exposed to Coal Tar was characterized by the changes in the lamellae sections where necrotic erosion was observed as well as tissue rupture and distortion. The Experimental group 3 showed sloughing of dead skin layer of cells and advanced necrotic erosion.

Mantle

The light micrographs of the control group mantle were observed with Magnified portion of mantle tissue with normal arrangement of muscle fibres and columnar epithelial cells. In Experimental group 1 exposed to Coal Tar showed slight ruptures in the columnar epithelial cells along with distortion of the tissue. In Experimental group 2 columnar epithelial cells were ruptured along with distortion of the tissue and the muscle fibers were shrinked. The last Experimental group 3 of bivalves exposed to the highest dose of Coal Tar was seen to show fragmented muscle fibres and completely damaged columnar epithelial cells.

Effect of Coal Tar on histopathology of gills section (Fig 1)



Effect of Coal Tar on histopathology of gills section (Fig 1)

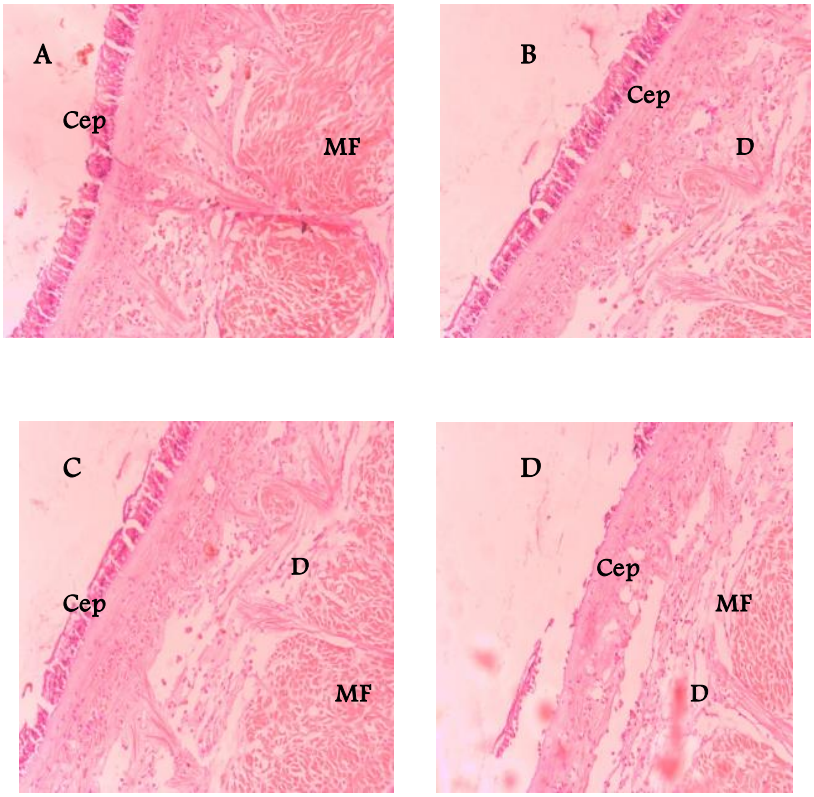
A- Control: Basic Structure showing lamellae

B- Exp 1: V- vacuolated regions, Tr- tissue rupture and D- distortion.

C- Exp 2: NE-Necrotic erosion, V- vacuolated regions, Tr- tissue rupture, D-distortion.

D- Exp3: S- Sloughing (shedding of dead skin), NE- Necrotic erosion.

Effect of Coal Tar on histopathology of Mantle tissue (Fig 2)



Effect of Coal Tar on histopathology of Mantle tissue (Fig 2)

A- Control: Magnified portion of mantle tissue with normal arrangement of muscle fibers and columnar epithelial cells.

B- Exp1: Cep- columnar epithelial cells were slightly ruptured, D- distortion of the tissue.

C- Exp2: Cep- Columnar epithelial cells were ruptured D-distortion of the tissue, MF- shrinked muscle fibers.

D- Exp3: Cep-Completely damaged columnar epithelial cells, D- distorted tissue, MF- fragmented muscle fibers.

Coal Tar effect on the biomolecule's estimation

The changes in the concentration of different biomolecules and enzymes exposed to Coal Tar for 28 days were revealed from fig _3____ to __15___.

The Protein concentration showed significant increase dose dependently in the experimental 2 and 3 groups of the mantle tissue (F= 25.82, P≤0.01) Whereas in gill tissue there was slight significant increase dose dependently was observed in experimental 3 (F=4.694, P≤0.01) and experimental 2 and experimental 1 were insignificant (fig 3-4)

The Carbohydrates concentration showed a significant decrease dose dependently in the experimental 3 group of the mantle tissue (F=7.780, P≤0.05) and experimental 1 and experimental 2 were insignificant Whereas in gill tissue there was slight significant decrease dose dependently was observed in experimental 2 (F=5.529, P≤0.05) and experimental 1 and experimental 3 were insignificant. (Fig 5-6)

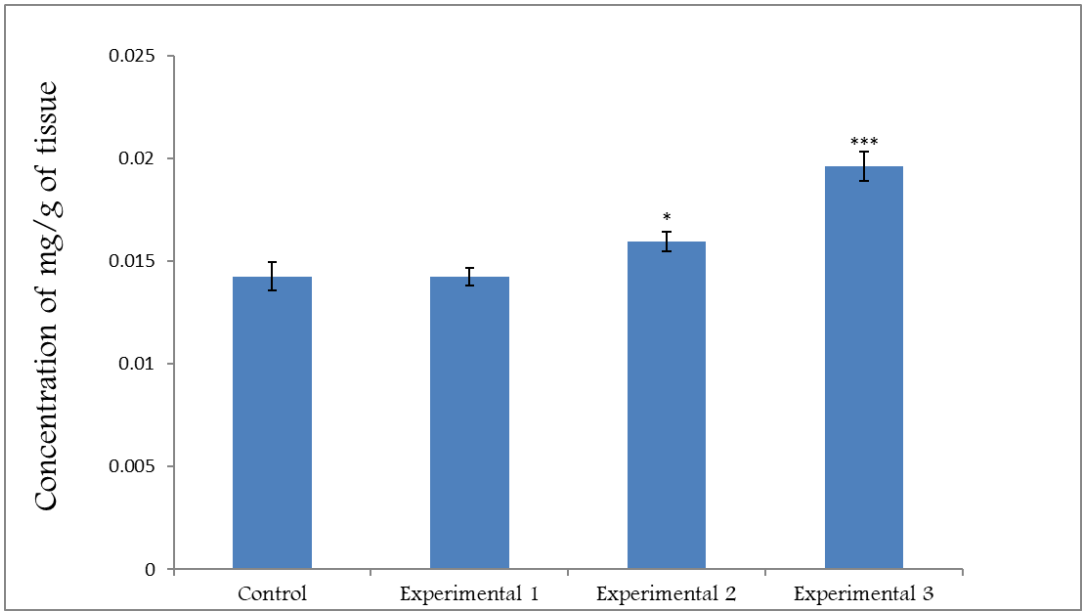
The Reduced Glutathione Concentration showed a significant increase dose dependently in all the experimental groups of the mantle tissue (F=1.16, P≤0.01) Whereas in Gill tissue there was slight significant increase dose dependently was observed in experimental 2 and experimental 3 (F=13.43, P≤0.01) and experimental1 was non-significant. (Fig 7-8)

The Free Sugars Concentration showed a significant deccrease dose dependently in all the experimental groups of the mantle (F= 5.613, P≤0.001) and also similar significance was observed in the gill tissue (F= 4.213, P≤0.001) (fig9-10)

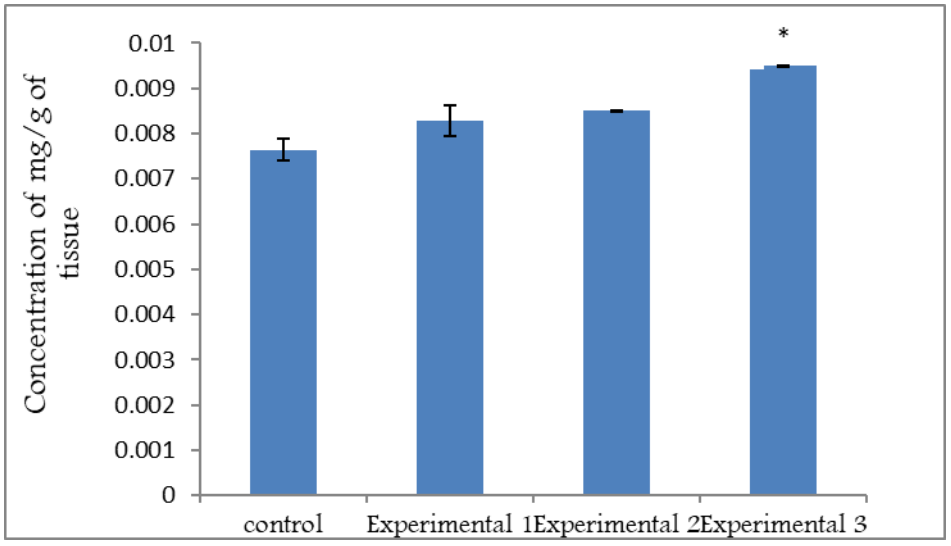
The Catalase enzyme activity showed a significant increase dose dependently in experimental 2 and experimental 3 groups of the mantle tissue ($F=6.542$, $P\leq0.001$) Whereas in the gill tissue there was significant increase dose dependently was observed in experimental 2 and experimental 3 groups ($F=9.035$, $P\leq0.001$) and experimental 1 was insignificant. (fig11-12)

In Na/K+ ATPases: The mantle tissue of experimental groups showed a significant decrease dose dependently in experimental 3 group ($F=5.518$, $P\leq0.05$) Whereas in the gill tissue there was a significant decrease dose dependently was observed in experimental 2 and experimental 3 groups ($F=44.55$, $P\leq0.001$) and experimental 1 group was insignificant(fig12-13)

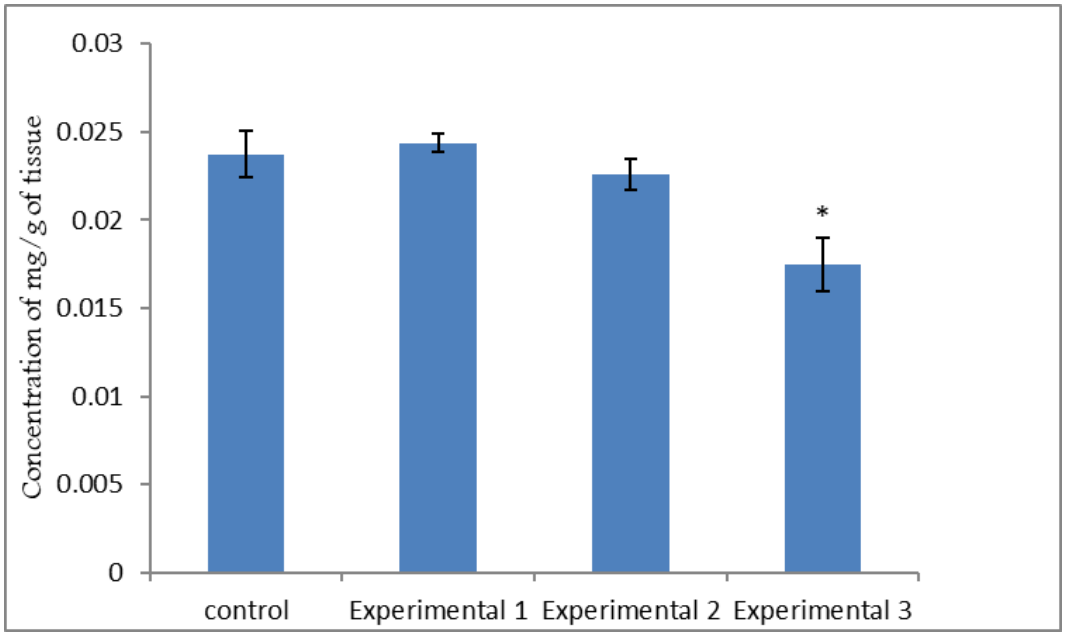
In Mg+ ATPases: In both mantle ($F=66.07$, $P\leq0.001$) and gill tissue ($F=52.41$, $P\leq0.001$) a significant increase dose dependently was observed in all the experimental groups (14-15)



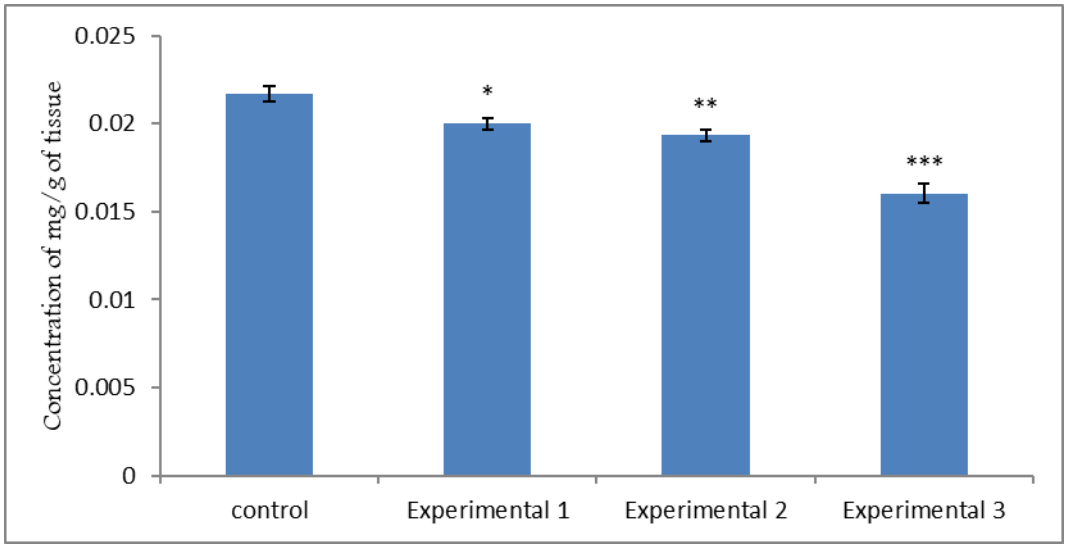
(Fig 3) Effect of Coal Tar on Total Protein content in the mantle of Bivalve. NS (Non-significant) * P≤0.05 significant ***P≤0.001highly significant.



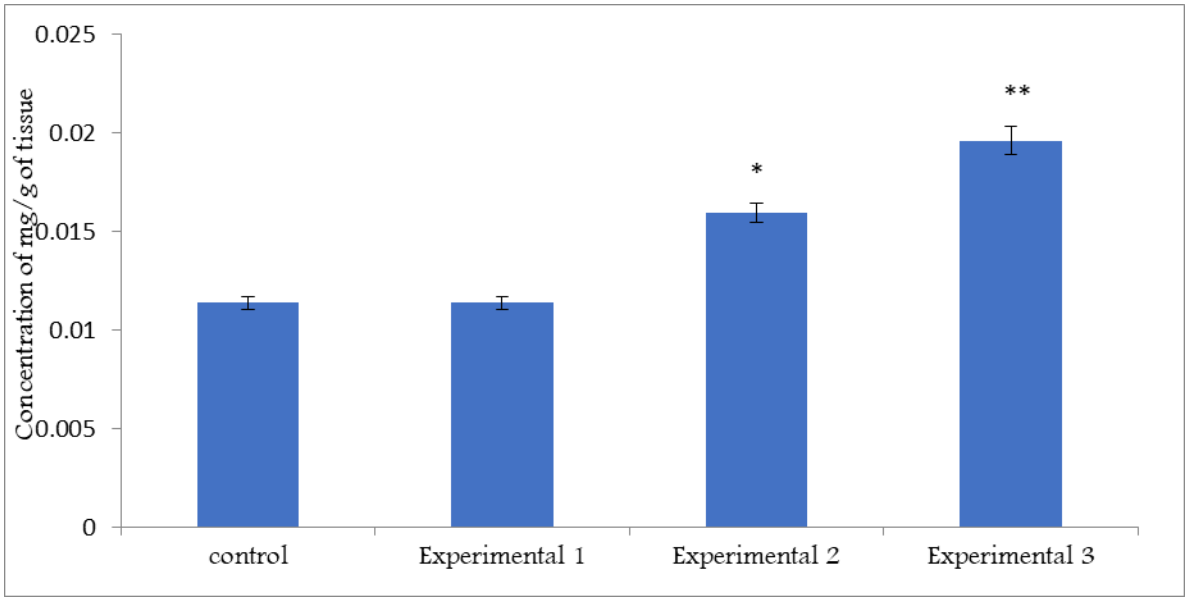
(Fig 4) Effect of Coal Tar on Total Protein content in the gills of Bivalve. NS (Non-significant), * P≤0.05 significant



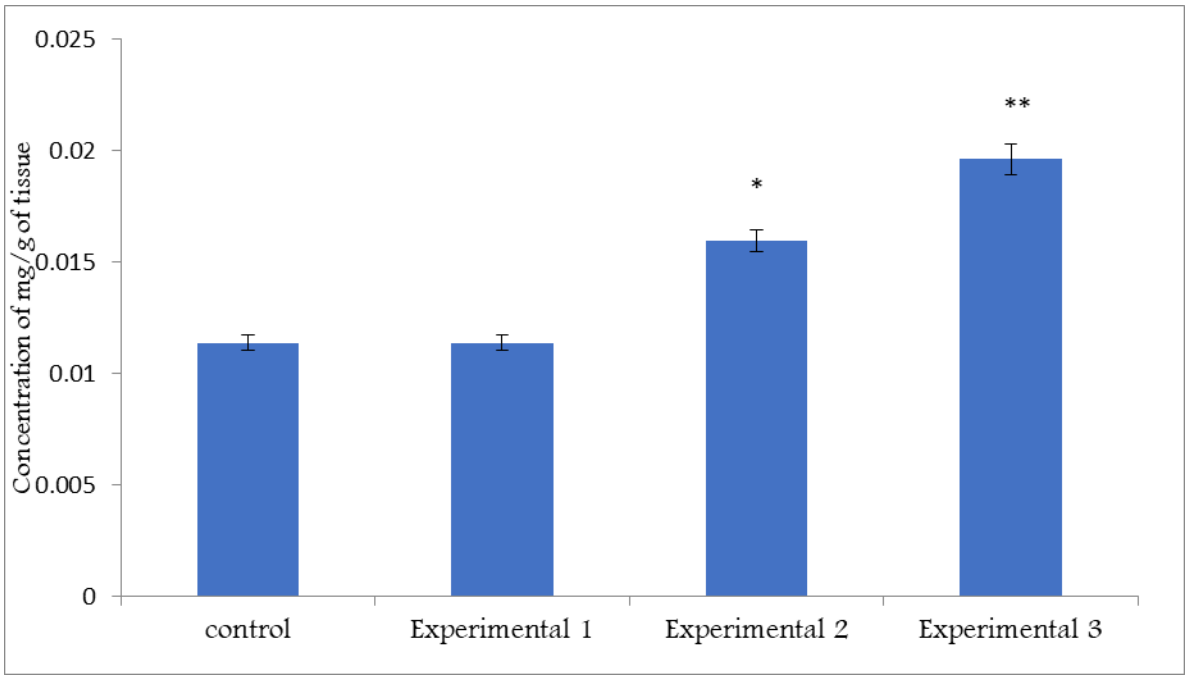
(Fig 5) Effect of Coal Tar on Total Carbohydrates content in the mantle of Bivalve. NS (non-significant), * P≤0.05 significant.



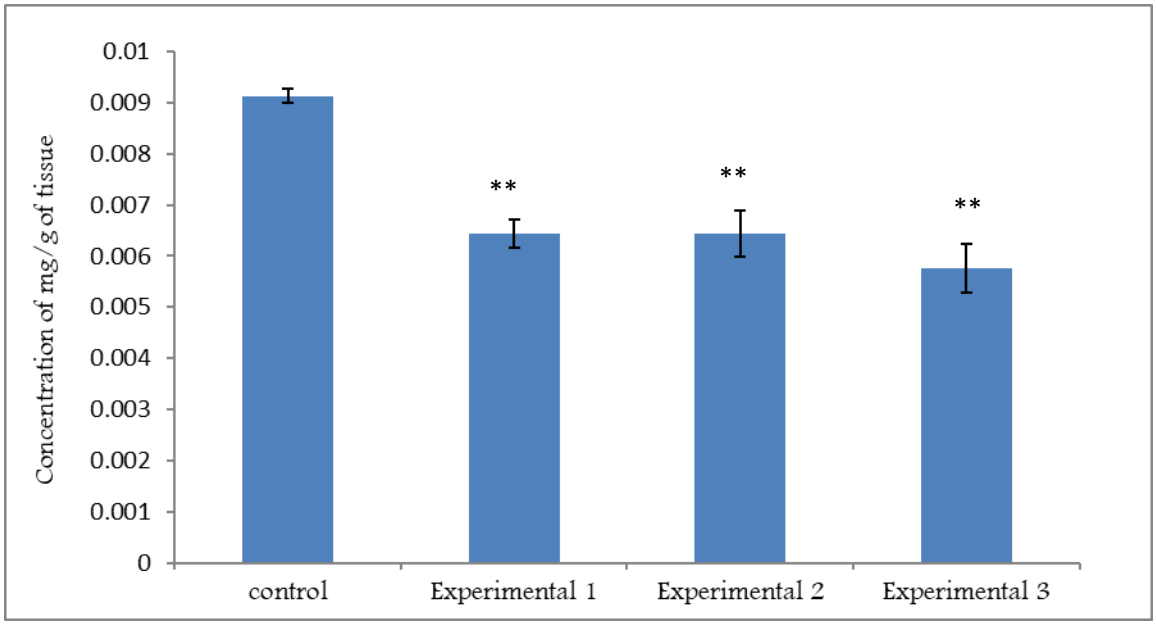
(Fig 6) Effect of Coal Tar on Total Carbohydrates content in the gills of Bivalve. NS (Non-significant) * P≤0.05 significant, **P≤0.01highly significant. ***P≤0.001 very highly significant.



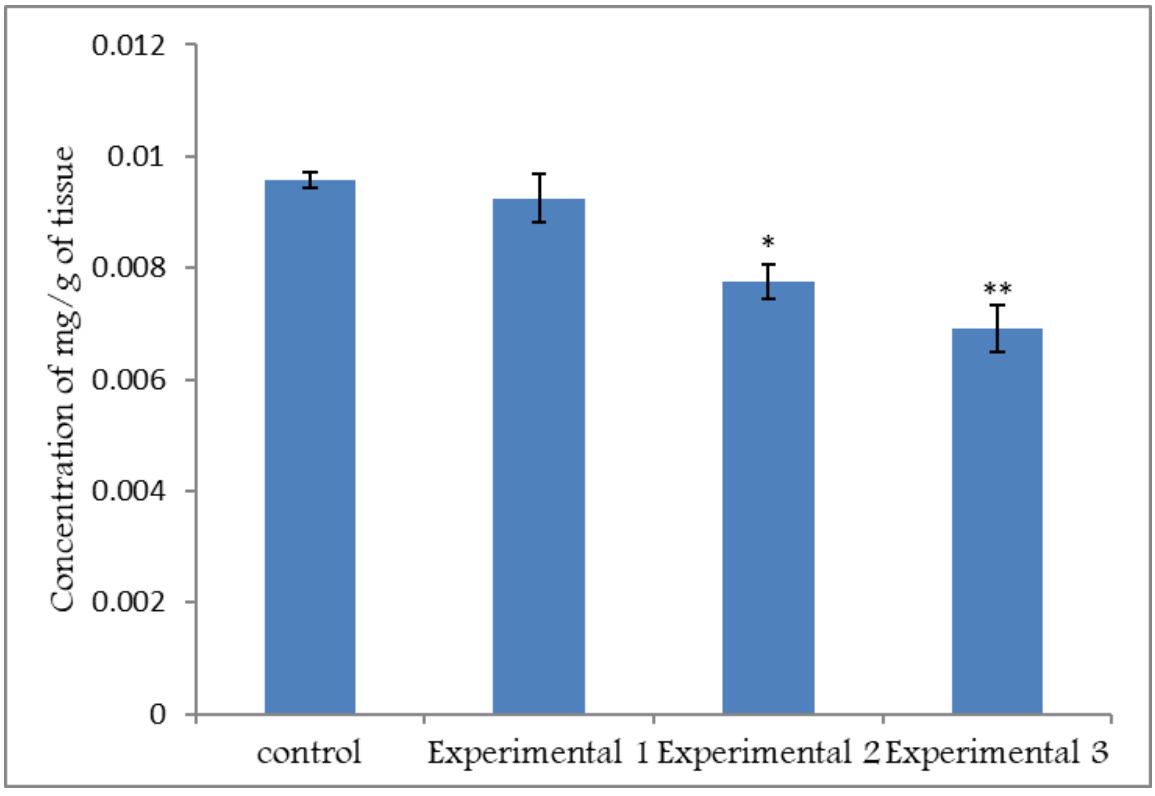
(Fig 7) Effect of Coal Tar on Reduced Glutathione content in the mantle of Bivalve. *P≤0.05 significant, ** P≤0.01 highly significant



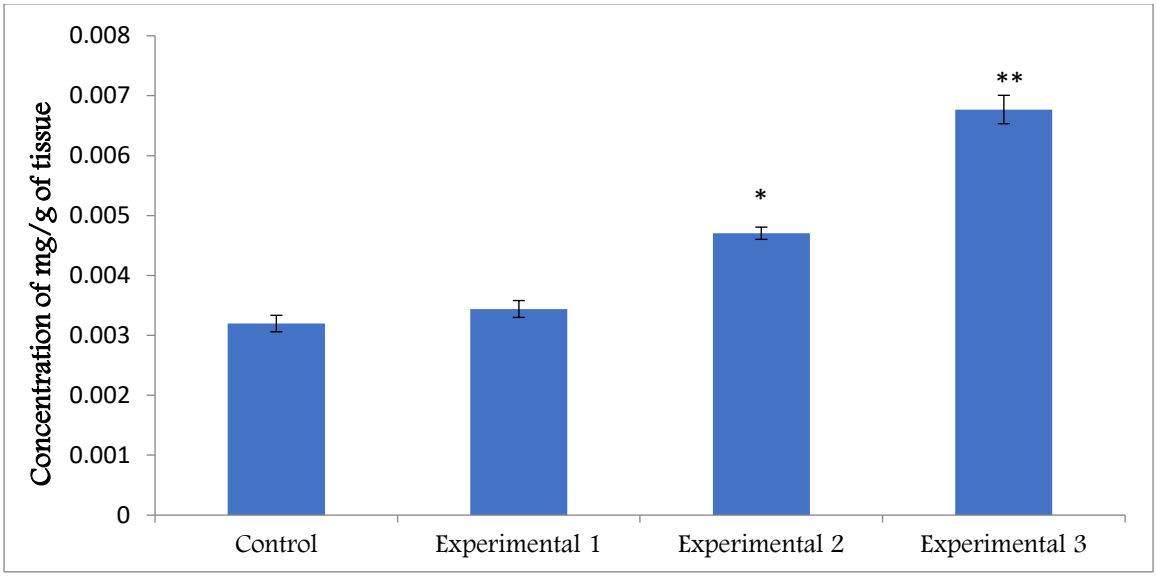
(Figure 8) Effect of Coal Tar on Reduced Glutathione content in the gills of Bivalve. NS (non – significant), *P≤0.05 significant, ** P≤0.01 highly significant.



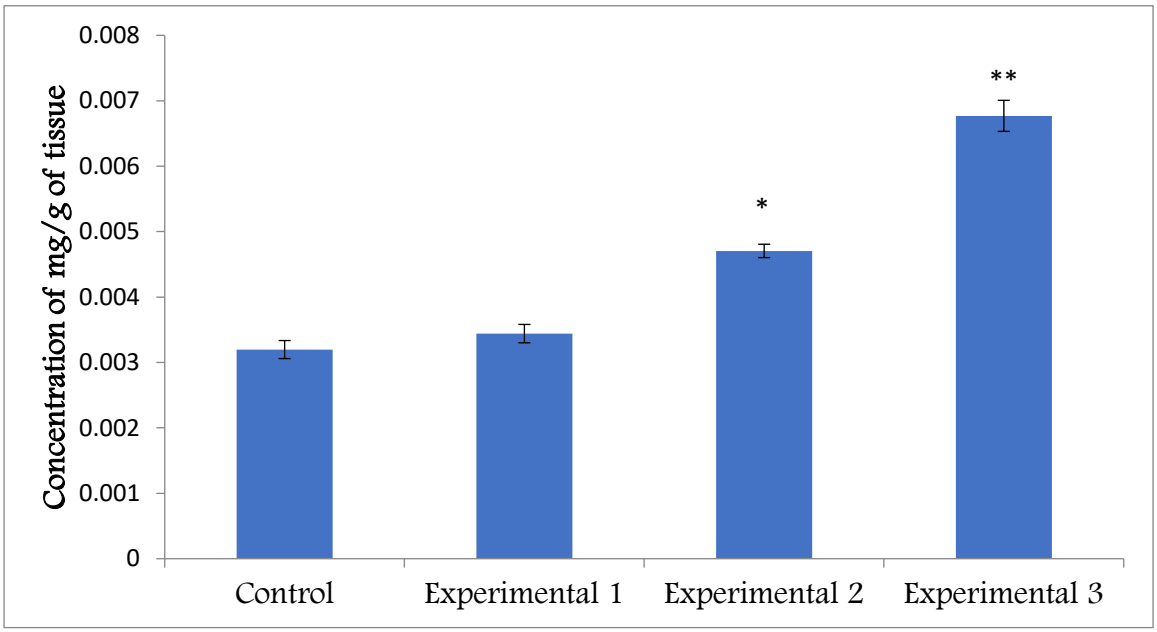
(Fig 9) Effect of Coal Tar on Free Sugars content in the mantle of Bivalve
*P≤0.05 significant, ** P≤0.01 highly significant



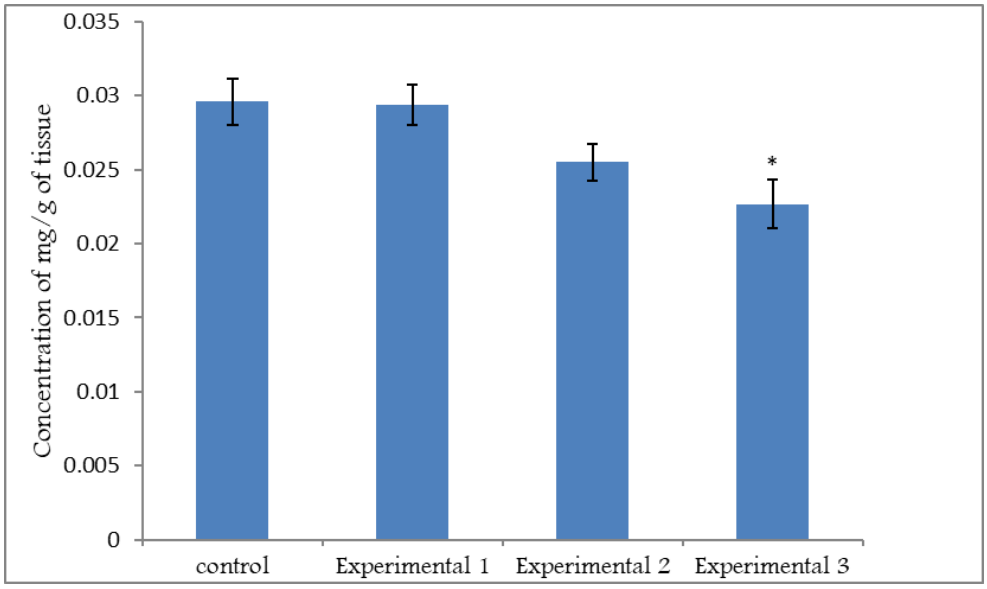
(Fig 10) Effect of Coal Tar on Free Sugars content in the gill of Bivalve P≤0.05 significant, ** P≤0.01 highly significant



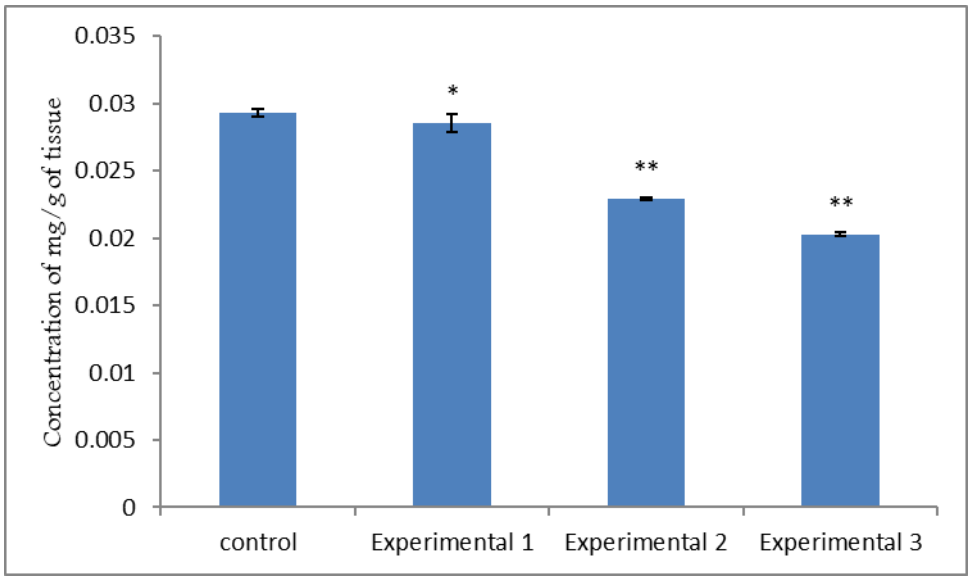
(Fig 11) Effect of Coal Tar on Catalase content in the mantle of Bivalve. NS (non-significant) * P≤0.05 significant, ** P≤0.01 highly significant



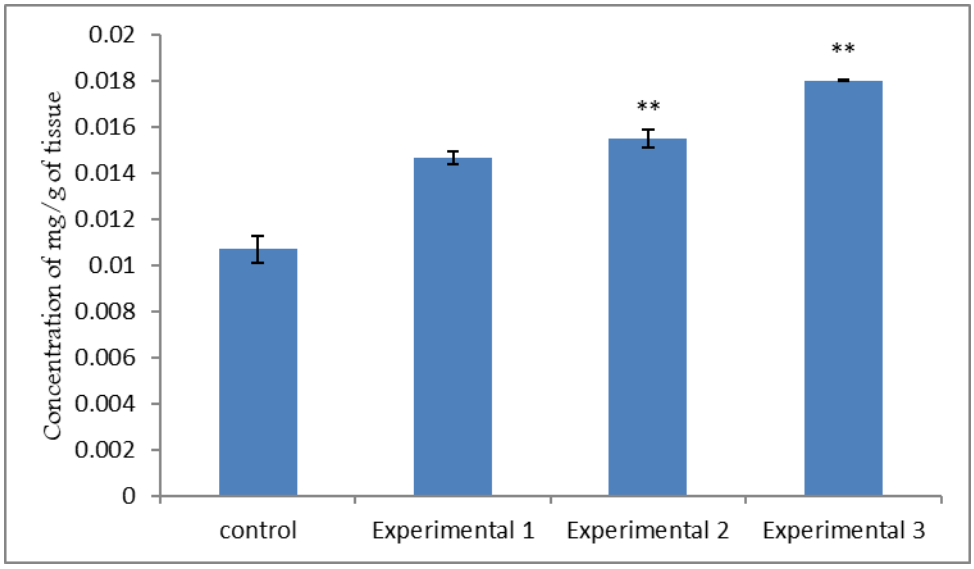
(Fig 12) Effect of Coal Tar on Catalase content in the gills of Bivalve. NS (non-significant) * P≤0.05 significant, ** P≤0.01 highly significant



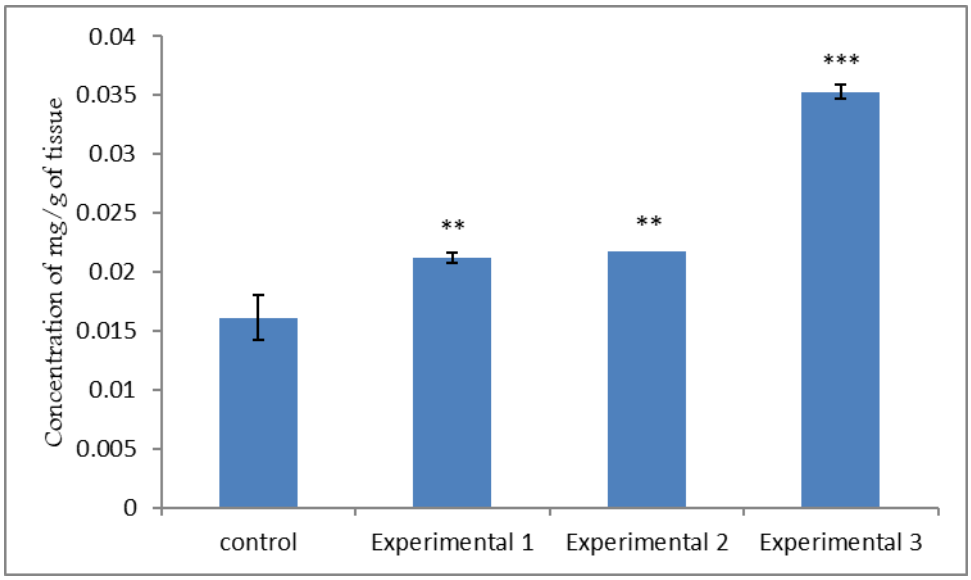
(Fig 13) Effect of Coal Tar on Na/K+ atpases activity in mantle of the bivalve. NS (non- significant) *(P≤ 0.05 significant).



(Fig 14) Effect of Coal Tar on Na/K+ atpases activity in gill of the bivalve. NS (non- significant) *P> 0.05 significant, **P≤0.001 highly significant.

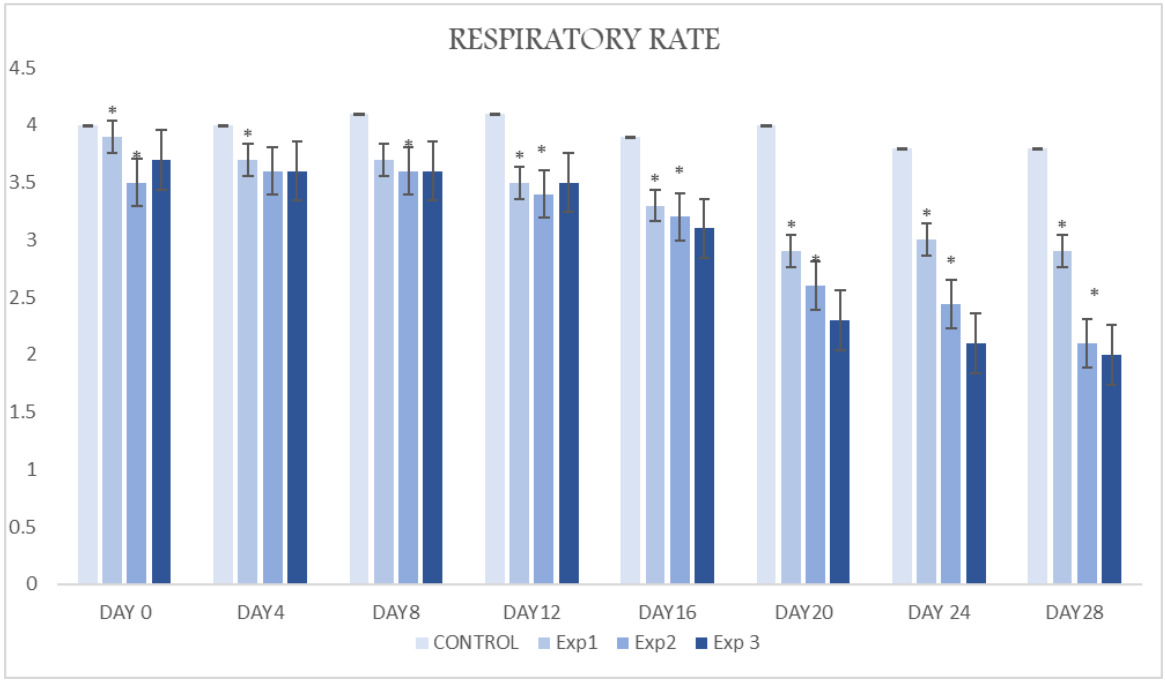


(Fig 15) Effect of Coal Tar on Mg+ ATPases activity in mantle of the bivalve. Ns(non significant)**P≤0.001 highly significant

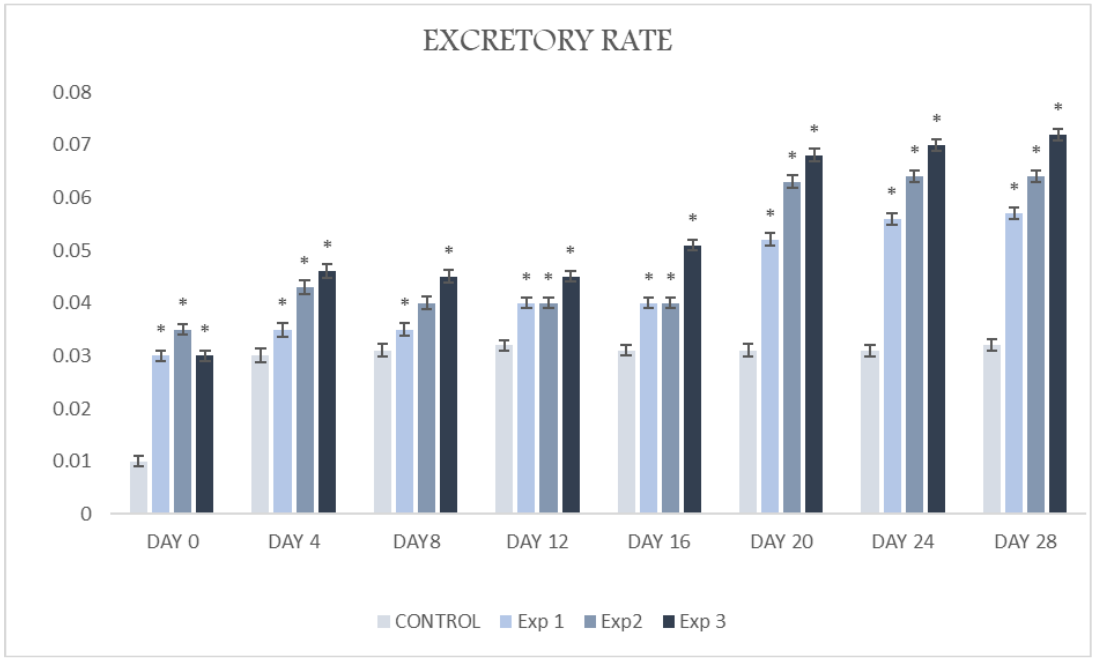


(Fig 16) Effect of Coal Tar on Mg+ ATPases activity in gill of the bivalve **P≤0.001 highly significant, *** P≤0.001 highly significant

Effect of Coal Tar on the Respiratory rate and Excretory rate



(Figure No 17) Effect of Coal Tar on oxygen consumption ns (nonsignificant), *(P≤0.05, significant)



(Figure No 18) Effect of Coal Tar on excretion ns(non-significant), *(P≤0.05, significant)

The oxygen consumption rate decreased significantly with days (F= 265.4, P≤0.001) and also declined significantly with respect to doses (F=603.5, P≤0.001). The interaction between the days and the doses was showing significant decrease (F=3.863, P≤0.001) (fig 17)

The excretory rate increased significantly with days (F= 321.5, P≤0.001) and also inclined significantly with respect to doses (F=51.6, P≤0.001) The interaction between the days and the doses was showing significant increase (F=3.125, P≤0.001) (fig 18)

DISCUSSION

5.DISCUSSION

PAHs from coal tar possess a unique structure that can lead to long-term negative effects on the health of the organism with its interference. Hence the present study documented the varying toxicity effects elicited by PAHs from coal tar via histopathological studies, biochemical estimates, and changes in physiological functions of the bivalves Polymesoda erosa.

Bivalve histopathology is considered a useful tool for biomonitoring coastal ecosystems. Histopathological changes in target tissues occur due to exposure to a wide range of contaminants (Au, 2004), and they indicate the status of target tissues, providing a general view of the damage received by the organism. In bivalves, the gills and the mantle are directly exposed to environmental contaminants and play an important role in food collection, absorption and protection. The gills filter large volumes of water in order to obtain nutrients, and are therefore in near continuous contact with pollutants present in the water column whereas the mantle provides complete protection to the internal organs along with respiration (Canes et al., 2010).

The Observations from Light Microscope histopathology of the Gill demonstrated vacuolated regions, tissue rupture along with distortion of cells, advanced necrotic erosion and sloughing of dead skin layer. Similar Histopathological changes were observed in manila clam exposed to heat and cold stress (Menike et al., 2013). Histopathological sections of the mantle tissue demonstrated ruptures and loss of columnar epithelial cells, tissue ruptures and distortion of cells, shrinked and fragmented muscle fibers. These results are

supported by studies that treated bivalves with PAHs induced substances (Shenawy et al., 2008) (Hashem et al., 2017). Bivalves have a single pair of gills, each of which is composed of a curtain of filaments and attached dorsally within the mantle cavity. The filaments are covered with cilia, whose beating in unison creates currents that move water over the gills, and during the filtration process, all filtered particles are trapped on the gills and consumed as source of food. Similarly, the respiratory organ mantle in association with the gills helps in filter feeding and protecting the internal organs (Goaling et al., 2008). In the present study both gills and mantle tissue showed a significant decrease in the concentrations of total carbohydrates and free sugars which in the form of glucose and glycogen serves as important source of energy for body activities. This might be due to the immediate utilization of reserve food under coal tar stress in the tissues (Venkateshwarlu et al., 1995). The findings were comparable with that of the effect of heavy metal, mercury chloride on the activity of biochemical components of the freshwater bivalve *Lamelledis marginalis* (Muniv et al., 2020).

Proteins constitute the major part of gill tissue transferring ions and water along with the exchange of oxygen, carbon dioxide, acids, and ammonia. A significant increase in the total protein concentration was observed both in the gills and mantle decreased catabolism and increased anabolism of protein along with cellular stress to meet the energy demands during stressed conditions caused by coal tar hence also affecting the bio mineralizing property of the mantle (Muniv et al., 2020, Freer et al., 2014). This data kept par with that reported by Mahajan et al., (2001) wherein the elevation of the protein content in different tissues such

as gonad, gill, and hepatopancreas of bivalve occurred after exposure to HgCl2 and CuSO4.

Catalase is one of the major enzymes along with reduced glutathione that decomposes H202 to less-reactive molecular oxygen and water and thereby protecting cells from oxidative damage. In the present study, PAHs were able to induce significant oxidative stress in both the gill and mantle of the bivalves with an increase in the concentrations of both catalase and reduced glutathione. This could be due to oxidative stress via the formation of ROS that may lead to cell membrane damage in the gills as well as the mantle tissue but most prominent in the gill tissue as it has higher exposure to coal tar comparatively. (Yiin and Lin, 1995, Canesi et al., 2012). These findings are similar to that of Boudjema et al. (2014) in which a significant elevation of CAT was reported in the brown mussel (Perna perna) experimentally exposed to Pb.

A negative relationship between oxygen consumption rate and shell size was observed in bivalve species. The results for the oxygen consumption rate decreased significantly with days and showed significant decline with respect to doses. The interaction between the days and the doses was showing significant decrease. A study reported by (Pourmozaffar et al., 2019) stated that oxygen consumption rate decreased with increasing body size at both salinities, because high metabolic rate of the small size is higher than large- and medium-sized clams. Thus, proving that there is a significant drop in respiration with respect to doses. Ammonia as the end product of protein metabolism of aquatic invertebrates is highly toxic but it diffuses rapidly into seawater because its high solubility in water and small molecular size. The results showed that there was a significant increase in the excretion rate with respect to the doses. The

interaction between the days and the doses was showing significant increase. Similar study was been done by (Pourmozaffar et al., 2019) reported that, to survive in stressful (hyper and hypoosmotic) conditions, increase in energy cost was likely related to ammonia excretion and oxygen consumption. Bivalve species are considered to be euryhaline organisms due to effective adaptation to fluctuations of environmental salinity. The results for osmoregulation were observed that the Na/K+ ions concentrations were decreased on exposure while the Mg+ ions were increased. Similar study was reported by (Lima et al., 2012) stated that reduced Na + and K + levels, when compared with untreated controls from samples of fluids. The decrease may work as a compensatory mechanism to maintain internal osmolarity, thus making up for increases in Mg 2+ ionic concentrations. Hence proving the above results. The present study revealed that the bivalves Polymesoda erosa collected fromChorao, Tiswadi Goa showed significant changes in the concentration of different biomolecules, respiratory rate, and excretion rate as well as histopathology which might have been induced by the interference and accumulation of PAHs from coal tar. This can lead to the deterioration in the health of the fish and clam populations and consequently result in the decline in fish catches around this area. Further, this may also pose a threat to the health of fish consumers as well as the health and livelihood of the fishing communities at Chorao, Tiswadi Goa.

SUMMARY

6.SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) are widespread across the globe mainly due to long-term anthropogenic sources of pollution. PAHs are a class of chemicals that occur naturally in coal, crude oil, and gasoline. They result from burning coal, oil, gas, wood, garbage, and tobacco. They can bind to or form small particles in the air. High heat when cooking meat and other foods will form PAHs. Naphthalene is a manmade PAH used in the United States to make other chemicals and mothballs. Cigarette smoke contains many PAHs. The fact that coal tar NAPLs are combinations of hundreds of molecules, predominantly polycyclic aromatic hydrocarbons (PAHs). Due to its increasing demand and versatility, its exposure to humans is on a rapid momentum, making its investigations the need of the hour.

The follow-up work was to understand the toxicity of Coal Tar on Polymesoda erosa. The bivalves were divided into 4 groups with 1 control group and 3 Exp. Groups. They were subjected to Coal Tar dose dependently for 28 days. The effects of these Coal Tar were evaluated on the anatomy, and Gill and Mantle functioning of bivalves. Different anatomical changes were also of focus relaying the damage caused by Coal Tar in both gill and mantle via necrotic erosion, tissue rupture and distorting the tissue.

The Gill functioning was affected by the dysfunctioning of different enzymatic (catalase) as well as non-enzymatic antioxidants (Reduced glutathione). Different biomolecule concentrations like total carbohydrate, total proteins and free sugars were also altered with significant differences affecting the metabolic

activity and, in the gill, and mantle tissue. Thus, the current study report as a whole reveals the adversities caused by Coal Tar when exposed to the bivalves.

7.CONCLUSION

In conclusion, the research on coal tar's effects on the mud clam *Polymesoda erosa* offers crucial information on the possible effects of pollutants on marine life. The study's findings suggest that *Polymesoda erosa's* survival, growth, and metabolic reactions may be adversely affected by exposure to coal tar. These findings imply that the health and wellbeing of marine ecosystems may be seriously threatened by coal tar contamination.

To preserve the survival of our seas and the numerous species that call them home, it is crucial to keep track of how coal tar and other contaminants affect marine life. To stop future damage to our seas and marine life, it is important to prioritise efforts to minimise the quantity of coal tar and other pollutants that are discharged into the environment by industry and human activity.

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