

Coal Tar Toxicity in the Mangrove Clam *Polymesoda erosa*

Feazel R. Dias^A, Sarita Rebelo^A, Dr. Shamshad M. Shaikh^{A*}

^AZoology Discipline, School of Biological Sciences and Biotechnology, Goa University,
Taleigao Plateau, Goa, India 403705

Corresponding author email: ^{A*}shamshad.shaikh@unigoa.ac.in

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are persistent, bioaccumulative contaminants derived largely from anthropogenic combustion sources, and coal tar a dense, chemically complex non-aqueous phase liquid comprising hundreds of PAH and heterocyclic PAH constituents represents one of their most concentrated environmental sources. While PAH toxicity in bivalves is well documented, no prior study has examined the effects of coal tar on the mangrove clam *Polymesoda erosa*, an ecologically important filter-feeder and a food source for coastal communities. This study evaluated the chronic effects of dose-dependent coal tar exposure on the anatomy, histology, biochemistry, and physiology of *P. erosa* gill and mantle tissue over 28 days, using three exposure doses against a control group (n = 6 per group). Histopathological analysis revealed progressive, dose-dependent damage in both tissues, ranging from vacuolation and epithelial rupture to necrotic erosion, sloughing, and fragmentation of muscle fibres. Biochemical assays showed significant depletion of total carbohydrate and free sugar reserves alongside elevated total protein content, indicating metabolic reallocation under toxicant stress. Antioxidant enzyme activity (catalase) and reduced glutathione increased significantly, reflecting oxidative stress, while Na⁺/K⁺-ATPase activity declined and Mg²⁺-ATPase activity rose, suggesting compensatory ionoregulatory adjustment. Whole-organism respiratory rate declined, and excretory (ammonia) output increased significantly with both dose and exposure duration. Collectively, these findings demonstrate that coal tar exposure induces cumulative structural, biochemical, and physiological impairment in *P. erosa* within a short exposure window, highlighting a previously undocumented ecotoxicological risk. Given the species' ecological and economic importance, these results highlight the need for continued monitoring, bioaccumulation studies, and human health risk assessment in coal-tar-affected coastal habitats.

Keywords: Coal tar, Polycyclic aromatic hydrocarbons, ecotoxicity.

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are lipophilic, fused-ring organic compounds occasionally arising from natural processes such as forest fires and organic matter diagenesis (Wolska et al., 2012), are now dominated in the environment by anthropogenic inputs. Incomplete combustion of coal, petroleum, and biomass generates pyrogenic PAHs that reach marine and estuarine systems chiefly through atmospheric deposition, while petrogenic PAHs enter water bodies directly via fuel discharge and oil spills, the two classes distinguishable by their relative proportions of parent and alkylated congeners (Lima et al., 2005). Individual PAHs partition, persist, and bioaccumulate differently depending on their physicochemical properties and the receiving matrix (Mackay and Callcott, 1998), and non-polar, high-molecular-weight congeners are consistently associated with the greatest long-term toxicity. Given their recognised carcinogenic and non-carcinogenic effects and the disproportionate exposure of coastal and seafood-dependent communities (Yender et al., 2002), PAH contamination of coastal ecosystems continues to warrant close ecotoxicological scrutiny.

Coal tar is among the most concentrated and chemically complex environmental sources of PAHs. This dense non-aqueous phase liquid (NAPL), a legacy residue of manufactured gas plant (MGP) operations, remains immobilised in soils long after the industry's decline in the 1950s, slowly and continuously releasing its constituents into groundwater and sediment (Illangasekare et al., 1995; Ghoshal et al., 1996). It comprises hundreds of compounds, predominantly PAHs and heterocyclic PAHs, alongside phenolics and monocyclic aromatics, which together can account for up to 40% of its total mass, making full characterisation of its toxicological profile inherently difficult (Peters et al., 1993). Coal tar contamination is not purely historical: coal-tar-based sealants and paints remain an active, contemporary PAH

source, with documented reductions in aquatic macroinvertebrate abundance and diversity and elevated tissue PAH burdens in exposed organisms (Bommarito et al., 2010; Mougnot et al., 2022). Beyond its well-established human carcinogenicity, notably via benzo(a)pyrene (Bickers and Kappas, 1978; Nijsten and Stern, 2003), coal tar and its PAH constituents are broadly mutagenic, teratogenic, and immunotoxic across taxa (Burchiel and Gao, 2014; Rangarajan et al., 2015), with pronounced bioaccumulation and ecotoxic effects reported in benthic and bottom-feeding aquatic organisms (Honda et al., 2020; Abdel-Shafy and Mansour, 2016). Because the bulk of contaminant loading entering estuaries and coastal seas via riverine and direct discharge ultimately settles into bottom sediments (McIntyre, 1992), sediment-dwelling, filter-feeding bivalves represent one of the most persistently exposed and ecologically consequential faunal groups at risk from coal tar contamination.

Bivalves are widely used as sentinel organisms for coastal pollution owing to their limited mobility, broad distribution, and strong capacity to bioaccumulate contaminants from both water and sediment, and their role as keystone filter-feeders means that impaired physiological function in these organisms can propagate through nutrient cycling and the wider food web (Duarte et al., 2008; Gallardi, 2014). Species- and habitat-specific exposure patterns are well documented: mussels occupying the air-water interface accumulate predominantly low-molecular-weight PAHs, whereas sediment-associated clams accumulate heavier, particle-bound congeners (Thuy et al., 2018; Varanasi et al., 1992). Within bivalve tissues, PAHs tend to accumulate preferentially in the mantle owing to its direct contact with the surrounding environment, followed by the hepatopancreas, gonads, gills, and adductor muscle (Wang et al., 2020), and histopathological studies report PAH-induced necrosis and oedema of branchial lamellae, degeneration of gill filaments, and loss of inter-filament junctions in exposed oysters (Al-Hashem, 2017). Coal tar specifically has been shown to drive combined metal and PAH bioaccumulation in oyster tissue alongside lysosomal membrane damage in haemocytes (Chiovatto et al., 2021), while coal-tar sealant runoff has been linked to measurable declines in benthic macroinvertebrate community health (Bryer et al., 2010).

The mangrove clam *Polymesoda erosa*, a burrowing, filter-feeding bivalve inhabiting the

sediment-rich margins of mangrove forests, is continuously exposed to porewater- and sediment-bound contaminants and supports coastal livelihoods as an economically important edible species. Existing contaminant research on this genus has, however, focused almost exclusively on heavy metals and microplastics: studies report *Polymesoda* tissue concentrations of Zn, Pb, Cd, and Ni exceeding WHO permissible limits, with clear seasonal and environmental modulation of uptake (Yusoff et al., 2021; Yaakub et al., 2019; Cruz et al., 2020), and microplastic ingestion has been identified as an additional physical and chemical stressor in the genus (Daud et al., 2021). Notably, sediment and tissue profiling of *P. erosa* has confirmed the presence of multiple PAHs at sites prompting consumption advisories (Azis et al., 2021), yet no study to date has directly assessed the toxic effects of coal tar, arguably the most concentrated and environmentally persistent PAH-bearing matrix, on this species.

This gap is significant given the scale of coal tar's documented toxicity in other aquatic taxa and the ecological and economic importance of *P. erosa* to mangrove-associated coastal communities. The present study addresses it by evaluating the chronic effects of dose-dependent coal tar exposure on the anatomy and physiology of *P. erosa*, with particular focus on gill and mantle tissue as primary sites of contaminant contact and metabolic activity. We hypothesise that coal tar exposure induces measurable anatomical damage and enzymatic, biochemical, and physiological dysfunction in these tissues, and accordingly examine gross anatomical changes, gill and mantle structural and functional integrity, and key physiological endpoints; respiratory rate, excretory rate, and osmoregulation across a 28-day dose-dependent exposure regime.

II. MATERIALS AND METHODS

A. Collection and Maintenance of Test Organisms

Specimens of the mud clam *Polymesoda erosa*, with uniform body weights of 50-55 g, were collected from Chorao, Tiswadi, Goa, India. All experimental procedures were carried out with prior approval from the Animal Ethics Committee, Goa University (Ref. No.

GU/ZOO/2021/Q/35), and in accordance with CPCSEA guidelines. A total of 24 bivalves were maintained in the animal house of Zoology Discipline, School of Biological Sciences and Biotechnology under a 12 h light/dark cycle and acclimatised for two weeks prior to exposure. Clams were housed in aquarium tanks (six individuals per tank) containing mud substrate, aerated water maintained at constant temperature, and microalgae as a food source; water was renewed and aerators cleaned on alternate days.

B. Experimental Design and Coal Tar Exposure

Following acclimatisation, bivalves were randomly allocated to four groups of six individuals each: one control and three coal-tar-exposed groups. Chronic dose-dependent exposure was conducted by suspending cylindrical pipes coated with coal tar paint of increasing surface area (123.15 m², 153.94 m², and 197.92 m² for Experimental groups 1-3, respectively) within each tank for a 28-day exposure period, following the exposure design of Chiovatto et al. (2021). The control group was maintained under identical conditions without coal-tar-coated pipes.

C. Behavioural and Morphological Observations

Clams in all groups were monitored daily throughout the exposure period for behavioural changes, including burrowing activity and pedal (foot-assisted) locomotion, and for gross morphological changes such as siphon retraction and altered foot extension, to assess sub-lethal responses to coal tar exposure.

D. Euthanasia and Tissue Isolation

At the end of the 28-day exposure period, clams were euthanised by immersion in ice-cold water, after which the gill and mantle tissues were aseptically dissected, rinsed in physiological saline, and weighed.

E. Histological Analysis

Isolated gill and mantle tissues were fixed in 10% formalin and subsequently transferred to 70% ethanol for storage. Fixed tissues were processed, embedded in paraffin, sectioned at 0.1 µm, and stained with haematoxylin and eosin. Sections were examined and photomicrographed under a light microscope at 20× magnification to assess coal-tar-induced histopathological changes.

F. Biochemical and Enzymatic Estimations

Gill and mantle tissue homogenates were used to estimate biomolecule content and enzyme activity relevant to metabolic and oxidative status. Total carbohydrate and free sugar content were estimated following Roy et al. (1991), using the Anthrone method of Carroll (1956) and the alkaline copper-arsenomolybdate method of Nelson (1944), respectively. Total protein content was determined by the Folin-Ciocalteu method of Lowry et al. (1951). Reduced glutathione (a non-enzymatic antioxidant) was estimated using the DTNB method of Moron et al. (1979). Catalase activity was assayed using the dichromate-acetic acid method described by Sinha (1972). Ion-transporting ATPase activities (Na⁺/K⁺- and Mg²⁺-ATPase) were estimated by quantifying released inorganic phosphate following ATP hydrolysis, based on the method of Fiske and Subbarow (1925), with Mg²⁺-ATPase activity isolated using ouabain inhibition.

G. Estimation of Respiratory and Excretory Rate

Aquatic respiration was estimated by determining dissolved oxygen consumption in tank water using the Winkler titrimetric method (Welsh and Smith, 1960). Excretory rate was estimated by measuring ammonia excretion using the phenol-hypochlorite colorimetric method of Chaney and Marbach (1962). Both parameters were measured across control and experimental groups to assess coal-tar-induced physiological alterations.

H. Statistical Analysis

All data are expressed as mean ± standard deviation. Statistical analyses were performed using GraphPad Prism 9. One-way analysis of variance (ANOVA) was used to compare differences among group means, and Student's *t*-test was used to compare individual experimental groups against the control. Statistical significance was set at *P* < 0.05, with *P* < 0.001 considered highly significant.

III. RESULTS

A. Effect of Coal Tar on Gill and Mantle Histoarchitecture

Gill sections from the control group showed intact primary and secondary lamellae with no structural abnormalities (Fig. 1A). Coal tar exposure produced dose-dependent histopathological deterioration: Experimental group 1 exhibited vacuolated regions accompanied by early tissue rupture and distortion (Fig. 1B); Experimental group 2 additionally showed necrotic erosion of the lamellar epithelium (Fig. 1C); and Experimental group 3, receiving the highest coal tar dose, displayed sloughing of the dead epithelial layer alongside advanced necrotic erosion (Fig. 1D), indicating progressive loss of lamellar integrity with increasing exposure.

A parallel dose-dependent pattern was observed in mantle tissue. Control sections showed a normal arrangement of muscle fibres and columnar epithelial cells (Fig. 2A). Experimental group 1 showed slight rupture of columnar epithelial cells with mild tissue distortion (Fig. 2B); Experimental group 2 showed more pronounced epithelial rupture, tissue distortion, and shrinkage of muscle fibres (Fig. 2C); and Experimental group 3 exhibited complete disruption of columnar epithelial architecture together with fragmented muscle fibres (Fig. 2D). Overall, both tissues showed a consistent severity gradient across experimental groups, with the gill epithelium progressing toward necrosis and sloughing, and the mantle progressing toward complete loss of cellular architecture.

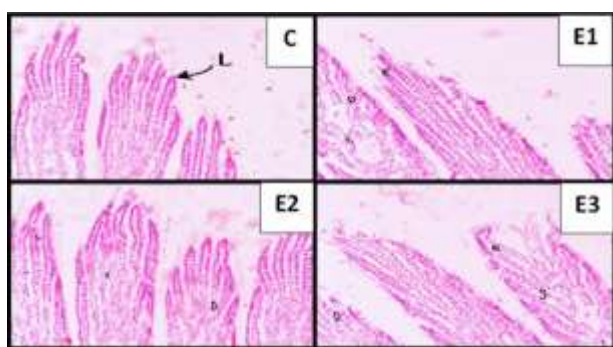


Fig. 1. Effect of coal tar on histopathology of the gill section. C - Control: basic structure showing lamellae. E1 - Exp. 1: V, vacuolated regions; Tr, tissue rupture; D, distortion. E2 - Exp. 2: NE, necrotic erosion; V, vacuolated regions; Tr, tissue rupture; D, distortion. E3 - Exp. 3: S, sloughing (shedding of dead skin); NE, necrotic erosion.

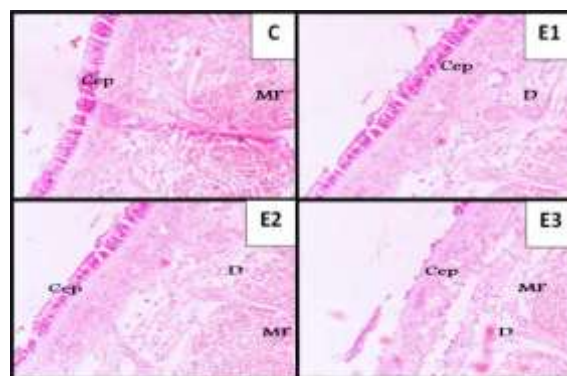


Fig. 2. Effect of coal tar on histopathology of the mantle tissue. A - Control: magnified portion of mantle tissue with normal arrangement of muscle fibres and columnar epithelial cells. B - Exp. 1: Cep, slightly ruptured columnar epithelial cells; D, distortion of the tissue. C - Exp. 2: Cep, ruptured columnar epithelial cells; D, distortion of the tissue; MF, shrunken muscle fibres. D - Exp. 3: Cep, completely damaged columnar epithelial cells; D, distorted tissue; MF, fragmented muscle fibres.

B. Effect of Coal Tar on Biomolecule Content and Enzyme Activity

Biomolecule concentrations and enzyme activities in gill and mantle tissue were significantly altered following 28 days of coal tar exposure, with the direction and magnitude of change varying by parameter, tissue, and dose (Table 1; Figs. 3-16).

Total protein content increased significantly in a dose-dependent manner in the mantle of Experimental groups 2 and 3 ($F = 25.82$, $P \leq 0.01$), while in the gill a comparatively modest but significant increase was observed only in Experimental group 3 ($F = 4.694$, $P \leq 0.01$), with Experimental groups 1 and 2 showing no significant change (Figs. 3, 4). Total carbohydrate content, in contrast, declined significantly with dose, reaching significance in the mantle of Experimental group 3 only ($F = 7.780$, $P \leq 0.05$), and in the gill of Experimental group 2 only ($F = 5.529$, $P \leq 0.05$) (Figs. 5, 6). Free sugar content showed a significant, dose-dependent decrease across all experimental groups in both mantle ($F = 5.613$, $P \leq 0.001$) and gill tissue ($F = 4.213$, $P \leq 0.001$), representing the most consistent biomolecule response across tissues (Figs. 9, 10).

Reduced glutathione levels rose significantly with dose in all experimental groups of the mantle ($F = 1.16$, $P \leq 0.01$), whereas in the gill the increase reached significance only in Experimental groups 2 and 3 ($F = 13.43$, $P \leq 0.01$) (Figs. 7, 8). Catalase activity likewise

increased significantly with dose in Experimental groups 2 and 3 of both mantle ($F = 6.542$, $P \leq 0.001$) and gill tissue ($F = 9.035$, $P \leq 0.001$), with Experimental group 1 remaining unaffected in either tissue (Figs. 11, 12).

The two ATPase isoforms responded in opposite directions. Na^+/K^+ -ATPase activity decreased significantly with dose, reaching significance in the mantle of Experimental group 3 alone ($F = 5.518$, $P \leq 0.05$) but in the gill of both Experimental groups 2 and 3 ($F = 44.55$, $P \leq 0.001$), indicating greater sensitivity of gill ion-transport machinery to coal tar exposure (Figs. 13, 14). Mg^{2+} -ATPase activity, conversely, increased significantly with dose across all experimental groups in both mantle ($F = 66.07$, $P \leq 0.001$) and gill tissue ($F = 52.41$, $P \leq 0.001$) (Figs. 15, 16).

TABLE 1. SUMMARY OF DOSE-DEPENDENT CHANGES IN BIOMOLECULE CONCENTRATION AND ENZYME ACTIVITY IN GILL AND MANTLE TISSUE OF *POLYMESODA EROSA* FOLLOWING 28-DAY COAL TAR EXPOSURE.

Parameter	Tissue	Trend	Groups significant	F-value	P-value
Total protein	Mantle	Increase	Exp. 2, Exp. 3	25.82	≤ 0.01
Total protein	Gill	Increase	Exp. 3	4.694	≤ 0.01
Total carbohydrate	Mantle	Decrease	Exp. 3	7.780	≤ 0.05
Total carbohydrate	Gill	Decrease	Exp. 2	5.529	≤ 0.05
Free sugars	Mantle	Decrease	Exp. 1, 2, 3	5.613	≤ 0.01
Free sugars	Gill	Decrease	Exp. 1, 2, 3	4.213	≤ 0.01
Reduced glutathione	Mantle	Increase	Exp. 1, 2, 3	1.16	≤ 0.01
Reduced glutathione	Gill	Increase	Exp. 2, Exp. 3	13.43	≤ 0.01
Catalase	Mantle	Increase	Exp. 2, Exp. 3	6.542	≤ 0.01

Catalase	Gill	Increase	Exp. 2, Exp. 3	9.035	≤ 0.01
Na^+/K^+-ATPase	Mantle	Decrease	Exp. 3	5.518	≤ 0.05
Na^+/K^+-ATPase	Gill	Decrease	Exp. 2, Exp. 3	44.55	≤ 0.01
Mg^{2+}-ATPase	Mantle	Increase	Exp. 1, 2, 3	66.07	≤ 0.01
Mg^{2+}-ATPase	Gill	Increase	Exp. 1, 2, 3	52.41	≤ 0.01

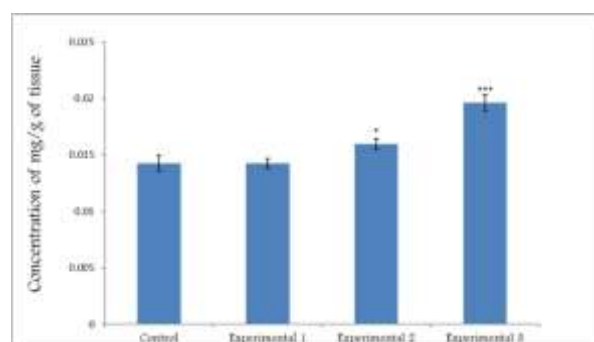


Fig. 3. Effect of coal tar on total protein content in the mantle of the bivalve. NS, non-significant; * $P \leq 0.05$, significant; *** $P \leq 0.001$, highly significant.

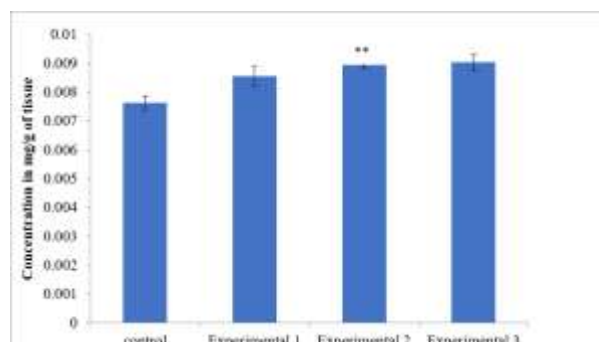


Fig. 4. Effect of coal tar on total protein content in the gills of the bivalve. NS, non-significant; * $P \leq 0.05$, significant.

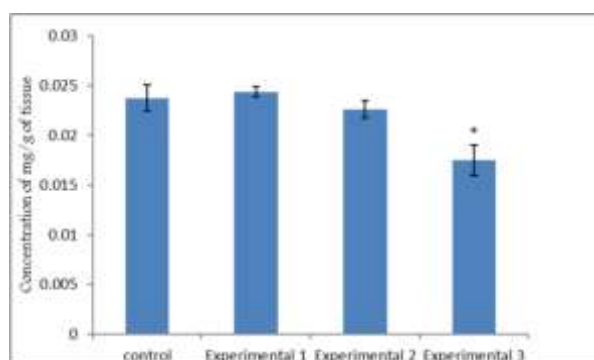


Fig. 5. Effect of coal tar on total carbohydrate content in the mantle of the bivalve. NS, non-significant; * $P \leq 0.05$, significant.

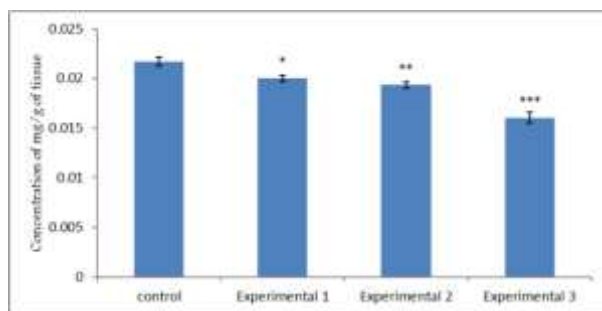


Fig. 6. Effect of coal tar on total carbohydrate content in the gills of the bivalve. NS, non-significant; * $P \leq 0.05$, significant; ** $P \leq 0.01$, highly significant; *** $P \leq 0.001$, very highly significant.

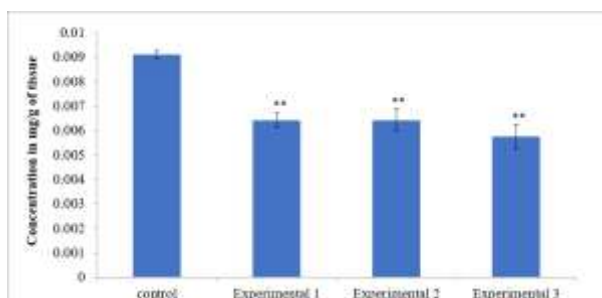


Fig. 7. Effect of coal tar on reduced glutathione content in the mantle of the bivalve. * $P \leq 0.05$, significant; ** $P \leq 0.01$, highly significant.

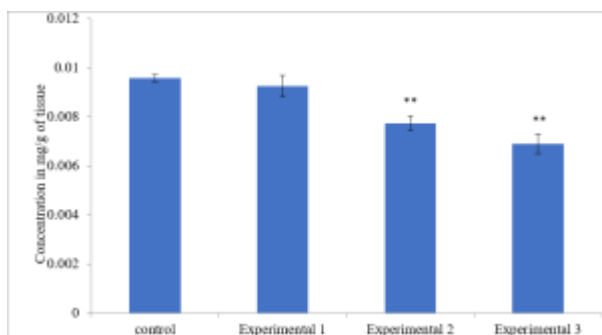


Fig. 8. Effect of coal tar on reduced glutathione content in the gills of the bivalve. NS, non-significant; * $P \leq 0.05$, significant; ** $P \leq 0.01$, highly significant.

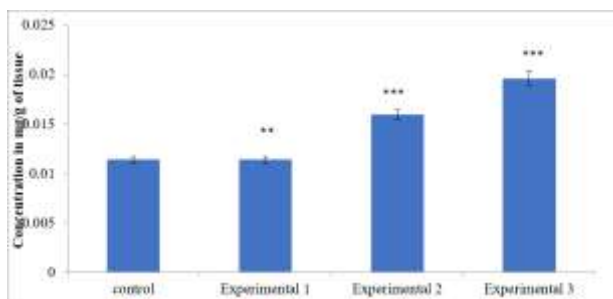


Fig. 9. Effect of coal tar on free sugars content in the mantle of the bivalve. * $P \leq 0.05$, significant; ** $P \leq 0.01$, highly significant.

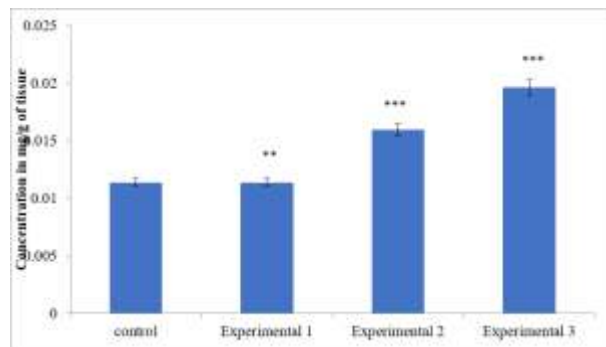


Fig. 10. Effect of coal tar on free sugars content in the gill of the bivalve. * $P \leq 0.05$, significant; ** $P \leq 0.01$, highly significant.

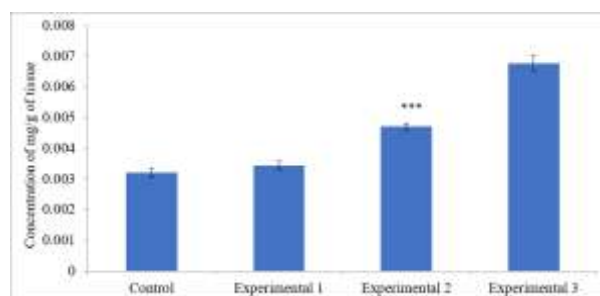


Fig. 11. Effect of coal tar on catalase activity in the mantle of the bivalve. NS, non-significant; * $P \leq 0.05$, significant; ** $P \leq 0.01$, highly significant.

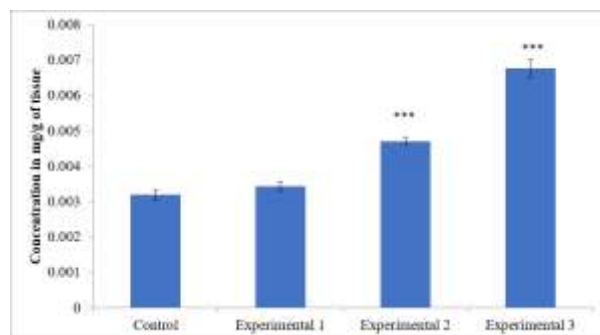


Fig. 12. Effect of coal tar on catalase activity in the gills of the bivalve. NS, non-significant; * $P \leq 0.05$, significant; ** $P \leq 0.01$, highly significant.

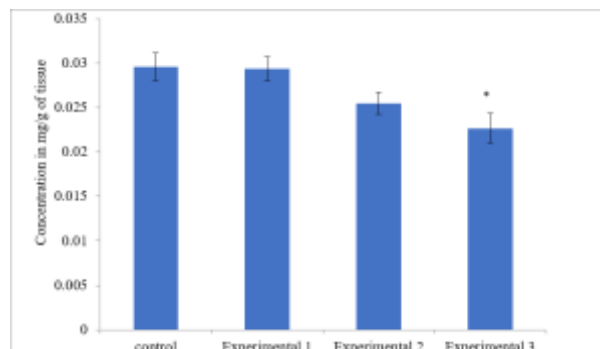


Fig. 13. Effect of coal tar on Na^+/K^+ -ATPase activity in the mantle of the bivalve. NS, non-significant; * $P \leq 0.05$, significant.

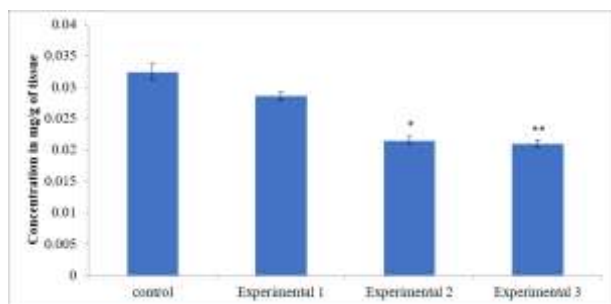


Fig. 14. Effect of coal tar on Na^+/K^+ -ATPase activity in the gill of the bivalve. NS, non-significant; * $P \leq 0.05$, significant; ** $P \leq 0.001$, highly significant.

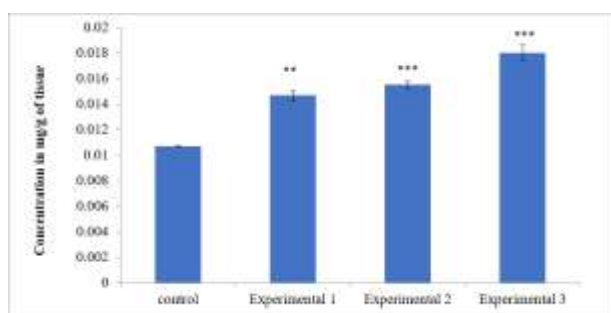


Fig. 15. Effect of coal tar on Mg^{2+} -ATPase activity in the mantle of the bivalve. NS, non-significant; ** $P \leq 0.001$, highly significant.

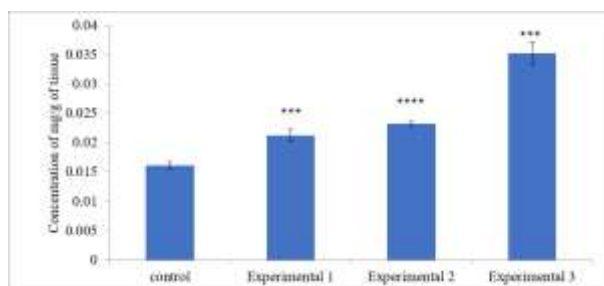


Fig. 16. Effect of coal tar on Mg^{2+} -ATPase activity in the gill of the bivalve. ** $P \leq 0.001$, highly significant; *** $P \leq 0.001$, highly significant.

C. Effect of Coal Tar on Respiratory and Excretory Rate

Oxygen consumption declined significantly over the 28-day exposure period ($F = 265.4$, $P \leq 0.001$) and with increasing coal tar dose ($F = 603.5$, $P \leq 0.001$), with a significant day $\times$ dose interaction ($F = 3.863$, $P \leq 0.001$), indicating a progressive suppression of respiratory activity with both exposure duration and concentration (Fig. 17). Excretory rate showed the inverse trend, increasing significantly with exposure duration ($F = 321.5$, $P \leq 0.001$) and dose ($F = 51.6$, $P \leq 0.001$), with a significant day $\times$ dose interaction ($F = 3.125$, $P \leq 0.001$) (Fig. 18). Together, the decline in oxygen uptake and concomitant rise in ammonia excretion

point to a coal-tar-induced disruption of normal respiratory-excretory physiology in *P. erosa*.

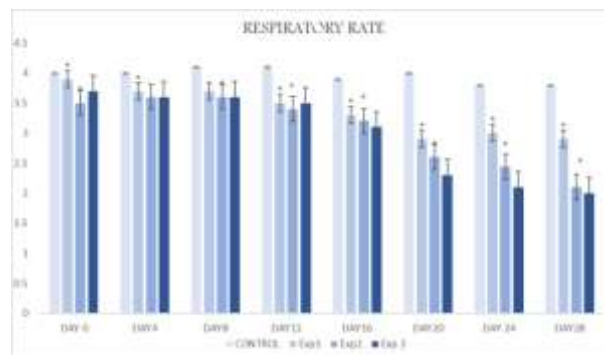


Fig. 17. Effect of coal tar on oxygen consumption. NS, non-significant; * $P \leq 0.05$, significant.

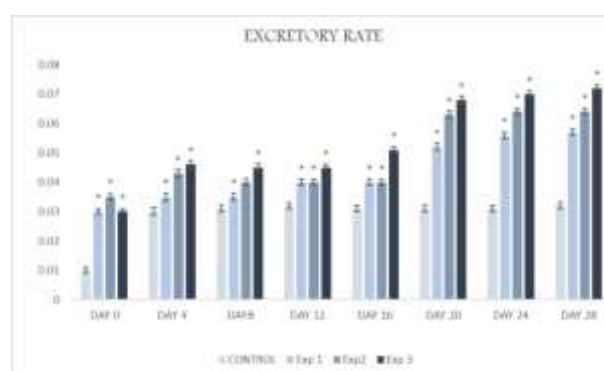


Fig. 18. Effect of coal tar on excretion rate. NS, non-significant; * $P \leq 0.05$, significant.

IV. DISCUSSION

PAHs derived from coal tar possess ring structures and physicochemical properties that favour persistence and bioaccumulation, and the present study demonstrates that this translates into measurable, interrelated toxicity at the histological, biochemical, and physiological levels in *Polymesoda erosa*. Because the gill and mantle are the two tissues in most direct and continuous contact with the surrounding water column the gill through its filtration of large water volumes for nutrient capture, and the mantle through its protective and respiratory role for internal organs (Canesi et al., 2010) both were expected, and confirmed, to be primary targets of coal tar toxicity, with the severity and nature of the response in each tissue offering complementary evidence of a common underlying stress.

The histopathological findings provide the clearest structural evidence of this damage. Gill sections progressed from vacuolation and early

tissue rupture to necrotic erosion and, at the highest dose, sloughing of the dead epithelial layer a pattern of injury consistent with that reported in Manila clams subjected to thermal stress (Menike et al., 2013), suggesting that coal tar exposure activates cellular damage pathways broadly comparable to other forms of environmental stress rather than a wholly PAH-specific injury cascade. The mantle showed a parallel dose-dependent trajectory, from mild rupture of columnar epithelial cells to complete epithelial disruption and fragmentation of muscle fibres, findings that align with prior reports of PAH-induced bivalve histopathology (El-Shenawy et al., 2008; Al-Hashem, 2017). Because gill filaments are ciliated structures responsible for generating the feeding currents on which the animal depends, and the mantle simultaneously mediates protection and gas exchange (Gosling, 2008), damage of this severity in both tissues implies compounding, rather than independent, functional impairment a link borne out by the biochemical and physiological results that follow.

At the biochemical level, the significant depletion of total carbohydrate and free sugar reserves in both gill and mantle points to an energetic cost of coal tar exposure: glucose and glycogen reserves appear to have been mobilised to meet elevated metabolic demand under toxicant stress, consistent with the interpretation offered for other pollutant-stressed bivalves (Venkateshwarlu et al., 1995) and comparable to biochemical disruption reported in the freshwater bivalve *Lamellidens marginalis* exposed to mercuric chloride (Muniv et al., 2020). The concurrent, dose-dependent rise in total protein content in both tissues is consistent with this energy-mobilisation narrative rather than contradicting it: proteins are central to gill ion and gas exchange function, and their accumulation likely reflects a shift toward increased protein anabolism and reduced catabolism as the tissue attempts to meet stress-induced cellular demands, potentially at some cost to the mantle's biomineralising capacity (Muniv et al., 2020; Freer et al., 2014). A comparable elevation in tissue protein content following metal exposure (HgCl_2 , CuSO_4) has been reported across gonad, gill, and hepatopancreas tissue in other bivalves (Mahajan et al., 2001), lending support to protein upregulation as a conserved stress response rather than an artefact specific to this study.

The antioxidant response reinforces this picture of oxidative stress. Catalase and reduced glutathione act jointly to neutralise reactive oxygen species

(ROS) and limit peroxidative membrane damage, and both showed significant, dose-dependent increase in gill and mantle tissue, most pronounced in the gill consistent with its greater direct exposure to coal-tar-derived contaminants relative to the mantle (Yiin and Lin, 1995; Canesi et al., 2012). This antioxidant upregulation mirrors findings in the brown mussel *Perna perna* following lead exposure, where catalase activity rose significantly under metal stress (Boudjema et al., 2014), further supporting oxidative stress as a common toxicological mechanism across structurally distinct contaminants.

These cellular and biochemical disruptions are reflected at the whole-organism level in the respiratory and excretory responses. Oxygen consumption declined significantly with both exposure duration and coal tar dose, indicating progressive suppression of aerobic metabolism; this is consistent with reports that oxygen consumption in bivalves is inversely related to metabolic burden, with disproportionately higher metabolic demand in smaller individuals driving greater relative oxygen use under stress (Pourmozaffar et al., 2019). Concurrently, excretory (ammonia) output rose significantly with dose and duration ammonia being the principal nitrogenous end-product of protein catabolism in aquatic invertebrates and highly diffusible in seawater a pattern similarly linked by Pourmozaffar et al. (2019) to the elevated energetic cost of maintaining physiological homeostasis under stress. Together, declining respiration and rising excretion describe an organism reallocating limited metabolic resources toward compensatory and detoxification demands at the expense of routine metabolic function.

The ionoregulatory results add a further dimension to this compensatory picture. Na^+/K^+ -ATPase activity decreased while Mg^{2+} -ATPase activity increased across experimental groups in both tissues, mirroring reductions in Na^+ and K^+ levels reported alongside compensatory increases in Mg^{2+} concentration in other bivalve osmoregulatory studies (Lima et al., 2012). This inverse relationship suggests that the observed decline in Na^+/K^+ -ATPase-mediated ion transport may represent a compensatory mechanism to preserve internal osmotic balance as Mg^{2+} -dependent processes

are upregulated, rather than a simple, undirected loss of ionoregulatory capacity.

Taken together, the histopathological, biochemical, and physiological findings of this study converge on a consistent toxicological narrative: coal tar exposure in *P. erosa* triggers oxidative stress and structural tissue damage in the gill and mantle, which in turn drives compensatory shifts in energy reserves, protein metabolism, and ion regulation, ultimately manifesting as suppressed respiration and elevated nitrogenous excretion at the organismal level. Given that *P. erosa* is both an ecologically significant filter-feeder and an economically important food source for coastal communities such as those in Chorao, Tiswadi, Goa, the deterioration in health documented here carries implications beyond the individual organism extending to bivalve population viability, broader estuarine food-web integrity, and potentially to the health and livelihood of communities dependent on this resource.

V. CONCLUSION

This study provides the first evidence that coal tar, one of the most concentrated and chemically complex sources of PAHs, inflicts serious, dose-dependent damage on the mangrove clam *Polymesoda erosa*. Within just 28 days of exposure, coal tar drove progressive necrosis and structural collapse in gill and mantle tissue, disrupted energy reserves and protein metabolism, forced antioxidant systems into overdrive, destabilised ion regulation, and suppressed the animal's very ability to breathe and excrete waste normally. That a single pollutant can compromise an organism at every level from cell to tissue to whole-body physiology within such a short exposure window highlights how little resilience these bivalves have against sustained coal tar contamination. *P. erosa* is a keystone filter-feeder and a food source that coastal communities such as those in Chorao, Tiswadi, Goa, depend on directly. Continued or expanding coal tar contamination of these habitats, whether from legacy manufactured-gas-plant residues or ongoing sources such as coal-tar-based sealants and paints, places bivalve populations, estuarine food webs, and ultimately human health and livelihoods on a trajectory toward silent, cumulative harm. The absence of prior toxicological data on coal tar in this species is itself a warning: a widely consumed, ecologically vital organism has been at risk without documentation until now. Future work should extend this exposure period to assess whether these

effects intensify, plateau, or become irreversible over longer timescales, and should quantify tissue-level PAH bioaccumulation and depuration kinetics directly. Molecular investigations into oxidative stress and apoptotic pathways, alongside reproductive and developmental endpoints, would clarify whether coal tar threatens not just individual health but population persistence. Parallel human health risk assessments, evaluating PAH transfer through consumption of contaminated bivalves, are urgently needed to translate these findings into actionable guidance for coastal communities and regulatory bodies alike.

VI. REFERENCES

- Abdel-Shafy, H. I., & Mansour, M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, 25(1), 107–123.
- Al-Hashem, M. A. (2017). Gill histopathological effects of PAHs on adult pearl oyster, *Pinctada radiata* at Al-Khiran coast in Kuwait. *Journal of Environmental Protection*, 8(2), 109–119.
- Au, D. W. T. (2004). The application of histo-cytopathological biomarkers in marine pollution monitoring: A review. *Marine Pollution Bulletin*, 48, 817–834.
- Azis, M. Y., Setiyanto, H., Salim, A., Vita Hidayati, N., Asia, L., Piram, A., Doumenq, P., & Syakti, A. D. (2021). Evidence of micropollutants in sediment and mud clams (*Polymesoda erosa*) from one of mangrove biodiversity hotspots in Indonesia. *Polycyclic Aromatic Compounds*, 42(7), 4448–4465.
- Batchamen Mougnot, J. B., Waanders, F., Fosso-Kankeu, E., & Al Alili, A. R. (2022). Leaching of polycyclic aromatic hydrocarbons from the coal tar in sewage wastewater, acidic and alkaline mine drainage. *International Journal of Environmental Research and Public Health*, 19(8), 4791.
- Bickers, D. R., & Kappas, A. (1978). Human skin aryl hydrocarbon hydroxylase: Induction by coal tar. *Journal of Clinical Investigation*, 62(5), 1061–1068.
- Boudjema, K., Kourdali, S., Bounakous, N., Meknachi, A., & Badis, A. (2014). Catalase activity in brown mussels (*Perna perna*) under acute cadmium, lead, and copper exposure and depuration tests. *Journal of Marine Biology*, 2014, 1–9.
- Bryer, P. J., Scoggins, M., & McClintock, N. L. (2010). Coal-tar based pavement sealant toxicity to freshwater macroinvertebrates. *Environmental Pollution*, 158(5), 1932–1937.
- Burchiel, S. W., & Gao, J. (2014). Polycyclic aromatic hydrocarbons and the immune system. In H.-W. Vohr (Ed.), *Encyclopedia of immunotoxicology*. Springer.
- Canesi, L., Ciacci, C., Fabbri, R., Marcomini, A., Pojana, G., & Gallo, G. (2012). Bivalve molluscs as a unique target group for nanoparticle toxicity. *Marine Environmental Research*, 76, 16–21. <https://doi.org/10.1016/j.marenvres.2011.06.005>
- Canesi, L., Fabbri, R., Gallo, G., Vallotto, D., Marcomini, A., & Pojana, G. (2010). Biomarkers in *Mytilus galloprovincialis*

- exposed to suspensions of selected nanoparticles (nano carbon black, C60 fullerene, nano-TiO₂, nano-SiO₂). *Aquatic Toxicology*, 100, 168–177.
- Carroll, N. V., Longley, R. W., & Roe, J. H. (1955). The determination of glycogen in liver and muscle by use of anthrone reagent. *Journal of Biological Chemistry*, 220(2), 583–593.
- Chaney, A. L., & Marbach, E. P. (1962). Modified reagents for determination of urea and ammonia. *Clinical Chemistry*, 8(2), 130–132.
- Chiovatto, A. C. L., et al. (2021). Effects of substances released from a coal tar-based coating used to protect harbor structures on oysters. *Marine Pollution Bulletin*, 166, Article 112221. <https://doi.org/10.1016/j.marpolbul.2021.112221>
- Cruz, T. C., Nayak, G. N., Tiwari, A. K., & Nasnodkar, M. R. (2020). Assessment of metal pollution and bioaccumulation of metals by edible bivalve *Polymesoda erosa* in the Zuari Estuary, west coast of India. *Marine Pollution Bulletin*, 158, Article 111415.
- Daud, A., Mallongi, A., Bahar, B., & Hidayat. (2021). Mussel fish (*Polymesoda erosa*) and microplastics in Tallo River, Makassar, Indonesia. *Indian Journal of Forensic Medicine & Toxicology*, 15(3), 4539–4546.
- El-Shenawy, N. S., Moawad, T. I. S., Mohallal, M. E., Abdel-Nabi, I. M., & Taha, I. A. (2009). Histopathologic biomarker response of clam, *Ruditapes decussatus*, to organophosphorous pesticides Reldan and Roundup: Laboratory study. *Ocean Science Journal*, 44, 27–34.
- Fiske, C. H., & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry*, 66(2), 375–400.
- Freer, A., Bridgett, S., Jiang, J., & Cusack, M. (2014). Biomineral proteins from *Mytilus edulis* mantle tissue transcriptome. *Marine Biotechnology*, 16, 34–45.
- Gallardi, D. (2014). Effects of bivalve aquaculture on the environment and their possible mitigation: A review. *Fisheries and Aquaculture Journal*, 5, Article 105.
- Ghoshal, S., Ramaswami, A., & Luthy, R. G. (1996). Biodegradation of naphthalene from coal tar and heptamethylnonane in mixed batch systems. *Environmental Science & Technology*, 30(4), 1282–1291.
- Gosling, E. (2008). *Bivalve molluscs: Biology, ecology and culture*. John Wiley & Sons.
- Honda, M., & Suzuki, N. (2020). Toxicities of polycyclic aromatic hydrocarbons for aquatic animals. *International Journal of Environmental Research and Public Health*, 17(4), 1363.
- Illangasekare, T. H., Ramsey, J. L., Jr., Jensen, K. H., & Butts, M. B. (1995). Experimental study of movement and distribution of dense organic contaminants in heterogeneous aquifers. *Journal of Contaminant Hydrology*, 20(1–2), 1–25.
- Lima, A. L. C., Farrington, J. W., & Reddy, C. M. (2005). Combustion-derived polycyclic aromatic hydrocarbons in the environment—A review. *Environmental Forensics*, 6(2), 109–131.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265–275.
- Mackay, D., & Callcott, D. (1998). Partitioning and physical chemical properties of PAHs. In A. H. Neilson (Ed.), *PAHs and related compounds* (The handbook of environmental chemistry, Vol. 3-I, pp. 325–345). Springer-Verlag.
- Mahajan, & Zambare. (2001). Ascorbate effect on copper sulphates and mercuric chloride induced alternations of protein level in freshwater bivalve, *Corbicula striolata*. *Asian Journal of Microbiology, Biotechnology and Environmental Science*, 3(2-1), 95–100.
- McIntyre, A. D. (1992). The current state of the oceans. *Marine Pollution Bulletin*, 25, 1–4, 28–31.
- Menike, U., Lee, Y., Oh, C., Wickramaarachchi, W. D. N., Premachandra, H. K. A., Park, S. C., Lee, J., & De Zoysa, M. (2014). Oligo-microarray analysis and identification of stress-immune response genes from Manila clam (*Ruditapes philippinarum*) exposure to heat and cold stresses. *Molecular Biology Reports*, 41, 6457–6473.
- Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta*, 582(1), 67–78.
- Muniv, Y., Supanekar, S., & Khan, A. (2020). Biochemical studies on fresh water bivalves exposed to the mercury chloride. *Asian Journal of Advances in Research*, 11–17.
- Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry*, 153(2), 375–380.
- Nijsten, T. E. C., & Stern, R. S. (2003). The increased risk of skin cancer is persistent after discontinuation of psoralen+ultraviolet A: A cohort study. *Journal of Investigative Dermatology*, 121(2), 252–258.
- Peters, C. A., & Luthy, R. G. (1993). Coal tar dissolution in water-miscible solvents: Experimental evaluation. *Environmental Science & Technology*, 27(13), 2831–2843.
- Pourmozaffar, S., Tamadoni Jahromi, S., Rameshi, H., Sadeghi, A., Bagheri, T., Behzadi, S., & Abrari Lazarjani, S. (2020). The role of salinity in physiological responses of bivalves. *Reviews in Aquaculture*, 12(3), 1548–1566.
- Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., & Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine*, 5(3), 182–189.
- Roelofzen, J. H., Aben, K. K., Van Der Valk, P. G., Van Houtum, J. L., van de Kerkhof, P. C., & Kiemeneij, L. A. (2007). Coal tar in dermatology. *Journal of Dermatological Treatment*, 18(6), 329–334.
- Rohalin, W. M., Yaakub, N., & Nordin, R. H. (2019). Bioconcentration of cadmium and nickel in mud clams (*Polymesoda expansa*) at Sungai Balok, Pahang. *Journal of Applied Science & Process Engineering*, 6(2), 362–368.
- Roy, R., & Dattatraya Pujari, P. (2010). Diets rich in n-3 polyunsaturated fatty acids are associated with amelioration of D-galactosamine induced hepatitis in mice. *Recent Research in Science and Technology*, 2(1). www.recent-science.com
- Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*, 47(2), 389–394. [https://doi.org/10.1016/0003-2697\(72\)90132-7](https://doi.org/10.1016/0003-2697(72)90132-7)
- Thuy, H. T. T., Loan, T. T. C., & Phuong, T. H. (2018). The potential accumulation of polycyclic aromatic hydrocarbons in phytoplankton and bivalves in Can Gio coastal wetland, Vietnam.

Environmental Science and Pollution Research, 25(18), 17240–17249.

Varanasi, U., Stein, J. E., Reichert, W. L., Tilbury, K. L., Krahn, M. M., & Chan, S.-L. (1992). Chlorinated and aromatic hydrocarbons in bottom sediments, fish and marine mammals in US coastal waters: Laboratory and field studies of metabolism and accumulation. In C. H. Walker & D. R. Livingstone (Eds.), *Persistent pollutants in marine ecosystems* (pp. 83–115). Pergamon Press.

Venkateshwarlu, M., & Sunita, A. (1995). Impact of monocrotophos on tissue carbohydrates of the freshwater crab, *Barytelphusa guerini* (M. Edwards). *Indian Journal of Comparative Animal Physiology*, 13, 35–36.

Wang, H., et al. (2020). Occurrence and potential health risks assessment of polycyclic aromatic hydrocarbons (PAHs) in different tissues of bivalves from Hainan Island, China. *Food and Chemical Toxicology*, 136, Article 111108. <https://doi.org/10.1016/j.fct.2019.111108>

Welsh, J. H., & Smith, R. I. (1960). *Laboratory exercises in invertebrate physiology*. Burgess Publishing Company.

Yender, R., Michel, J., & Lord, C. (2002). *Managing seafood safety after an oil spill*. Hazardous Materials Response Division, Office of Response and Restoration, National Oceanic and Atmospheric Administration.

Yiin, S. J., & Lin, T. H. (1995). Lead-catalyzed peroxidation of essential unsaturated fatty acid. *Biological Trace Element Research*, 50, 167–172.

Yusoff, A. H., et al. (2021). Heavy metals in marsh clam (*Polymesoda expansa*) as bioindicators for pollution in industrial and sand mining area of Kelantan River Basin, Malaysia. *Trends in Sciences*, 18(20), 1–9.

CRediT authorship contribution statement

Feazel R. Dias: Investigation, Methodology, Formal analysis; Sarita Rebelo: Methodology, Formal analysis, and writing original draft; Shamshad M. Shaikh: Validation, Writing, review & editing, Supervision, and Project Administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge Goa University, DST-FIST (Department of Science & Technology-Fund for Improvement of S&T Infrastructure), and RUSA (Rashtriya Uchchatar Shiksha Abhiyan) for providing infrastructure.