

Impact of Coastal Pollution on Microbial and Mineral Profile of Edible Oyster (*Crassostrea rivularis*) in the Coastal Waters of Andaman

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Abstract The impact of coastal pollution was studied using edible oysters, *Crassostrea rivularis* as an indicator at two sites viz., North Wandoor (NW) and Phoenix Jetty (PJ) in Port Blair, Andaman. The hydrographic parameters showed that nitrite, nitrate and phosphate concentration were less and dissolved oxygen were more at NW compared to PJ. The oysters were collected from the study sites and biochemical, microbial, mineral profiles and ATPase activities were estimated. ATPase activity was inhibited in the gill tissue of oysters ($p < 0.05$) of PJ sample. Total microbial load in the water and oyster, and coliform bacteria (MPN) in the water were significantly ($p < 0.05$) higher at PJ compared to the NW. There was no significant difference ($p > 0.05$) in the mineral profile of water collected from both the sites. However, calcium and magnesium were more in the oysters collected from NW ($p < 0.05$), and Cu, Zn and Cd were more in PJ samples ($p < 0.05$).

Keywords Oyster · *Crassostrea rivularis* · ATPases · Coliform bacteria · Mineral · Pollution

Andaman and Nicobar groups of Islands, situated in the Bay of Bengal sea, is reconized for its pristine environmental conditions, vast coral reef ecosystem, rich species biodiversity harbouring various life forms. In recent times due to rapid urbanization and increased human influx, lots of

sewages are generated and ultimately the quality of sea water is deteriorated. Polluted effluents have adverse effects on coastal and marine ecosystems (Bose et al. 2012). Microbial profile in coastal environment is an essential and integral parameter to predict coastal pollution (Swarnakumar et al. 2008). Pollution of aquatic ecosystem by heavy metals has raised significant concern due to increased industrial and mining activities. Many aquatic organisms have the tendency to accumulate heavy metals and pesticides present in the effluents and deposit into their body. Bioaccumulation of metals in aquatic organisms can have longterm implications on the human health and ecosystem (Fernandes et al. 2007; Xia et al. 2011). Biochemical responses in aquatic organisms have been used in several environmental monitoring programs to characterize anthropogenic pollution (Burgeo et al. 1996; Cajaraville et al. 2000). Many biomarkers are now extensively being used for pollution monitoring programme (Livingstone 1993; Kaaya et al. 1999). Oysters are sensitive aquatic organisms, extensively used as bio-indicators for environmental monitoring programme (Domingos et al. 2007; Ricciardi et al. 2006). Oyster (*C. rivularis*) is a sessile filter feeding aquatic organism distributed throughout the Andaman waters. The present study has been undertaken in order to assess the impact of city effluents on water and oysters (*C. rivularis*) collected from Phoenix Jetty (PJ), a polluted area and compare it with an unpolluted and undisturbed site [North Wandoor (NW)] with least human interference.

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Materials and Methods

The Andaman and Nicobar Islands (ANI) are the largest archipelago in Bay of Bengal (BoB) situated between 14°–16° N latitude and 92°–94° E longitude. Biodiversity

of these islands is high due to its climatic condition, location and rich biotic resources. Three dominant species of edible oysters (*Saccostrea cucullata*, *Crassostrea rivularis* and *C. gryphoides*) are found in Andaman and Nicobar Island (ANI) (Ahlawat et al. 2002). Among them *C. rivularis* is widely distributed in the coastal waters of ANI, and hence, *C. rivularis* has been selected as bio-indicator in the present study. Port Blair, the capital city of ANI, generates large quantities of sewage and other effluents, which are discharged into the sea through small drainage channels. PJ (92°44'12.96"E, 11°40'27.63"N) is not only the discharge point of sewage, but also a harbour area where inter-island ships are stationed, repaired and dry dock activities are performed. NW (92°36'59.53"E, 11°36'43.24"N) is located about 30 km from the PJ adjacent to marine protected areas rich in coral reef and is relatively undisturbed.

The samples (water and oysters) were collected during high tide from two different sites for analysis during February to May 2011. Around 35 oysters were collected from each site and used for the experiment. The oysters were removed slowly using a shovel and scupper and brought to the laboratory in labeled plastic bags placed in an ice chest in aerated condition. Shell length, height and meat yield were measured to the nearest 0.1 mm using a vernier caliper, and each specimen was weighed in grams (total wet weight). The water samples were collected in plastic bottles and brought to the laboratory in an ice box. Temperature, pH, salinity, alkalinity and turbidity of the water samples were analyzed in situ using a thermometer, pH meter and Model 611 Intelligent Water Quality Analyzer. Dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), phosphate, nitrite and nitrate were estimated following APHA, (1998). For the mineral estimation, water and oyster tissue samples were prepared by the nitric acid digestion method (APHA, 1998). The final samples were analyzed using Atomic absorption spectroscopy and Flame photometer model 1381E.

For estimating ATPase activities in gill and mantle tissues, the oysters were dissected out, weighed and homogenized in chilled sucrose (0.25 M) using Teflon coated mechanical homogenizer. 5 % homogenate was prepared on wet weight basis. Centrifugation was done at 5000 rpm for 10 min at 4°C. The supernatant was transferred to sample vials and stored at -20°C. The whole procedure was done under ice cold condition. The supernatant was used for estimation of enzyme activity. ATPase activity was assayed following the method of Cotou et al. (2001).

For total culturable count (TCC), serial dilution was done followed by spread plating. The nutrient agar plates (Lapage et al. 1970) were incubated at 37°C for 24 h and

the colonies were counted in colony counter after incubation. The coliform bacteria in water samples were enumerated following MPN method (APHA 1998; Oblinger and Koburger 1975). The microbiological aspect of the study was performed under absolute aseptic condition in order to minimize contamination and false results.

The data obtained were subjected to student 't' test using statistical package, SPSS (ver. 11) to find out the differences in treatment means at $p < 0.05$. Also average monthly values were used for *t* test to determine significant difference between two locations (NW and PJ) and two way ANOVA test was used to determine significant difference within locations as well as among different months (Fig. 1).

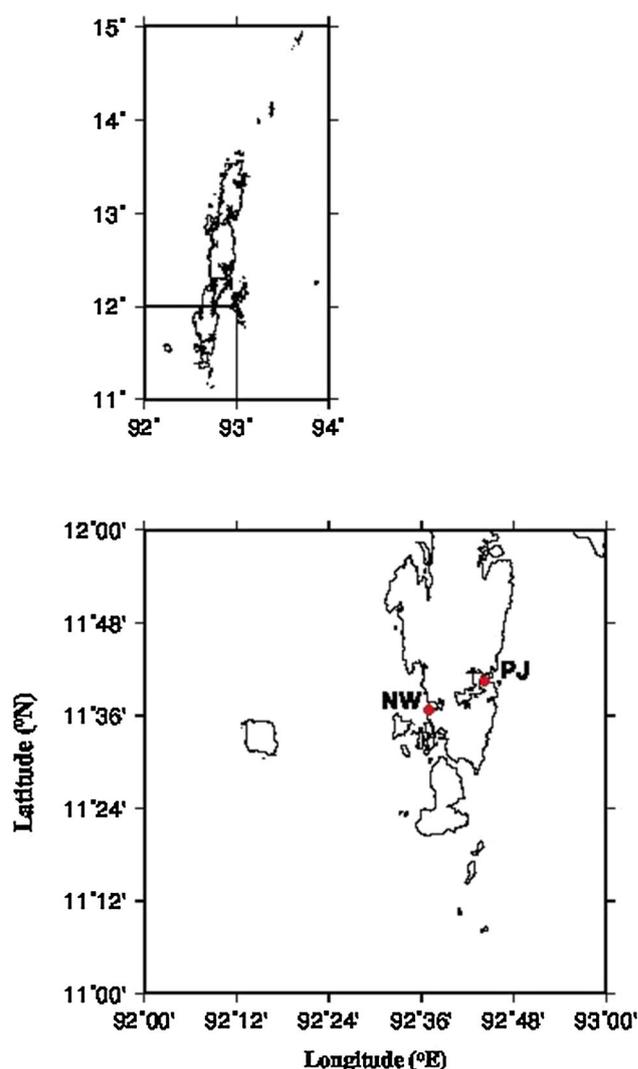


Fig. 1 Water and oyster sampling sites at Phoenix Jetty (PJ) and North Wandoor (NW)

Results and Discussion

Biochemical responses of oysters and mussels to pollutants are used in several environmental monitoring programs (Romeo et al. 2003; Domingos et al. 2007). In the present study, *C. rivularis* was selected for the study owing to their wide distribution in the coastal waters of ANI.

Data on the length and weight of *C. rivularis* collected from PJ and NW are presented in Table 1. In North Wandoor, the size of oysters ranged between 75 and 155 mm and in Phoenix Jetty, it was between 61 and 110 mm and are legal harvestable size for human consumption. There were significant differences ($p < 0.05$) in the length and weight of oysters collected from both sites. Oysters collected from NW were clean, bright and bigger in size (both length and weight) unlike those from PJ which had debris on the shell top, dark in colour and smaller in size. The darker colour of oyster was due to exogenous materials deposited above the shell of the oysters such as clay, silt as well as algae. Other biological parameters such as width, average meat yield, percentage of shell yield and, percentage of meat yield were also found to be higher in oysters from NW compared to those from PJ.

Adenosine triphosphatase (ATPases) is a membrane-bound enzyme responsible for immediate release of energy (Smitha and Philip 2011) and plays a strategic role in regulating oxidative phosphorylation, ionic transport and several other membrane transport phenomena (Reddy and Philip 1994). Hence, measurement of ATPase activity can

be used as a surrogate biomarker to assess the exposure to chemical pollutants (Parvez et al. 2006). In the present study, ATPase activity in gill tissue of the oysters collected from PJ was significantly inhibited ($p < 0.05$) compared to NW oysters (Table 1). However, in mantle tissue the inhibition effect was not significant ($p > 0.05$). Domingos et al. (2007) reported that sessile organisms like oysters after settlement in the estuarine waters, were chronically exposed to several contaminants and ATPase activities in those organisms might be inhibited or enhanced. Oysters are filter feeders and their gills are constantly exposed to the outside environment for filtration and respiration, and thereby coming in contact with different pollutants present in the waters. Reduction in total ATPase activities in gill tissues due to exposure to paper mill effluent (Parvez et al. 2006), heavy metal and phenols (de la Torre et al. 2000), benzo[a]pyrene exposure (Xu et al. 2001) and lindane (Gopal et al. 1993) has already been reported. Shad et al. (2012) recorded reduction of Na^+ , K^+ -ATPase activity in the gill tissue of freshwater mussels *Elliptio complanata* on 28th day exposure to lead. Reduction in ATPase activities in oysters collected from PJ could be due to alterations in the structure and functions of the membrane or may be due to direct inhibition owing to long-term exposure of gill to stressors present in the waters.

The coastal population of India consumes large quantities of oysters. Uncooked oysters can serve as vectors for many food borne pathogenic microorganisms, including *Vibrio* (Altekruse et al. 2000; Feldhusen 2000). Even

Table 1 Biological, microbiological and enzymatic activities in oysters and waters collected from two different sites

Parameters		North Wandoor	Phoenix Jetty
<i>Biological parameters</i>			
Oyster	Length (cm)	9.86 ± 0.28 ^a (7.50–15.50)	8.57 ± 0.235 ^b (6.1–11.0)
	Weight (g)	181.35 ± 8.37 ^a (92.03–273.1)	89.34 ± 5.44 ^b (29.9–157.5)
	Width (cm)	7.89 ± 0.16 ^a (5.7–9.4)	6.93 ± 0.228 ^b (4.00–8.70)
	Average meat yield	12.273 ± 0.45 ^a	5.14 ± 0.30 ^b
	Shell yield (%)	92.92 ± 0.33	94.09 ± 0.23
	Meat yield (%)	7.08 ± 0.33	5.91 ± 0.23
<i>Microbiological parameters</i>			
Oyster	Total culturable count	3.8 × 10 ⁴ CFU mL ^{-1a}	4.6 × 10 ⁴ CFU mL ^{-1b}
Water	Total culturable count	4.3 × 10 ³ CFU mL ^{-1a}	5.1 × 10 ³ CFU mL ^{-1b}
Water	MPN	23 MPN 100 mL ^{-1a}	460 MPN 100 mL ^{-1b}
<i>ATPase activities</i>			
Oysters	Mantle	2.6 ± 0.35 ^a	1.93 ± 0.33 ^a
	Gill	4.35 ± 0.60 ^a	1.61 ± 0.30 ^b

Values are mean ± standard error. Different superscripts in a row differ significantly ($p < 0.05$). Figures in parenthesis indicate the range. CFU Colony Forming Unit. ATPase activities expressed in μg of phosphorous released/mg protein/min at 37°C (n = 6)

recreational waters may be a health hazard for bathers if several microbial pathogens such as bacteria, viruses, fungi and protozoa are present (Moe 1997). As per the Central Pollution Control Board (CPCB) (1986) total coliform organisms should be 100 MPN/100 mL or less for bathing, contact water sports and commercial fishing. Similarly, total culturable count (TCC) provides a quantitative idea about the presence of microorganisms (colony forming units) such as bacteria, yeast and mold in a sample. Thus, enumeration of the viable or metabolically active bacterial number is important for understanding the marine ecosystem (Altug and Balkis 2009). In this study, TCC was more in oyster tissue than in the water sample and the number was more in the PJ sample (Table 1) compared to NW. Oyster being a filter feeder organism, more and more colony forming organisms might have accumulated in the body during filtration of water. Similarly, MPN results also demonstrated that coliform are significantly higher ($p < 0.05$) at PJ (460/100 mL) compared to NW (23/100 mL). Mary and Jansi (2014) recorded 64MPN/100 mL and 39MPN/100 mL of total coliform from the waters of Colachel and Kadiyapatnam fish landing centre of India, which is more than that observed in the samples collected from NW and less than that reported from PJ, which indicates that NW area can be suitable for bath and other recreational activities. Higher load of TCC and MPN in PJ might be due to the discharge of sewage into PJ areas.

Water quality can undergo major changes due to human activities (Goudie 1990). In the present study, alkalinity ($p < 0.05$), nitrite ($p > 0.05$), nitrate ($p < 0.05$) and phosphate ($p < 0.05$) level were higher in the samples collected from PJ compared to NW, whereas, dissolved oxygen ($p < 0.05$) level and percentage of dissolved oxygen (DO%) ($p < 0.05$) were always less saturated compared to NW. Similarly, low salinity ($p < 0.05$) and higher COD ($p < 0.05$) at PJ compared to NW, is due to discharge of fresh and sewage water from the city area which imparted higher oxygen demand (Table 2). There were no variations of other water quality parameters collected from the selected sites as also recorded in earlier studies (Sarma et al. 2012). The consistent pattern observed in the current study indicated that PJ is constantly becoming more polluted compared to NW.

The present study recorded poor water quality parameters and higher microbial load in waters at PJ compared to NW. Oysters at PJ are smaller in size with poor ATPase activity and high body burden of heavy metals, which indicated that oysters living at PJ are under stress due to the combined effects of several stressors present in the waters.

Essential minerals such as copper, zinc and manganese play important roles in biological systems; whereas mercury, lead and cadmium are toxic, even in trace amounts.

Table 2 Different hydrological parameters of water from North Wandoor and Phoenix Jetty

Parameters	Feb-11		Mar-11		Apr-11		May-11		Average	
	NW	PJ	NW	PJ	NW	PJ	NW	PJ	NW	PJ
Temperature (°C)	29.56 ± 0.27	30.73 ± 0.4	33.1 ± 3.4	30.66 ± 0.65	31.7 ± 0.1	31.12 ± 0.73	33.0 ± 0.0	32.68 ± 0.0	31.84 ± 1.7	31.30 ± 0.9
pH	8.31 ± 0.03	8.30 ± 0.07	8.38 ± 0.10	8.29 ± 0.11	8.92 ± 0.1	8.57 ± 0.20	8.53 ± 0.03	8.75 ± 0.3	8.53 ± 0.3	8.48 ± 0.2
Salinity (g L ⁻¹) ^b	33.04 ± 0.67	31.92 ± 2.25	35.06 ± 0.1	32.13 ± 3.07	33.5 ± 0.0	31.29 ± 2.67	29.17 ± 0.02	23.9 ± 11.9	32.69 ± 2.5	29.8 ± 3.9
Turbidity (NTU)	31.3 ± 0.0	30.20 ± 0.34	41.81 ± 3.98	46.84 ± 5.71	55.7 ± 0.8	47.30 ± 5.71	66.05 ± 3.95	53.02 ± 9.0	48.71 ± 15.3	44.34 ± 9.8
Alkalinity (mg L ⁻¹) ^b	103 ± 1.0	116.8 ± 7.29	107 ± 1.0	112.0 ± 6.78	80.0 ± 4.0	98.80 ± 9.76	74.00 ± 2.0	103.2 ± 12.0	91.0 ± 16.4	107.7 ± 8.2
DO (mg L ⁻¹) ^{a,b}	5.9 ± 0.50	4.16 ± 1.91	5.80 ± 0.2	3.64 ± 1.71	6.15 ± 0.2	4.14 ± 1.98	6.75 ± 0.05	3.80 ± 2.52	6.15 ± 0.4	3.94 ± 0.3
DO% ^{a,b}	110.2 ± 2.80	78.44 ± 9.94	102.3 ± 2.7	77.44 ± 10.3	105.4 ± 3.2	68.56 ± 32.2	113.9 ± 0.6	63.82 ± 46.9	107.9 ± 5.2	72.06 ± 7.1
Nitrite (mg L ⁻¹)	0.007 ± 0.0	0.008 ± 0.00	0.007 ± 0.0	0.016 ± 0.02	0.007 ± 0.0	0.008 ± 0.00	0.007 ± 0.0	0.008 ± 0.0	0.007 ± 0.0	0.01 ± 0.0
Nitrate (mg L ⁻¹) ^{a,b}	0.43 ± 0.05	0.57 ± 0.08	0.61 ± 0.05	0.86 ± 0.54	0.32 ± 0.0	0.476 ± 0.13	0.271 ± 0.04	0.588 ± 0.2	0.407 ± 0.15	0.624 ± 0.2
Phosphate (mg L ⁻¹) ^{a,b}	0.03 ± 0.00	0.04 ± 0.01	0.04 ± .005	0.05 ± 0.02	0.05 ± 0.0	0.053 ± 0.02	0.035 ± 0.0	0.046 ± 0.01	0.038 ± 0.01	0.05 ± 0.0
COD (mg L ⁻¹) ^b	0.4 ± 0.08	0.51 ± 0.38	0.32 ± 0.20	0.32 ± 0.11	1.2 ± 0.4	2.24 ± 0.67	2.00 ± 0.4	2.40 ± 1.0	0.98 ± 0.79	1.37 ± 1.1

^a Significant different between locations ($p < 0.05$) when monthly average is taken into consideration (students *t* test)

^b Significant different between months as well as locations ($p < 0.05$) (ANOVA test)

The essential metals can also produce toxic effects at high concentrations (Nielsen 2000; Turkmen et al. 2009). It has been reported that oysters can accumulate higher Cu, Zn, Cd, and Cr than the mussels, but lower Hg, Pb, and As, which indicates that the animals accumulate different heavy metals at different levels in the body from the surrounding seawater (Mok et al. 2015). Accumulation of heavy metals in marine organisms depends on both their uptake and elimination rates (Mok et al. 2015) and may also be affected due to their feeding method and habitat. It is well documented that oysters have the ability to tolerate high concentration of metals such as copper and zinc in their body tissue without showing deleterious effects (Lin and Hsie 1999; Soto-Jimenez et al. 2001). Rojas de Astudillo et al. (2005) hypothesized that the ability of oysters to bioaccumulate copper and zinc could be used to reflect longterm exposure to environmental contamination by these metals.

In the present study, Ca and Mg level ($p < 0.05$) were low in oysters at PJ compared to NW, inspite of having higher concentration of these minerals in water at both the sites. These two minerals are responsible for oyster shell formation. The pollutants in PJ might have imparted severe stress and would have interfered the mineral uptake and metabolism in oysters, which in turn might have resulted in smaller size of oysters in PJ compared to those in NW (Table 1). In contrast Cu, Zn and Cd levels were significantly higher ($p < 0.05$) in oysters collected from PJ compared to NW (Table 3). Goksu et al. (2005) studied heavy metal deposition (Cu, Zn and Cd) on bivalvia species from Akkuyu Bay and found similar results as recorded in the oyster samples collected from the NW. However, oysters from coastal area of Trinidad and Venezuela (Rojas de Astudillo et al. 2005) and oysters from Karwar coast (Kumar et al. 1990) recorded relatively higher concentration of Cu, Zn and Cd. Heavy metals present in the water bodies gains entry through feeding or

by direct diffusion, and accumulate in the body. In addition, due to continuous exposure of higher concentration of heavy metals, ability of oysters to excrete out these metals from their body might have been reduced. Rojas de Astudillo et al. (2005) hypothesized that the ability of oysters to bioaccumulate copper and zinc could be used to reflect longterm exposure to environmental contamination by these metals. In the present study, Ca and Mg level ($p < 0.05$) were low in oysters at PJ compared to NW, inspite of having higher concentration of these minerals in water at both the sites. These two minerals are responsible for oyster shell formation. The pollutants in PJ might have imparted severe stress and would have interfered in the mineral uptake and metabolism in oysters, which in turn might have resulted in smaller size of oysters in PJ compared to those in NW (Table 1). In contrast, Cu, Zn and Cd levels were significantly higher ($p < 0.05$) in oysters collected from PJ compared to NW (Table 3). Goksu et al. (2005) studied heavy metal deposition (Cu, Zn and Cd) on bivalvia species from Akkuyu Bay and found similar results as recorded in the oyster samples collected from the NW. However, oysters from coastal area of Trinidad and Venezuela (Rojas de Astudillo et al. 2005) and oysters from Karwar coast (Kumar et al. 1990) recorded relatively higher concentration of Cu, Zn and Cd. Heavy metals present in the water bodies gains entry through feeding or by direct diffusion, and accumulate in the body. In addition, due to continuous exposure of higher concentration of heavy metals, ability of oysters to excrete out these metals from their body might have been reduced. A world wide compilation on legal limit of hazardous substance in fish and fishery products (Nauen 1983) revealed that legal limit of cadmium, copper and zinc in mollusks and mollusc products are 2.0, 70.0 and 1000.0 ppm in Australia, while in India copper and zinc are 10.0 and 50.0 ppm and in Germany limit 0.5 ppm for cadmium. Similarly for cadium limit is 1.0 mg/kg wet weight as suggested by the European

Table 3 Concentration of minerals in water ($n = 3$) and oyster muscle ($n = 10$) from North Wandoor and Phoenix Jetty

Minerals	Water			Oyster*		
	Unit	North Wandoor	Phoenix Jetty	Unit	North Wandoor	Phoenix Jetty
K	mg L ⁻¹	381.67 ± 3.28	373.00 ± 7.51	mg g ⁻¹	2.567 ± 0.123	2.4374 ± 0.131
Ca	mg L ⁻¹	330.82 ± 5.71	368.89 ± 36.55	mg g ⁻¹	0.2125 ± 0.008 ^a	0.0769 ± 0.007 ^b
Mg	mg L ⁻¹	871.74 ± 11.05	909.08 ± 34.96	mg g ⁻¹	1.3176 ± 0.193 ^a	0.8512 ± 0.052 ^b
Na	–	–	–	mg g ⁻¹	3.64 ± 0.12	3.42 ± 0.16
Mn	µg L ⁻¹	1.12 ± 0.03	1.07 ± 0.03	µg g ⁻¹	3.884 ± 0.27	3.407 ± 0.30
Cu	µg L ⁻¹	5.46 ± 0.007	5.45 ± 0.013	µg g ⁻¹	0.0271 ± 0.002 ^a	0.2296 ± 0.0156 ^b
Zn	µg L ⁻¹	42.79 ± 5.34	49.48 ± 2.60	µg g ⁻¹	0.158 ± 0.019 ^a	0.743 ± 0.044 ^b
Cd	µg L ⁻¹	0.102 ± 0.014	0.098 ± 0.008	µg g ⁻¹	0.057 ± 0.005 ^a	0.399 ± 0.057 ^b

* Wet weight basis. Values with different superscripts in a row differ significantly ($p < 0.05$)

Commission (2001), 2.0 mg/Kg by Codex Alimentarius Commission (2006), 2.0 lg/g for both Australia, New Zealand (FSANZ 2008) and 1.5 ppm for fish and fishery products by Food Safty and Standard Authority of India (FSSAI 2012). In the present study, all the hazardous metals are much below the maximum permissible limit of different countries, which indicates that the intake of metals via consumption of this animal is not hazardous to humans (Mok et al. 2015.)

This study concludes that indiscriminate release of effluents at PJ has to be checked and there is a need for proper treatment. However, a more comprehensive study on sewage profile released in the Phoenix Jetty along with bioaccumulation pattern of different minerals and heavy metals on time series in organism, sediments and water would provide better insight on the mineral dynamics and bioaccumulation pattern in Andaman.

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Compliance with Ethical Standards

Conflict of interest For this manuscript, authors declare that they have no conflict of interest. The present work is a part of a dissertation work of the first author for the award of M.Sc. Degree and the work was carried out at Central Agricultural Research Institute, Port Blair. In the acknowledgement section, authors have acknowledged the facilities received from the Director of Central Agricultural Research Institute, Port Blair.

Ethical Standard It is hereby declared that the experiments conducted (as mentioned in the manuscript) comply with the current laws of the country, India.

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