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Fluorescein diacetate hydrolysis assay on copepod *Tisbe furcata* as a new rapid bioassay to assess marine sediment quality

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Toxicity bioassays measure the direct impacts of contaminants on aquatic environment. Sediment toxicity bioassay using resident species with analysis of priority chemicals accomplish the reliable risk assessment. Copepods are sensitive to environmental contaminants and widely applied for toxicity bioassays. Therefore, present study demonstrates sediment toxicity bioassay on meio-benthic harpacticoid copepod *Tisbe furcata*. Sediment quality parameters, metals, and total petroleum hydrocarbons (TPHs) are measured in the sediment. Pollution load index (PLI) and potential ecological risk index (PERI) are determined from metal concentration. Biological responses of copepod and sediment microbe are measured by hydrolysis of fluorescein diacetate (FDA). Sediment quality is assessed by the rate of FDA hydrolysis in comparison with sediment quality parameters. Absorbance of fluorescein in sediment extracts measured between 0.0437 and 0.0846 by copepods. Sediment toxicity response of copepods exhibited that the estuarine sediments are highly toxic with considerable ecological risk attributing higher PLI and PERI respectively. Interestingly, the toxicity bioassay exhibits moderate toxicity in the sediment samples of bar mouth and off-shore of the estuary. However, PLI and PERI reveal that these sediments are unpolluted with low ecological risk and even the impact from unknown emerging contaminants can be captured by sediment toxicity using copepod. High hydrolytic activities by sediment microbes at main stream of estuary and coastal sediments are attributed to sewage discharges. These assays are more environmentally relevant, reliable and cost-effective, and numerous tests can be conducted with basic laboratory equipments to regulate pollution.

Keywords. Pollution; sediment toxicity; fluorescein diacetate; risk assessment; environmental quality assessment.

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1. Introduction

Coastal ecosystems are under serious threat by anthropogenic pollution from activities like urbanization and industrialization (Edem *et al.* 2018). The total input of pollutants in coastal waters of India estimates that there is 1.11×10^{10} m^3/year of sewage, 1.35 million m^3/day of industrial waste, 34,500 ton/day of solid waste, agriculture and aquaculture based effluent is around 2.37 million m^3/day, 1.56×10^{12} m^3/year of riverine discharges, and 18.37 million tons/year of fertilizers (GESAMP 2001; Zingde 2001). These pollutants ultimately sink into the coastal sediments from the water column. Most of the coastal sediments are reported with higher levels of metal and organic contaminants exceeding the sediment quality guidelines (Rokade 2009). Thereby it affects the water, sediment quality and physiological activities of the organism and leads to alteration in community structure by loss of sensitive species and proliferation of tolerant species (Catala *et al.* 2016). Accumulation and presence of contaminants in sediments differ spatially due to the influence of discharge points, load, the flow of water, depth and sediment texture (Cornelissen and Gustafsson 2005). Release of nutrients enhances the algal growth and leads to eutrophication (Pei *et al.* 2020). Sediment microbes are playing major role in decomposing of organic matter, however, higher load of organic matter causes hypoxia which leads to anaerobic degradation and release of toxic gases like ammonia and hydrogen sulfide (Jiang *et al.* 2016). In this context, many studies focused on measuring the level of priority pollutants in sediment rather than water columns since they are present at detectable levels due to accretion and higher residential period (Fent 2004). Measuring pollutant levels and derivation of ecological risk indices is a key to categorization of polluted locations and chemical of concern. However, these are not appraising the impacts of pollutants on resident organisms and ecosystem (Allan *et al.* 2006).

Apparently many emerging chemicals are continuously being produced and reaching the coastal environment. Those are not measured due to the absence or the inadequacy of suitable analytical techniques and/or facilities. Analytically, undetectable toxic chemicals that go unmeasured can pose environmental risks and they should be considered for sediment quality/risk assessment along with priority chemicals. This can be done by adopting a comprehensive risk assessment strategy by the inclusion of toxicity bioassay data along with monitoring of pollutant levels and micro/-macro benthic biodiversity indices (Simpson and Batley 2016). Sediment toxicity bioassay is an emerging tool to assess the impact of pollutants accumulated in the sediment irrespective of toxic chemicals and organic enrichment (Schweizer *et al.* 2018). Toxicological analysis provides information about bioavailability, cumulative effects, interaction and stress on the organism (Schlekat *et al.* 2016). Whole sediment toxicity bioassays are reliable for assessing coastal environments that are influenced by multiple anthropogenic sources. Determination of biological responses is a challenging task at environmental levels of contaminants in the fraction of sediment. This is conquered by the application of biomarker fluorescent dye like fluorescein diacetate (FDA) and dichloro fluorescein diacetate (DC-FDA). These stains are widely used to measure the physiological stress in test organisms induced by stressors at environmentally reliable levels (Guilherme 1997). FDA is a non-fluorescent, non-toxic chemical to living cells, hydrolyzed to fluorescent chemical fluorescein by a group of enzymes. FDA is hydrolyzed by the enzymes of proteases and esterases in the living cell and the amount of activity can be measured in the living organism and/or extracellular media (Welschmeyer and Maurer 2012; Jiang *et al.* 2016; Edem *et al.* 2018). In this context, the sediment toxicity bioassays on sensitivity species from different groups, e.g., microbes, rotifers, copepod and polychaete worms help to assess realistic impact of pollutants on the ecosystem. Toxicity of sediment is determined using FDA on microbes and microalgae (Welschmeyer and Maurer 2012; Jiang *et al.* 2016; Edem *et al.* 2018). Bioassays involving resident sensitive species of ecological significance of the particular ecosystem provide reliable risk assessment (Fontvieille *et al.* 1992). Copepods are important component in benthic food-web and sensitive to various pollutants. Hence, the sediment toxicity bioassays on benthic copepods may enhance the reliability of risk assessment in addition to the microbial assays (Marco *et al.* 2016; Edem *et al.* 2018). The microbial luminescence inhibition assays are applied as a preliminary test mostly for chemical toxicity and freshwater systems along with a battery of bioassays on other species (Parvez *et al.* 2006). Sediment toxicity testing kits available using the bioluminescent bacteria *Vibrio fischeri* is useful for a limited

number of tests. However the microbial activity differs with different factors other than the toxicants like media composition, revival of freeze dried microbe, temperature, etc. (Strotmann *et al.* 2020). Another fluorescence biomarker, dichloro-FDA is applied widely for quantifying the ROS due to oxidative stress. Unlike DC-FDA, the FDA captures the impacts of pollutants/stressors on physiological inhibition along with oxidative stress.

An epibenthic harpacticoid copepod *T. furcata* is chosen for assessing sediment toxicity. This species is selected based on wide distribution, benthic habitat, ecological importance, sensitivity to contaminants, and amenability to laboratory culture (Hagopian *et al.* 2001; Victor and Simpson 2011; Sivaleela and Venkataraman 2014; Isabella *et al.* 2018). This would help in cost and time cutting in procuring the commercial assay kits for limited number of assays with reliable results. The bioassay using mixed species would provide more reliable results but increased complexity may vary the results of replicated experiment. Hence, the advantage of mono-species toxicity test is easy, less complex and reproducibility of results. Thus, the present study aims to (1) develop a rapid sediment toxicity bioassay using FDA hydrolysis by benthic copepod, and (2) compare the results of toxicity bioassay with sediment quality parameters sediment texture, bulk density, total organic carbon, chlorophyll, metals, total petroleum hydrocarbons and pollution load index.

2. Materials and methods

2.1 *Study area*

Sediment samples were collected from 10 sampling sites at north Chennai coast and Ennore estuary covering the area from Kasikovilkuppam (13°.1870E; 80°.3190N) in the south to Buckingham canal (12°.27248E; 80°.32170N) in the north. Five sites are selected in the estuary and five sites in the coastal and offshore regions (figure 1). Anthropogenic activities influencing in the sampling stations are (S1) port activities and fly ash; (S2) handling of crude oil and coal in Kamarajar port for thermal power plants; (S3 and S4) thermal effluent discharges, dredging and operation of fishing boats; (S5 and S6) receiving the effluents from industrial, domestic activities and fishing of polycheate worms; (S7) impact of collective discharges from Ennore estuary in offshore sediment at 15 m water depth; (S8–S10) receiving effluents from marine outfalls, fishing and non-point domestic discharges.

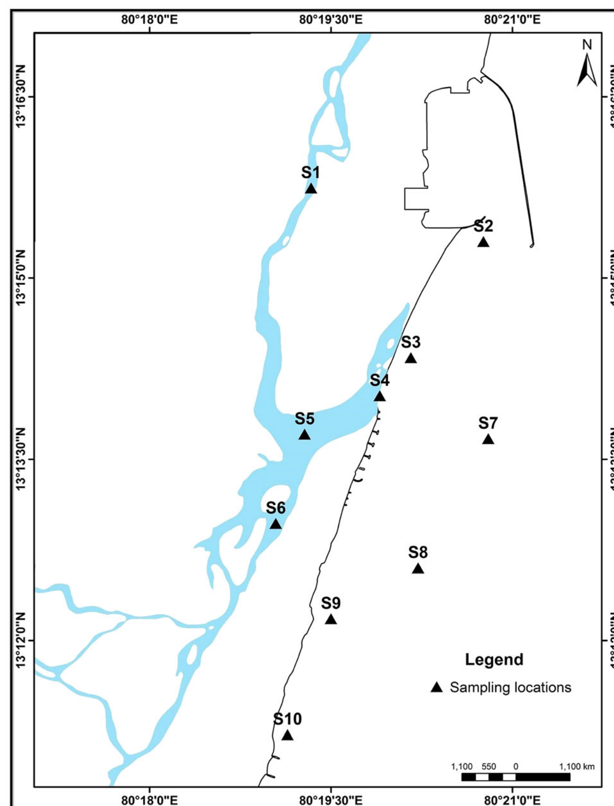

Figure 1. Map showing the study area around Ennore estuary with sampling locations.

2.2 *Sampling and analysis of sediment quality parameters*

Surface sediment samples were collected using Van-Veen grab (area 0.01 m^2) in March 2019. Totally, 30 sediment samples were collected in three replicates from each sampling sites. Collected samples were transferred into polythene bags, transported to the laboratory in an ice box and stored in a deep freezer at –20°C until the bioassay and analysis. Aliquots of sediments were dried at 65°C for 24 h in a hot-air oven for analysis of sediment quality parameters, viz., texture, TOC, chlorophyll-*a* and pheophytin, TPH and metals.

2.2.1 *Sediment texture, bulk density and TOC*

Sediment texture was analyzed using a sieve shaker (Retsch AS 200) for sandy sample and particle size analyzer (Malvern Hydro 2000G) for clayey samples. The biogenic calcareous portion in the sediment samples were removed using concentrated

hydrochloric acid before texture analysis. The proportion of sand, silt and clay was derived based on the quantity of different grain sized portions in the sediment (Folk and Ward 1957; Friedman 1979). The dry bulk density (BD) was calculated using the following equation of Dadey *et al.* (1992). Volume and moisture content were measured gravimetrically using 5 g of fresh sediment sample.

$$\text{Dry BD} = (1 - \varphi) \times Ps,$$

where φ is the porosity as volume, and Ps is the grain specific gravity as weight.

Total organic carbon was analyzed by the wet oxidation method described by Walkley (1935). Briefly, 0.5 g of dried sediment sample is added into 500 ml Erlenmeyer flask. Then, 10 ml of 1N of $K_2Cr_2O_7$ was added and mixed by swirling followed by addition of 20 ml of concentrated H_2SO_4. This mixture was incubated for 20 min at room temperature, and then diluted to 200 ml with distilled water. Following by 10 ml of phosphoric acid, 0.2 g of sodium fluoride and 15 drops of diphenylamine indicator were added into the diluted sediment mixture. The solution becomes a dark brown colour and this sample was titrated with 0.5N ferrous ammonium sulphate until the appearance of brilliant green colour. The TOC in the sample was calculated using the following equation:

$$\%\ \text{Organic Carbon} = 10(1 - T/S)[1.0\text{N}(0.003)(100/W)],$$

where T is the Titre volume (ml) for sample and S is the Titre volume (ml) for blank. 0.003: factor value converting to carbon, 1.0N: normality of $K_2Cr_2O_7$, 10 volume (ml) of $K_2Cr_2O_7$, W: weight (g) of sediment sample.

2.2.2 *Sediment chlorophyll-a and pheophytin*

The chlorophyll-a content in sediment samples is determined by the method described by Lorenzen (1967). One gram of fresh sediment sample incubated at 4°C under dark conditions overnight after mixing with 4 ml of 90% acetone. Then the samples were centrifuged at 4000 rpm for 5 min and absorbance was measured in supernatants using an UV-Vis spectrophotometer (Hitachi, model F-2700) at 665 and 750 nm before and after acidification. The chlorophyll-a and pheophytin were calculated following the equations below:

$$\text{Chl-}a\ (\text{mg/g}) = \frac{A \times K \times (665o - 66a)}{g \times l} \times v,$$

$$\text{Pheopigment (mg/g)} = \frac{A \times K(R[665a] - 665o)}{g \times l} \times v,$$

where A is the absorption coefficient (11) of chlorophyll-a, K: normalization factor (2.43), $665o$: absorbance before acidification, $665a$: absorbance after acidification, v: volume (ml) of acetone, g: weight (gm) of sediment, l: path length (cm) of cuvette, R: ratio (1.7) of $665o$:$665a$ in the absence of pheophytin.

2.2.3 *Total petroleum hydrocarbons (TPH)*

TPH concentrations in the sediment samples were determined using the method described by IOC-UNESCO (1982). Eighty gram of fresh sediment was saponified with 100 ml of methanol and 3 g of KOH and heated in a reflux condenser apparatus at 75°C for 90 min. The mixture was filtered through Whatman No.1 filter paper after cooling into room temperture and transferred into separating funnels. The TPHs in filtrates were extracted into *n*-hexane. The fluorescence of the extracted sample was measured in a fluorescence spectrophotometer (Hitachi, F-2700) at 364 nm of emission and 310 nm of excitation wavelengths. Chrysene was used as a calibration standard and results were expressed as chrysene equivalent. The extraction and analytical methods were validated by spiked recovery of known concentrations of chrysene. *n*-hexane used as reagent and solvent blank after undergoing the same extraction procedure without sediment sample. Negligible fluorescence value for blank was observed and the recovery of 93–104% was obtained within 10% standard deviation of mean value.

2.2.4 *Metals*

The metals in sediment samples were determined after the acid digestion (Tessier *et al.* 1979). Briefly, 0.2 g of powdered dry sediment samples were mixed in nitric and perchloric acids in a ratio of 4:1 and heated at 190°C for 1 h. The sample was filtered through Whatman No. 1 filter paper and made up to 25 ml using ultrapure water. Metal concentrations in the extracted sediment were measured using the Atomic Absorption

Spectrometer in flame mode except for selenium in hydride vapor method (Varian, SpectrAA 220FS).

2.3 *Conduct of sediment toxicity bioassay*

2.3.1 *Copepod collection and culture maintenance*

Copepods were collected from shallow backwaters in Chennai using a zooplankton net (150 μ mesh). Samples were packed in polythene bag with ambient seawater and oxygen gas. Multi-species copepod culture was established in glass aquarium tanks with seawater and mixed microalgae diet. Meio-benthic harpacticoid copepod *T. furcata* was isolated and identified using morphological identification key characters (Dahms *et al.* 1991). Monospecies culture of *T. furcata* was established under laboratory controlled conditions of 24 ± 2°C of temperature, 30 psu of salinity, 5.5 ± 1 mg/l of dissolved oxygen and photoperiod of 16-h light and 8-h dark adjusted with 2500 ± 500 lux of light intensity. The mixed microalgae and diatom cultures consisting of *Isochrysis galbana* and *Thalassiosira* sp. were fed during the culture of copepod.

2.3.2 *Preparation of whole sediment extract for toxicity bioassay*

A fresh sediment of 2 g was mixed with 40 ml of filtered seawater in 50 ml centrifuge tubes in six replicates. Replicated tubes were marked alphabetically from A to F. The sediment mixture in the tubes was shaken well using a reciprocal shaker for 1 h at 280 rpm speed. Tubes marked with A, B and C were heated in a hot water bath for 1 h at 80°C to seize the microbial activities in the sediment extract and allowed to cool to room temperature (Everaert *et al.* 2016). Then, all the tubes (A–F) were kept undisturbed for settlement for 24 h. Absorbance values of all the sediment extracts were measured as blank (Abs_{Blank}) and 10 ml aliquots of water from each sediment extract was transferred into another set of 15 ml centrifuge tubes for measuring the blank. Sediment toxicity bioassay was initiated by the addition of 40 μM FDA into the sediment extracts in four different combinations (table 1). Tubes A and D were used for measuring the chemical hydrolysis (combination 1) and sediment microbial activities, respectively (where the copepods are not exposed, combination 2). A customized toxicity test chamber was used for exposing the copepods to sediment extracts. Fifteen (15) ml plastic centrifuge tubes were modified as copepod exposure chambers by fixing a piece of zooplankton net at the bottom of tube as shown in figure 2. Ten copepods were added into each tube while half portions of tubes were immersed with filtered seawater in a beaker. These tubes with copepod were exposed in the B, C, E and F tubes for 1 h to determine the FDA hydrolysis by copepod activities alone (combination 3) and along with microbial activities and autolysis (considered as total hydrolysis by chemical hydrolysis, microbial and copepod, combination 4) (table 1 and figure 2). Ten copepods were exposed to 10 ml of filtered seawater as positive control for activity of copepods. The copepods were removed from the sediment extracts after the exposure period of 1 h. Then, FDA hydrolysis reaction was terminated by the addition of 1 ml acetone in the sediment extracts. The fluorescence was measured at 495 nm in the extracts after centrifugation at 4000 rpm for 10 min (Green *et al.* 2006; Jiang *et al.* 2020) and noted as absorption in sample (Abs_{Sample}). The rate of hydrolysis among different combinations is calculated as follows.

$$\text{FDA hydrolysis by sediment chemicals } (H_{\text{Chem}}) = Abs_{\text{Sample(A)}} - Abs_{\text{Blank(A)}}$$

$$\text{FDA hydrolysis by sediment microbe } (H_{\text{Microbe}}) = \left[Abs_{\text{Sample(D)}} - Abs_{\text{Blank(D)}}\right] - (H_{\text{Chem}})$$

$$\text{FDA hydrolysis by copepods } \left(H_{\text{Copepod}}\right) = \left[Abs_{\text{Sample(B or C)}} - Abs_{\text{Blank(B or C)}}\right] - (H_{\text{Chem}})$$

$$\text{Total hydrolysis } (H_{\text{Total}}) = Abs_{\text{Sample(E or F)}} - Abs_{\text{Blank(E or F)}}.$$

2.4 *Statistical analysis*

The results of sediment quality parameters and sediment toxicity tests are presented from triplicate measurements. Principal component analysis (PCA) was performed to identify the influencing sediment quality parameters and contaminants like metals and TPH on sediment toxicity using the statistical tool, Primer (version 6). The relationships among the parameters and toxicity of sediment were determined using correlation coefficient. Sediment toxicity data are analysed for their difference

Table 1. *Details of whole sediment toxicity bioassay to capture the impact of sediment chemical composition, microbes, copepod in the hydrolysis of FDA.*

Test composition	Sample processing	FDA hydrolysis by
2g sediment + 40 ml seawater +40 µM FDA	Heat treated at 80°C for 1 h to arrest microbial activity	Sediment chemical composition
2g sediment+40 ml seawater +40 µM FDA	Sediment mixture maintained at room temperature	Sediment chemicals and microbial activities
2g sediment+40 ml seawater +10 copepod +40 µM FDA	Heat treated at 80°C for 1 h to arrest microbial activity	Sediment chemicals and copepods
2g sediment+40 ml seawater +10 copepod +40 µM FDA	Sediment mixture maintained at room temperature	Sediment chemicals, microbial, and copepod activities

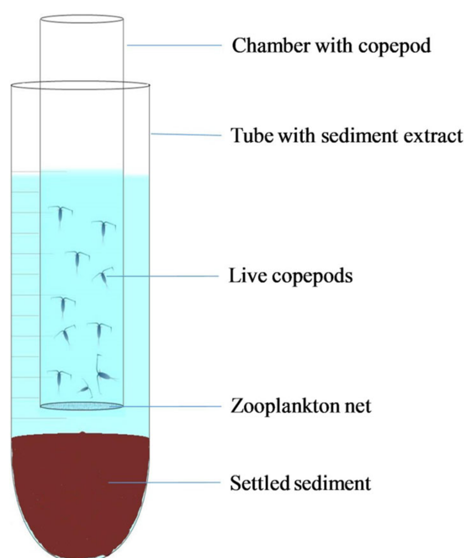

Figure 2. Customized test chamber used for whole sediment toxicity bioassay on copepods.

among the sampling stations by ANOVA using SPSS (version 16).

3. Results

3.1 *Sediment quality parameters*

Results of texture, bulk density, TOC, TPH, chlorophyll-*a*, pheophytin and metals are presented in table 2 and figure 3(a–d). Coastal sediments are sandy with silt-like texture at Ennore port (S2), bar mouth of estuary (S4) and southern coastal stations (S9 and S10). Coastal sediments had lesser bulk density (1.45 g/cm^3) than estuarine sediments (2.17 g/cm^3). Overall, sediment chlorophyll-*a* observed between 1.95 (S7) and 10.68 (S5) mg/g and pheophytin from 2.33 (S3) to 21.37 (S4) mg/g among the sampling stations. Higher levels of total organic carbon are observed at mid-estuarine sediments with 1.79 ± 0.72% than coastal and off-shore sediments with 0.25 ± 0.08%. TPHs determined along the Ennore estuary and coastal sediments ranged from 0.15 to 12.14 µg/g. Estuarine sediments are observed with higher TPHs compared to the coastal sediments in the range from 0.44 ± 0.12 (S1) to 12.14 ± 0.10 µg/g (S5). The highest concentration was observed in mid-estuary, estuarine mouth, and Kamarajar port. Metal concentrations (µg/g) were found in the range of 5.76 ± 0.9 to 114 ± 3.1 for Cr, 5.7 ± 1.2 to 39.5 ± 1.7 for Cu, 0.45 ± 0.05 to 5.93 ± 0.9 for Cd, 6.7 ± 0.4 to 60.53 ± 2.0 for Pb, 7.2 ± 0.5 to 48.5 ± 2.7 for Ni and 0.17 ± 0.04 to 1.63 ± 0.08 for Se. Metal concentrations across all the stations are illustrating the order of Cr > Pb > Ni > Cu > Cd > Se. Sediment quality indices like pollution load index (PLI) and potential ecological risk index (PERI) were derived from the spatial metal concentrations to assess the level of metal pollution and their ecological risk (figure 3b). PLI (1.32–1.93) indicates the main stream of estuary (S3–S6) is polluted by metal contamination (figure 3b). This reflects in the PERI between 24.6 and 348.2 among the sediments. In the Buckingham canal (S5; PERI: 348.2) and urban sewage (S6; PERI: 167.3) drains into the mid-estuary, those sediments are under considerable to moderate potential ecological risk (figure 3b). Strong correlation between PLI and PERI shows the relationship between the metal concentration and the ecological risk.

3.2 *Sediment toxicity bioassay*

Whole sediment toxicity bioassay results with three different components, *viz.*, sediment chemical hydrolysis, microbial hydrolysis, and benthic harpacticoid copepod *T. furcata* are related with sediment quality parameters. Hydrolysis of FDA by copepod *T. furcata* on exposure to whole

Table 2. *Details of sampling stations and sediment quality parameters (mean ± SD; n = 3).*

Stations	Sampling stations	Latitude	Longitude	TOC (%)	Chl-*a* (mg/g)	Pheo (mg/g)	TPH (µg/g)	Chromium (µg/g)	Copper (µg/g)	Cadmium (µg/g)	Lead (µg/g)	Nickel (µg/g)	Selenium (µg/g)
S1	Buckingham canal	13.27248	80.32170	0.82±0.04	5.58±1.35	4.34±1.24	0.44 ± 0.12	20.44±1.27	15.13±1.3	2.44±0.86	22.61±2.3	16.53±0.7	0.81±0.01
S2	Kamarajar Port (Ennore)	13.2550	80.3460	0.53±0.04	6.84±1.50	5.36±0.68	3.36±0.49	37.3±2.03	5.7±1.21	2.48±1.2	37.33±1.24	19.8±1.1	0.17±0.04
S3	Estuarine mouth North	13.2340	80.3320	0.47±0.06	6.51±1.28	2.33±0.97	10.14±0.11	55.8±3.02	24.26±3.1	2.78±0.94	48.36±2.2	35.63±1.8	0.45±0.02
S4	Estuarine mouth	13.23326	80.32891	0.53 ±0.11	8.48±0.92	21.33±1.18	10.39±0.13	114.4±3.16	15.91±2.46	2.8±0.5	54.23±1.6	48.5±2.7	0.64±0.01
S5	Mid-estuary North	13.2390	80.3360	1.79±0.72	10.68±1.90	18.46±0.98	12.14±0.10	84.5±2.22	39.5±1.79	5.93±0.92	60.53±2.0	36.56±1.9	1.63±0.08
S6	Mid-estuary South	13.21180	80.31215	0.79±0.04	4.64±0.65	9.36±1.19	1.59±0.54	69.97±3.58	28.63±1.57	3.81±0.61	64.62±2.75	36.51±1.3	0.62±0.03
S7	Ennore Off	13.2329	80.3544	0.35±0.06	1.95±0.95	6.83±0.53	1.01±0.11	5.76±0.97	10.7±1.1	1.5±0.06	10.53±1.1	5.53±0.8	0.42±0.01
S8	Periyakuppam Off	13.2100	80.3370	0.33±0.05	5.56±0.74	6.58±0.56	0.15±0.04	7.0±1.08	10.86±0.95	0.45±0.05	13.56±1.41	10.43±0.6	0.11±0.01
S9	Periyakuppam coast	13.2030	80.3250	0.35 ±0.07	5.68±1.02	7.5±0.56	0.61±0.38	6.06±1.97	11.56±0.68	1.43±0.13	6.7±0.43	7.26±0.5	0.33±0.05
S10	Kasikovilkuppam coast	13.1870	80.3190	0.25 ±0.08	5.41±1.35	3.57±0.45	0.45±0.05	17.8±1.63	12.16±1.25	0.63±0.01	18.55±1.24	11.99±0.9	0.39±0.01

sediment extracts demonstrates that the estuarine sediments are toxic. The higher FDA hydrolysis by copepod and microbes in the estuarine sediments was related with TOC, TPH, metal, PLI and PERI. However, higher hydrolysis by copepod and sediment chemical at offshore (S7) sediment with lesser PLI is indicating the presence of unknown stressor. Highest microbial activity in southern coastal sediment (S10) indicates the discharge of sewage waters. Lesser microbial hydrolysis in the sediments of coastal and offshore sediments (S2 and S7–S10) was accompanied with lesser TOC and sediment algal pigments than the estuarine sediments (1–5 fold). High hydrolytic activity by sediment chemicals in the mid-estuarine and coastal station of Periyakuppam (S9) infers the impact of wastewaters and marine outfalls. The correlation between hydrolytic activities and TOC, chlorophyll-*a* and pheophytin confirmed the relationship of microbial activities and discharges of wastewaters. Cluster analysis such as similarity index and MDS analysis reveals the spatial variations of sediment toxicity influencing sediment quality parameters. Similarity index separates the Ennore offshore sediment (S7) between contaminated toxic sediments and unpolluted sediments. MDS also revealed the similarity index by categorizing the sediments into two groups based on sediment toxicity and influencing parameters. Among these two groups, sediment of S7 is set aside from contaminated toxic sediments.

4. Discussion

4.1 *Sediment quality parameters and pollution gradient*

The estuarine sediments constitute more silt fractions compared to coastal samples due to the continuous fluvial input, landfilling and dredging (Khan *et al.* 2012; Kunte *et al.* 2013). Sand varies between 85% and 97.4% in the harbour and river mouth sediments indicating that these locations witness frequent erosion and accretion. Higher density in estuarine sediment is due to tightly packed organic matter with sediment. Looser porous sandy texture with lesser bulk density in coastal sediments facilitates re-suspension causing lower accumulation of organic matter and contaminants. Photosynthetic production and degradation in sediment are determined by the ratio

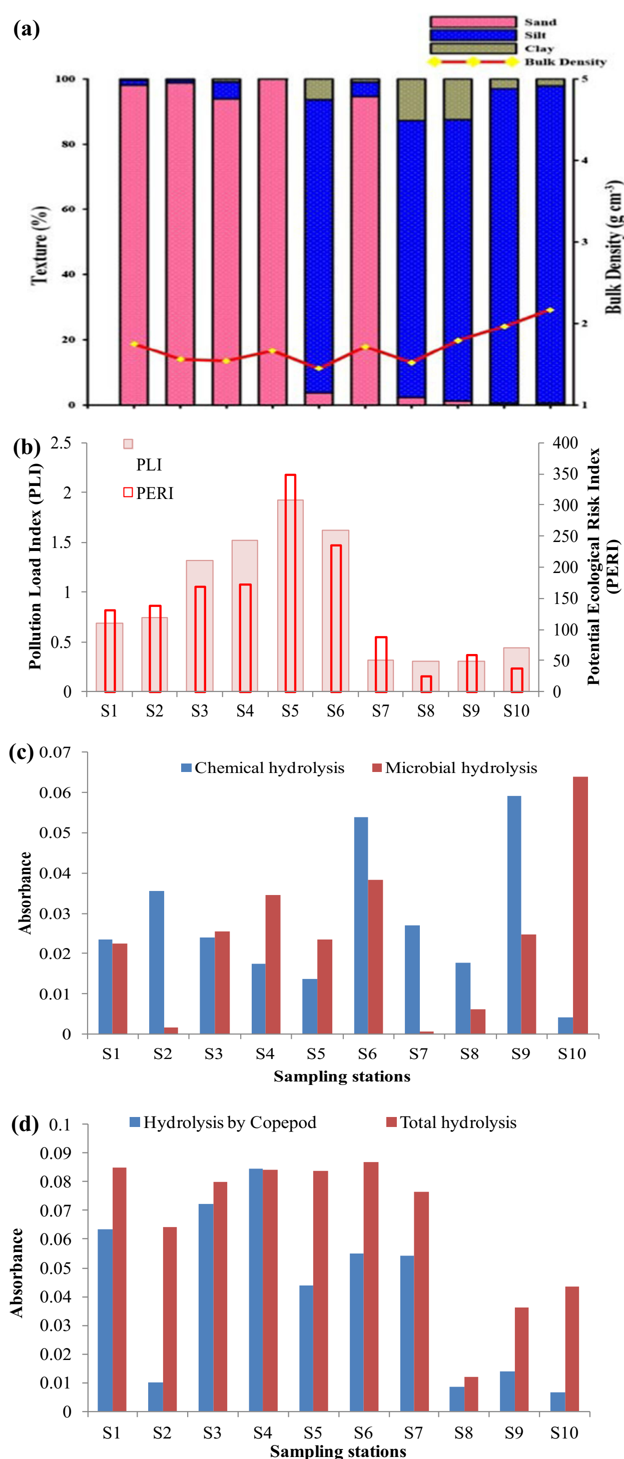

Figure 3. Graphs demonstrating spatial variations of sediment quality parameters, geochemical, ecological risk indices and sediment toxicity in and around Ennore estuary. **(a)** Sediment texture and bulk density, **(b)** Pollution Load Index (PLI) and Potential Ecological Risk Index (PERI), **(c)** whole sediment toxicity in terms of FDA hydrolysis by sediment chemical, microbe; and **(d)** hydrolysis by copepod and total hydrolysis after exposed to different combinations of sediment extracts.

between Chl-*a* and pheo-pigment in the sediment. This ratio more than 1 is indicating higher production with lower degradation. Higher production in coastal sediment near the bar-mouth of the estuary attributes the optimum nutrients from the discharge and adequate dilution.

The distribution pattern of the TPHs is related with release of untreated sewage and industrial waste waters. The hydrodynamic characters such as water currents and tides influence the distribution in addition to sediment texture (Ran *et al.* 2014; Gurumoorthi and Venkatachalapathy 2017). Similar ranges of TPH were recorded in the sediment samples across the Indian coast (Venkatachalapathy *et al.* 2010, 2013; Rajan *et al.* 2019). The distribution of TPH in sediments is attributed to the discharges from ports and harbours, industries, and urban sewage.

Higher concentrations of metals were observed at estuarine and northern coastal (Ennore port) sediments due to the influence of urban and industrial wastewater discharges and their residential time. Southern coastal and offshore sediments were recorded with lesser metal concentrations, indicating the effect of northward shore currents and dispersion of metals in offshore sediments.

TOC is highly related with PERI indicating the metal accumulation with organic matter in the sediments.

The source of increasing metal concentration in sediments is due to the fluvial input from Kosasthalaiyar River and Pulicat channel in addition to anthropogenic inputs (Gonnelli and Renella 2010; Magesh *et al.* 2013; Jayaprakash *et al.* 2016). Metal concentrations observed in this study (table 2) are well within the range of previously reported concentrations along the Indian coastal sediments, *viz.*, Ennore estuary, Kallar estuary, Korampallam Creek, Punnakayal estuary, Pulicat lake, Cuddalore coast and Ganga River (Jayaprakash *et al.* 2008, 2016; Muthuraj and Jayaprakash 2008; Magesh *et al.* 2013; Khan *et al.* 2017; Kavita and Jitendra 2019). Higher concentration of metals in main stream of the estuary are from the industries and petrochemicals, and these are controlled by organic matter and grain size of sediment (Kumar *et al.* 2001; Lin *et al.* 2002; Loska *et al.* 2004; El Nemr *et al.* 2006).

4.2 FDA hydrolysis and sediment quality parameters

FDA hydrolysis by sediment chemical, microbe and copepod is used as a tool describing the rapid sediment toxicity bioassay to amplify the reliability of sediment quality assessment.

Totally, four different batches of sediment extracts were prepared for measuring the hydrolysis of FDA by sediment chemical, microbes, copepod and total hydrolysis. The sediment was heated up at an optimum temperature of 80°C and 1-h time to arrest the microbial activity. Sediment processing to arrest the microbial activities by heating is an efficient method and has an effect on hydrolysis of FDA by release of various chemical forms (Gumprecht *et al.* 1995; Jiang *et al.* 2016). Considering this, study was conducted by incubating the sediment extracts at room temperature (28 ± 2°C) for 1 h to obtain optimum absorbance values. Total FDA hydrolysis in the sediment extracts by chemical, microbial and copepod ranges from 0.0121 to 0.0867 with higher values at mid-estuary and bar-mouth after 1-h incubation. The total hydrolysis of FDA is dissimilar to the sum of hydrolysis by chemical, microbial and copepod. This is attributed to spatial variability of biogeochemical properties in sediment by the influence of land drains, tidal and wave forces of the sea. Ionic strength and fluxes in sediment with organic carbon and alkaline condition have been reported for increased hydrolysis of FDA (Young *et al.* 2008).

Sediments from estuarine (S6) and coastal (S2 and S9) beds are observed with higher rates of chemical hydrolysis. These stations are directly influenced by different activities like Kamarajar port (S2), urban wastewater discharge (S6) and marine out-fall (S9). Higher levels of TOC are recorded at S1, S5 and S6 (Buckingham canal and mid-estuarine stations) did not accompany the chemical hydrolysis except mid-estuarine sediment by inputs of urban wastewater in the mainstream of the estuary (Jayaprakash *et al.* 2016). Higher levels of abiotic hydrolysis with varying TOC levels need further exploration to identify specific indicators through FDA hydrolysis (Segnini *et al.* 2014; Gurumoorthi and Venkatachalapathy 2017). Sediment carbon, nitrogen and phosphorus have strong influence on FDA hydrolysis by sediment microbial activities in the polluted and unpolluted sites of River Ganges (Jaiswal and Pandey 2018). Hydrogen ion concentration is an important parameter on FDA hydrolysis which affects protein and enzyme reactions in the organisms (Green *et al.* 2006). Pathogenic bacterial contamination and infusion through sewage discharges are hazardous to the environment and human health (Avnimelech *et al.* 2001; Segnini *et al.* 2014). Thus, sediment microbial activity is used to assess the impact of nutrient pollution on health of aquatic ecosystem (Redelstein *et al.* 2015). The absorbance of fluorescein was measured across the estuarine and coastal sediments from 0.0006 to 0.0382 by microbial activity. Higher microbial activity was recorded at coastal sediment of Kasikoilkuppam station followed by mid-estuarine and bar-mouth sediments. Sandy substratum in offshore sediments (S7 and S8) with minimal organic carbon resulted in limited microbial activity. This is confirmed by cluster analysis and negative correlation co-efficient with off-shore and estuarine sediment clusters (table 3 and figure 4). Minimum absorbance value was recorded by chemical hydrolysis at southern sampling station (S10; Kasikoilkuppam coast) ascribed by dispersal through shore current, wave and tidal activities. In contrast, this station (S10) is characterized by higher microbial activity than

Table 3. *Correlation coefficient between the sediments quality parameters, and sediment toxicity bioassay.*

Parameters	TOC	Chl-*a*	Pheo	TPH	PLI	PERI	Copepod	Total	Chemical
Chl-*a*	0.67								
Pheo	0.55	0.64							
TPH	0.56	0.78	0.63						
PLI	0.73	0.67	0.64	0.80					
PERI	0.89	0.63	0.59	0.72	0.93				
Copepod	0.25	0.12	0.39	0.55	0.59	0.49			
Total	0.51	0.19	0.34	0.52	0.70	0.73	0.81		
Chemical	−0.12	−0.30	−0.13	−0.32	−0.06	0.01	−0.07	0.02	
Microbe	−0.02	0.16	0.08	0.06	0.25	0.04	0.05	0.06	−0.21

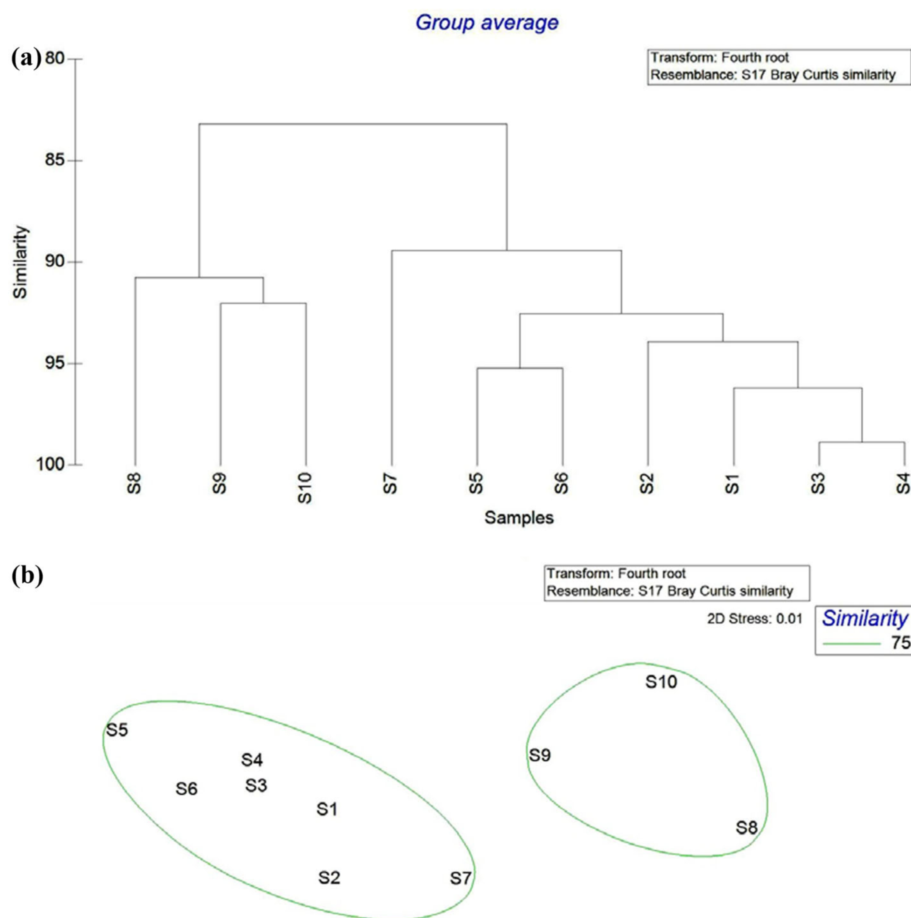

Figure 4. Cluster dendrogram **(a)** and MDS diagram **(b)** showing similarity index between the sampling stations according to the quality and toxicity of sediment.

the other sediment samples that are attributed to discharges of domestic untreated sewage waters (Bharathi *et al.* 2018). Decreasing microbial activity along the northern coastal sediments (S9, S8 and S7) is caused by dispersal of suspended sewage particles along the seashore sediment by northward shore current in the season (Pandian *et al.* 2004).

4.3 *FDA hydrolysis by copepod and sediment quality indices*

The hydrolytic assay results are based on stress of living copepods, and metals in sediments affect the activity of meiobenthic copepods. The sampling stations are categorized into three groups, viz., high toxic sediments (S4 > S3 > S1), moderate toxic sediments (S6 > S7 > S5) and non-toxic sediments (S2, S8, S9 and S10) based on the response of copepod on exposure to sediment extracts. However, the mid estuarine sediments (S3–S6) polluted by metals are under moderate to considerable risk (figure 3b). Cluster analysis and MDS are reflecting this contrasting relationship among the risk indices and sediment toxicity bioassay results (figure 4). High toxicity of sediments in estuarine mouth (S3 and S4) and Buckingham canal (S1) are due to the influence of industrial and thermal plant activities. The moderate toxicity in mid-estuarine sediments (S5 and S6) is explained by the discharges of urban sewage and accumulation of higher amounts of organic load. Maximum phyto-pigments at stations 4 and 5 (Chl-*a*: 8.48 and 10.68 mg/g; Pheo: 21.33 and 18.46 mg/g) indicate continuous supply of nutrients stimulating excessive growth of tolerant phytoplankton and perhaps remediates metal toxicity (Neelam and Ramaiah 1998). However these sediments are measured with higher metal, TOC and TPH concentrations, while the moderate sediment toxicity is ascribed by lesser bioavailability and/or negative effect on copepod by inhibition of activity or mortality. However, mortality of copepods in the

sediment extracts at higher levels of metals and pollutants interfere in the hydrolysis of FDA (Hagopian *et al.* 2001). The higher microbial hydrolysis at the coastal sediment (S10), besides lower hydrolysis by copepod with less metal and TPH concentration, perhaps an indication of anthropogenic sources for microbial contamination by sewage discharges.

4.4 *Precautions in FDA hydrolysis assay using the copepod*

Every experiment has their inherent uncertainties through matrix interferences and certainly precautions will aid in improving the analytical quality. Likewise, the sediment toxicity bioassay involves complex matrix and biological activity. Even then, the accuracy of the bioassay results could be ensured by clean test vessels, healthy individuals of copepods, fresh stock of FDA, adequate replicates and blanks for each sediment samples. Higher temperature has the effect on FDA hydrolysis, hence, the experiment should be conducted in room temperature. The reaction should be arrested by the addition of acetone in all the test vessels after the exposure period. Selection of adequate quantity of sediment based on the pollution level may be useful to survival of copepod during the experiment to precisely record the response.

5. Conclusion

This study demonstrates whole sediment toxicity bioassay using benthic copepod *T. furcata* along with the activity of sediment microbe and influence of sediment chemical composition. Responses of copepod on exposure to sediment extracts exhibited the level of sediment toxicity. Estuarine sediments are more toxic and coastal sediments are moderately toxic. High microbial activity is identified by FDA hydrolysis at coastal sediment. Sediment toxicity bioassay is a direct measure of ecological impact even from the traces of contaminants, unknown emerging contaminants and hence for reliable ecological risk assessment, one must include this parameter in monitoring.

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Author statement

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