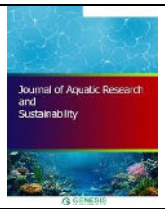
Journal of Aquatic Research and Sustainability 2 (1): 23-30

Content available at Genesis Publishing Consortium Limited

Journal of Aquatic Research and Sustainability

Journal homepage: www.genesispcl.com/journals/jars



Original Research

Sediment toxicity on the survival of *Capitella capitata* (Fabricius, 1780) and early life stages of *Echnometra mathaei* (Blainville, 1825) in Dar es Salaam coastal marine waters, Western Indian Ocean

Benard Mwakisunga[1], Pratap Harishchandra Bhagwanj[2]

[1] *Department of Environmental Health Sciences, Ruaha Catholic University, Iringa, Tanzania*
[2] *Department of Zoology and Wildlife Conservation, University of Dar es Salaam, Tanzania*

Article info

Article history
Received: 06 December 2024
Revised: 11 January 2025
Accepted: 12 January 2025
Published: 12 March 2025

Keywords
Embryo development
Fertilization success
Heavy metals
Sediment toxicity
Survival success

Abstract

Coastal marine pollution is a growing problem worldwide and ascertaining its impacts to aquatic biota is a growing concern. This study investigated the toxicity effects of sediments from Dar es Salaam harbor on the survival of *Capitella capitata* (Fabricius, 1780), fertilization and embryo development of *Echinometra mathaei* (Blainville, 1825) benthic biota along Dar es salaam harbor. Dar es salaam harbor is the main effluent receiving area along Tanzanian coastal marine waters associated with port operations. Sediment samples were collected at 35 sampling stations, *C. capitata* were collected at Msasani bay, 40 km from the harbor and *E. mathaei* were collected at Oysterbay rock shore about 10 km from the harbor. Sediments were collected along Dar es Salaam harbor using 50 cm long light piston corer and analyzed for heavy metals and total petroleum hydrocarbon levels and were used for toxicity testing. Higher concentrations of Cu, Cd, Pb, Zn, Cr, As, Ni, Fe, Ba, Mn were observed in the southern and central part of the harbor as compared to the northern par. However, Pb, Cr, Cd and Zn had the highest concentrations at the center of the harbor. Hydrocarbons included tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane and total petroleum hydrocarbons (TPH), their levels were low though was higher in the southern part of the harbor than in the central and northern part. Survival toxicity bioassay of *C. capitata* was conducted in sediments and seawater made from 1:4 sediments: seawater and 1:20 ratio, in a static and 48 hours' replaceable seawater, similarly fertilization and embryo development bioassay of *E. mathaei* were conducted in elutriates made from similar sediment – seawater ratios in a static and 24 hours' replaceable seawater. Higher mortality of *C. capitata*, lower fertilization and embryo development of *E. mathaei* were observed in sediments from the central part of the harbor and in the southern part, corresponding with the observed higher heavy metals and hydrocarbons levels. Revealing that higher heavy metal and hydrocarbons levels were the cause of the observed toxic effects. Comparison of the observed toxic effects showed that fertilization bioassay is the most sensitive test, thus making it suitable as bioindicator for the tropical costal marine pollution monitoring.

1. Introduction

Most of the unwanted products of anthropogenic activities on land and in the atmosphere ultimately end up into the aquatic environment with their effects mostly revealed in aquatic ecosystems. This induces increased stress which in turn challenges the survival and fitness of aquatic biota (Rumisha *et al.*, 2016). The main sources of contaminants in developing countries are from the discharge of untreated industrial, domestic wastes and agricultural runoff (FAO, 2017). Furthermore, mining and coastal development which involves shipping, dredging operation and resource exploitation append significant contaminants in aquatic systems. Contaminants eventually accumulate in sediments, making it more toxic to benthic organisms (Custodio *et al.*, 2019; Mihale, 2019; Mahugija and Sheikh, 2018; Sivakumar *et al.*, 2016). Dredging operation, suspension of materials in water column prior settlement and disposal of dredged material release contaminants back in the water column which may affect the development of early life stages of most invertebrates whose fertilization and embryo development takes place in the water column. Marine ecosystems in Tanzania and the Western Indian Ocean countries are facing increasing trends of pollution in coastal waters (Sawe *et al.*, 2019; Mihale, 2017). Dar es Salaam harbor is associated with shipping and port operation activities and is known as the main effluent receiving zone from major rivers flowing through industrial areas of Dar es Salaam City. Contaminants from these sources ultimately accumulate in sediments, thus threatening reproduction, development and the survival of benthic organisms. Higher heavy metal concentrations have been reported in sediments and some aquatic biota along Dar es Salaam coastal waters (Mihale, 2019; Sawe *et al.*, 2019; Rumisha *et al.*, 2015). High concentrations of Pb, Cd and Zn were observed in coastal sediments (Sawe *et al.*, 2019; Rumisha *et al.*, 2016). The ecological effects of contamination have been evident by the decreased biodiversity and coastal algal blooms (Gu, 2015). Considerable ecological risks indices to aquatic biota of heavy metals in sediments at Dar es Salaam harbor channel, Mtoni and Msimbazi estuaries in the vicinity have been reported (Mwakisunga *et al.*, 2021; Mihale, 2019; Rumisha *et al.*, 2015).

However, information on the toxic effects of heavy metals in sediments to the survival and development of early life stages of macrobenthic fauna at species level are scarce and vary with space

Corresponding authors
Email address: bmwakisunga@rucu.ac.tz (Benard Mwakisunga)

doi: https://doi.org/10.69517/jars.2025.02.01.0004

ISSN: 3079-5826

based on the variation in physiochemical conditions of ecosystems, the pollution load and the adapted biological responses of aquatic species (Rumisha *et al.*, 2015; Gu, 2014). Leaving a gap of knowledge on how benthic biota responds to sediment contaminants especially heavy metals in tropical marine waters and what could be the suitable bioindicator for monitoring tropical coastal marine water pollution? Therefore, this study aimed at assessing the survival responses of the benthic polychaetes *Capitella capitata* as well as in-vitro fertilization and embryo development success of sea urchins *Echinometra mathaei* to sediments collected at Dar es Salaam harbor. The findings of this study provides a sensitive bioindicator which will be used in pollution biomonitoring in the tropical coastal marine waters especially in the Western Indian Ocean regulatory authorities.

Polychaetes (*C. capitata*) was used because it is one of the infaunal benthos, inhabiting a wide range of sediment types from mud to sand, with a short life span and are non-selective deposit feeders, thus forming the base of aquatic food web (Musale and Desai, 2017). They are important prey for crustaceans, Molluscs, fish and wading birds (Rengaiyan *et al.*, 2017), majority are used as fish baits by fisher men. Likewise, Sea urchins (*E. mathaei*) are abundant and readily available species in the Western Indian Ocean having a complex life cycle which involves a sessile benthic adult phase and planktonic larval phase (Mishra, 2013). The wide habitat range, feeding behaviour, short life span and abundance of these benthic macrofauna makes them suitable bioindicators of contamination and for eco-toxicological studies in the tropical marine waters.

2. Materials and Methods

2.1 Ethical approval

Not applicable.

2.2 Study area and sampling design

Samples were collected from the southern to the northern part of the Dar es Salaam harbor, from January to May, 2020 (Figure 1).

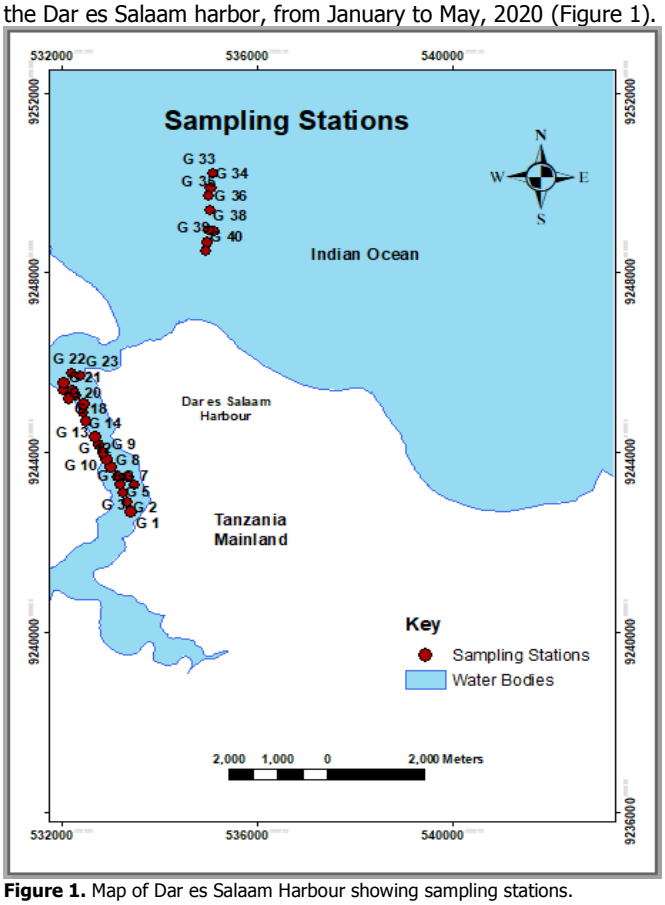


Figure 1. Map of Dar es Salaam Harbour showing sampling stations.

Sampling stations were established at an interval of 50 m apart where sediments for heavy metals and hydrocarbons analysis and toxicity bioassay were collected using the light piston corer at an average water depth of 15 m. Sediment samples for toxicity testing were transferred into zip lock plastic bags. Polychaetes (*C. capitata*) were collected at Msasani Bay, a non-polluted site about 10 km north of Dar es Salaam harbor. Sampling of polychaetes was performed according to USEPA (2001) and Goh *et al.* (2014). Polychaetes were stored in 1 L wide mouth glass bottle containing sieved field sediments and overlaying seawater. On the other hand, Sea urchins (*E. mathaei*) were collected during low tide by hand picking at Oysterbay rocky shore located north of the Dar es Salaam harbor. Organisms were identified using identification key (Raymond, 2002). Following standard procedures detailed in USEPA (2001), sea urchins were kept in a plastic container containing pebbles, natural kelp (*Ulva fasciata*) and overlying seawater collected from the same site to mimic their natural environments. Organisms were then transported to the laboratory for acclimatization and toxicity bioassays. In the laboratory, sediment samples were kept at 4 °C until used for toxicity tests. *In situ* determination of physical chemical parameters (pH, temperature, salinity and dissolved oxygen) was carried out during collection of organisms using Palintest MACRO 900 Water Quality Multi-parameter kit. The established average values of pH 8.55, temperature 30.04 °C, salinity 34.4‰ and DO 97.1% were similarly monitored during acclimatization of organisms and toxicity testing to mimicked the natural environment of test organisms.

2.3 Acclimatization of test organisms

Polychaetes (*C. capitata*) were introduced into 50 cm (L) x 30 cm (W) x 50 cm (H) aerated glass chambers containing sediments sieved through 2 mm nylon sieve and overlying seawater collected from the same site. The holding chambers were aerated and water replaced after every two days. Sea urchins (*E. mathaei*) were introduced into similar glass chambers containing pebbles and coralline sand with overlying seawater collected from the same site where sea urchin were collected and acclimatized for for a minimum of seven (7) days. Sea urchins were fed with natural kelp (*U. fasciata*) collected from the same site where sea urchins were collected and overlying seawater was daily changed (24 h).

2.4 Extraction of pepsin soluble collagen

Elutriate preparation was performed in accordance to USEPA (2001). Elutriate is the solution left after purifying by washing and decanting or after separation of the light and heavy particles off by washing. Elutriates were prepared using 800 ml unfiltered seawater and 200 ml sediment sub sample to maintain 1:4 volume of sediment-seawater ratio, well-mixed in 1 L beakers at 27±2 °C room temperature. The mixture was stirred vigorously for 30 min with a mechanical stirrer. After stirring, the mixture was then allowed to settle for 1 hour. The visually clear supernatants (about 300 ml) were siphoned off by using 50 ml glass syringe without disturbing the settled material. The supernatant represented 100% liquid plus suspended particulate phase for the whole sediment toxicity bioassay (USEPA, 2002). A volume of 50 ml of the supernatant was kept for toxicity bioassay and the remaining were filtered through Millipore filter to remove particulate matters then the filtrate was used for heavy metals and hydrocarbons analysis. Elutriates were then stored in glass bottle at ±4 °C before analysis and toxicity testing.

2.5 Heavy metals analysis in sediments and elutriates

The analysis of heavy metals in sediments followed the USEPA Hazardous Waste Test Methods (SW-846 Method 6010D) for Inductively Coupled Plasma-Optical Emission Spectrometry protocol at the Government Chemist Laboratory Authority (ISO 9001:2015 Certified). Sediment samples were air dried for two days and grinded using motor and pestle. One gram of the sediment sample was weighed and placed into Kjeldahl tubes, thereafter 10 ml of aqua-regia (3:1 mixture of HCl and HNO_3) was added. The mixture was

then digested under Kjeldahl system (TR Gerhardt No. 4021853) for two hours at 180 °C. Samples were allowed to cool at room temperature (25 °C), and filtered using Whatman filter paper No 41 into 100 ml volumetric flask and then filled up to the mark with deionized water. Elutriate samples (95 ml) were digested using 5 ml of HNO_3 in a Pyrex conical flask and heated on an electrical plate at 150 °C for two hours, then allowed to cool to room temperature. Thereafter filtered into 100 ml conical flask and filled with Milli-Q water up to the mark and analyzed for heavy metals. Heavy metals were analyzed by using PerkinElmer® Optima™ 4300DV dual via inductively coupled plasma optical emission spectrometer (ICP-OES), equipped with crossflow nebulizer, Scott spray chamber, and Echelle grating, segmented array charge-coupled device detector (SCD) (PerkinElmer, Inc., Shelton, CT, USA) which was optimized for a sensitivity of about 50,000 counts/sec for a 1-ng/mL Rh solution. Each element emitted multiple wavelengths, but a single wavelength for a given element was selected which its intensity was proportional to the concentration of that element in the analyzed sample.

2.6 Acute toxicity bioassay bioassay

Acute toxicity tests for *in-vitro* fertilization and embryo development success of *E. mathaei* were conducted in 10 ml glass beaker containing 1 ml of elutriates. A male and female Sea urchin were washed thoroughly with distilled water to remove epiphytes on spines and placed on a Petri dish separately. According to Mishra (2013) and USEPA (2001), induced spawning of male and female Sea urchin was carried out by injecting 1 ml of 0.5 M KCl into the coelomic cavity with a disposable syringe. Sperms and eggs were collected using micropipette. *In-vitro* fertilization and embryo development of *E. mathaei* were conducted using elutriates made from 1:4 and 1:20 sediment-seawater ratios. Comparison of relative sediment toxicities of multiple sites and control treatments were desired. The tests were conducted by making 1 ml sperm and egg stock solutions using control seawater. With modification to USEPA (2002), after 10 minutes' gametes acclimatization, 10 µl of sperms and 100 µl of eggs were mixed into 1 ml of elutriates and the control seawater, that was left for 30 minutes' fertilization period (Mishra, 2013). Thereafter, 10 µl of 1% formalin was added to stop fertilization process. Then 10 µl sub sample was observed under stereo microscope. Number of fertilized eggs per 100 eggs were counted and recorded, the percentage of which was established as fertilization success.

For the embryo development bioassay, similar procedures were adopted as in fertilization, however after 30 min fertilization period the process was not stopped, instead 100 µl of fertilized eggs were added into 10 ml elutriates contained in 20 ml glass container and then left for 48 hours for embryo development. The tests were conducted in dark since larvae are photopositive, with no water change but with mild aeration. After 48 hours, triplicate samples of 10 µl each were observed under the stereo microscope for the development of two arms of the embryo.

2.7 Chronic toxicity bioassay

Sediment toxicity tests was adopted from USEPA (2001), using polychaete worms (*C. capitata*) to determine the survival in field collected sediments for a period of 14 days. A total of 35 sediment samples collected from the harbor and the controls were tested for toxicity evaluation on the survival of polychaetes (*C. capitata*). Each of the recorded value was a mean of 5 replicates and the test organisms had an average length of 4.45 cm, which ensured that all of the test organisms had similar physiological state and were relatively from the same cohort. Every treatment contained 10 specimens of polychaetes in 1 L HDPE container each containing 200 ml of sediments filled with 800 ml of overlying seawater with five replicates and a control. Physio-chemical parameters in each container was maintained at pH 8.0 ± 0.4, temperature 29.03 ± 2 ºC, DO 68 – 73.3 % saturation, salinity 36.2 ± 2.0 ‰ in static condition with 50 % water renewal every 48 hours without feeding. Mortality was recorded at an interval of 2 hours for the first 12 hours and then after 24 hours. After 14 days, sediments were sieved through 1 mm metal sieve and survived organisms counted while unobserved organisms were considered dead and the dead emerged specimen were daily recorded and removed from the test.

2.8 Data analysis and quality control

Variation in the levels of heavy metals and total hydrocarbons between sampling stations were tested by using student t-test and toxicity tests data were tested for normality using Kolmogrov-Smirnov (KS) test and found normally distributed (KS = 0.22; P>0.10) for mortality and embryo development bioassays but fertilization test was not normally distributed. Therefore, evaluation of *C. capitata* mortality in different sediment samples were performed by using F-test. Mean mortalities between treatments and controls was tested by using paired two tailed t-test while variation in mortality with sediment sizes was tested by using one way ANOVA. Fertilization success in treatments and controls were tested using unpaired two tailed non parametric Mann-Whitney and fertilization success between samples were tested using one sample two tailed t-test. Assessment of the correlation and agreements between fertilization success in 1:4 and 1:20 ratios were performed using Pearson correlation coefficient and Kohen's Kappa test respectively. Differences of embryo development among sampling stations and between treatments and controls were tested by one sample two tailed t-test. Comparison of the fertilization success and embryo development success of *E. mathaei* in elutriates was performed by using paired *t*–test.

Quality control included triplicate sampling of sediments, analysis, and toxicity tests, procedural blanks, and measurement of the Certified Reference Materials (IAEA - 356). Test chambers, glassware and other equipment used to store and prepare test seawater, stock solutions and test sediments were thoroughly cleaned before use by phosphorus free detergent, distilled water, 10% HCl acid and two distilled water rinses. Glassware used only for live animals not exposed to toxicants were cleaned by distilled water. The percentage recoveries of metals obtained from Certified Reference Materials (IAEA - 356) ranged from 81.2% to 118.6%. The precision of ICP-OES analysis using relative standard deviation (%RSD) was < 5%. The results indicated good agreement between the certified and the obtained values. Polychaetes and sea urchin mortality during acclimatization period in holding tanks was < 5%. Test organism's survival, fertilization success and embryo development in controls was above 86%, making it acceptable for toxicity bioassays comparison with test treatments.

3. Results and Discussion

3.1 Total heavy metal concentrations in sediments

Heavy metal concentrations in sediments varied significantly with sampling stations along the harbor (Table 1). Higher heavy metal concentrations in sediments were observed in the southern part of the harbor (G1 to G10) as compared to the central (G11- 21) and northern part (G 31-40) of the harbor (Table 1) and had significant (t = 35.715, df = 31, P < 0.0001 at 95% confidence interval) variations between sampling stations. The higher heavy metal concentrations in the southern part of the harbor corresponded with Mihale (2019) findings in Mtoni river proximate to the southern part of the harbor, revealing that the area is highly contaminated with heavy metals brought in to the shore by rivers such as Mzinga and Kizinga rivers which converge at Matoni estuary which then flows to the Indian ocean through the harbor channel.

3.2 Total petroleum hydrocarbons in sediments

The concentrations of total petroleum hydrocarbons (TPH) (∑(n-alkanes) in sediments varied from 0.23 to 4.612 mg/kg with an average of 1.607 ± 1.117 mg/kg. Nonadecane and Heptadecane had relatively higher concentration (Table 2).

Table 1. Heavy metal concentrations (mg/kg) in sediments along Dar es Salaam harbor channel.

Stations	Cu	Zn	Pb	Cr	Cd	As	Ni	Co	Fe
The Southern harbor area									
G1	3.8	8.7	8.3	11.7	1.1	2.3	1.0	1.5	5321.7
G2	47.3	144.5	34.6	64.9	0.4	9.0	17.5	12.0	32895.7
G3	49.7	132.1	34.9	48.4	2.6	9.5	13.8	10.6	26530.0
G4	20.1	69.2	22.3	43.1	2.0	8.3	10.0	6.0	21540.4
G5	56.1	124.6	68.7	55.5	2.6	10.9	14.8	9.5	28117.3
G6	36.8	86.2	31.7	46.0	2.0	10.1	16.6	11.1	24165.6
G7	40.8	88.4	23.0	51.7	2.5	9.6	15.0	9.1	25418.1
G8	62.5	130.9	29.8	60.6	1.0	8.1	14.0	9.7	26964.1
G9	24.9	75.9	58.8	48.6	2.7	11.8	12.4	6.1	26510.0
G10	2.7	15.7	0.7	11.6	0.5	4.5	1.2	1.6	5371.6
mean	34.5	87.6	31.3	44.2	1.7	8.4	11.6	7.7	22283.5
The Central harbor area									
Stations	**Cu**	**Zn**	**Pb**	**Cr**	**Cd**	**As**	**Ni**	**Co**	**Fe**
G11	24.8	57.4	24.5	36.4	2.0	5.3	7.3	5.0	15010.1
G12	42.0	162.1	42.6	72.4	3.4	8.8	20.4	14.0	36324.0
G13	10.3	36.4	27.7	17.8	0.7	3.3	1.5	2.3	7857.4
G14	7.3	125.8	84.7	24.0	1.7	6.1	2.1	2.3	9830.3
G15	22.7	188.3	124.8	34.4	2.0	7.8	7.3	3.8	16185.6
G16	11.1	40.5	9.3	31.7	1.0	6.0	3.9	4.0	15178.0
G17	21.9	52.9	14.5	27.7	1.0	4.5	4.7	3.8	11511.2
G18	15.0	38.8	7.3	28.9	0.6	4.3	4.4	3.6	11709.1
G19	28.0	72.6	15.4	37.3	1.4	5.9	8.3	5.2	17464.2
G20	24.3	58.3	13.9	32.3	0.9	5.4	5.2	4.3	13397.9
G21	3.8	6.9	0.2	6.7	0.2	0.9	bdl	0.7	2457.5
mean	19.2	76.4	33.2	31.8	1.4	5.3	6.5	4.4	14265.9
The Northern harbor area									
Stations	**Cu**	**Zn**	**Pb**	**Cr**	**Cd**	**As**	**Ni**	**Co**	**Fe**
G31	4.7	15.7	5.2	18.5	0.6	4.9	0.2	2.0	8273.8
G32	3.7	8.4	bdl	13.7	0.2	4.5	bdl	1.2	6616.9
G33	4.4	12.6	4.4	13.4	1.1	4.2	bdl	1.3	6505.3
G34	5.1	13.7	4.8	15.7	0.3	4.1	bdl	1.6	6682.8
G35	11.6	36.5	8.4	31.5	1.2	6.0	4.4	3.7	13529.2
G36	13.7	49.8	10.6	38.7	1.3	7.1	7.9	4.6	17869.3
G37	17.0	58.5	18.9	38.3	1.6	7.0	8.0	4.0	17055.8
G38	24.4	55.9	18.6	46.5	2.3	8.3	10.9	5.9	20918.6
G39	13.7	53.0	10.9	50.2	1.8	8.4	12.5	7.0	21775.2
G40	3.0	6.0	3.8	12.1	0.5	2.8	bdl	0.9	4258.7
mean	10.1	31.0	9.5	27.9	1.1	5.7	7.3	3.2	12348.6

bdl = below detection limit

Table 2. Hydrocarbon concentrations in sediments along Dar es Salaam harbor channel.

Hydrocarbon	Concentrations of hydrocarbon (mg/kg)
Tetradecane	0.164
Pentadecane	0.84
Hexadecane	0.339
Heptadecane	2.47
Octadecane	0.546
Nonadecane	4.7
Mean value	1.607

Petroleum hydrocarbons assumed a similar trend with heavy metals, higher levels of hydrocarbons were observed in southern part of the harbor followed by the central part of the harbor and decreasing northwards. The differences between sampling stations were significant (t = 34.714, df = 31, $P < 0.0001$ at 95% confidence interval), revealing that the main source of petroleum hydrocarbons at the harbor is from the southern part of the harbor, possibly from municipal and industrial wastes, river runoff from the city entering the ocean through Mtoni estuary and storage facilities at the harbor. However, levels were lower than those reported elsewhere (Adeniji *et al.*, 2017) and according to Toluna *et al.* (2001), the observed values were of no toxicological effect.

3.3 Eco-toxicological assessment of sediments and elutriates to benthic biota

Sediment and elutriate toxicity bioassay were performed to elicit the potential toxic effects of sediment contaminants to the survival of benthic macrofauna and their early life stages in water column. Linkage of the levels of heavy metals and organic contaminants in sediments and elutriates were the basis for confirming whether the observed toxicities are due to the levels present in the sediments and their subsequent elutriates. However, the concentrations of petroleum hydrocarbons were too low to express toxicity effects.

3.3.1 Chronic toxicity bioassay of field sediments on the survival of C. capitata

The physiochemical parameters of the overlying seawater were daily recorded and maintained at DO 73.26 ± 5.0%, temperature 29.03 ± 2 ºC, pH 8.01 ± 0.4 and salinity 36.2 ± 2.0 ‰ mimicking the range of parameters observed in the field where test organisms were collected. Physiochemical conditions were in a range proposed by Saes *et al.* (2018), suitable for toxicity bioassay with tropical marine species.

Evaluation of *C. capitata* mortality in sediments from different sampling stations showed that, higher mortalities were observed in the southern part, the central and northern part of the harbor and expressed a significant differences ($F_{25, 88} = 11.858$, $P < 0.001$) in mortalities between sampling stations. This showed existence of spatial variation in sediment toxicities along the Dar es Salaam harbor (Figure 2). Variation in mortality with sediment grain sizes revealed no significant variation (ANOVA, F = 30.479, df = 30, $P = 0.143$ at 95% confidence interval) indicating little influence of sediment grain sizes in *C. capitata* mortality.

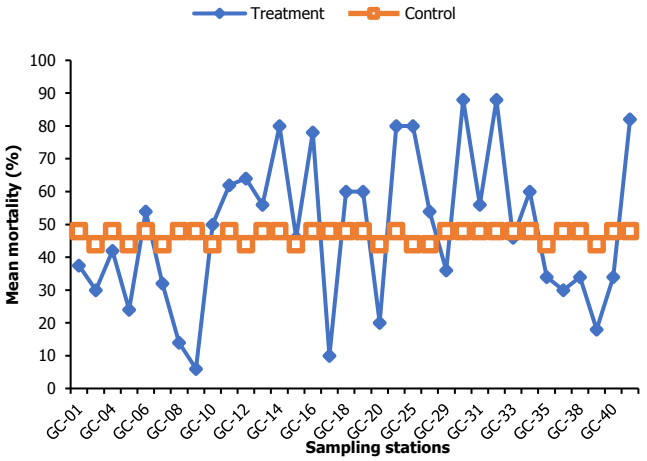


Figure 2. Comparison of *C. capitata* mortality in treatment and control experiments in sediments [*GC = Gravity corer and the numbers are the sampling stations].

Variation in *C. capitata* mortality with sampling stations was probably due to differences in inherent sediment contamination levels, types of contaminants, micro-environmental conditions and presence of non-point sources of contaminants along the harbor. Higher mortalities were observed in the southern part of the harbor, at the central harbor area and areas proximal to Msimbazi river mouth in the northern part, corresponding to the observed trend of heavy metal concentrations in similar areas (Figure 3). However, the paired sample two tailed t-test indicated that, the population mortality means of treatments and controls were not significantly different ($t_{33} = 0.4307$, $P > 0.05$). Indicating that, *C. capitata* was not sensitive to the observed levels of contaminants or has higher bioaccumulation capacity of the observed contaminants.

The observed spatial variation in sediment toxicities to *C. capitata* along Dar es Salaam harbor was similarly revealed by Bocchetti *et al.* (2004), Bat (2005), Haring *et al.* (2010), caused by spatial differences in contaminant levels and types, and the apparent specific environmental condition in different microhabitats (Mwakisunga *et al.*, 2020). The higher mortalities of *C. capitata* in southern and the harbour area might have been attributed by the observed higher heavy metal contamination and potential ecological risks in the two areas (Mwakisunga *et al.*, 2021). Likewise, high mortalities in samples collected from the southern and northern parts might have been associated with the effect of fine sediments (Saes *et al.*, 2018) from Mtoni and Msimbazi estuaries which sediments were more compacted and had low porosity hence providing difficult media for *C. capitata* to burrow through (Bat *et al.*, 2019). It could as well be due to the feeding behaviour of *C. capitata*, which preferably feed on fine sands,

mud and detritus and thus uptake more heavy metals adsorbed to fine sediments especially Cu and Zn (Van der Oost *et al.*, 2003).

Other studies revealed a significant correlation between biomarkers activity and heavy metal concentrations in invertebrates. Glutathione S-transferase (GSTx) and conjugates electrophilic metabolites with glutathione secretion were induced by both metals and organic contaminants (Greco *et al.*, 2010). Low concentrations of Cu, Cd and Zn ions (10 mg/l) caused a significant decrease in the enzymatic and amylolytic activity in tissues of aquatic invertebrate tissues (de Lima *et al.*, 2019). Said *et al.* (2017) observed inhibition of acetylcholinesterase enzyme (AChE) activity in invertebrate species when exposed to heavy metals. Likewise, Pagano *et al.* (2017) observed granular deposition of heavy metals in tissues of *Nereis succinea* related to the heavy metal concentrations from different sites. Thus, high heavy metal contamination in sediments puts more stress to the survival and health of marine benthic invertebrates. However, in this study, sediments were considered toxic when mean mortality of *C. capitata* in treatment were significantly greater than in controls. Nevertheless, mean mortality between treatments and controls were not significantly different, revealing that, sediments from Dar es Salaam harbor were either not contaminated enough to pose significant mortality effects to *C. capitata* or the test organism was insensitive to the observed levels of heavy metals which is due to the fact that, most *C. capitata* bio-accumulate heavy metals in their body tissues (Saes *et al.*, 2018; Bat, 2019). Hence more studies are required to determine the rate of bioaccumulation of heavy metals in *C. capitate* to ascertain its bioaccumulation capacity and establish the threshold levels.

3.3.2 Acute toxicity bioassay of elutriates on fertilization and embryo development of *E. mathaei*

The physicochemical parameters of elutriates were recorded and the ranges established, DO saturation ranged from 68 - 77.5 %, pH 7.6 – 8.24, temperature 26.5 – 31.7 °C and salinity 28.8 – 39.7 ‰. Data for toxicity bioassay were expected to be binomial (i.e. an egg is fertilized or not fertilized counted per 100 eggs). Successful fertilization was observed by the development of a ring as an outermost layer of an egg.

Mean fertilization success in 1:4 ratio treatments ranged between 49.66 and 94.55% with an average of 75.8%, while in control the range lied between 80.0 and 92.3% with an average of 87.01%. Comparison between fertilization success in treatments and controls and between sampling stations were significantly different (Mann-Whitney (U' = 831, $P < 0.0001$ and t = 35.715, df = 31, $P < 0.0001$ at 95% confidence interval respectively). Lower fertilization success was observed in samples collected in the southern and at the harbor area (Figure 3), indicating that higher levels of heavy metals and hydrocarbons contamination in the southern and at the harbor area have influenced the observed lower fertilization (Mwakisunga *et al.*, 2021).

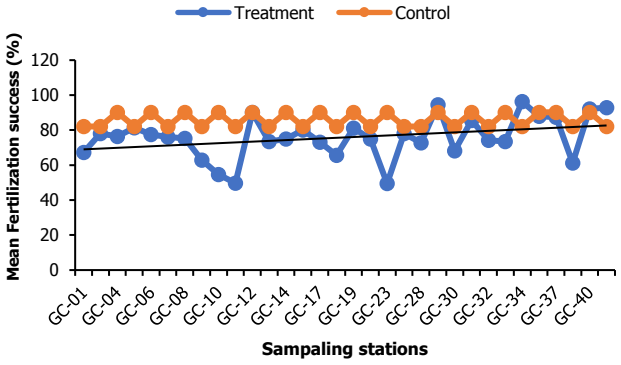


Figure 3. Fertilization in 1:4 sediment to seawater ratio of *E.mathaei* [*GC = Gravity corer and the numbers are the sampling stations].

Similarly, fertilization success of *E. mathaei* in more dilute elutriates of 1:20 sediment to seawater ratio treatments ranged between 31.38 to 71.73 % with an average of 51.49 % while in controls ranged between 86.6 to 88.4 % with an average at 87.25% (Figure 4). Analysis of the fertilization success in 1:20 ratio showed a similar trend of toxicities with those of 1:4 ratios. Likewise, mean fertilization success between treatments and controls and between sampling stations revealed significant differences (Mann-Whitney test, U' = 676.0, $P < 0.0001$ and t = 26.95, df = 25, $P < 0.0001$ at 95% confidence interval respectively).

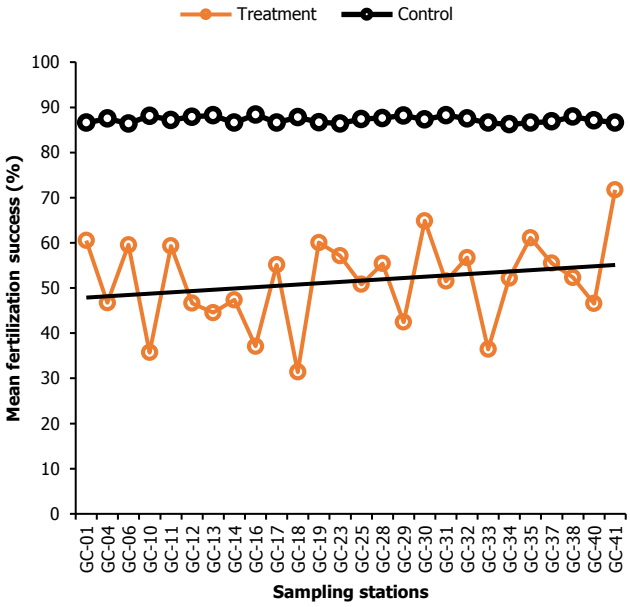


Figure 4. Fertilization in 1:20 sediment to seawater ratio of *Echinometra mathaei* [*GC = Gravity corer and the numbers are the sampling stations].

Fertilization success was lower in samples collected in the southern area and at the harbour area than in the northern part of the harbour. This is due to the observed higher heavy metal contamination status and potential ecological risk in those areas (Mwakisunga *et al.*, 2021) and higher hydrocarbons levels in the two sites.

Aassessment of the correlation and agreements between fertilization success in 1:4 and 1:20 ratios were performed using Pearson correlation coefficient and Kohen's Kappa test respectively. Tests revealed that the two ratios had insignificant correlation with no agreement (R = 0.0045, $P = 0.93$ at 95% confidence interval) and (Kappa = 0.0001). Indicating that one of the ratio was more toxic than the other (Figure 5). According to Novelli *et al.* (2006), 1:20 sediment – seawater ratio was more toxic that 1:4 ratios due to dilution effect.

According to Resgalla *et al.* (2020), the ranges of physiochemical parameters monitored during *in-vitro* fertilization bioassays were at a range not causing fertilization inhibition, decreased mitotic activity and embryo development defects. The disagreement of the fertilization success in the two ratios (1:4 and 1:20) was due to the fact that 1:4 sediment-seawater ratio was less toxic as compared to 1:20 ratio due to dilution effect (Novelli *et al.*, 2006). Higher sediment-seawater ratio toxicities are due to larger volumes of seawater to the volume of sediments which in turn influence toxicants passing from the solid phase in sediments to liquid phase. Thus in this study, 1:20 sediment-water ratio was more toxic than 1:4 ratios and the observed lower fertilization success was due to higher contamination of heavy metals and hydrocarbons and not the variations in physical-chemical conditions in the laboratory.

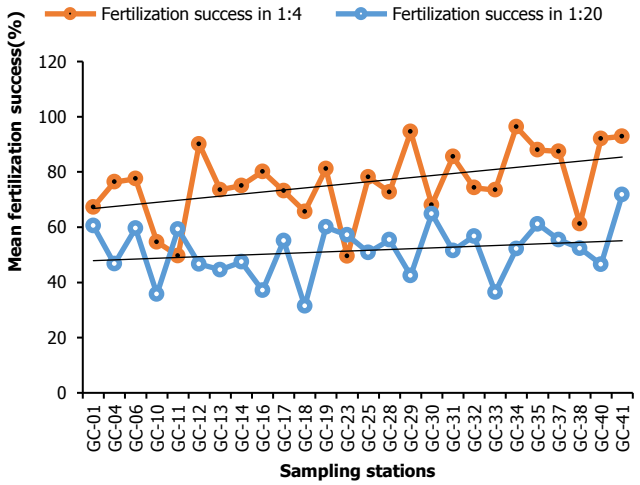


Figure 5. Trend of fertilization success in 1:4 and 1:20 ratios. [*GC = Gravity corer and the numbers are the sampling stations].

3.3.3 Embryo development bioassay of E. mathaei in elutriates

Similar to fertilization success, in embryogenesis bioassay, sediment samples from multiple sites were compared with a control in which data were binomially observed including embryo development of two arms or no development of two arms in 48 hours of *E. mathaei*. Observations were done per 100 embryos which developed two arms and without. Embryo development success of *E. mathaei* in treatments ranged from 11.4 to 96.4% with an average of 54% while in controls ranged between 78.9 and 84.8 with an average of 82.3%. Embryo development varied with sampling stations (Figure 6), low development was observed in samples collected in the southern and at the harbor area. Data were normally distributed using Kolmogorov-Smirnov (KS) test (KS = 0.11, $P > 0.1$) and the differences between means of embryo development between sampling stations were significantly different (t = 12.442, df = 30, $P < 0.0001$ at 95% confidence interval). Comparison between means of treatments and controls using paired two tailed *t*-test (t = 6.442, df = 30, $P < 0.0001$ at 95% confidence interval) revealed significant differences, indicating spatial variation.

The sediments were considered toxic when the differences in embryo development between treatments and the control were significant. Thus, the attested embryo development successes between treatments and the control showed significant differences, hence presenting significant sediment toxicity to the early stage's development of *E. mathaei*. The observed higher toxicities in the southern and at the central harbor area might have caused by the observed moderate to very high contamination of heavy metals (Mwakisunga *et al.*, 2021) and the higher hydrocarbons levels. Comparison of the fertilization success and embryo development bioassys passed normality test ($P > 0.1$) using Kolmogorov-Smirnov test and showed significant differences using paired t test (t = 4.169, df = 55, $P = 0.0001$, CI = 95%), revealing that, fertilization sucess was more sensitive bioassay as compared to embryo development. The findings corresponds to previous studies, Trifnoggi *et al.* (2017) found little variation in *Lytechinus variegatus, Echinometra lucunter, Arbacia lixula* and *Encope emarginata* embryo development sensitivities to Pb, Cu, Cd, Cr elements but Pb and Cu were relatively more toxic to the embryo development of *Lytechinus variegatus*. Indicating that, toxicity effects of heavy metals to sea urchin embryo development varies slightly with species and the type of metal and animal species sensitivity to the contaminants. The higher sensitive to Pb, Cu and Zn is a reason for the observed lower embryo development in the samples collected from the southern and harbour areas of this study which had higher contamination of Cu, Pb and Zn.

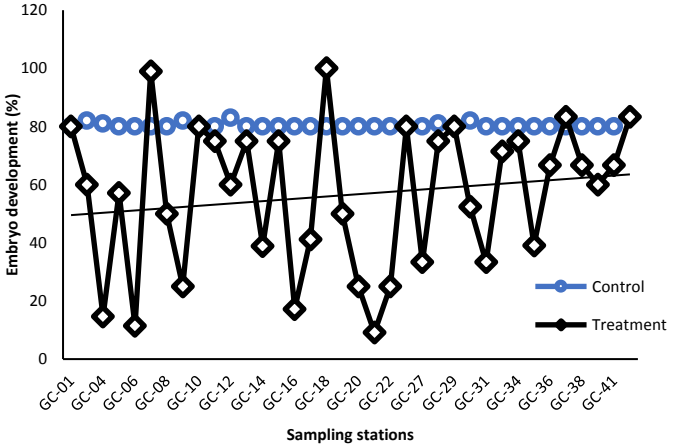


Figure 6. Embryo development success in 1:4 sediments to seawater ratio elutriates.

Reasons to low fertilization and embryo development of Sea urchins exposed to contaminants has been explained by previous studies. Ruocco *et al.* (2016) found doubling malformation of *Paracentrotus lividus* embryo at increasing concentrations of Cd, Cu, Zn and Pb. Likewise, pluteus, prism and gastrula stages of embryo development (48-96 hours) of *E. mathaei* were inhibited when exposed to 200 µg/l, 400µg/l and 800 µg/l of Pb (Gambardella *et al.*, 2021). Therefore, Sea urchin fertilization and embryo development are more sensitive to Pb, Cu, and Zn and the effects varies with concentrations, thus higher levels of Pb, Cu and Zn in coastal marine waters endanger the proliferation of sea urchins generation and its ecological food web.

On the other hand, physio-chemical conditions in the laboratory affects the fertilization and embryo development of Sea urchins. Mamindy *et al.* (2011) observed that, fertilization of sea urchin in warm, acidified and high pCO_2 seawater was only affected by sperm density. Resgalla *et al.* (2020) reviewed that pH variation in <7 and > 8.5 induced fertlization inhibition, decreased mitotic activity and impaired embyo development in sea urchins. Carballeira *et al.* (2012) showed that sediment particle size and amount of organic matter have significant effect to elutriate toxicities. Contrary to Mamindy *et al.* (2011), Rahman *et al.* (2004) found that the optimal range of fertilization and embryo development biassay in Sea urchins varies with species. However, Rahman *et al.* (2004) noted that fertilization success and consequent embryonic development are affected much by a factor of egg size in the species *E. mathaei.* This makes *E. mathaei* life stage more appropriate bioindicator for heavy metal contamination assessment and biomonitoring in coastal marine waters. Therefore, the observed lower embryo development was caused by the higher contamination of heavy metals along the harbor area.

4. Conclusions

This study tried to advance the knowledge from the previous understanding of the levels of heavy metals and hydrocarbons in tropical marine waters and the established comparative concentrations standards of contaminants. The toxicity effects of sediments to the survival of *C. capitata* in treatments and control showed no significant differences, indicating that *C. capitata* was not sensitive enough to the observed levels of contaminants or had higher bioaccumulation capacity of the contaminants, thus not a suitable bioindicator for tropical coastal marine pollution biomonitoring. However, in early life stages of coastal marine biota, the observed contaminant's concentrations lowered embryo development and

fertilization success of *E. matheae*. Revealing that, heavy metals and hydrocarbons contamination in tropical coastal marine waters pose a significant threat to sustainability of benthic biota whose fertilization and embryo development takes place in the water column. Nevertheless, fertilization success bioassay was more sensitive than embryo development, thus making it suitable as bioindicator for the evaluation and biomonitoring of heavy metals and hydrocarbons contamination in tropical coastal marine waters. The insensitivity of *C. capitata* to heavy metals might be due to its capacity to bio-accumulate heavy metals, thus requiring more studies to ascertain the rate of bioaccumulation and the potential of heavy metals and hydrocarbons transfer from polychaetes to higher trophic levels through food chain and the associated health effects to higher trophic level organisms.

Abbreviations

USEPA=United States Environmental Protection Agency; FAO=Food and Agricultural Organization; DO=Dissolved oxygen; HDPE=High Density Poly Ethylene.

Acknowledgments

Sincere thanks go to TECHNITAL Ltd, Italy for financial support of the field work which was part of the contracted work for the "Assessment of the effects of dredged materials at Dar es Salaam harbour to marine ecosystems".

Funding information

TECHNITAL Ltd, Italy provided the fund to conduct the study.

Data availability

All data generated and analyzed during this study are included in this article.

Informed consent statement

Not applicable.

Conflict of interest

Authors declare that, they have no known competing financial or personal interests that plausibly appeared to influence the work on this paper.

Authors' contribution

Conceptualization: Benard Mwakisunga and Pratap Harishchandra Bhagwanj; **Data collection**, **analysis and manuscript preparation:** Benard Mwakisunga. All authors critically reviewed the manuscript and agreed to submit final version of the published article.

References

Adeniji AO, Omobola O, Oko, OO, Anthony I and Okoh AI, 2017. Petroleum hydrocarbon profiles of water and sediment of Algoa Bay, Eastern Cape, South Africa. Interantional Journal of Environmental Research and Public Health, 14(10): 1263. https://doi.org/10.3390/ijerph14101263

Bat L, 2005. A review of sediment toxicity bioassays using the Amphipods and Polychaetes. Turkish Journal of Fisheries and Aquatic Sciences, 5(2): 119–139.

Bat L, Şahin F and Öztekin A, 2019. Assessment of heavy metals pollution in water and sediments and polychaetes in Sinop shores of the Black Sea. KSU Journal of Agriculture and Nature, 22(5): 806–816. https://doi.org/10.18016/ksutarimdoga.v22i45606.535882

Bocchetti R, Fattorini D, Gambi MC and Regoli F, 2004. Trace metal concentrations and susceptibility to oxidative stress in the polychaete *Sabella spallanzanii* (Gmelin) (Sabellidae): Potential role of antioxidants in revealing stressful environmental conditions in the Mediterranean. Archives of Environmental Contamination and Toxicology, 46(3): 353–361. https://doi.org/10.1007/s00244-003-2300-x

Carballeira C, De Orte MR, Viana LG, Del Valls TA and Carballeira A, 2012. Assessing the toxicity of chemical compounds associated with land-based marine fish farms: The sea urchin embryo bioassay with *Paracentrotus lividus* and *Arbacia lixula*. Archives of Environmental Contamination and Toxicology, 63(2): 249–261. https://doi.org/10.1007/s00244-012-9769-0

Custodio M, Huaraca F, Espinoza C and Cuadrado W, 2019. Distribution and accumulation of heavy metals in surface sediment of Lake Junín National Reserve, Peru. Open Journal of Marine Science, 9: 33–48. https://doi.org/10.4236/ojms.2019.91003

de Lima BR, Lima JM, Maciel JB, Valentim CQ, Nunomura RS, Lima ES, Koolen HHF, de Souza A, Pinheiro MB, Cass QB and da Silva FMA, 2019. Synthesis and inhibition evaluation of new Benzyltetrahydroprotoberberine alkaloids designed as acetylcholinesterase inhibitors. Frontiers in Chemistry, 7: 629. https://doi.org/10.3389/fchem.2019.00629

FAO, 2017. The future of food and agriculture: Trends and challenges. Food and Agriculture Organization, Rome, Italy. pp. 180.

Gambardella C, Marcellini F, Falugi C, Varrella S and Corinaldesi C, 2021. Early-stage anomalies in the sea urchin (*Paracentrotus lividus*) as bioindicators of multiple stressors in the marine environment: Overview and future perspectives. Environmental Pollution, 287: 117608. https://doi.org/10.1016/j.envpol.2021.117608

Goh BPL, Lai CH, Tan LT, Yap NWL and Dissanayake A, 2014. Handbook of marine ecotoxicology techniques. Goh BPL (Eds.). National Institute of Education, Nanyang Technological University, National Parks Board Singapore. pp. 110. http://dx.doi.org/10.13140/2.1.3101.5205

Greco L, Serrano R, Blanes MA, Serrano E and Capri E, 2010. Bioaccumulation markers and biochemical responses in European sea bass (*Dicentrarchus labrax*) raised under different environmental conditions. Ecotoxicology and Environmental Safety, 73: 38–45. https://doi.org/10.1016/j.ecoenv.2009.09.011

Gu JD and Wang Y, 2015. Coastal and marine pollution and ecotoxicology. Ecotoxicology, 24(7-8): 1407–1410. https://doi.org/10.1007/s10646-015-1528-3

Gu JD, 2014. Assessment of ecosystem health and ecotoxicology through chemical analysis and modeling. Ecotoxicology, 23(4): 475–479. https://doi.org/10.1007/s10646-014-1206-x

Haring HJ, Smith ME, Lazorchak JM, Crocker PA, Euresti A, Wratschko MC and Schaub MC, 2010. Comparison of bulk sediment and sediment elutriate toxicity testing methods. Archive of Environment Contamination and Toxicology, 58(3): 676-683. https://doi.org/10.1007/s00244-009-9447-z

Mahugija JAM and Sheikh HM, 2018. Status of selected heavy metals dispersion from top soil in and around automobile workshop areas in Zanzibar Municipality, Tanzania. Tanzania Journal of Science, 44: 12–23.

Mamindy PY, Hamer B, Roméo M, Géret F, Galgani F, Durmiši E, Hurel C and Marmier N, 2011. The toxicity of composted sediments from Mediterranean ports evaluated by several bioassays. Chemosphere, 82(3): 362–369. https://doi.org/10.1016/j.chemosphere.2010.10.005

Mihale MJ, 2017. Environmental health risks due to anthropogenic metals in Mtoni estuary ecosystem and its environs, Tanzania. Huria: Journal of the Open University of Tanzania, 24: 1–24.

Mihale MJ, 2019. Multivariate assessment of metal contamination in Mtoni sedimentary environment, Tanzania. Tanzania Journal of Science, 45(2): 158–172.

Mishra A, 2013. Developmental biology of the sea urchin, *Echinometra mathaei* (Blainville, 1825) (Echinodermata: Echinoidea) and its larval settlement mechanism. PhD thesis. Pondicherry University, India.

Musale AS and Desai DV, 2011. Distribution and abundance of microbenthic polychaetes along the South Indian coast. Environmental Monitoring Assessment, 178(1-4): 423–436. https://doi.org/10.1007/s10661-010-1701-3

Mwakisunga B, Machiwa JF and Pratap HB, 2020. Assessment of sediment and benthic macrofauna distribution at Dar es Salaam harbour channel**.** Journal of Geoscience and Environment Protection, 8(3): 133–147. https://doi.org/10.4236/gep.2020.83010

Mwakisunga B, Pratap HB, Machiwa JF and Stephano F, 2021. Heavy metal contamination and potential ecological risks in surface sediments along Dar es Salaam harbour channel. Tanzania Journal of Science, 47(5): 1606–1621. https://dx.doi.org/10.4314/tjs.v47i5.11

Novelli AA, Losso C, Libralato G, Tagliapietra D, Pantani C and Ghirardini A, 2006. Is the 1:4 elutriation ratio reliable? Ecotoxicological comparison of four different sediment: water proportions. Ecotoxicology and Environmental Safety, 65(3): 306–313. https://doi.org/10.1016/j.ecoenv.2005.08.010

Pagano G, Thomas P, Guida M, Palumbo A, Romano G, Oral R and Trifuoggi M, 2017. Sea Urchin bioassays in toxicity testing: II. Sediment evaluation. Expert Opinion Environmental Biology, 6:1. https://doi.org/10.4172/2325-9655.1000141

Rahman SM and Uehara T, 2004. Effects of egg size on fertilization and embryonic development of sibling tropical sea urchins (genus, *Echinometra*). Science Asia, 30: 75-80.

Raymond MD, 2002. Field guide book to the seashores of eastern Africa and the western Indian Ocean Islands. Raymond MD (Eds.). SIDA. pp. 464.

Rengaiyan P, De K and Ingole B, 2017. Polychaete assemblage driven by substrate composition along the coastal waters of the South-eastern Arabian Sea. Regional Studies in Marine Science, 16: 208–215. https://doi.org/10.1016/j.rsma.2017.09.004

Resgalla Jr C, Friedrichsen JC, Souza RC, Vieira DC, Deucher K and da Silva MAC, 2020. Assessment of sea urchin spawning induction and oocyte conservation applied to ecotoxicological assays. Ocean and Coastal Research, 68(1-2): e20288. https://doi.org/10.1590/S2675-28242020068288

Rumisha C, Mdegela RH and Leermaker MK, 2016. Trace metals in the giant tiger prawn *Penaeus monodon* and mangrove sediments of the Tanzania coast: Is there a risk to marine fauna and public health? Ecotoxicology and Environmental Safety, 132: 77–86. https://doi.org/10.1016/j.ecoenv.2016.05.028

Rumisha C, Shukuru H, Lyimo J, Maganira JD and Nehemia A, 2015. Benthic macroinvertebrate assemblages in mangroves and open intertidal areas on the Dar es Salaam coast, Tanzania. African Journal of Aquatic Science, 40(2): 143–151. https://doi.org/10.2989/16085914.2015.1051504

Ruocco N, Costantini M and Santella L, 2016. New insights into negative effects of lithium on sea urchin *Paracentrotus lividus* embryos. Scientific Reports, 6: 32157. https://doi.org/10.1038/srep32157

Saes RVST, Moreira LB, Davanso MB, Perina FC and Abessa DMS, 2018. Developing a protocol whole sediment toxicity testing with the polychaete *Armandia agilis*. Ecotoxicology and Environmental Contamination, 13(2): 85–97. https://doi.org/10.5132/eec.2018.02.11

Said REM, Abdallah AT, Mostafa MA and El-Shimy NA, 2017. Efficiency of polychaete *Nereis* (*Neanthes*) *succinea* as biomonitor for heavy metals pollution in the Red Sea, Egypt. Advances in Bioscience and Bioengineering, 5(5): 86-91. https://doi.org/10.11648/j.abb.20170505.13

Sawe SF, Shilla DA and Machiwa JF, 2019. Assessment of enrichment, geo-accumulation and ecological risk of heavy metals in surface sediments of the Msimbazi mangrove ecosystem, coast of Dar es Salaam, Tanzania. Chemistry and Ecology, 35(9): 835–845. https://doi.org/10.1080/02757540.2019.1663344

Sivakumar S, Chandrasekaran A, Balaji G and Ravisankar R, 2016. Assessment of heavy metal enrichment and the degree of contamination in coastal sediment from south east coast of Tamilnadu, India. Journal of Heavy Metal Toxicity and Diseases, 1(2): 11. https://doi.org/10.21767/2473-6457.100011

Toluna LG, Okay OS, Gaines AF, Tolay M, Tüfekçi H and Kıratlı N, 2001. The pollution status and the toxicity of surface sediments in İzmit Bay (Marmara Sea), Turkey. Environment International, 26(3): 163–168. https://doi.org/10.1016/S0160-4120(00)00096-9

Trifuoggi M, Pagano G, Guida M, Palumbo A, Siciliano A, Gravina M, Lyons DM, Burić P, Levak M, Thomas PJ, Giarra A and Oral R, 2017. Comparative toxicity of seven rare earth elements in sea urchin early life stages. Environmental Science and Pollution Research, 24(25): 20803–20810. https://doi.org/10.1007/s11356-017-9658-1

USEPA, 2001. Methods for collection, storage and manipulation of sediments for chemical and toxicological analyses: Technical manual. Office of Science and Technology. Washington DC, USA.

USEPA, 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. U.S. Environmental Protection Agency Office of Water, Washington DC, USA.

Van der Oost D, Beyer J and Vermeulen NPE, 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology, 13(2): 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6