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Lyphira perplexa Galil, 2009 photo by Tariq H.Y. Al-Maliky

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Journal:

Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. Biodiversitas 7: 154-158.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta***.** 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

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Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com. DOI:10.1038/msb.2008.24

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Short Communication: New records of Leucosiid crabs *Lyphira perplexa* Galil, 2009 (Crustacea; Decapoda; Leucosiidae) in the northwest of the Arabian Gulf, Iraq

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Manuscript received: 12 March 2020. Revision accepted: 11 May 2020.

Abstract. *Al-Maliky THY. 2020. Short Communication: New records of Leucosiid crabs* Lyphira perplexa *Galil, 2009 (Crustacea; Decapoda; Leucosiidae) in the northwest of the Arabian Gulf, Iraq. Ocean Life 4: 1-3.* The present study reports the occurrence of one leucosiid crabs species, *Lyphira perplexa Galil*, 2009, from the Iraqi coast, Northwest of the Arabian Gulf. So considered, this was a recording of crabs for the first time from the Iraqi waters and the Arabian Gulf. Previously, *L. perplexa* was described from various sites in the Arabian Gulf. Therefore, the taxonomic morphological details of the specie described and clarified are; the carapace is carried granules close to its dorsal surface, while the hepatic and the branchial carry granules slightly larger and in behind front were granules irregularly beaded cumference in behind the front, there are differences in size.

Keywords: Arabian Gulf, Crustacea, Leucosiidae, *Lyphira perplexa*

INTRODUCTION

The Genus *Lyphira* crab was important in coastal biological diversity and artisanal fisheries of all coastal countries, including Iraq. So far, research is continuing to record species affiliated with it. Leucosiidae of crab is a common faunal of littoral and sublittoral smooth sediment habitats and is more diverse than all brachyuran families (Sudharma et al. 2014). In recent years, interest has been in recording crustaceans, especially the crab of Brachyura, on the Iraqi coast from the Arabian Gulf (Al Khafaji et al., 2017; Al Khafaji and Al Maliky, 2019); also Maliky and Al-Maliky recorded *Lyphira heterograna* (Ortmann, 1892) one of the types of this family, in the preparation. Recorded *Lyphira perplexa* and *Acrania erinacea* in the Gulf Arabian waters by Ebadi et al. (2018). And Sudharma et al. (2014) recorded *L. perplexa* crab in Indian waters.

This report is the first record of *L. perplexa* from the northwest of the Arabian Gulf.

MATERIALS AND METHODS

A total of eight specimens (four males and four females) of *L. perplexa* crab were investigated and revised during the present study. First, samples were collected from the waters of the Iraqi coast northwest of the Arabian Gulf using trawl fishing: N29°53'35.9736", E48°35'28.9212", in-depth between 6-13 m (Figure 1). The collected specimens were immediately preserved in 70-80% Alcohol. Next, the crabs have diagnosed in the laboratory, Department Marine Biology, Marine Science Center (MSC), University of Basrah, Iraq.

The collected and examined specimens were photographed and identified according to the findings of Galil (2009) and Ebadi et al. (2018). All the measurements were taken in mm. Abbreviations: C.W.: carapace width; CL: carapace length; Male G1: first left gonopods, Coll.: collector.

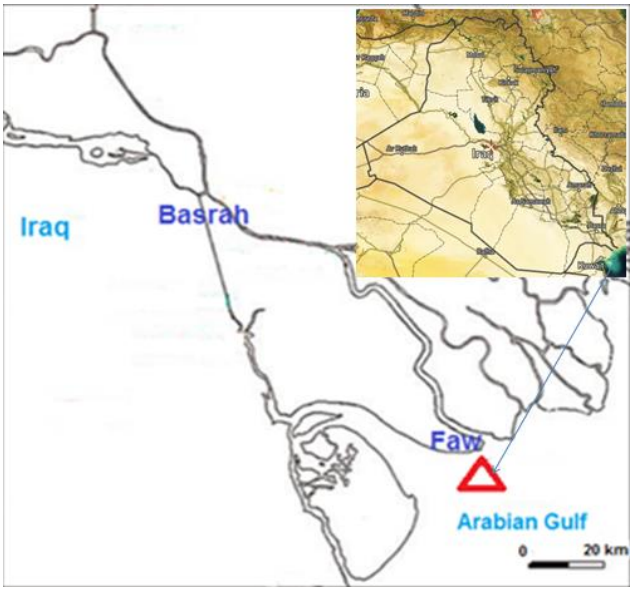

Figure 1. Map representing the occurrence of *Lyphira perplexa* northwest of the Arabian Gulf

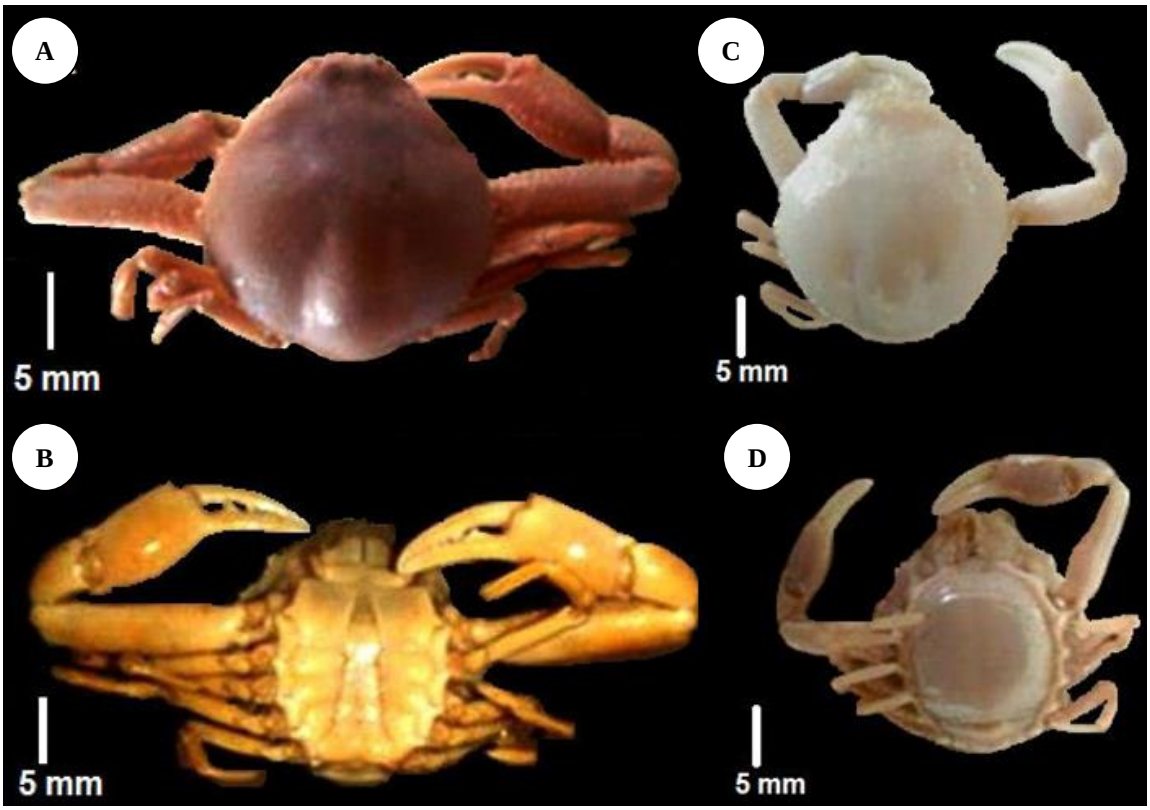

Figure 2. *Lyphira perplexa*: A, B, ♂; C, D, ♀. Scales:5 mm.

Figure 3. *Lyphira perplexa*: first male G1. Scales:2 mm

RESULTS AND DISCUSSION

Identification
Systematic accounts
Order: Decapoda Latreille, 1802
Family: Leucosiidae Samouelle, 1819
Subfamily: Philyrinae Rathbun, 1937
Genus *Lyphira* Galil, 2009
Species *Lyphira perplexa* Galil, 2009
(Figures 2. A-D)

Only first in the male abdominal segment of articulate, and transversely yoke-shaped; first pleopod plain, as Second-6th abdominal segments of bearing abdominal denticle, while Apical process of the first pleopod flattened, squat, also, carapace taller than cheliped merus (Galil, 2009).

Examined material

Coll. AL-Maliky,four ♂♂♂♂, CL 23.48, 19.64, 18.32, 17.80; CB 22.20, 19.25, 17.78, 17.53 mm; four ♀♀♀♀, CL 18.95, 16.99, 16.76, 16.62 mm; CB 18.42, 15.66, 16.19, 16.36 mm, (MSC).

Diagnosis

The carapace is carried granules close to its dorsal surface (Figure 2.A). At the same time, the hepatic and the branchial carry slightly larger granules, and behind the

front were granules irregularly beaded cumference in the front, different in size. And granules of minutely in both the frontal margin from carapace and external maxillipeds. Anterior margin of epistome is mindless. The Pterygostomian region is prominently granulated. Anterior margin of the abdominal sulcus in both males and females with prominently granulate (Figure 2.B, 2.D). Fused male abdominal segments 2-6 carry granular basal knots separated by concave. The margins of the abdominal segments are fused in the granules of the female, in which the granules of the regions are prominent (Figure 2.C, 2.D). Carpus with a row of granules on the inner margin; a piece of the granules are extremely fine on the upper margin.

The upper and lower surfaces of the propodus and the lower conical granules with an inner surface. Grooved fingers, the most prominent granulation on the outer margins; the inner margin of polyex with a triangular dental margin. Male granulation more than female. G1 has long hair in the apical portion of the Gonopod, and its tip is hairless with a large, pointed opening (Figure 3).

Remarks

Lyphira perplexa (Galil, 2009) can be easily distinguished and diagnosed from other types of Leucosiidae found in the waters of the Arabian Gulf. *L. perplexa* can be diagnosed from *Lyphira heterograna* by its smaller granules on the surface and margin of the carapace. The present specimen represents the new record of the specie L. perplexa its distribution range in the Arabian Gulf, Iraq.

Distribution

It spreads on the Indian and Pakistani coasts, the Gulf of Oman, the Iranian and Kuwaiti coasts, and the current study on the Iraqi coast of the Arabian Gulf.

ACKNOWLEDGEMENTS

I thank everyone who helped me complete my work with my colleagues at the Marine Science Center, Basra University, Iraq.

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Polycyclic aromatic hydrocarbons in fishes and some environmental samples on the coast of Ghana

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Abstract. *Osei-Yeboah E, Appiah-Opong R, Ofosuhene M. 2020. Polycyclic aromatic hydrocarbons in fishes and some environmental samples on the coast of Ghana. Ocean Life 4: 4-16.* The shift in industrialization in our society, including mining of coal and minerals, drilling for oil has led to the accumulation of xenobiotic products and natural chemical substances. The activities from mining and drilling have continuously contributed to the release of polycyclic aromatic hydrocarbons (PAHs) and lead to their deposition in coastal environments. The process ultimately leads to bioaccumulation in plants and animals, creating dangerous pollutants. PAHs is a well-known cause of cancer, mutation, and embryonic pathologies. In Jubilee oil fields, Ghana, oil drilling is ongoing. However, onshore baseline environmental assessment of PAHs has not yet been performed. In this study, we performed an environmental assessment of some communities bordering the oil drilling fields to establish (i) levels of fish DNA adduct formation of fishes, (ii) levels of PHA in soil, plants, water, and fish. Several fishes from three study areas showed the presence of micronuclei as evidence in the examination of blood smears, although their mean micronucleated frequencies were below the threshold frequency of 15%. There was no statistical difference between their mean frequencies using one-way ANOVA analysis with a p-value < 0.05 except that of the data from one species, *Chloroscombrus chrysurus* (Linnaeus, 1766). Reverse-phase HPLC analysis of fish, water, plant and soil samples collected from six study sites was performed. Two fish species *Pomadasys incisus* (Bowdich, 1825) at Aboadze and *Thunnus alalunga* (Bonnaterre, 1788) from Dixcove recorded four and one PAH compound, respectively with concentrations above the maximum contaminant levels of 30 µg/kg set by the USEPA. The mean concentration of PAHs in water samples from two of the four study areas ranged from 1.4 to 1255 µg/L. All samples recorded concentrations above the threshold limit value of 50 ng/L set by the World Health Organization. Sixty plant samples were collected across the six study areas, and only *Erythrina senegalensis* DC. and *Ficus umbellata* Vahl recorded the presence of PAHs with concentrations in the range of 0.15-4.70 mg/kg. Soil samples were collected from two different depths of 0-15 cm and 15-30 cm. The mean concentration of PAHs in surface soils (0-15cm) ranged from 0.1 to 95 mg/Kg, with that at 15-30 cm ranging from 0.12 to 105 mg/kg. The PAH composition profile in all the samples was similar, with 2- 3 ring PAHs being dominant, which is suggestive of a petrogenic source.

Keywords: biomarker, DNA adduct, fish, polycyclic aromatic hydrocarbon

INTRODUCTION

Crude oil is commonly regarded as one of the highest valued resources a country can possess because it plays a very important role in modern society. The high value of oil is because this commodity is currently the dominant energy source and is expected to remain so over the next several decades (Wang et al. 2006). Moreover, the oil demand kept increasing, thanks to the burgeoning populations worldwide, more industrialized countries, and manufacturing places with high demand for energy and crude oil (Madlener and Sunak 2011).

In huge quantities, Ghana has discovered crude oil off the shores of its Western Atlantic Coast. This discovery came after a century of exploration activities with financial support from various resources (Ecobank 2014). However, Ghana must wait three decades to claim the oil discovery in 2007 by the Jubilee partners because the discovery coincided with the Jubilee anniversary after Ghana declared independence from the UK in 1957. Thus, the first well was christened Jubilee and the Jubilee partners include Sabre Oil, Tullow Ghana Limited, and Gas Limited, and Kosmos Energy Ghana. In October 2009, the reserves of the Jubilee fields were estimated at 490 million barrels of high-quality oil, and currently, an average of 102,630 barrels of oil are drilled daily (World Bank 2009). Total oil revenue from January to September 2013 amounted to GH¢1,150.2 million, against a target of crude oil production from the Jubilee field, averaging 102,503 barrels of oil per day (bopd).

The toxic composition of crude oil cannot be ignored because crude oil is a complex mixture of hydrocarbons containing more than 17,000 compounds (Pampanin and Sydnes 2013). Crude oil is composed of a group of substances called polycyclic aromatic hydrocarbons (PAHs) from two to eight conjugated ring systems and exhibits all the chemical properties of aromatic compounds. PAHs can have a range of substituents such as nitro, alkyl, and amino groups in their structure (Pampanin and Sydnes 2013). The precursors for PAHs found in crude oil are natural products such as steroids that have been chemically converted to aromatic hydrocarbons over time (Feng et al. 2009). PAHs can be formed naturally by low-temperature, high-pressure reactions of natural organic matter and, in

this way, make up a significant fraction of petroleum hydrocarbons (Latimer and Zheng 2003; Mishra and Das 2017).

In substantial concentrations, PAHs in the marine environment are divided into two groups based on their origin, namely pyrogenic and petrogenic (Pampanin and Sydnes 2013). The petrogenic PAH is derived from oil and drilling activities such as oil spills, disasters, and effluence from industrial sites, oil refineries, and the majority from traffic exhaust emissions. Meanwhile, the pyrogenic PAH is derived from volcanic eruptions, forest fires, and incineration. Pyrogenic PAHs are usually composed of larger ring systems (above 6 rings), while petrogenic PAHs are composed of 2 to 6 rings. The PAHs pollutants from the coastal areas and their water bodies primarily originated from effluent released by industries, runoff from roads, smelter industries, and oil spills, as well as water that is generated from drilling routine activities.

Oil seeps refer to natural springs where liquid and gaseous hydrocarbons trickle out of the ground, scattering all over the globe with a higher concentration in some regions of the world. The presence of a natural oil seep often has resulted in the discovery of oil reservoirs that are large enough to supply commercial oil production (Devold 2013).

During oil drilling, various chemicals and materials are released into the environment, e.g., drilling mud, drill cuttings, oil, and chemicals injected into the drilling machine to control corrosion or assist the separation of oil from water as well as general industrial waste (Adewole et al. 2010). Spillage or discharge of these wastes into the sea and/or land has been associated with adverse environmental and public health effects (Lyons et al. 1999). Their adverse environmental effects include the dead and moribund marine animals, the oil coating of shorelines and other water bodies and PAH contamination (particularly coastal waters, plants, and other biological organisms). The percentage of PAH in crude oil ranges between 0.2% and 7%. Some PAHs are bioaccumulative, persistent and toxic to humans and other organisms. In addition, the substances are carcinogenic, mutagenic, or teratogenic. Substances that combine these characteristics raised a serious concern for public health and the environment (German Federal Environment Agency 2012).

Therefore, tracking the levels of PAH in coastal zones is necessary. In this regard, biomarkers become crucial as they serve as indicators of PAH pollution. Fishes are suitable model organisms for environmental genotoxic bio-indicator organisms due to their role in the aquatic trophic chain. In addition, fish are sensitive to low concentrations of genotoxic substances (Al-Sabti and Metcalfe 1995). Several assays among them can evaluate the genotoxic effects of PAH on fish is micronuclei analysis in peripheral blood erythrocytes (Al-Sabti and Metcalfe 1995). The micronucleus assay is a quick, sensitive and reliable assay to determine damage to the DNA. Micronuclei can originate both from whole chromosomes delayed during cellular division anaphase and from acentric fragments resulting from chromosomal breaks which are not incorporated into the main nucleus (De Lemos et al. 2008). Genotoxicity results from DNA damage and can happen through the formation of DNA adducts. DNA adduct formation is the covalent binding of highly electrophilic chemicals to DNA. Thus, altering DNA structure and making it unable to undergo normal processes of replication, transcription, and repair. If the proper DNA synthesis is not restored by repair mechanisms and the adducts persist, these alterations may cause mutations and cancer development.

Specific objectives of this study were: (i) To assess hepatic DNA adduct formation in selected fish species. (ii) To determine the PAH levels in fish harvested in the communities. (iii) To assess the levels of PAH in water, plants, and soil samples from the selected communities.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals and reagents used in this study include PAH Mix 14 Standard (from Dr. Ehrenstorfer GMBH Augsburg, Germany), Silica Gel (Merck, Germany), Acetone (Prolabo VWR BDH, Singapore), Dichloromethane (DCM), Hexane, Ethanol, Methanol, Maygrunnwald, DPX Mountant, Acetonitrile, and Giemsa Stain (Sigma Aldrich St Louis, USA), Copper (II) Sulfate Pentahydrate [$CuSO_4.5H_20$], Sodium Sulfate Anhydrous [Na_2SO_4], Phosphoric Acid [H_3PO_4], were obtained from Wako Pure Chemicals, Japan.

Study area

The study was conducted in six districts bordering the marine environment of the Jubilee Oil field, Western Region of Ghana (Figure 1). The Western Region forms about 10% of Ghana's total land area; it covers an area of 23,921 km^2 (Western - Government of Ghana 2015). It is located in the south-western part of Ghana and shares borders with the Republic of Cote D'Ivoire to the west, Central Region to the east, Ashanti and Brong Ahafo Region to the north, and the Gulf of Guinea to the south. The Cape Three Points, which is the southernmost part of Ghana where crude oil was discovered in commercial quantities in June 2007, is located in this region. The western coastline covers approximately 95 km of stable shoreline, which extends from the Republic of Cote D'Ivoire borders to the estuary of Ankobra.

The 2010 population and housing census estimated the region's total population to be 2,376,031, representing 9.6% of the total population of Ghana (Ghana Statistical Service Report 2012). The region has about 75% of its vegetation within the high forest zone of Ghana, and its south-western parts are noted for the rainforest, interspersed with patches of mangrove forest along the coast and coastal wetlands (Western-Government of Ghana 2015). The region lies in the equatorial climatic zone characterized by moderate temperatures from 22°C to 34°C at night and day, respectively. The region is the wettest part of Ghana, with a double maxima rainfall pattern averaging 1,600 mm per year. The two rainfall peaks fall between May to July and September to October. It also experiences intermittent minor rains all year round. The humidity of the region ranges from 70-90%.

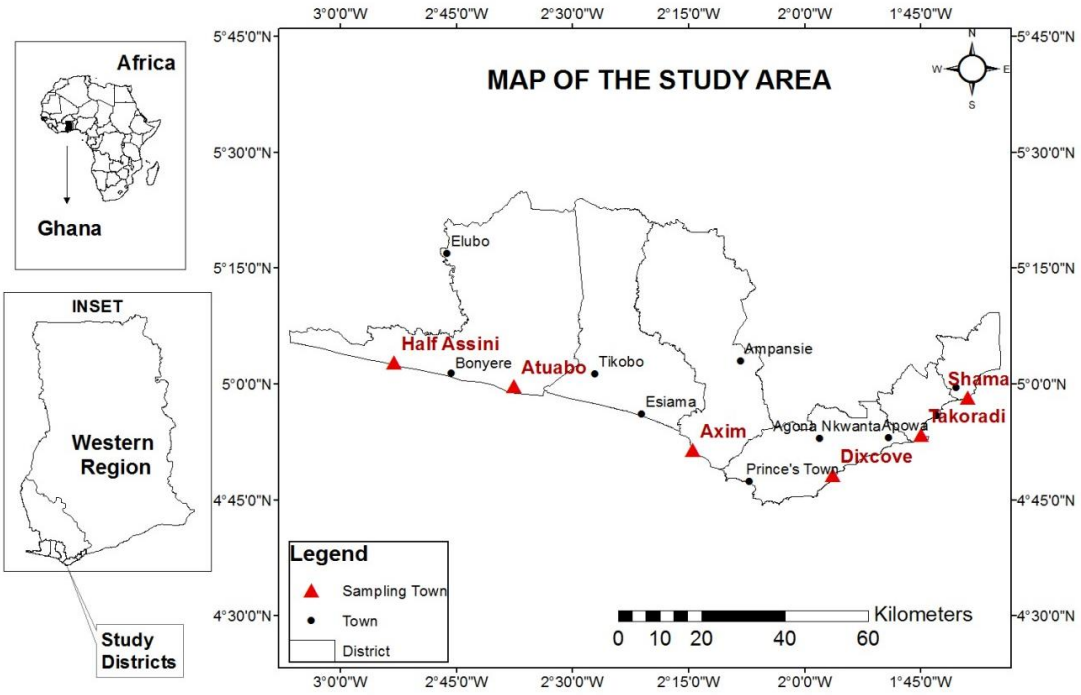

Figure 1. A map of the study areas in the Western coast of Ghana

The major industrial activities in the region are petroleum industry, oil and gas exploration, services, agriculture, excluding fishing but including forestry and hunting, mining and quarrying, manufacturing, and wholesale and retail trade (Western-Government of Ghana 2015). The region is also endowed with a wide variety of minerals, including gold, bauxite, iron, diamond, and manganese, and thus the region is an attractive investment destination for numerous small and large-scale gold mining companies.

Ellembelle District

The district covers a total land area of 1468 km^2 and is located in the southern end of the region between longitudes 2º05' W and 2º35' W and latitudes 4º40N and 5º20N. It shares boundaries with Jomoro District to the west, Nzema East Municipal to Tarkwa - Nsuaem Municipal to the East, Wassa Amenfi West District to the north, and a 70 km stretch of sandy beach to the south. The district lies within the wet semi-equatorial climate zone. According to the 2010 Population and Housing Census, the district's population is 87,501 (Ghana Statistical Service Report 2012). The area experiences rainfall throughout the year, with the highest monthly mean rainfall occurring between May and June. The mean annual rainfall is 1,600 mm, and the average temperature is about 29°C. Fishing is the major industry in this district. The area's vegetation comprises moist semi-deciduous rainforest and secondary forest southward with savannah vegetation along the coastal area. The topography is generally undulating, with the highest point at about 450ft above mid-sea level.

Jomoro District

The district, covering a land area of 1344 km^2, is located between latitudes 040 55' N and 050 15' N and longitudes 020 15' W and 020 45' W. It shares boundaries with Aowin-Seaman and Wassa Amenfi to the north, Ellembelle District to the east, La Cote D'Ivoire to the west and the Gulf of Guinea to the south. The population of Jomoro District, according to the 2010 population census, is 150,107 (Ghana Statistical Service Report 2012). The heavy rainfall occurs from April to July and September to November. The average annual rainfall is 1732 mm. There is also a short dry spell in August and a long dry period from December to January. The climate of the area is classified as equatorial monsoon. The vegetation is a tropical rainforest with coastal vegetation, largely mangrove swamps (Western-Government of Ghana 2015). Jomoro District is basically an agricultural one, and arable farming and livestock rearing constitute the backbone of her economy. Agriculture engages the majority of the population who obtain their livelihood from farming and other agro-related activities such as deep-sea and freshwater fishing as well as coconut oil extraction.

Nzema East Municipality

Nzema is located at the southern end of the region between longitudes 2 05‖ and two 35‖ W and latitudes 4 40‖ and five 20‖ N of the equator. It is bordered on the west by Ellembelle District, on the north by Wassa Amenfi West District, on the east by the Tarkwa Nsuaem Municipal, Prestea Huni Valley, and Ahanta West Districts, and on the south by the Gulf of Guinea. It covers a land area of 2194 km^2. The population of the district is 60,828, according to

the 2010 Population and Housing Census (Ghana Statistical Service report 2012). It lies between the wet semi-equatorial climatic zones. Rainfall is experienced throughout the year, with the highest monthly mean occurring around May and June each year. The average temperature is about 29°C. The surrounding vegetation is made up of moist semi-deciduous rainforest, mainly in the northern part, with secondary forest southwards. The topography is generally undulating, with the highest point of about 100 m above sea level. This district is predominantly a fishing community. The main landing beaches are Lower Axim, Ahobre, and Effasu.

Ahanta West District

The district lies between latitude 4°.45 "N and longitude 1°.58 "W. It covers a total land area of 591 km^2, and 106,215 people occupy it, according to the 2010 Population and Housing Census report (Ghana Statistical Service Report 2012). It is bounded on the east by the Sekondi Takoradi Metropolitan Assembly (STMA), on the west by the Nzema East Municipal, and on the north by Mpohor Wassa East and Tarkwa Nsuem Districts and the Gulf of Guinea to the south. The area's climate is within the south-western equatorial climate zone, with the highest mean temperature of 34°C recorded between March and April and the lowest mean temperature of 20°C in August. It experiences a double maxima rainfall of over 1700 mm with an average relative humidity of about 75%. The vegetation falls largely within the high rainforest vegetation zone. The topography is generally low-lying.

Shama District

It covers a land area of 215 km^2 and is bordered to the north by the Mpohor Wassa East District, to the south by the Gulf of Guinea, to the west by Sekondi-Takoradi Metropolitan District and the east by Komenda Edina Eguafo-Abirem District in the Central Region. The district has a population of 81,906 (Ghana Statistical Service Report 2012). It lies within the tropical climatic zone. The area experiences two rainfall maxima each year. The major rainy season starts from March to mid-July, followed by a short dry spell that runs till August. The period from early September to mid-November marks the minor rainy season. The annual rainfall varies from 1000 to 1700 mm, with the mean annual rainfall of about 1380 mm. The annual temperature ranges from 25 to 28°C. The vegetation is mainly coastal thicket, thin to dense shrubs. The northern part of the district is made up of thick bushes with other small-sized trees. In the coastal area, the thicket is intermingled with tall grass species, including mangrove swamps and raffia groves at the estuary of the river Pra. It lies within a low-lying area, and the landscape is generally undulating with an elevation in most parts less than 80 m above sea level. The geological formations are made up of Lower Birimian and granite soils. The coastal areas are parent materials with faulty shelves and sand of various types resting on granite, gneiss, and schist (Western-Government of Ghana 2015). The Volta River Authority's thermal generation plant, which generates electricity from diesel and gas, is located in this district.

Sekondi-Takoradi Metropolitan District

The district, which covers a land area of about 49.78 km^2, is located in the southwestern part of Ghana. It is bordered to the north by Mpohor Wassa East District, east by Shama District, west by Ahanta West District, and south by the Gulf of Guinea. The metropolis's population is 559,548, according to the 2010 Population and Housing Census (Ghana Statistical Service Report 2012). The climate is equatorial, with an average annual temperature of about 25°C. Rainfall is bi-modal, with the major season occurring between March and July and the minor season occurring between August and November. The mean annual rainfall is about 1,380 mm. The natural vegetation is mainly woodland with thickets interspersed with tall grass species along the coastal areas (Western-Government of Ghana 2015). The area has a varied landscape and is undulating with ridges and hills. The area's geology is underlain with faulty shale and sandstone on granite, gneiss, and schist (Western-Government of Ghana 2015).

Experimental design

Communities were clustered into three according to distance from the Jubilee field: over ten kilometers (Aboadze in Shama District and New Takoradi in Sekondi-Takoradi Metropolis) onshore of the oil fields, 5-10 kilometers (Atuabo in Ellembelle District and Half Assini in Jomoro District) and under-five kilometers (Dixcove in Ahanta West District and Lower Axim in Nzema east Municipality). Samples were collected from a randomly selected community in each district. Five sampling sites were randomly selected in the community for plant and soil collection. Water samples were collected from five different sources in each community (where surface water or wells were found), and fish samples were obtained from landing beaches in each community.

Inclusion and exclusion criteria

This study site covered the coastal communities bordering the Jubilee oil field in the Western region of Ghana. We exclude water samples from water bodies because they did not serve any domestic purpose in each particular study area. Instead, leaves were collected from dominant plant species in each community. Consequently, only fresh fish were obtained from the various landing beaches in each study area.

Sample collectionSoil samples

Soil samples were obtained from 5 different locations from the in-land and shores of selected susceptible communities at depths of 0 to 15 cm and 15 to 30 cm (each study area represented by 10 soil samples). The samples were stored in plastic containers for PAH extraction and analysis.

Water samples

Water was collected from 5 different locations except for Shama and Sekondi-Takoradi Metropolitan Districts, transferred into clean bottles and stored at 4°C until PAH analysis was performed. Shama and Sekondi-Takoradi Metropolitan Districts were excluded because they did not

meet the criteria as water from these sources was not used for domestic purposes. A total of 20 water samples were collected from five sources in each of the four study areas.

Plant and fish samples

The dominant plant samples were randomly taken from areas in selected communities near the in-land and shores. They were kept in sealed plastic bags and stored at 4°C until utilized for PAH analysis. For each sampling cluster, two different plant species were collected, thus, 10 plant samples were collected from each study area, and a total number of 60 plants were taken for this project.

The number of fish species sampled differed from district to district, but in all 38 fish samples (three per each species making a total of 114) were collected from the entire selected cluster or fishing community for PAH extraction and micronucleus test. The samples were transported on ice to the laboratory and stored at -20°C until use. A total of 27 fish species were collected for this project, but some species were found in more than one study area, thus the total number of 38 fish samples.

Determination of PAHs concentration

Polycyclic aromatic hydrocarbons (PAHs) concentration in plants, soil, and water samples were analyzed according to the method described elsewhere (Li et al. 2008). Meanwhile, the PAH content in fish was modified from the method described by Denton et al. (1999).

Preparation of samples

Plants

The plant sample was ground into a homogenous mixture using a pestle and mortar, then two grams of the samples were placed in a conical glass flask containing 5 mL acetone, 5 g of anhydrous sodium sulfate, and 0.1 g copper powder. Extraction was done in an ultrasonic water bath at a temperature of 30°C for 15 min. The mixtures were centrifuged at 2190 rpm for 10 min. Next, the supernatant was collected into fresh 15 mL centrifuge tubes. The extraction process was repeated by adding 5 ml of acetone to the residues, and the content was transferred into glass flasks before sonicating. The supernatants were then pooled together and evaporated in a rotary evaporator, then subjected to further extraction by column chromatography.

Soil

Soil samples were air-dried at room temperature (25°C), gently crushed, and filtered using a 1 mm mesh sieve to remove the larger size of particles. One gram of each sieved soil sample was transferred in a 100 mL glass beaker containing 5 mL acetone, 5 g of anhydrous sodium sulfate, and 0.1 g copper powder. The extraction process was the same as done in section 2.2.5.1.1.

Fish

Fish livers (1.5 g) were thawed prior to extraction. Subsequently, 10 g anhydrous, 10 ml of dichloromethane, and granular sodium sulfate were added to each tissue sample before homogenization using a mortar and pestle. The mixture was centrifuged at 3500 rpm for 20 min. Next, the supernatant was collected into a new 50 mL centrifuge tube. The process was repeated and the supernatants for each sample were pooled. After reducing the volume to approximately 0.5 ml using rotary evaporation, the extract was transferred to a 15 mL centrifuge tube with two 0.5 mL rinses of dichloromethane. Next, the tube was placed in a water bath and the extract volume was reduced to 0.25 mL under a gentle stream of nitrogen. Each extract was then dissolved in 2 mL of hexane, and a further reduction in volume to 1 mL was done before further extraction by column chromatography.

Water

Before extraction, the C18 cartridges were pre-conditioned with 5 mL acetonitrile, followed by vacuum drying for 10 sec and washed with 5 mL of HPLC grade ultra-pure water PAHs extraction using a solid-phase extraction (SPE) cartridge system. Each water sample (100 mL) was then percolated through the cartridges at a flow rate of 5 mL/min with a vacuum pump. The column was then eluted isocratically with 10 ml hexane and dichloromethane (1:1 v/v). The eluent of each sample was then subjected to HPLC analysis.

Column chromatographic extraction of PAHs

All the extracts from plants, soil, and fish samples were subjected to further extraction of PAHs by column chromatography.

Plant, soil and water samples

Ten glass columns (Ø1.5 mm) were packed with cotton wool by positioning the cotton securely in the narrowest part of the column using a long glass rod. The columns were securely clamped and supported on a stand, after which the taps were closed. This step was followed by filling the columns to one-third of their volume with dichloromethane and hexane (1:1 v/v) as a mobile phase. First, the silica slurry was prepared by mixing 70 g of silica gel and 200 mL of mobile phase in a glass beaker. The slurry was then pipetted into the column with a Pasteur pipette, and the solvent was allowed to drain to prevent overflowing. Next, the columns were gently tapped with a rubber bung to free them of air bubbles. This step was followed by draining the solvent until its level was even with the surface of the stationary phase, after which the tap was closed. The concentrated samples were then dissolved in 2 mL of the mobile phase. Next, 1 mL of each sample was loaded into a separate packed column. Next, each sample was eluted with 8 ml of mobile phase, and the eluents were collected in labeled test tubes. This was followed by evaporating the eluted samples to dryness under a gentle flow of nitrogen gas. Finally, the residue was reconstituted with 0.5 ml of acetonitrile for HPLC analysis. Further extraction of PAHs in soil and fish samples by column chromatography was done as described above.

HPLC analysis of samples

All samples were analyzed by the HPLC (Shimadzu from Japan) using a C18 reverse-phase column coupled with an ultraviolet detector. The separation of analytes was achieved by adopting the following conditions, a flow rate of 1.0 mL/min at 40°C. The injection volume was 20 µL. The column was stabilized at 40°C for 1 hour before chromatography. The mobile phase comprised water, phosphoric acid (component A), and acetonitrile (component B). Details of the mobile phase are given in Table 1. Analyte (PAHs) peaks were identified by their retention times compared to the corresponding retention times of the PAH standards used.

Genotoxicity assessment

Genotoxicity assessment of fish samples was done using the Micronucleus assay of Garg et al. (2012).

Micronucleus assay

Blood smears samples were made using peripheral blood samples from the caudal vein of the fish specimen. The slides were then air-dried at room temperature for 24 h. Dipping slides fixed slides into methanol for 5-10 min. The slides were then smears were stained with Maygrunwalds stain solution-I for 2-3 min, washed with double distilled water and dried. Upon drying, smears were stained with Maygrunwalds stain solution-II for 3-6 min, washed with double distilled water and dried. Finally, all the slides were then stained with Giemsa stain (10%) in phosphate buffer for 30 min and washed with double distilled water to remove all Giemsa particles. The prepared slides were fixed with DPX mountant and dried overnight. Normal and abnormal cells and micronucleus were observed and counted under a microscope (Leica, Germany) at a magnification of 100×.

The average micronuclei per 1000 cells of each fish species were determined based on the following scoring criteria; (i) The micronucleus resembles the staining characteristics and possesses similar morphology to the main nuclei. (ii) Any micronuclei present in the cytoplasm or only just touching the main nucleus. (iii) Micronuclei were smooth-edged and smaller than one-third the diameter of the main nuclei. Then, the frequencies of micronuclei were calculated for each species using the equation below.

$$\text{MNE } (^{0}/_{00}) = \frac{\text{Number of cells containing micronuclei}}{\text{Total number of cells counted}} \times 1000$$

Statistical analysis

Results were expressed as means + standard deviation (SD). Concentrations of plants and soils were expressed as mg/kg wet weight, whereas concentrations of fish were expressed as µg/g. Water concentration was shown as µg/L. The results of the micronuclei test were expressed as mean micronuclei frequency/1000 cells. Comparisons of micronuclei frequency/1000 cells between species across study areas were made using one-way analysis of variance (ANOVA) with the statistical package SPSS 14.0.2 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Concentration of PAHs

Concentration of eighteen PAH compounds namely, naphthalene, 1- methylnaphthalene, 2-methylnaphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, fluorene, fluoranthene, pyrene, anthracene, benz[a]-anthracene, benz[a]fluoranthene, benz[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, chrysene, and indeno[1,2,3,c,d] pyrene were determined in water, fish liver, plants and soil samples.

PAHs in fish livers

The number of analyzed fish species from each district differed from the various landing beaches in the selected communities. The means and standard deviation of PAHs in all the fish species were tabulated from the data obtained from duplicate HPLC analysis of composite samples of each fish species (each species represented with three fish). For HPLC analyses, the liver of three fishes for each species was pooled together for extraction.

Nine different fish species(three per each species) namely: *Illisha africana, Sardinella aurita, Sardinella eba, Brachydeuterus auritus, Dentex congoensis, Pomadasys incises, Pseudupe prayensis, Lutjanus fulgens* and *Katsuwonus pelamis* were sampled from Shama District in the Aboadze landing beach. Only *P. incises* detected acenaphthylene (15.267 µg/g), naphthalene (6.933 µg/g), 2-methylnaphthalene (5.667 µg/g) and acenaphthene (0.333 µg/g) (Figure 2). No PAHs were detected in the other fish species from the community. From Dixcove in the Ahanta West District, the fish species *Thunnus albacores, Trichiurus lepturus, Thunnus alalunga,* and *K. pelamis* were sampled. However, only fluorene with a concentration of 0.180 µg/g was detected in *T. alalunga* fish (Figure 2).

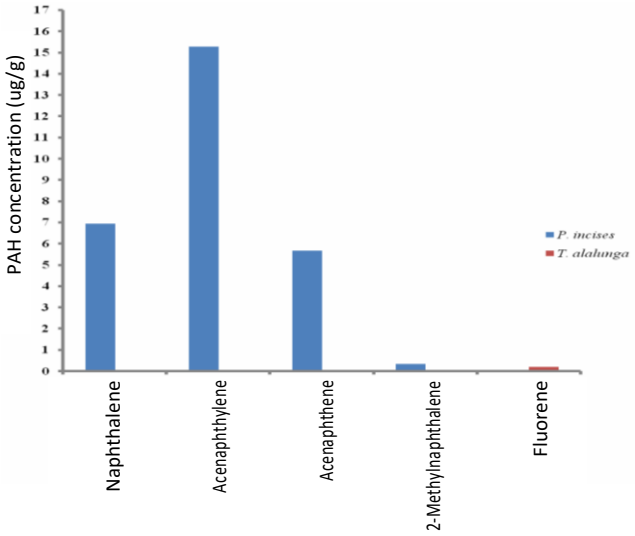

Figure 2. PAH concentrations in *P. Incises* liver from Aboadze in Shama District and *T. alalunga* fish from Dixcove in Ahanta West District, Ghana

Table 1. The mobile phase composition

Time/(min)	0.1% Phosphoric acid in water (%)	Acetonitrile (%)
0	50	50
5	60	40
10	80	20
25	80	20
30	95	5

Fish species: *Pentanemus quinquarius, Galeoides decadactylus, Pseudotholitus senegalensis, I. africana,* and *B. auritus* from New-Takoradi landing beach in the Sekondi-Takoradi Metropolitan District showed no detectable PAHs. No PAHs were detected in the fish species: *B. auritus, Dentex angolensis, Chloroscombrus chrysurus, Sphyraena sphyraena, Stromateus fiatola* and *Pseudotolithus typus* sampled in Lower Axim in Nzema East District.

Furthermore, no PAHs were found in *Seriola dumurili, Caranx chrysurus, Ethmalosa dorsalis, S. sphyraena, Caranx hippos,* and *Selene dorsalis* fish species sampled from Atuabo in the Ellembelle District. Likewise, no PAHs were detected in fishes: *B. auritus, S. Sphyraena, Hemiramphus brasiliensis, S. aurita, Pomadasys incisus* (Bowdich, 1825) and *Acanthurus monroviae* from Half Assini in the Jomoro District.

Livers from each three *P. incises* and *T. alalunga* were pooled and extracted with dichloromethane (DCM), then subjected to silica gel column chromatography prior to duplicate reverse phase HPLC analysis. Concentrations were calculated relative to PAH standards. Results are means of n= 2.

PAHs in water samples

Inland water bodies, including rivers and dug wells used for domestic purposes, were analyzed for the water PAHs. Samples were collected from four out of the six study sites/ communities. These four communities are Atuabo in Ellembelle, Lower Axim in the Nzema East, Dixcove in Ahanta West District and Half Assini in the Jomoro District. However, water samples from Atuabo and Half Assini had no PAH detected in them.

In Dixcove, 1-methyl naphthalene, naphthalene, acenaphthylene, fluorene, phenanthrene, benz[a] fluoranthene, fluoranthene, and chrysene were detected with an average concentration of 21.20 ± 29.85, 178.50 ± 253.95, 1255.30 ± 2015.40, 2.40 ± 2.55, 124.30 ± 92.55, 723.40 ± 685.90, 3.10 ± 6.95 and 2.20 ± 4.90 µg/L, respectively (Figure 3). Water bodies from Half Assini had the following PAHs: 1-methyl naphthalene, naphthalene, fluorene, fluoranthene, phenanthrene, detected with an average concentration of 11.4 ± 25.45, 380.3 ± 850.0, 1.4 ± 3.1, 36.7±82.05, 185.5 ± 415.00 µg/L, respectively (Figure 4).

Water samples from five water bodies in this study site were subjected to solid-phase extraction and prior to reverse phase HPLC analysis after which concentrations of each PAH compound was calculated relative to concentrations of PAH standards. Results are means ± SD. N=5 (Figure 3-4).

PAHs in plants samples

A total of sixty plants (ten from each district) were collected for PAHs analysis with the common ones being *Alchornea cordifolia, Chromolaena odorata, Avicennia nitida, Erythrina senegalensis, Ficus sagittifolia, Ficus exasperata, Ficus umbellata, Terminalia catappa, Spondias mombin, Thespesia populnea,* and *Senna siamea* various sampling clusters in the study sites. The means and standard deviation of PAHs in plant leaves were computed from the data obtained from duplicate HPLC analysis of composite samples of leaves of each plant species.

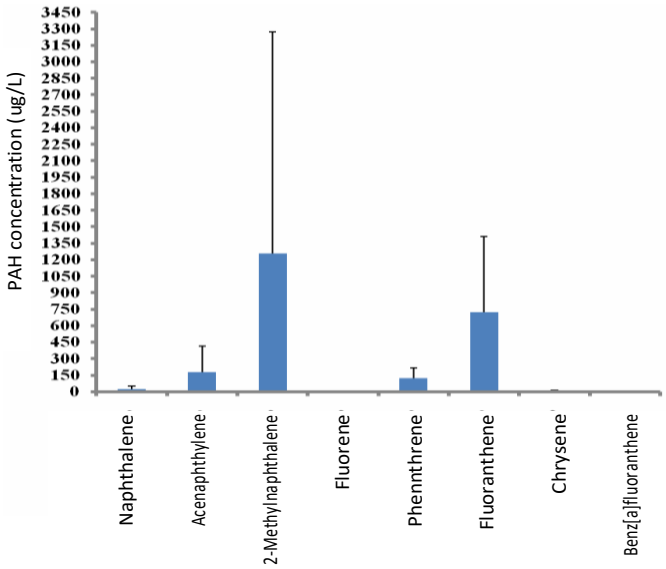

Figure 3. PAH concentration in water samples from Dixcove in Ahanta West District, Ghana

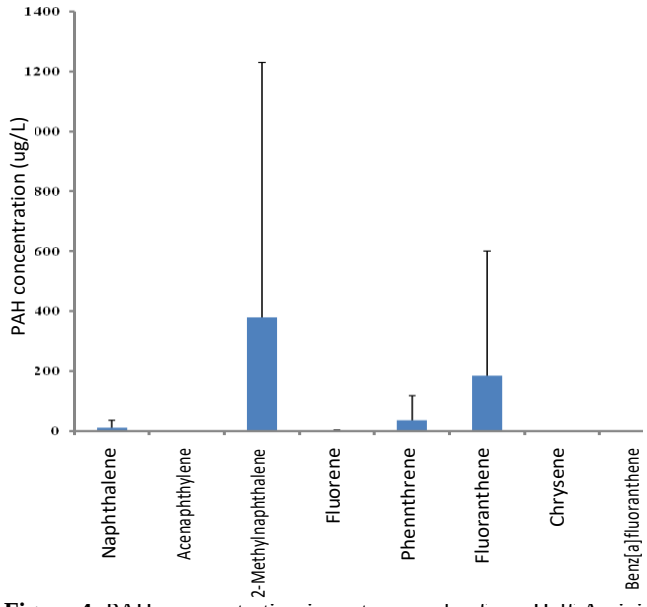

Figure 4. PAHs concentration in water samples from Half Assini in Jomoro District, Ghana

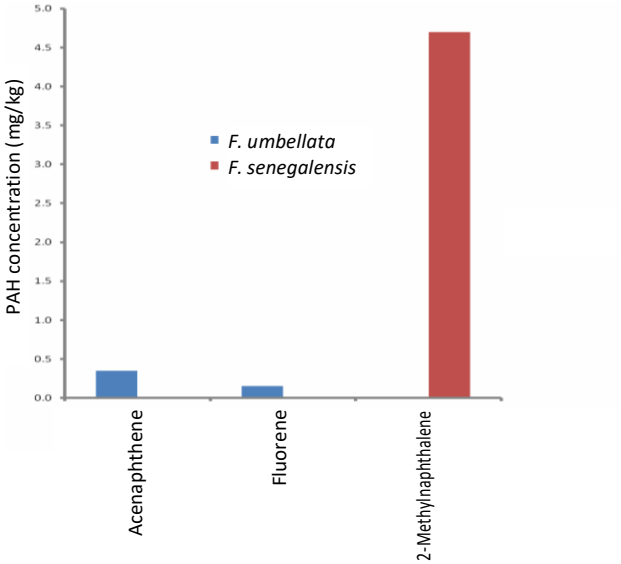

Figure 5. PAHs concentration in plant samples from Lower Axim in Nzema East District, Ghana

From all samples, *E. senegalensis* and *F. umbellata* from Lower Axim in Nzema East District had some PAHs detected. 2-methylnaphthalene was detected in the leaves of *E. senegalensis* with a concentration of 4.70 mg/Kg. Acenaphthene and fluorene with their respective concentrations of 0.35 mg/kg and 0.15 mg/kg were detected in the leaves of *F. umbellata* (Figure 5).

Plant samples from five different sampling clusters in this study site were taken through an acetone extraction and then subjected to silica gel column chromatography prior to reverse phase HPLC analysis. Concentrations were computed relative to concentrations of PAH standards. Results are means of n= 2.

PAHs in soil samples

Soil samples were taken from two different depths of 15-30 and 0-15 cm and used for the analysis in this study. The composite sampling of 3 different spots per location of 2 different depths, therefore the total number of soil sampled for this study was 60). The average concentrations of the various PAHs were calculated from all the sampling sites. Samples from Aboadze in the Shama district recorded seven PAHs from soil depth of 0-15 cm (Figure 6). The compounds were naphthalene (0.260 ± 0.580 mg/kg), acenaphthylene (3.360 ± 7.420 mg/kg), 2-methylnaphthalene (16.080 ± 35.870 mg/kg), acenaphthene (0.100 ± 0.220 mg/kg), fluorene (1.220 ± 1.800 mg/kg), phenanthrene (7.100±15.870 mg/kg) and fluoranthene (7.300 ± 16.320 mg/kg).

Figure 7 shows mean concentrations of PAHs in soil samples from Dixcove in the Ahanta West District. The compounds (with concentrations at 0-15 cm and 15-30 cm, respectively) were as follows: acenaphthene (0.600 ± 1.475 and 1.100 ± 2.459 mg/kg), 1-methylnaphthalene (26.600 ± 59.500 and 105.800 ± 236.570 mg/kg), 2-methylnaphthalene (0.700 ± 1.565 and 0.220±0.492 mg/kg), and anthracene (0.700 ± 1.565 and 0.86 ± 1.923 mg/kg). However, acenaphthylene (12.680 ± 28.350 mg/Kg), naphthalene (0.220 ± 0.490 mg/Kg), fluorene (0.14 ± 0.313 mg/Kg), phenanthrene (2.82 ± 6.30 mg/Kg) and fluoranthene (8.626 ± 19.288 mg/kg) were detected at the depth of 15-30 cm. Only Pyrene (0.220 ± 0.492 mg/Kg) was found at depth of 0-15 cm.

Soil PAHs levels for Lower Axim in the Nzema East District are shown in Figure 8. Only 2-methylnaphthalene concentrations of 0.460 ± 1.020 and 0.66 ± 1.475 mg/kg at soil depth of 0-15 cm and 15-30 cm, respectively and pyrene at concentrations of 0.120 ± 0.268 and 0.180 ±0.402 mg/Kg were detected. On the other hand, acenaphthene (0.420 ± 0.939 mg/kg) was detected only at depth of 15-30cm. PAHs were detected only at depths of between 0-15 cm in Half Assini of the Jomoro District. As shown in Figure 9, the PAHs were acenaphthene (0.160 ± 0.357 mg/kg), phenanthrene (1.580 ± 0.353 mg/kg), fluorene (0.100 ± 0.223 mg/kg), anthracene (0.200 ± 0.447 mg/kg) and fluoranthene (95.360 ± 21.322 mg/kg).

Soil samples from five different sampling clusters in this study site were taken through an acetone extraction and then subjected to silica gel column chromatography prior to reverse phase HPLC analysis. Concentrations were computed relative to concentrations of PAH standards. Blue bars indicate soil taken from 0-15cm. Red bars indicate soil taken from 15-30cm. Results are means ± SD. N= 5 (Figure 6-9).

Micronuclei in fish erythrocytes

Erythrocytes were observed using an optical microscope at 100× magnification from ten different fields were counted and then averaged as a representative value before being normalized using equation 2.1. Only cells with intact cellular and nuclear membranes were scored. The mean and SD of micronuclei frequency per 1000 cells for each fish species was then computed from normalized micronuclei values from three fishes. The mean and standard deviation of micronucleated erythrocytes (MNEs) per 1000 cells observed in the different fish species from Atuabo is shown in Figure 10, and from Lower Axim is shown in Figure 11. Six fish species from Atuabo, namely *S. dumurili, Ca. chrysurus, S. sphyraena, E. dorsalis, S. dorsalis, C. hippos* were analysed for the presence of the micronuclei. Only the three species *S. sphyraena, Ca. chrysurus,* and *S. dorsalis* had micronuclei with their mean MNEs/1000 cells of 0.51 ± 0.88, 0.13 ± 0.22 and 0.113 ± 0.22, respectively (Figure 10). Figure 11 shows that in Lower Axim, all the six fish species, namely *D. angolensis, B. auritus, S. sphyraena, Ch. chrysurus, S. fiatola* and *P. typus* had mean MNEs/1000 cells of 0.41 ± 0.72, 0.92 ± 1.59, 6.98 ± 12.09, 0.62 ± 1.07, 1.36 ± 2.36, 2.34 ± 4.06, respectively. However, of the six fish species, namely *S. aurita, B. auritus, S. sphyraena, H. brasiliensis, P. incisus* and *A. monroviae* from Half Assini, only *B. auritus* recorded MNEs/1000 cells of 2.21 ± 3.38 (Table 2).

At Aboadze, *I. africana, B. auritus, P. incises, D. congoensis, S. aurita, S. eba, P. prayensis, K. pelamis,* and *L. fulgens* recorded no MNEs. Likewise, fish species *P. quinquarius, G. decadactylus, P. senegalensis, B. auritus* and *I. africana* from New-Takoradi landing beach, Sekondi-Takoradi Metropolitan District had no MNEs. Moreover, the sampled fish species from Dixcove of the

Ahanta West District, e.g., *T. albacores, K. pelamis, T. lepturus*, and *T. alalunga*, recorded no MNEs.

Data of thin smears of peripheral blood presented as the mean frequency of micronuclei ± SD of N =3. These data were obtained from three fish per six different fish species were stained with Giemsa and Maygrunnwald solutions. ND means not detected. MN-Micronuclei, SD-Standard Deviation, MAX-Maximum, MIN-Minimum.

Table 2. Mean of micronuclei frequency/1000 cells from Half Assini in the Jomoro District of Western region of Ghana

Samples	Mean MN	SD	Max value	Min value
Brachydeuterus auritus	2.21	3.83	6.64	0.00
Sphyraena sphyraena	ND	ND	ND	ND
Sardinella aurita	ND	ND	ND	ND
Hemiramphus brasiliensis	ND	ND	ND	ND
Pomadasys incisus	ND	ND	ND	ND
Acanthurus monroviae	ND	ND	ND	ND

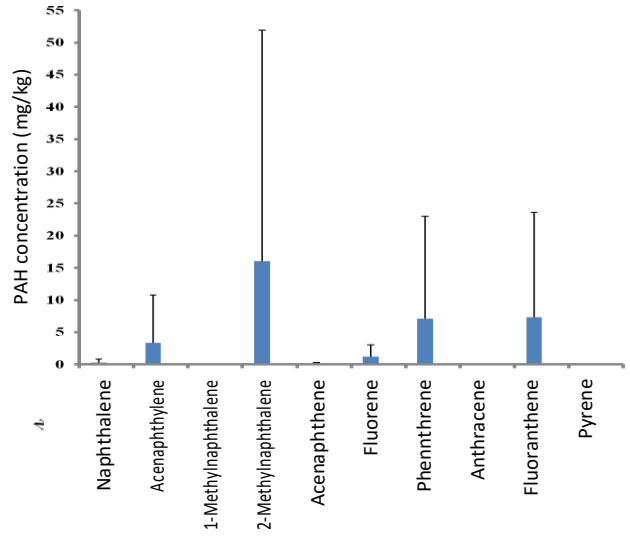

Figure 6. PAHs in soil samples from Aboadze in the Shama, Ghana

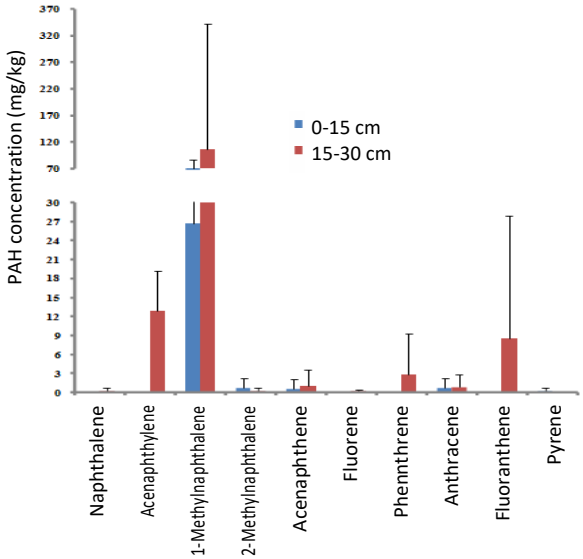

Figure 7. PAHs in soil samples from Dixcove in the Ahanta West District, Ghana

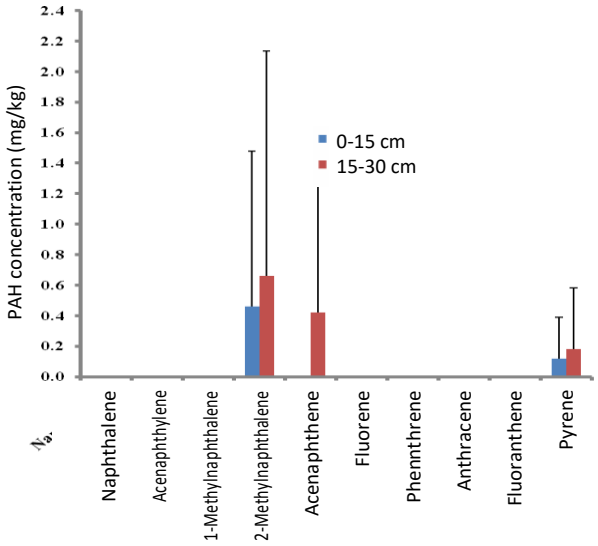

Figure 8. PAHs in soil samples from Lower Axim in the Nzema East District, Ghana

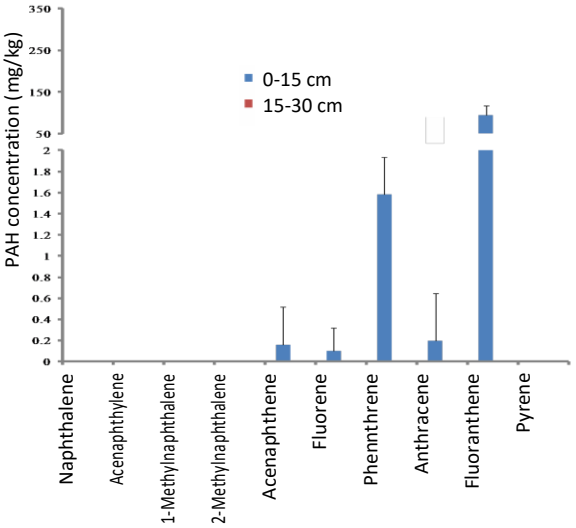

Figure 9. PAHs in soil samples from Half Assini in the Jomoro District, Ghana

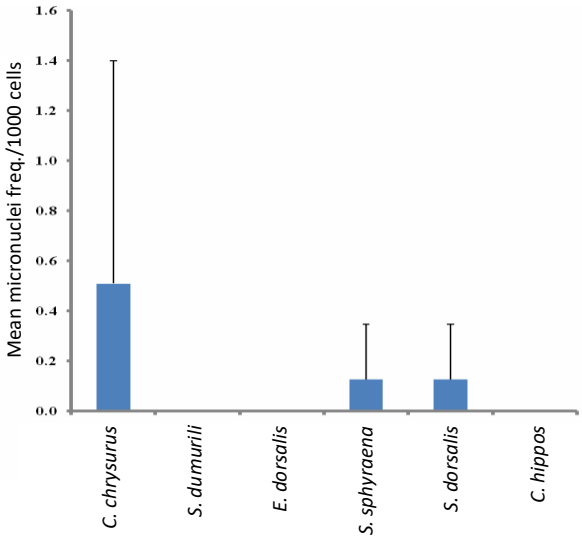

Figure 10. Mean micronuclei observed in fish species from Atuabo in Ellembelle District, Ghana

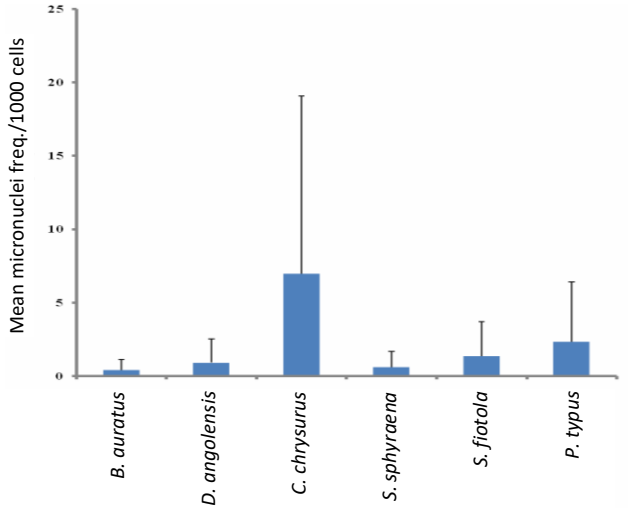

Figure 11. Mean micronuclei counts in different fish species from Lower Axim in Nzema East District, Ghana

Discussion

Many human activities contributed significantly to environmental pollution, e.g., oil drilling, industry, land reclamation, and continuous urbanization. The pollution released to the environment could have adverse consequences for human health when they are exposed to high levels of such toxicants from the air, animals, plants, and water bodies. These toxic substances can persist in the environment for some time because water bodies and land (all together with their fauna and flora), play a role in harboring, retaining and depositing these toxic substances (Li et al. 2008). In addition, some volatile toxic substances can readily contaminate atmospheric air. The most frequent contaminants surrounding oil drilling fields are oil spills, mineral oil, and polycyclic aromatic hydrocarbons (PAHs). Although there are hundreds of kinds of PAHs, the group of PAHs that pose health risks generally includes 1-methylnaphthalene, 2- methylnaphthalene, acenaphthene, naphthalene, acenaphthylene, fluorene, phenanthrene, fluoranthene, anthracene, pyrene, benz[a]anthracene, benz[a]fluoranthene, chrysene, and benz[k]fluoranthene. Here, we assessed the baseline assessment of biomarkers of pollution resulting from oil drilling of the Jubilee Oil Fields in the Western region of Ghana. The biomarkers of interest were the DNA adduct formation in fish samples. Accordingly, we also measured the presence of PAHs.

The presence of high levels of PAHs in fish tissues is suggestive of PAH contamination of coastal water from sources such as discharges from the crude oil drilling site, oil slicks, dumping of domestic wastes on the coast, accidental discharges of oil from ships and fishing boats, and smoke emissions from coastal industries (Neff and Burns 1996). Fishes are a good model of bioindicators for detecting the presence of pollutants in the aquatic environment because of their sensitivity to low concentrations of genotoxic substances (Al-Sabti and Metcalfe 1995). Fishes take up these toxicants and bio-concentrate them in organs such as gills, liver, and to a lesser extent, muscle tissues (Baussant et al. 2001). Moreover, the hydrophobic nature of PAHs can accumulate in the fish's fatty tissues following ingestion (Bouloubassi et al. 2001). The distribution of PAHs in the water column depends on their lipophilic nature.

In most cases, high and low molecular weight PAHs from sediments from the water column by particles that tend to settle at the bottom of water bodies and volatilization (Neff and Burns 1996). Therefore, it would be expected that the tissue of pelagic fishes, which are surface water dwellers, would indicate the absence of high molecular weight PAHs or even if they are present, their concentration will be very low. In each of the six study sites selected, a number of fish species, pelagic and demersal, showed the presence of PAHs. Figure 2 indicates the presence of PAHs (naphthalene, acenaphthylene, 2-methylnaphthalene and acenaphthene) in demersal fish species *P. incises*. All of these substances are low molecular weight PAHs. Their uptake by this particular demersal species may be due to high PAHs concentrations in the sediments where this species dwells.

The predominance of lower molecular weight PAHs points to petrogenic sources; thereby, these low molecular weight PAHs in *P. incises* can be attributed to petrogenic sources, including crude oil drilling activities (Saha et al. 2009). Fluorene was the only PAH in the species *T. alalunga* caught from Dixcove. On the other hand, the absence of PAH in fish with micronuclei detected in their erythrocytes can be attributed to their being faster metabolizers of PAH than species such as *P. incises* and *T. alalunga* (Varanasi et al. 1989). Although we found very low levels of PAHs, such a low concentration of contaminants can trigger the genotoxicity effects of PAHs in fish and, by extension, in coastal dwellers (White 2002). However, PAHs concentrations above the threshold limit value of 30 µg/Kg are deemed toxic (US-EPA 2000). Fish consumption is part of a routine diet for people in Ghana as it constitutes a major source of animal protein (Asamoah et al. 2012). People living in the coastal region such as the study sites tend to consume large quantities of fish and hence could be at a greater risk of being exposed to PAHs and their toxic effects such as growth reduction (Reynaud and Deschaux 2006), malformations of the embryo, endocrine alteration, DNA damage and congenital disabilities (Choi et al. 2006).

Two major influx of PAHs ended up in the aquatic environment: the water movement, which contains dissolved and particulate constituents derived from watersheds, and atmospheric deposition both in precipitation and dry deposition from airsheds of the coastal ocean (Latimer and Zheng 2003). Additionally, oil spills and leaks during the transport and production of petroleum also account for significant levels of PAHs in coastal marine environments (Wang et al. 2007).

Currently, petroleum is the dominant energy source in the world, and it is expected to remain so over the next several decades (Kharaka and Dorsey 2005). Consequently, the continued release of PAHs into the environment, as the adverse effect of activities related to petroleum production, is inevitable. A baseline assessment of PAHs in water bodies used for domestic purposes in these study areas

revealed fluoranthene as the only PAHs detected significantly.

World Health Organization stated that the limit of concentrations of individual PAHs in water bodies is generally found to be 50 ng/L and hence any concentration above this level indicates rather toxic levels (WHO 2008). However, it must be pointed out that the study sites which closest to the drilling sites, harbor no manufacturing industries (they are mainly coastal fishing communities); thus, leaching and atmospheric deposition may directly account for the elevated levels of fluoranthene recorded, particularly in Dixcove. Leaching of PAH in water bodies is the primary route for contamination of water bodies because the compounds tend to be strongly adsorbed on soil organic matter (Oros et al. 2007). Previous reports stress out the elevated concentration of PAHs predominantly, fluoranthene, pyrene and phenanthrene in water bodies (WHO 2008), probably as a result of the adsorption of the compounds to air particulate matter, which is finely dispersed into the water during wet deposition and ultimately leaching into the water bodies. These PAHs are classified as heavy molecular weight PAH, thus they are easily leached as water solubility decreases with increasing molecular weight (Verweij et al. 2004). The baseline results in this study compare with the level of PAH contaminations in water bodies reported by England and Wales, where PAH levels are above the set standard of 0.2 μg/L in water bodies (Kirby et al. 1998). The factors contributing to the low levels of PAHs in water bodies, whether surface or groundwater, can be attributed to their hydrophobic nature, the ease with which they are taken up by aquatic organisms and their subsequent bioaccumulation and strong interaction with sedimentary organic carbon (Thorsen et al. 2004).

Plant leaves were also assessed for the existence of PAHs because leaves are considered important sinks for atmospheric PAHs and are involved in the periodic cycling of PAHs (Slaski et al. 2000; Zygmunt and Namiesnik 2003). Plants have demonstrated a good quantitative indicator of exposure to both gaseous and solid-phase PAHs in the environment (Korury et al. 1999; Zare-Maivan 2011). Plant leaves are the primary sinks of airborne PAH compounds, whereas absorption of PAHs from soils through the root system is minimal (De Nicola et al. 2011). The study sites detected PAHs in only two dominant species, *F. umbellata* and *E. senegalensis* from Lower Axim. Therefore, the plant species *E. senegalensis* and *F. umbellata* can be used as a good indicator for evaluating PAH pollution in the future. Accumulations of PAHs are aided by leaf surface area. Low molecular weight and volatile PAHs partition between the atmosphere and vegetation (Binet et al. 2000). At high ambient temperatures, the low molecular weight PAHs can re-volatilize into the atmosphere and affect the concentration of PAHs in plants. A wide variety of plant species have also been used as indicators to assess PAH pollution levels in heavily polluted industrial regions as well as for the identification of unknown points of emission (Baud-Grasset et al. 1993). These include *Lemna gibba, Brassica napus, Betula pendula* and *Morus rubra* (Baud-Grasset et al. 1993; Duxbury et al. 1997; Vácha et al. 2010). Other reports have indicated a direct impact of soil and air pollution on PAHs content in plants (Salanitro et al. 1997). The very insignificant levels of PAHs detected in leaf samples suggest a very low concentration of PAHs in the atmosphere. This inference is supported by the fact that there were no manufacturing industries in the study sites, hence eliminating the inclusion of pyrogenic PAHs sources.

Some PAHs such as 2-methylnaphthalene, 1-methylnaphthalene, acenaphthene and anthracene were found in the different soil depths from Dixcove. However, acenaphthylene, naphthalene, fluorene, and phenanthrene were only found at depths of 15-30 cm, which is indicative of the leaching of these compounds to this depth, perhaps via petrogenic sources. Pyrene was the only high molecular weight PAH compound found in Dixcove in the 0-15 cm depth.

From the study site Lower Axim, acenaphthene, 2-methylnaphthalene, and pyrene were the only PAH compounds detected. Both acenaphthene and 2-methylnaphthalene are low molecular weight PAHs found at a depth of 15-30 cm. Pyrene, the only high molecular weight PAH found in Lower Axim, was also detected at both depths (surface and deep). At Half Assini, fluorene, acenaphthene, phenanthrene, fluoranthene, and anthracene were the PAHs detected, with all except fluoranthene being detected at a depth of 0-15 cm. Fluoranthene, the only high molecular weight PAH detected in this town, was found at both depths. Dixcove and Lower Axim are coastal towns close to the oil drilling site, thus these areas are at risk of PAH contamination. The predominant occupation in this community is fishing. Interestingly, all the PAHs found in Aboadze were low molecular weight PAHs detected at a depth of 0-15 cm. However, none of these low molecular weight PAHs had leached to a deeper depth, or the concentrations at this depth probably were too low to be detected. Aboadze is a coastal town with a thermal plant belonging to the Volta River Authority (VRA). Thermal plants burn diesel, a component of crude oil, to generate electricity. Thus, the PAH compounds in this study site could be partly attributed to the pyrogenic contaminants from energy generation via the thermal plant. Reasons such as high volatilization or dissolution could be assigned for the non-detection of very low molecular weight PAHs such as acenaphthylene, naphthalene, and acenaphthene on soil surfaces (depth of 0-15 cm). It is also possible that activities in the area have not yet generated any detectable PAHs levels. The results revealed fluoranthene and 1-methylnaphthalene as compounds with high concentrations in soil samples from Half Assini and Dixcove.

The frequency of over 15% micronucleus in an organism's peripheral erythrocytes indicates the presence of genotoxic agents such as PAHs in the environment (De Lemos et al. 2008). Thus, a baseline and periodic assessment of micronucleus are imperative to help regulate the release of these chemical pollutants into the aquatic environment and initiate the bioremediation process to curtail the potential toxic effects. Therefore, the micronucleus assay can be adopted for bio-monitoring

because it is a fast and sensitive indicator of structural alterations, DNA loss, and numerical chromosomal abnormalities (Galindo and Moreira 2009). In this environmental study, we employed different fish species from the communities bordering the oil drilling field to establish the baseline values for the biomarker in these sentinel organisms. No micronucleus was observed in fish species from these control sites (Aboadze, New-Takoradi and Dixcove) except the species *B. auritus* from the Jomoro District, where the mean frequency was (2.21 ± 3.83) MNE/1000. Similar studies carried out in Slovenia to establish baseline micronucleus frequency in fish species also gave low frequency (Al-Sabti and Metcalfe 1995; Bolognesi et al. 2006).

In addition, the low frequency of MNE and its variability in fish species determined in this study are similar to results found for *the Astyanax jacuhiensis* MNE baseline conducted in Brazil (De Lemos et al. 2008). However, there was no statistical significance between the mean micronuclei frequency of all the fish species analyzed, including the mean of *C. chrysurus* from Lower Axim, which showed the highest levels (6.98 ± 12.09). Though our study's results suggest minimal contamination, continuous monitoring should be done since any change in the environment could be easily detected by an increase in these parameters. Nevertheless, micronuclei were observed in peripheral erythrocytes from fish species caught in the communities close to the drilling sites, albeit no statistical significance when analyzed statistically. Results from other studies indicate a very low range of MNE frequency and a large range of variability in the frequency of other fish species (Bolognesi et al. 2006). The variability could be brought about by differences in metabolic and pharmacokinetic factors (Bolognesi et al. 2006). The MNE biomarker is a less expensive, quick and sensitive means to detect the damage induced by petrochemical discharges. The association between nuclear anomalies' frequency and genotoxic agents' exposure is well established (Al-Sabti and Metcalfe 1995).

In conclusion, this study has provided baseline data for assessing the pollution of six districts nearby the drilling activities at the Jubilee oil fields in the Western region of Ghana. DNA adducts identified by the presence of micronuclei and PAH levels are useful markers for pollution. Therefore, the presence of micronuclei from fish erythrocytes and PAHs in water, soil, plant, and fish livers, especially at sites close to the oil fields, may indicate pollution level from the drilling activities over the past three years since its commencement. Generally, insignificant levels of DNA adducts and PAHs were detected in the samples analyzed.

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Growth rate and survival rate of coral *Acropora* sp. transplanted on the artificial dead coral substrate in the waters of Baai Island, Bengkulu, Indonesia

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Abstract. *Andika D, Purnama D, Negara BFSP, Kusuma AB, Tapilatu RF. 2020. Growth rate and survival rate of coral* Acropora *sp. transplanted on the artificial dead coral substrate in the waters of Baai Island, Bengkulu, Indonesia. Ocean Life 4: 17-23.* Coral reefs are organisms that live on the bottom of the waters and are capable of producing limestone. Transplantation is a technique to accelerate the regeneration of coral reefs that can be used to protect coastal areas and marine life and improve the quality of coral habitat. This study aims to determine the survival rate, growth, and growth rate of height and width of *Acropora* sp. reared using transplantation technology. This research is useful as one of the basic information about the use of transplantation in maintaining coral reefs. The research location is on Baai Island at a depth of 2 m. This study used an experimental method utilizing a transplant technique with five treatments. At the end of the study, the survival rate of coral reefs *Acropora* sp. was 92%. For three months, the growth of coral reef height *Acropora* sp. ranged from 0.87 to 0.90 cm, while the width ranged from 0.84 to 0.94 cm. The growth rate of the average height of coral reefs *Acropora* sp. ranged from 0.72 to 0.82 cm, while the average width was 0.72 to 0.76 cm. Based on the ANOVA statistical test results, there was no difference in the growth rate in either height or width for each type of substrate used.

Keywords: *Acropora*, coral, survival rate, transplant

INTRODUCTION

Coral reefs are bottom-dwelling organisms capable of creating limestone (Kordi 2010; Tuhumena et al. 2019). Corals thrive on dead coral substrates that are sufficiently light and warm. Corals may live in bright waters as low as 10.6 m and warm at 23-25ºC (Sudiono 2008). Corals are considered to be slow-growing creatures (Muhidin 2012). Corals can grow at six to eight centimeters per year (Prameliasari et al., 2012; Tuhumena et al., 2019). Branching corals grow far faster than huge corals (Hairunizar et al., 2015; Tuhumena et al., 2019). Coral reef ecosystems are coastal ecosystems that perform important tasks and serve critical conditions directly and indirectly (Fadli 2009; Aditiyana 2012; Peck et al. 2021).

Coral reef ecosystems provide a dual purpose of protecting the shoreline and providing habitat for various marine species. According to Suryanti et al. (2011), coral reefs act physically as filters, ensuring water purity in coastal areas and wave absorbers. In addition, coral reefs serve as nurseries, feeding sites, and spawning grounds on an ecological level (Yunus et al., 2013; Algutomo et al., 2022). Coral reefs, on the other hand, are deteriorating at the moment. Wilkinson (2002) states that coral reef ecosystems have suffered a 10% to 50% decline in the last 50 years.

Coral reefs are harmed by coastal development activities such as agriculture, coastal dredging, and explosive fishing, which are the primary causes of coral reef degradation (Haruddin, 2011; Algutomo et al., 2022; Sitanala et al., 2022). As a result, additional work is required to rehabilitate damaged coral reef ecosystems.

Rehabilitation is a method of repairing damaged coral reef ecosystems. According to Callista and Sriwarno (2013), rehabilitation is the process of restoring damaged coral reef ecosystems. In addition, Priyono (2004) noted that numerous rehabilitation procedures, such as transplantation, have been used to enhance the condition of damaged or destroyed coral reefs.

Coral transplantation is a technique for accelerating coral reef regeneration that can preserve coastal areas and marine life while improving the quality of coral habitat (Pradana 2008). Coral reef transplantation has occurred in various locations, including Enggano and Tikus islands in Bengkulu Province, Indonesia.

Currently, Mukholladun et al. (2016) researched coral reef transplanting and reported that the growth rate of the coral reef species *Goniastrea* sp. is 16.33 mm/month. According to Subhan et al. (2014), the coral reef species *Acropora pocillopora* can grow up to 1.75 millimeters every month. However, no research on coral *Acropora* sp. transplantation using artificial dead coral substrate has been conducted on Baai Island, Bengkulu Province, Indonesia.

Baai Island, one of Bengkulu Province's ports, has the potential to provide waters for the rehabilitation of coral reef ecosystems. According to a preliminary survey, the

outer right side of Baai Island features healthy coral reefs. This study aims to determine the growth rate and survival rate of coral *Acropora* sp. transplanted onto a dead coral substrate in the waters off Baai Island, Bengkulu, Indonesia.

MATERIALS AND METHODS

Study area

Coral reef transplantation was carried out on Lentera Merah Waters of Baai Island, Bengkulu City, Bengkulu Province, Indonesia (Figure 1). Data analysis was carried out at the Fisheries Laboratory, Department of Marine Science, Universitas Bengkulu, Indonesia.

Method

The method in this research was the experimental method. The experimental method is research conducted to reveal a causal relationship between two variables by controlling the influence of other variables (Afriandi et al., 2012). This study used two types of substrates ratio, artificial substrates made from a mixture of cement: dead coral and a mixture of cement: sand. This substrate consisted of 5 treatments or 5 ratios, namely, 1:1 (A), 1:2 (B), 1:3 (C), 1:4 (D) (cement: dead coral reefs) and 1:1 (E) (cement: sand). The amount of cement used was equal, but the number of dead coral reefs differed. The control substrate was made of cement and sand, adding 25-30% water (Tjokrodimulyo 2007). The measurement and the growth recording were performed once a month for 3 months, and the measurement of physical and chemical parameters of waters was done twice a month for 3 months. The research station was an open ocean around Baai Bengkulu Island with a depth of 3 meters.

Coral transplantation on artificial media

Shelf making

The making of the shelves was adjusted to the water conditions and the selection of materials so that the shelves were not easily damaged or dragged by the current. The shelf size was 1 m^2 and a height of 40 cm with a capacity of 28 fragments/substrate. The iron used in making the shelves was 16 mm in diameter, and the space between one substrate to another was 20 cm (Figure 2).

Substrate making

The substrates were made of cement and dead coral reefs. The amount of water for mixing the substrate was 25-30% of the weight of the cement (Tjokrodimulyo 2007). The substrate size had a height of 2 cm and a diameter of 8 cm. The total number of substrates was 25 pieces, consisting of 5 pieces in a ratio of 1:1 (A), 5 pieces in a ratio of 1:2 (B), 5 pieces in a ratio of 1:3 (C), 5 pieces in a ratio of 1: 4 (D) and 5 control substrates made of cement and sand (Figure 3).

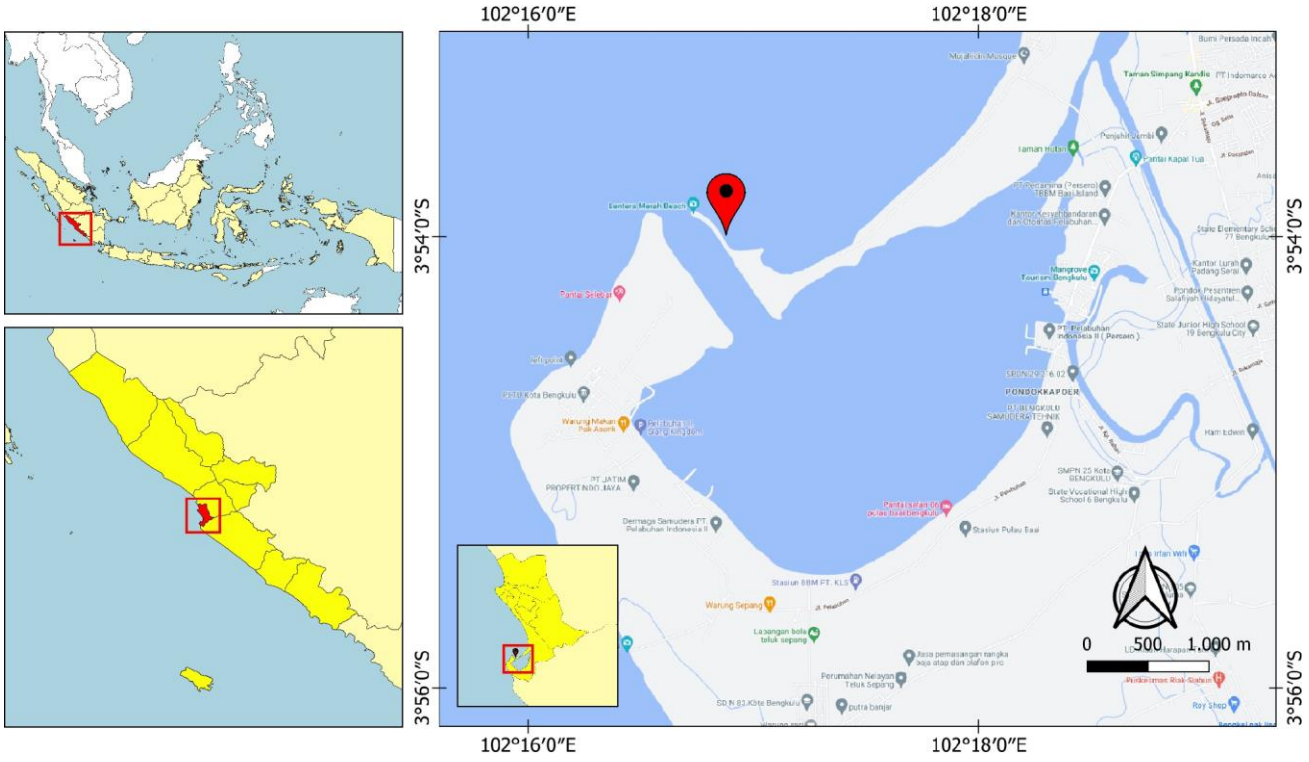

Figure 1. Map of the placement of *Acropora* sp. coral transplantation shelf transplanted on artificial dead coral reef substrate in the waters of Baai Island, Bengkulu, Indonesia

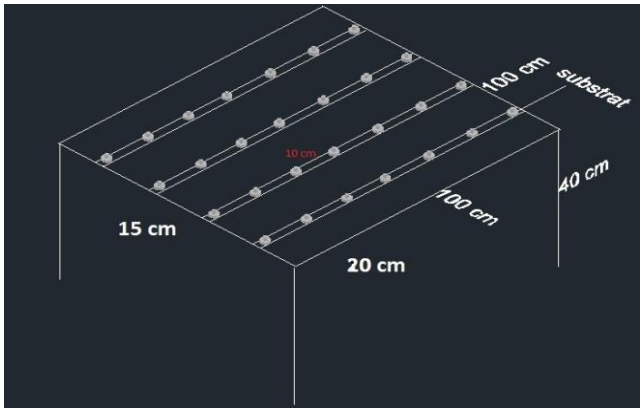

Figure 2. Transplantation shelf where the substrates ratio of cement: dead coral and cement: sand were attached

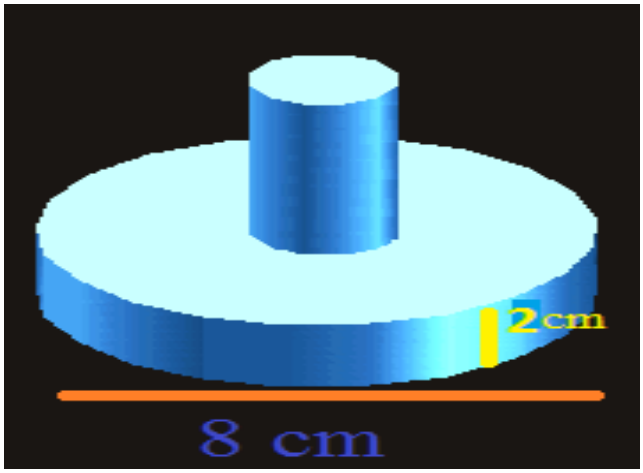

Figure 3. A substrate ratio of a mixture of cement: dead coral reef and cement: sand

Seed retrieval

Coral seedlings were taken around the waters of Baai Island with a depth of 3 m. The studied coral reefs were *Acropora* sp.

Transplant seed attachment

The seeds were attached to the substrates of a mixture of cement: dead coral reef and cement: sand and were tied with tirek to the pillars in the substrates so that the seeds could be arranged neatly and tightly bound.

Placement of transplant shelf

The placement of the transplant shelf at the study site was based on the availability of equipment on the transplant shelf using the SCUBA device so that there was no difficulty in performing the placement of the transplant shelf.

Measurement of coral reef growth and growth rate

Measuring growth on coral reef fragments/seedlings was carried out by measuring the growth rate, consisting of the increase in the height and width of corals. Measuring the height and width of coral reefs used a vernier caliper. The width of the coral reef *Acropora* sp. was measured from the width of the growing branches. The measurement process is carried out directly in the water using the help of SCUBA equipment. Dead or bleached corals will be counted and recorded to measure coral survival. The measurement interval of transplanted coral reefs was once a month, and the data collection on the growth of transplanted corals was conducted for three months (Figure 4).

Measurement of coral survival rate and growth rate

According to Sadarun (1999) to measure the growth rate and survival rate of transplanted corals uses the following formula:

Survival Rate (*SR*)

$SR = N_t / N_0 \times 100\%$

Where:

SR = Survival rate of hard corals (%),

N_t = number of transplanted corals at a certain time,

N_0 = number of transplanted corals at the beginning of the study.

Coral Reef Growth (β)

$\beta = L_t - L_0$

Where:

β = length/width growth of hard coral (cm)

L_t = length/width of hard coral at t time (cm)

L_0= length/width of hard coral at 0 time (cm)

Then the growth rate is calculated with the formula:

$$\alpha = \frac{(L_t + 1) - L_t}{(t_i + 1) - t_i}$$

Where:

α **:** growth rate of length or width of transplanted coral fragment

L_t+1 **:** average length or height of fragments at t+1 time

L_t **:** average length or height of fragments at i time

t_i+1 **:** observation at i+1 time

t_i **:** observation at i time

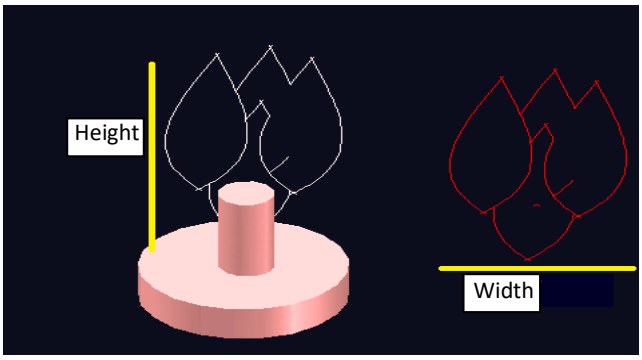

Figure 4. Measurement of coral reef growth and growth rate

Table 1. Water quality parameters

Parameter	Unit	Tool	Description
Temperature	°C	Thermometer	In situ
Salinity	‰	Refractometer	In situ
Brightness	%	Secchi disk	In situ
Current	m/s	Parachute Meter	In situ
pH	-	pH meter	In situ
TSS	Mg/L	Filter paper	In situ

Water quality measurement

The measurement of water quality parameters is shown in Table 1.

Data analysis

This study used the ANOVA test to determine growth differences between treatments used in transplanting *Acropora* sp. The calculated parameters were the vertical and horizontal sizes of each fragment.

RESULTS AND DISCUSSION

Survival Rate (SR) of *Acropora* sp.

The study results on the survival rate of *Acropora* sp. showed 80% on substrates A and C and 100% on substrates B, D, and E (Figure 5). However, the release of fragments from their holder in treatments A and C caused the survival rate in these treatments to be lower than in treatments B, D, and E. The release of reef fragments corals from the module is thought to be due to strong currents and waves and human activities such as snorkeling, diving, and fishing using fishing rods. Supriharyono (2007a,b) explains that damage to coral reef ecosystems is influenced by various biological, physical, and human factors.

Height and width growth of transplanted *Acropora* sp.

In each treatment, the measurement results of height growth of coral reef fragments of *Acropora* sp. showed an increase during three months of observation. At the end of the study, the average height growth of *Acropora* sp. ranged from 0.87-0.94 cm. The highest growth of *Acropora* sp. fragment height occurred in treatments B and C, 0.90 and 0.94 cm. In contrast, the lowest growth of *Acropora* sp. fragment height occurred in treatments D, A, and E with a size of 0.87 cm and 0.88 cm, respectively (Figure 6).

The growth rate of height and width of *Acropora* sp.

The measurement of the growth rate of coral reefs includes the growth rate of coral reef fragment height and growth rate of coral reef fragment width for three months. The results showed that the growth rate of the height and width of coral reefs *Acropora* sp. had the same increase pattern for all substrate types (Figure 7). Based on the data, the growth rate of height and width in the second month had the highest yield. Meanwhile, in the third month, the growth rate in height and width decreased compared to the second month, but there was still an increase.

Water parameters

The physical characteristics of the waters play an important role in determining the area's suitability for coral reef transplantation and are interrelated. Marine organisms have environmental requirements to live and grow well, the more suitable the conditions of the aquatic environment, the better the growth of an organism. Coral reefs are marine organisms that require environmental habitats to grow and reproduce. The growth of coral reefs is influenced by oceanographic factors such as physical and chemical parameters, as shown in Table 2.

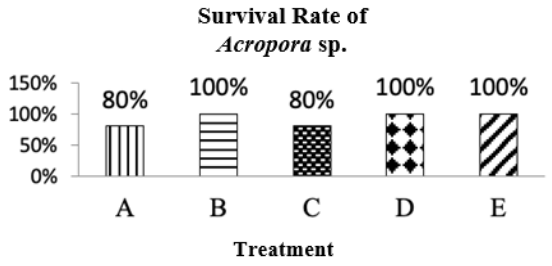

Figure 5. The survival rate of *Acropora* sp. in Lentera Merah Waters, Baai Island, Bengkulu, Indonesia; A=1:1, B=1:2, C=1:3, D=1:4 (cement: dead coral reefs), and E=1:1 (cement: sand)

Table 2. Observations of the physical and chemical conditions of Lentera Merah on Baai Island, Bengkulu, Indonesia

Parameters of the waters	Month				Average	Quality Standard
	I	II	III	IV		
Current (m/s)	0.04	0.08	0.04	0.06	0.05	-
Brightness (%)	100	100	100	100	100	-
Temperature (°C)	32	27.3	32	31.6	30.08	28-32.5 (Syaifullah 2015)
Salinity (‰)	35.6	30.3	35.3	36	33.99	33-34 (Dhahiyat et al. 2003)
pH	7.63	8.3	7.8	8	7.93	7.7-8.4 (Supriharyono 2007a,b)
TSS (mg/L)	0.05	0.15	0.1	0.1	0.1	0.16-0.21 (Andaris et al. 2015)

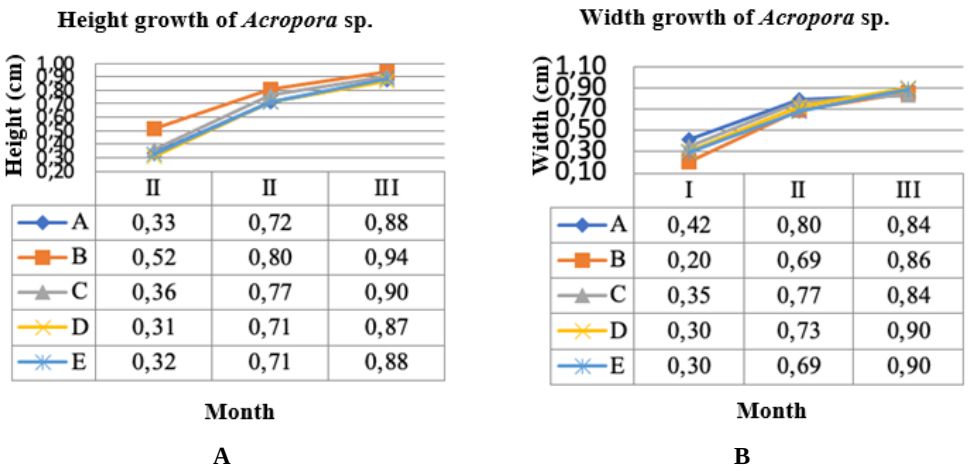

Figure 6. Growth of (A) height and (B) width of transplanted *Acropora* sp. in Lentera Merah Waters, Baai Island, Bengkulu, Indonesia

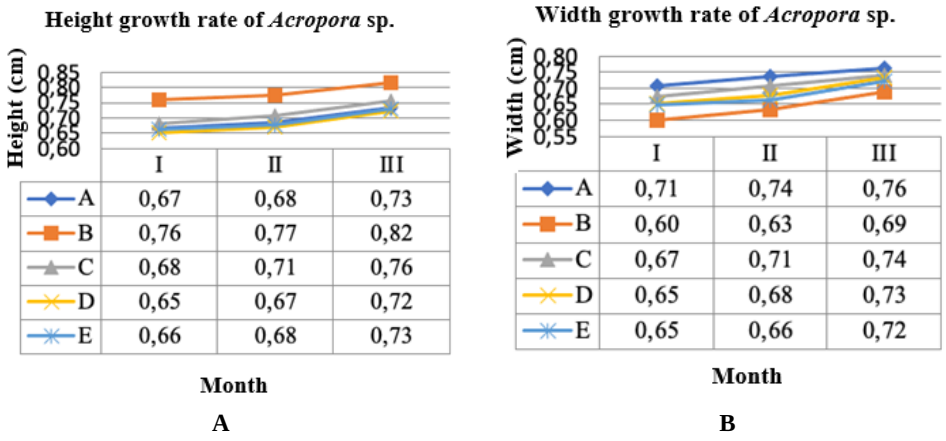

Figure 7. The growth rate of (A) height and (B) width of *Acropora* sp. in Lentera Merah Waters of Baai Island, Bengkulu, Indonesia

Discussion

Survival Rate (SR) of Acropora *sp.*

Overall, the survival rate of *Acropora* sp. of all types of treatment observed was still above 50%. Therefore, the transplant activity for coral reefs of *Acropora* sp., located in Lentera Merah, Baai Island, can be said to be successful. Harriot and Fisk (1988) in Pratama (2005) stated that coral transplantation was successful if the survival rate in most cases ranged from 50-100%, where corals were transplanted in the same or similar as their initial habitat. The survival rate of coral reefs transplanted in different habitats will be influenced by the ability of these coral reefs to adapt to their new environment.

The low survival rate of *Acropora* sp., namely 80%, in substrates A and C was caused by the detachment of the bonds of the transplanted coral fragments. It was possible because of the human factor that uses fishing gear around the research location. The results of field observations showed that many nets and fishing lines have tied up coral reef fragments. Supriharyono (2007a,b) explained that damage to coral reef ecosystems is influenced by various biological, physical, and human factors. Human factors that can cause damage to coral reefs include mining, bombing, and using non-environmentally friendly fishing gear. Biological factors that can cause damage to coral reefs are the presence of predation and disease, while physical factors that can cause coral damage are an increase in water temperature.

Growth height and width of transplanted Acropora *sp.*

From the observation, the growth width of *Acropora* sp. in each treatment continued to increase from months 1-3 (Figure 6). The average growth of the *Acropora* sp. fragment width at the end of the study ranged from 0.84 to 0.90 cm. The highest growth in the width of the fragments of *Acropora* sp. was found in treatments D and E, which was 0.90 cm. The lowest growth of *Acropora* sp. fragment width was found in treatments A and C, namely 0.84 and 0.84 cm. The growth in height and width of *Acropora* sp. in the first month was lower than in the second month. It was thought to be due to a non-suitable aquatic environment parameter for coral growth: temperature. It was also

possible because coral reefs were still in the process of adapting to the used substrate.

The low growth height of *Acropora* sp. presumably was due to many influencing organisms such as algae attached to coral reef fragments and transplantation substrates. According to Gomez and Yap (1984), algae are known to be competitors of corals in both space and light. Algae can cause damage to coral tissue and eventually the death of coral colonies

The results showed that the growth in height and width had the same pattern. However, they were lower in the first month than in the second. Although the low growth in the first month was thought to be due to low temperatures (Table 2), another possible cause was the adaptation process between coral reefs and the used substrate. Herdiana (2001) explains that adapting to coral reef growth takes 2-3 weeks.

In the second month, the growth of coral reefs, *Acropora* sp., increased significantly. In the second month, the coral reefs might have begun to adapt to the environment. In the third month, the growth rate increased but was lower than in the second, presumably due to a lack of energy. The energy of adapted coral reef growth was to strengthen the attachment of fragments to the transplanted substrate. According to Soedharma's (2008) statement, the energy of coral reef growth will affect the attachment of fragments to the substrate.

Growth in height and width of coral reefs in the first month with a mixture of cement substrate: dead coral and cement: sand got a small value, possibly due to environmental factors that did not match the quality standards. The brightness, temperature, salinity, and TSS were below the quality standard in the first month. It could have an impact on the growth of coral reefs. Brightness and TSS were related to the penetration of light into water. The turbidity of the waters would reduce the incoming light. Zooxanthellae used the light entering these waters to carry out the photosynthesis process. Without sufficient light, the photosynthesis process would be inhibited, which could seriously threaten growth (Insafitri and Nugraha 2006).

The growth rate of height and width of Acropora *sp.*

The decrease in the growth rate in height and width in the first month was thought to be due to a decrease in temperature, which was far from the optimum range for coral reefs to grow, namely 28-32°C (Syaifullah 2015). The maximum growth rate of height and width of *Acropora* sp. occurred in the third month, possibly due to the decrease of factors affecting the growth rate, such as the adaptation stage to the environment and the substrate in which coral reefs live. Overall, the growth rate of height and width of *Acropora* sp. continued to increase from months 1-3.

At the end of the observation, the highest growth rate for height was found in treatment B (cement: dead coral reefs) and C (cement: dead coral reefs), i.e., 0.82 cm and 0.76 cm, respectively. The lowest growth rate was found in treatments A, D, and E, which are 0.72 cm and 0.73 cm. At the end of the observation, the highest increase in width growth rate was seen in treatments A, C, D, and E with 0.76 cm, 0.74 cm, 0.73 cm, and 0.72 cm, respectively. At the same time, the lowest width growth rate in the final observation was in treatment B with 0.69 cm. The results of this study were lower when compared to Aditiyana's (2012) study, which got an average height growth rate of 1.04-2.69 cm/2 months.

The calculation using the ANOVA test showed Fcount was smaller than Ftable, namely 4.20 < 5.31, then H0 was accepted. It showed no difference in the growth rate in either height or width for each type of used substrates. The average measurement of the height and width growth rate for each type of substrate showed no different results.

Water parameters

The results of the current velocity measurement obtained an average value of 0.05 m/s (Table 2). This result was higher when compared to Insafitri and Nugraha's (2006) finding that the current velocity in small island waters was 0.04 m/s. The currents from the observations could be suitable for the growth of coral reef transplants. These results indicated that the current strength at the study site supported the rate of coral reef growth. The results of the brightness measurement at the research location with a depth of 2 m obtained an average value of 100% (Table 2). This result was higher when compared to research by Rasyid (2005) in Karimunjawa waters that obtained a brightness value of 71%. The observed value in the waters of Baai Island is much better for coral reef transplantation than in Karimunjawa waters. Brightness was one of the supports for the growth of coral reefs. The results of these observations indicated that the brightness at the study site supported the rate of coral reef growth.

The average temperature obtained during the observation was 30.08ºC (Table 2). This result is almost the same as in the study of Syaifullah (2015) conducted in the waters of Meranci Island with a temperature value of 31ºC. The result indicated that the temperature at the study site supported the growth of coral reefs. According to Supriharyono (2007a,b), the temperature range (30-31ºC) is the ideal temperature to help coral reefs grow optimally. Therefore, the temperature is one factor in determining the feasibility of coral reef cultivation sites. The observations showed that the temperature value was categorized as supportive of the growth rate of coral reef transplants. Based on the measurement of salinity at the study site, the average value was 33.99‰ (Table 2). The result was higher when compared to the study of Wildanun et al. (2016) in the waters of Mandingin Island, with a salinity value of 32‰. According to Dhahiyat et al. (2003), the stable salinity value as a place for transplanting coral reefs is 33-34‰. The results of this observation indicated that the salinity at the study site supported the growth of coral reefs.

The degree of acidity (pH) obtained during the observation was an average of 7.93 (Table 2). This result was higher than Cahyadi's (2001) research in Pari Island waters, with a pH value of 7. Although the pH value in this research location was higher than the study result by Haris et al. (2017), the pH value from observations could be suitable for the growth of transplanted coral reefs. These results indicated that the pH of the water at the study site

could support the growth of coral reefs. The results of the average TSS measurement obtained during the study were 0.01 mg/L (Table 2). This result was lower than the study result by Andaris et al. (2015) in Pari Island waters with a value of 0.16 mg/L. These results indicated that the value of the water parameters at the research site could support the rate of coral reef growth.

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Investigation on parasites of *Octopus cyanea* in Tanzania

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Abstract. *Msongole PL, Kimbita EN. 2020. Investigation on parasites of* Octopus cyanea *in Tanzania. Ocean Life 4: 24-36.* This study aimed to investigate parasites in *Octopus cyanea* (Gray, 1849) or Day octopus along the Indian Ocean of the Tanga region in Tanzania. Information from fishermen was required to determine their awareness of octopuses' parasitic infections. Twenty-five percent (25.3%) agreed on the presence of parasites in octopus skin and muscles, but these were not observed during laboratory investigation. Gamogony and sporogony of *Aggregata* sp. (Apicomplexa: Aggregatidae) were observed during histopathological examination of the digestive tracts of octopuses collected along the Tanga coastal area. The *O. cyanea* was infected with the coccidian parasite, *Aggregata* spp., with a prevalence of 41.1% (23 of 56 hosts examined). Oocysts were sub-spherical in the mucosa wall of the intestine and caecum, measuring 263-279 µm. Sporocysts were smooth-surfaced, dark-staining, spherical, typically 10-15 µm wide, and contained 9-22 banana-shaped sporozoites with a size of 2-6 µm long and 0.2-0.3 µm wide. The coccidian infection in octopuses is accompanied replacement of the infected cells with sporocysts. Parasite infection of *Aggregata* spp. was not significantly correlated with the weight of the octopus, study site, or sex of the host. Molecular analysis was used to confirm the parasite species, in which 68% of samples were positive. Molecular characterization revealed that the coccidian was the *Aggregata octopiana* (Schneider, 1875) Frenzel, 1885. Alignments revealed that the coccidian from *O. cyanea* resembled 89% with *A. octopiana* isolated from common Octopus in Spain. Despite the presence of *A. octopiana* in *O. cyanea*, the parasite does not cause effects on consumers unless the octopus is infected with another epizootiology agent.

Keywords: Coccidian infection, molecular characterization, octopus, parasite, Tanga

INTRODUCTION

Octopus fishing along the coast of Tanzania plays an important role as a source of protein and income that may improve fishermen's livelihood (Jiddawi and Ohman 2002). Octopus has been processed and sold in the local market and can be exported (Guard 2009). Local coastal communities of Tanzania mainly deal with artisanal fishing for White-spotted octopus (*Octopus chromatus* Heilprin, 1888), common octopus (*O. vulgaris* Cuvier, 1797), and Day octopus (*O. cyanea* Gray, 1849) as their source of employment and income generation (Guard and Mgaya 2002; Guard 2009; Mshana and Sekadende 2014) and protein source (Bultel et al. 1950; Estevez et al. 1996; Gestal et al. 2007).

Octopus belongs to the Family of Octopodidae (Emery et al. 2016), with the genus *Octopus* consisting of more than 100 species (Ignatius and Srinivasan 2006). Many *O. cyanea* is mostly found on the East African coast and Madagascar to south-eastern Asia and Hawaii (Guard and Mgaya 2002; Benbow et al. 2014). *O. cyanea* is a predatory cephalopod foraging and feeding on other invertebrates, such as crustaceans (Mshana and Sekadende 2014). The size of male and female *O. cyanea* differs at sexual maturity; females may reach 0.6 kg to 5 kg at spawning, while males may reach 300 g at sexual maturity (Van Heukelem 1973). Cannibalism can happen to small male octopuses during mating (Hanlon and Forsythe 2008).

Octopus fishing has been increasing in recent years due to demand and price increases in local and international markets (Sauer et al. 2011). However, global market growth can generally lead to heavy and unsustainable fishing of *O. cyanea* in Tanzania and East Africa (Guard and Mgaya 2002; Eriksson et al. 2012).

Despite the importance of octopuses in East Africa, there is not enough information concerning their threats. Most studies on octopus diseases and parasites have been conducted in European countries. Coccidiosis is among the most important diseases reported in octopuses, leading to malabsorption syndrome (Pascual et al. 2010). The disease is caused by the protozoa, an intracellular parasite that affects both wild and cultured octopus through food-web relationships (Gestal et al. 1999; Vidal and Haimovici 1999). The enteric infection does not directly cause the octopus's death, but it exposes the octopus to the risk of being affected by other disease-causing agents (Pascual et al. 2007).

Apart from protozoa, also octopus has been parasitized by isopods, digenean, nematodes, cestodes, and copepods (Pascual et al. 2007). Dicyemida, Spirurid nematodes, and *Cystidicola* sp. has been reported to cause infection in the stomach of octopus (Pascual et al. 1996; González et al. 2003). Ectoparasites like copepods (*Octopicola* spp. and *Pennella* spp.) can cause a serious pathological conditions in the gills. The parasite and cephalopod relationship are common in all oceans; cephalopod species have associated parasites (Pascual et al. 2007).

Despite the increased octopus fishing in East Africa in recent years, research in this field is still low, with limited published papers. Octopus diseases and parasites are not important (Vidal and Haimovici 1999). However, a parasitic infection (like coccidiosis) has been reported in

octopuses in the Mediterranean, Atlantic, and Pacific Oceans (Gestal et al. 2007). The *Aggregata* coccidian has been reported to infect the digestive tract of more than 13 species of octopus worldwide (Estevez et al. 1996).

This study aimed to investigate the possibility of *O. cyanea* hosting parasites, investigating the endoparasites and the ectoparasites along the coastal area of Tanga, Tanzania.

MATERIAL AND METHODS

Location and duration

Octopuses were purchased from fishermen in four sites in the Tanga Region, Tanzania districts from December 2017 to January 2018. The study sites were Kwale, Sahare, Kigombe, and Pangani Villages, located in Tanga Region (05004', 39006' E). The Tanga Region has four coastal districts, which were selected as the study area of this research (Figure 1).

Study design

A cross-sectional study design was used; with purposive sampling, four landing sites with n were calculated as sample size:

$n = Z\alpha^2 \times p\ (1-q)^2 / d^2$

Where, Zα (from the table) at Type I error of p= 0.05, q= 1.96, d= effect size.

Sampling strategy and sample collection

Samples were collected in four districts actively involved in octopus artisanal fishing. A total of 56 *O. cyanea* (about 12 from each site), with an average weight of 400.51 g, were collected from four districts (one coastal village per district). Artisanal gear used by local fishermen to catch octopuses. A small piece of kidney, liver, and caecum from each octopus was taken and fixed in 10% buffered formalin for histological purposes. Octopuses were frozen for preservation, transported in a sterile cool box to the laboratory, and thawed before necropsy at the Sokoine University of Agriculture, Morogoro, for parasite examination and identification.

Questionnaire administration

A total of 156 fishermen were selected randomly from four villages, Kwale, Sahare, Kigombe, and Pangani, for the questionnaire. Thirty-nine (39) fishermen over 18 from each selected site answered prepared questions concerning octopus parasites, fishing activities, habits of octopus consumption, type of octopus captured, and processing activities.

Although octopus fishing activities in these areas are seasonal, it was possible to visit fishermen and get some information concerning octopus fishing and parasites. The sites were chosen to gain information on fishermen's perceptions of the presence of parasites on octopuses, which octopus species they preferred, ways of octopus processing for consumption, and how they overcame any side effects caused by consuming octopus.

The structured questionnaire was in Swahili. For the fishermen who could not read and answer the questions on their own, each question was read to them to get some information about the fishing activities of octopuses and octopus parasites or diseases. The structured questions were open- and closed-ended, and the respondents were free to discuss during the interview.

Collected data were grouped according to the group age of the respondents and sex. Few respondents were female (age group 30-53) and mostly octopus dealers. The survey also asked about infectious agents, such as bacteria and fungi. Respondents were required to give their opinions on the effects of any parasites of octopuses on a human being. Some questions were asked on the measures they took to prevent parasites, bacterial or fungal effects.

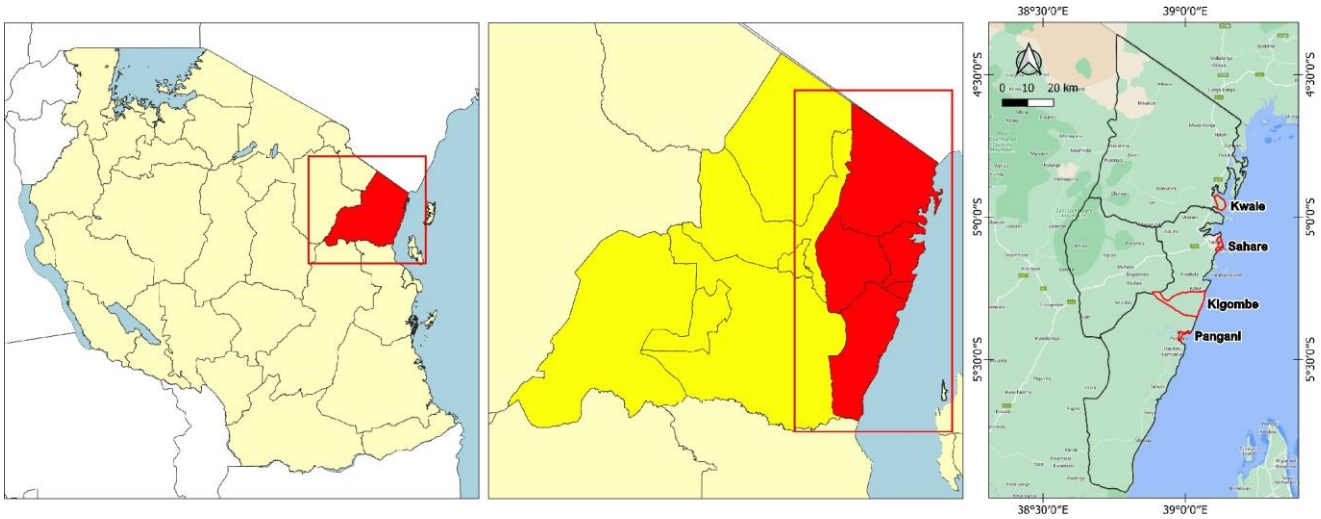

Figure 1. Maps showing the study sites in Tanga Region, Tanzania

Parasite examination

Gross examination

After mantle dissection, a macroscopic examination of parasites on the body surface and intestine for each octopus was done in the field. The entire octopus mantle and body surface were observed for the ectoparasites and endoparasites. The mantle was cut with scissors to observe endoparasites which the naked eye could see in the internal organs.

Laboratory work- examination for ectoparasites

Scrapings from the skin and gills of the specimen were smeared on clean glass slides, covered with cover slides, and examined under light microscopes for ectoparasites. Each sample was examined independently for parasites according to the protocol outlined previously by Obiekezie and Ekanem (1995). Skin scrapings and wet mounts from skin and gills were examined for abundance and distribution of the larval stage of worms and other ectoparasites like leaches. Gills were taken into a petri dish with tap water and placed under a dissecting microscope for larval worm examination.

Examination of intestinal parasites (endo-parasites)

In the laboratory, specimens were weighed using a weighing balance, and their weight was recorded in grams (g). Then, octopuses were dissected to get intestinal contents, caecum, liver, kidney, and fecal samples. The intestinal contents of the weighed octopus were taken into a beaker, mixed with concentrated salt solution, filtered, poured into a test tube then covered with a cover slip for the floatation process to recover the parasite's oocysts. After 10 minutes, a cover slip was removed for microscopic examination. The intestinal contents were also examined for adult worms.

Tissue parasites

The kidney, caecum, and liver were isolated in each dissected octopus. Small pieces of liver, kidney, and caecum were smeared on glass microscope slides, which were immediately fixed in methanol, and some pieces were stored in 70% ethanol. The smears were then stained in Giemsa solution (Bruno et al. 2006) for coccidian oocyst and sporocysts examination. Squashing was the means of detecting parasites from the octopus tissue. Squash preparations of fixed tissues from the intestine and caecum were examined by light microscopy. The parasites' sizes were measured using a calibrated ocular micrometer and expressed in micrometers (μm).

Tissue processing and histopathology

Heavily parasitized tissue of some samples' gills, kidneys, liver, and caecum were fixed in 10% buffered formalin and then trimmed in processing cassettes. The trimmed kidney, gills, liver, and caecum tissues in processing cassettes were fixed in 10% NBF for 48 hours, then processed routinely and embedded in paraffin wax using the standard paraffin procedure. The standard paraffin process (tissue processing) starts with fixation that preserves the tissue. That was followed by dehydration through graded ethanol (70%, 90%, and absolute), clearing in chloroform, and finally, infiltration of the tissue with molten paraffin wax. It was followed by embedding, and then sectioning was done using a rotary microtome (Baird and Tatlock) to produce 4 μm thin sections placed on paraffin section mounting bath (Electrothermal) microscope slides. Sections (4 μm) were stained with H & E following standard procedures (Culling et al. 1985). These sections were dried overnight in a paraffin oven (Electrolux) at 50°C.

Molecular identification

Isolation and purification of parasite

Infected digestive tracts were dissected and homogenized in 10 ml of distilled water with 1% Tween20 using a motor and paste. Tissue homogenates were filtered twice with nylon meshes of 100 μm and 41 μm, respectively, to remove tissue fragments, and then concentrated NaCl was added to make the sporocysts and oocyst float. The mixture was centrifuged at 1000 x g for 5 min in a centrifuge KUBOTA S100. The sporocyst was purified by density gradient centrifugation method according to Gestal et al. (1999) and preserved in 70% ethanol for DNA extraction.

DNA extraction

Genomic DNA was extracted from *Aggregata* parasite sporocysts isolated from the digestive tract of *O. cyanea* using the ZYMO Research DNA extraction kit following the manufacturer's procedures. Sporocysts were suspended in 500 μL of extraction buffer (NaCl 100mM, EDTA 25mM pH 8, SDS 0.5%) and opened by sonication on ice (5 cycles, 40W, 50 s) to release sporozoites. After Proteinase K (Sigma) digestion (1mg ml-1) at 37ºC overnight, the DNA was purified following the alcohol extraction method, as described by Sambrook et al. (1989). DNA was precipitated with ethanol and sodium acetate overnight at -20°C. Finally, the precipitated pellet was resuspended in 50 μL of Tris-EDTA (TE) buffer.

DNA amplification

The small subunit 18S rRNA gene with the size of 970 bp of extracted coccidian was amplified by PCR using conserved primers designed for *Aggregata* spp. (Kopečná et al. 2006), and derived from GenBank sequences: (*Aggregata* 1-F: 5 - ATGATGAAACTGCGAAGAGC-3; *Aggregata* 2-R: 5 - CGACGGTATCTGATCGTCTT-3); PCR reactions were performed in a total volume of 25 μL containing 1 μL of10mM dNTP mix, 0.25 μL Taq DNA polymerase (Roche), 2.5 μL Taq10x buffer, 1 μL 2.5 mM MgCl2, 1 μL of each primer and 1 μL of template DNA at 100 ng μL^{-1}. The temperature profile for primers 1-2 included an initial denaturation at 94°C for 10 min, 35 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min. For primers 3-4, we used an annealing temperature of 55°C. PCR products were separated on 1.5% agarose gels, stained with Gel Red including a 100- bp ladder size standard (Invitrogen), and visualized using a UV transilluminator.

DNA cloning and sequencing

PCR products were cloned using TOPO® Cloning Kit (Invitrogen) according to the protocol supplied by the manufacturers and transformed into TOP 10 F competent bacteria *Escherichia coli* (Mig., 1895) (Invitrogen). Screening of clones carrying 18S rRNA-coding region fragments was performed by PCR, adding the positive colony directly to the PCR mixture reaction using the corresponding *Aggregata* primers. Positive clones were purified by Microcon-centrifugal filter YM-50 (Millipore).

The purified PCR products were bi-directionally sequenced using BigDyeâ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) following manufacturers' procedures, using the 15 positive sequences from PCR. First, DNA sequencing was performed on an ABI 3500XL DNA Analyzer (Applied Biosystems), which uses a 24-capillary at Mbeya Zonal Referral Hospital. Next, the sequenced fragment was assembled and arranged using Genius Software into consensus sequences, then entered in a GenBank for alignment with other *Aggregata* spp.

Phylogenetic analysis

The aligned sequence products were used to construct a phylogenetic tree using sequences of other Apicomplexa taxa to determine the species of the *Aggregata* isolated from *O. cyanea*. The phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis version 7.0 (MEGA-7 software) (Kumar et al. 2016).

Data analysis

Questionnaire data

Survey data were collected, entered, and stored in the spreadsheet using Microsoft Excel version 12 and analyzed using a Statistical Package for Social Sciences (SPSS) version 16.0. Qualitative descriptive analysis was used to describe the awareness of respondents on octopus parasites. In addition, a chi-square test was done to test the goodness for the observed frequencies, such as the number of respondents on parasite existence, yielding statistical differences with the age group of fishermen at p-value of 0.05.

Parasite infection

To describe parasite infection in *O. cyanea*, the prevalence of parasite infection was calculated according to Mitchell et al. (2000).

$$\text{Prevalence} = \frac{\text{number of individuals of hosts infected with parasites}}{\text{Number of hosts examined}} \times 100$$

Oneway ANOVA (performed using the PAST program) was used for normally distributed data to compare the mean differences in parasite infection between sites. Two sample t-tests were performed to determine if there was a statistically significant difference in parasite infection between the host's sex (male and female). Linear regression analysis was carried out to determine if there is a significant relationship in the rate of infections between individuals (*r* applied to determine the degree of association between parasite intensity and host-related factors such as octopus weight). Quantitative analysis was performed for statistically significant ectoparasites and the site of infection. A p-value of ≤ 0.05 was considered statistically significant. Parasite populations between sites of sample collection were analyzed using prevalence (P, %) of infection as a parameter according to Mitchell et al. (2000). DNA sequence data analysis was done using the Predictive Analysis Software PASW Statistics 19.0 statistical program and presented as a percentage.

RESULTS AND DISCUSSION

Questionnaire administration (field survey)

Profile of the interviewed fishermen and octopus dealers

A total of 156 fishermen from four study sites were interviewed; most were men (94.87%). The respondent's mean age was 40.07 years, with most of them aged 31-53. According to their responses, it was observed that only 32% were able to read the questionnaire (25% primary education, 7% secondary education), while 68% were not educated and unable to read the questionnaire (Table 1). Most fishermen along the Tanga coastline were found to have poor knowledge of parasites in general. 80% of the fishermen responded that they fished only one species of octopus, which is *O. cyanea*, even though they didn't know the name of the octopus they fish. Most fishermen identified octopus by the local name "pweza."

Perception of fishermen on parasite existence in octopus

The results from fishermen and octopus dealers' responses were grouped according to the respondent's age and sex. Age and sex were used as one criterion. According to the data obtained from respondents, the issue of parasites in octopuses is not well known (Table 2).

During the survey, we observed that experienced fishermen understood some abnormal conditions in octopuses, including their diseases. Therefore, elders experienced in fishing were expected to give some good information about parasite infections and diseases in octopuses.

In Figure 2, respondents were asked if the octopus they caught harbored or if there was any observable parasite. Again, most respondents (97/156) didn't know octopuses could harbor parasites, while a small minority knew parasites on an octopus.

Table 1. General features of the respondents who participated in the survey

Characteristic	Sampling site N (% of respondents)				Total
	Sahare	Kigombe	Pangani	Kwale	
Age group					
Young (18-30)	7(17.1)	11(26.8)	16(35.6)	10(34.5)	44(28.2)
Middle (31-53)	23(56.1)	20(48.8)	24(53.3)	16(55.2)	83(53.2)
Old (>54)	11(26.8)	10(24.4)	5(11.1)	3(10.3)	29(18.6)
Sex					
Male	39(95.1)	41(100)	41(91.1)	27(93.1)	148(94.9)
Female	2(4.9)	-	4(8.9)	2(6.9)	8(5.12)
	41	41	45	29	156

Table 2. Fishermen's opinions on parasites in octopus (n=156) expressed as a percentage of respondents

Characteristic	Parasite status (% of respondents)			Total
	Present	Absent	Don't know	
Age group				
Young (18-30)	2(4.54)	23(52.45)	19 (43)	44
Middle (31-53)	5(6.02)	42(50.6)	36(43.4)	83
Old (>54)	8(2.8)	10(34.5)	11(37.9)	29
Sex				
Male	13(86.7)	69(92)	66(100)	148
Female	2(13.3)	6 (8)	0(0)	8
	15	75	66	156

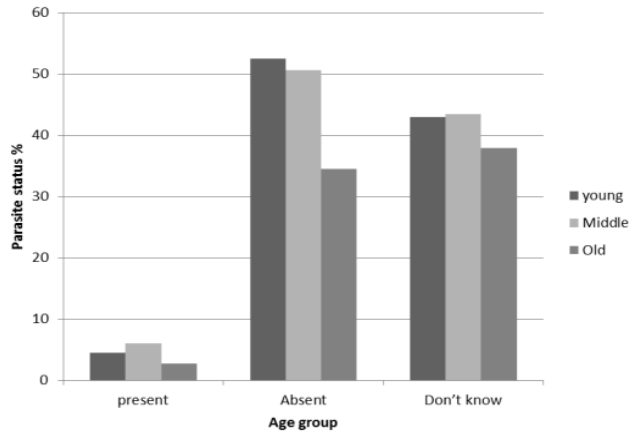

Figure 2. Respondent's opinions on the existence of parasites as the question answered by different age groups

Response on type of pathogen (parasite) occurring in octopus

Most fishermen in each age group had no idea about the kind of parasite infecting octopus. Fishermen were asked to describe the appearance of any parasites they saw in octopus during fishing and processing (Table 3). The respondents answered if the parasites have any effects on consumers.

Fishermen's perceptions of octopus disease in general

Fishermen and octopus dealers were asked to mention and describe any disease in octopuses. The responses to the question were recorded based on the age group of the respondents. The respondent age group was statistical significance at 0.05 levels (X2 = 2.72, p = 0.033) to octopus disease status.

Laboratory analysis of parasite infestation

Among fifty-six (56) *O. cyanea* collected, only 41% (23) were infested with parasitic protozoa, the coccidian *Aggregata* spp., all of which were found in the digestive gland (liver), kidney, and intestine. Table 4 shows the octopus samples examined and the number infested at each selected study site. Cysts were found on the octopus body and digestive tract (the cecum). As much as 96% of the infected octopuses weighed above 500g, while 89.3% below this weight were found uninfected by parasites. The bigger the octopus's size, the higher the probability of having a parasite, especially a gastrointestinal protozoan.

Table 3. Fishermen's responses on the kind of cause of disease harbored by octopus along the coastline of Tanga, Tanzania

Age group	Cause of disease (n=156)				Total
	Worms	Bacteria	Fungus	Don't know	
Young (18-30)	6	0	0	41	47
Middle(31-53)	5	1	3	71	80
Elders (>54)	3	1	1	24	29
Total	14	2	4	136	156

Table 4. Shows the number of octopuses examined for parasites, prevalence, and number of octopuses infected with parasites

Site	No. examined	No. infected	Prevalence %
Kwale	14	5	35.7
Kigombe	15	8	53.3
Sahare	13	3	23.1
Pangani	14	7	43.8
Total	56	23	41

External lesions were observed in some octopuses, while some had been injured with their arms chopped. The lesions could result from ectoparasite invasion having attached, fed, and detached, leaving some wounds on their host's body. Fishermen have blamed the predator eel fish for being the main reason for causing lesions and cutting the arms of octopuses.

Histopathological evaluation of octopus intestine, liver, kidney, and gills showed substitution of tissues with developed oocysts with sporocysts and gametes in some tissues. The gametes were mostly observed in the intestinal mucosa, especially the caecum, while no gametes were found in the gills, liver, and kidney tissues in the intestinal mucosa, substituted by many oocysts. Histological sections of the kidney and liver showed little protozoa infections compared to the digestive tracts, especially the caecum. However, the mucosa of many infected octopuses was found to have lesions caused by invasion of the protozoa oocysts, which replaced the host mucosal cells.

Gross examination

White cysts containing *Aggregata octopiana* (Schneider, 1875) Frenzel, 1885 were commonly found by gross observation in the wall of digestive tracts of some octopuses sampled from Kwale, Pangani, Kigombe, and Sahare (Figures 3 and 4). Most cysts were seen in the intestines, especially in the cecum. In Figures 3 and 4, cysts were seen as white spots in the wall of the intestine, and sometimes they were observed in the digestive gland of heavily infected octopus. Apart from the observable white cysts, no other parasite was observed on the mantle or intestine surface. No ectoparasites were found during the examination of parasites. A fresh smear of eyes, skin, and gills showed no worms in octopuses collected from all selected study sites.

Fresh smear examination

In fresh smear preparation, some of the sporocysts had already raptured, as shown in Figure 5, with the sporozoites being easily seen with a light microscope with different magnifications. The sporocysts were ovoidal and smooth, containing a large number of sporozoites.

Fresh squash examination

The results for Giemsa stain squash preparation of the intestine and caecum showed spherical sporocysts, ranging from 10-15 µm containing sporozoites (Figures 6 and 7). The Light Microscope observation of fresh tissue squash showed that the intestine and kidney were infected with parasites, while the liver was less infected.

Prevalence of parasite infection

The prevalence of parasites was calculated after a light microscope examination for the parasite using a smear, squash preparation, and histopathology examination. The results were grouped depending on the number of hosts examined concerning the site where samples were collected (Table 5).

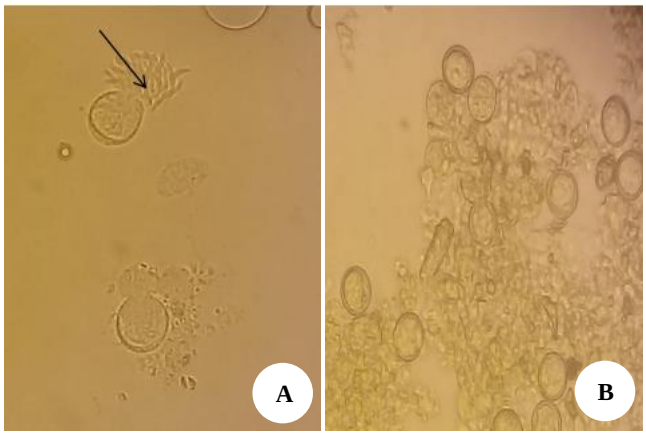

Figure 5. Light microscopic examination of the fresh smear of intestinal contents. A. showing the sporocyst raptured to release the banana-shaped sporozoites. B. Several sporocysts with sporozoites inside

Figure 3. A macroscopic view of dissected *Octopus cyanea* showing heavy infection with Coccidian parasite in the intestine, white cysts (arrow)

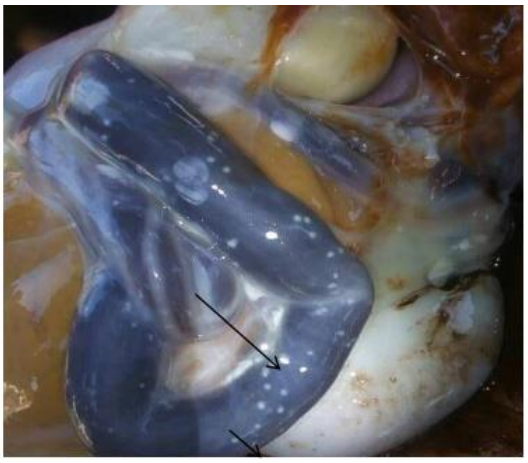

Figure 4. Photograph showing a macroscopic view of an octopus intestine with a heavily infected condition. Note the number of white cysts (arrows) infecting the intestine and caecum of the octopus

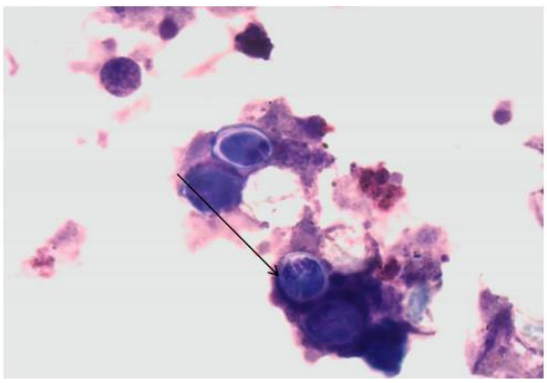

Figure 6. Fresh squash preparation of the octopus caecum infected with coccidian parasites. The figure shows the sporocysts with sporozoites (arrow)

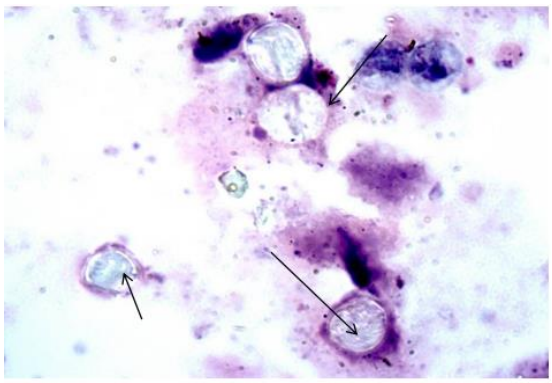

Figure 7. Fresh squash preparation of the intestine shows sporocysts with sporozoites inside (arrows)

Histopathology examination

Histopathological study showed that each organ of the infected octopus had several huge oocysts containing sporozoites and gametes. The gamogony stage was observed in the intestine and the caecum, where the microgametes and macrogametes were seen, as shown in Figure 8 (observe arrows). The parasites were mostly found in the submucosa and muscular layers (Figure 9). The detachment of epithelial cells was observed, especially during the sporogonic stage of *Aggregata* sp. (Figure 11). Some nuclear displacements were observed in gills and mucosal cells due to the presence of many sporocysts and developing macrogametes (Figure 10). However, there was less tissue destruction in the gills and kidneys compared to the intestine and caecum.

Sporocysts counting

During sporocysts isolation, large numbers were observed under the light microscope. With the aid of a light microscope, several sporocysts were recorded using the Neubers chamber, as shown in Table 5. Also, during sporocysts counting, some had already released the sporozoites (Figure 5).

Analysis results

Prevalence of infection by individuals

The comparison was conducted to any association between infections' density and individual octopuses' weight. The data were normally distributed, and a linear regression analysis was performed.

The correlation between the weight of an individual octopus and the number of sporocysts was not statistically significant at the 0.05 level (r= 0.18927, p= 0.16238). The plot of several sporocysts and the weight of hosts showed a weak positive correlation. A significant difference was not found. The number of sporocysts depends on the infective dose of parasites that the host ingested from the intermediate host and not its weight (Figure 12).

Regression equation:

$y = 7.157 – 2288.5x$,

$r = 0.18927$

$r^2 = 0.035825$ (the host's weight can explain, i.e., only 3.5825% of the variation in infection density).

$t = 1.4165$, $p = 0.16238$ (not significant).

Infestation between sites

A one-way ANOVA was performed on the normally distributed data to compare the means of sporocysts between study sites. The means of infections were not significantly different among sites at the 0.05 level (F= 1.63, p= 0.1936). A Tukey's Honest Significant Difference test indicated that parasite infestation in samples from Kigombe was higher than those from other sites. Since there was no statistical significance between parasite infestations in octopuses among sites, post hoc testing was not required. The parasite infection prevalence was positively and significantly correlated among sites (r= 0.98, p= 0.008, df= 3) (Figure 13).

Table 5. Details on octopuses examined, state of infection, body weight, and location of octopus collection

Host no.	BW (g)	Locality	HS	I	No. sporocyst/g	Site of infection
O 3	387	KW	F	+	2 x10^3	Caecum
O 5	586	KW	F	+	1.2 x 10^3	Digestive tract
O 1	962	S	M	+	3 x 10^3	Digestive tract
O 4	1532	KW	F	+	8 x 10^2	Digestive tract
O 5	1540	K	F	-		
O 6	442	K	M	-		
O8	261	P	M	-		
O 7	253.5	KW	M	-		
O 9	567	P	F	+	5 x 10^2	Caecum
O 10	262	P	M	-		
O2	317	P	F	-		
O1	217.6	K	F	-		
O5	255.4	P	F	-		
O1	250	P	M	-		
O7	261	P	M	-		
O2	263	S	M	-		
O9	567	KW	M	-		
O3	341.8	P	M	+	6 x 10^3	Caecum
O4	512	K	M	-		
O6	442	KW	F	-		
O11	511.4	P	M	+	8 x 10^2	Digestive tract
O6	347	S	F	-		
O3	712	K	F	+	1.6 x 10^3	Caecum
O7	422	K	F	+	6 x 10^2	Digestive tract
O 1	387	KW	M	-		
O10	262	KW	M	-		
O4	267	S	F	-		
O3	253	S	M	-		
O2	337	K	F	+	8 x 10^3	Digestive tract
O6	250.5	P	F	+	3 x 10^3	Digestive tract
O10	312.8	S	M	-		
O9	251.4	K	F	-		
O7	560	S	M	-		
O12	712.6	K	M	+	5 x 10^3	Kidney, digestive tract
O11	461	S	F	-		
O13	502.3	P	M	+	1 x 10^4	Digestive tract
O14	281	KW	F	-		
O15	637	P	F	+	4 x 10^3	Digestive tract
O16	511.9	P	F	-		
O4	252	P	F	-		
O12	251.8	P	M	-		
O10	524	K	M	+	6 x 10^3	Digestive tract
O9	378.2	S	F	-		
O12	428	KW	M	+	1.2 x 10^3	Digestive tract, kidney
O14	390.3	K	F	+	8 x 10^2	Digestive tract
O11	442	KW	F	-		
O12	571	S	F	+	1 x 10^3	Digestive tract
O15	419	K	F	+	8 x 10^2	Digestive tract
O5	267.6	S	M	-		
O13	704.7	KW	M	-		
O11	672	K	F	+	2 x 10^3	Digestive tract
O13	254.8	S	M	-		
O5	1540	KW	M	+	3.4 x 10^3	Caecum
O14	734.2	P	M	+	2.8 x 10^3	Digestive tract
O13	302	K	M	-		
O2	586	KW	M	-		

Note: M: male host; F: female host; O: host numbers; KW: Kwale; P: Pangani; S: Sahare; K: Kigombe; BW: Octopuses body weight; HS: host sex; I: Infection

Infection between male and female hosts

The results showed that the infection was higher in male octopuses than in female octopuses (Figure 14). However, the comparison of means between the two sexes of hosts was performed using two-sample t-tests, and there was no statistically significant difference in parasite infection between the two sexes.

Molecular analysis

A total of 25 samples of coccidian parasites isolated from the intestine of *O. cyanea* were run in a TAKARA PCR Thermo cycler machine using a pair of primers to confirm the presence of *Aggregata* species in the isolated intestinal parasites. Seventeen samples were positive for the 1F-2R primers, and eight were negative. In Figure 15 are representative samples that show positive results in the first pair of primers visible in 1.5% agarose gel.

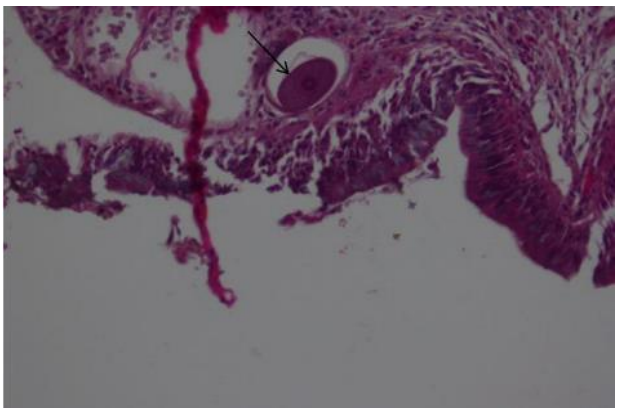

Figure 8. Macrogamete with spongy cytoplasm in the microvilli of the intestine of *Octopus cyanea*

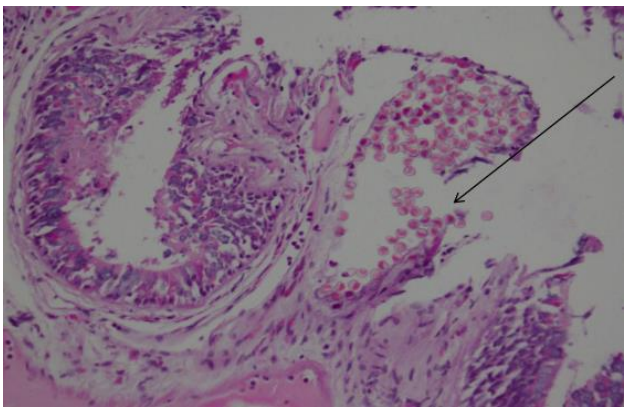

Figure 11. Detachment of the epithelial cell seen (arrow)

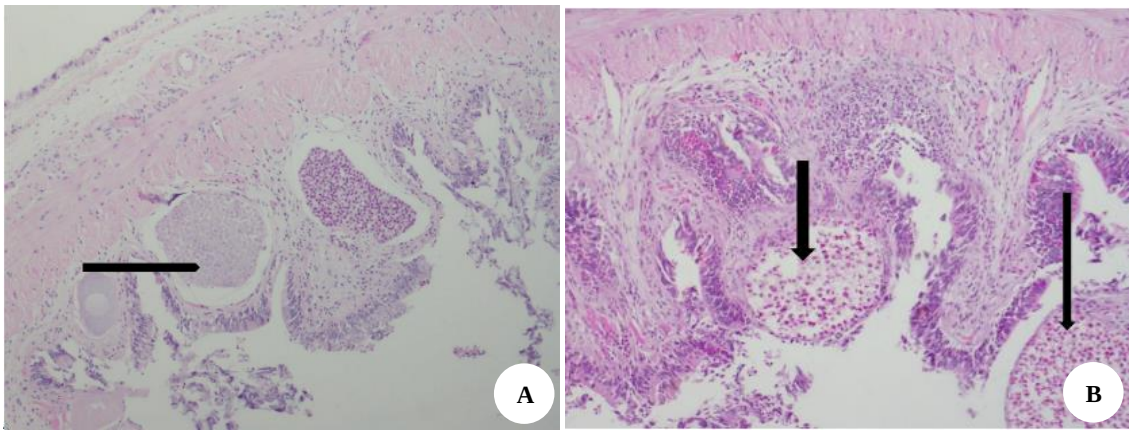

Figure 9. (A and B) H & E section showing the mucosal cells of the intestine replaced with large Oocysts containing many sporocysts (X10). An arrow shows Oocyst within the intestinal villi

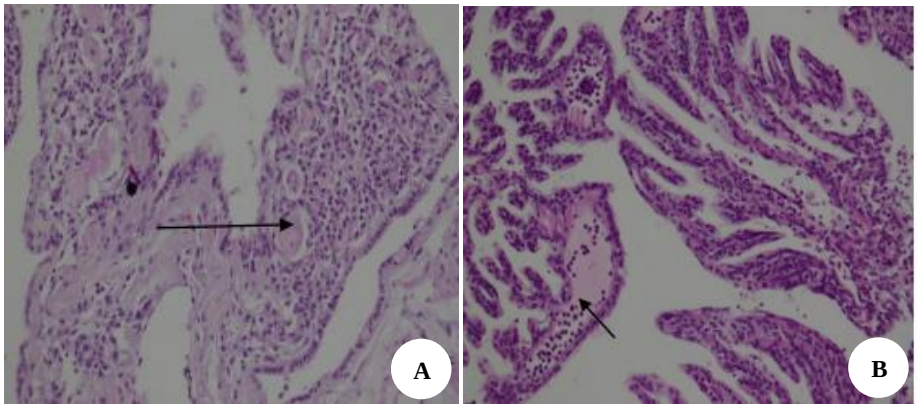

Figure 10. (A and B) H&E stain section LM examination of the octopus gill-infected oocysts of the coccidian parasite

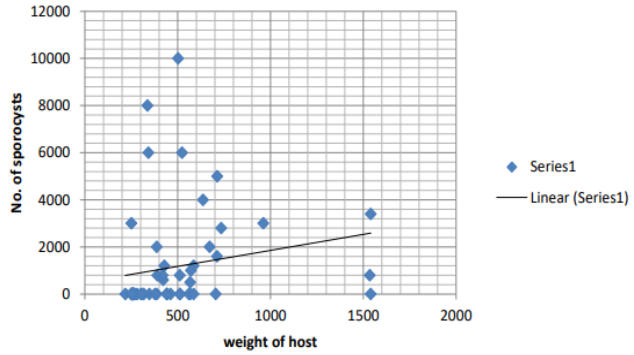

Figure 12. Relationship between the number of sporocysts in an individual host and the weight of the infected hosts

	Sum of sqrs	df	Mean square	F	p-value
Between groups:	2.21581E07	3	7.38602E06	1.63	0.1936
Within groups:	2.35562E08	52	4.53003E06		
Total:	2.5772E08	55			

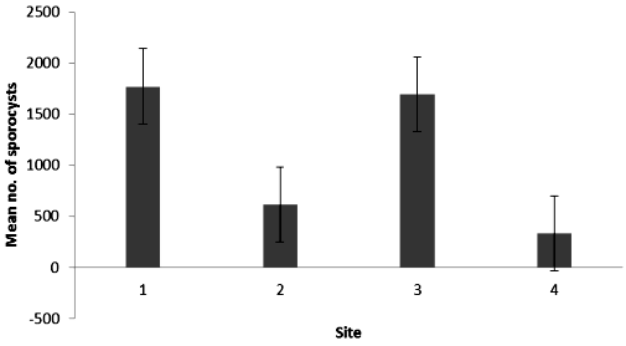

Figure 13. Prevalence of parasite infestation in octopuses among the sites where octopuses were collected (1- Kigombe, 2-Kwale, 3-Pangani, 4- Sahare)

Female		Male	
N:	27	N:	29
Mean:	974.07	Mean:	1317.2
95%:	(284.9 1663.2)	95%:	(360.44 2274)
Var.:	3.0351E06	Var.:	6.3272E06

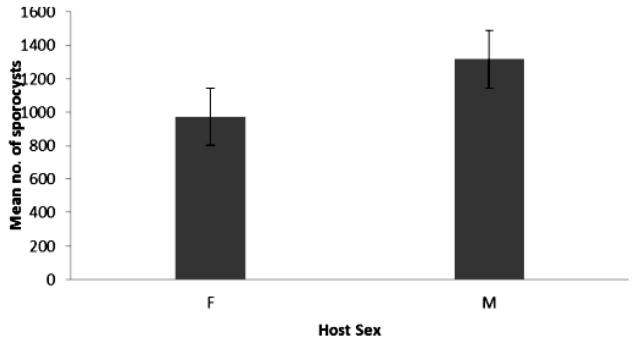

Figure 14. A variation on coccidian infection in male and female *Octopus cyanea*

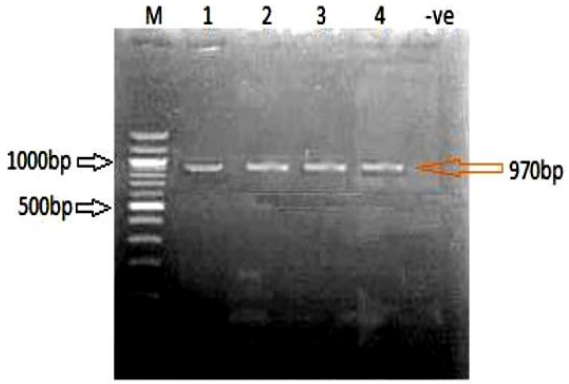

Figure 15. Photograph showing PCR amplification of *Aggregata* species, using universal primers, *Aggregata* primers 1F and 2R were used to amplify an 18S rRNA gene. The positive product 970bp is visible in 1.5% agarose gel. M is a 100bp Marker, and the gel was stained with Gel Red

.

Aggregata sp. DNA was found in pooled intestinal samples collected from 23 infected *O. cyanea* on the Indian Ocean coast of Africa. In proportion, 68% of DNA samples performed (amplified) in a PCR were positive. The positive PCR products were cloned for sequencing to confirm the *Aggregata* spp.

Seventeen of the 25 samples reacted positively with the designed *Aggregata* primer pair (Table 6). The remaining eight samples had no identifiable PCR amplicons with the designed primers. Of the 17 positive samples, two of them were weak, and they were not selected.

Sequencing identification of coccidian parasite

Histology and microscopic examination aided in the phylogenetic identification of *Aggregata* spp. Cloning and sequencing were done for the 15 strongly positive DNA samples to confirm the parasite species. The sequenced samples were edited and assembled in Genius Software to form a consensus sequence aligned in a GenBank. The alignment showed that all the sequences were identical to *A. octopiana* isolate H1 18S ribosomal RNA gene for 89% with Accession number KC188342 followed by *A. eberthi* gene isolate RV2 18S ribosomal RNA for 84% with Accession number KC188343.

Table 7 shows the summary of alignment of the consensus sequences during BLAST analysis and the percentage of identity with the *A. octopiana* sequences from a GenBank.

Discussion

Octopus cyanea represents an important economic artisanal fishery product around the Indian Ocean coast of East Africa, providing national income and a source of proteins to East African countries (Guard and Mgaya 2002). The *O. cyanea* artisanal catch comprises about 99% of octopus caught off the coast of Tanzania (Guard 2009).

The fishermen's community depends on octopus fisheries as their source of protein and employment. It was observed that a variety of people dealt with artisanal octopus fishing as their source of income and food, as reported by Jiddawi and Ohman (2002).

Even though octopus fishing has been a major activity in coastal areas, the community lacks knowledge of octopus parasites and diseases (Pascual and Guerra 2001). Furthermore, knowledge of the octopus-parasite relationship in the wild is poor since coccidian has not been reported to cause any harm to consumers (Bentacor et al. 2013). Even does not cause direct mortality in octopuses (Pascual et al. 1996; Pascual and Guerra 2001; Gestal et al. 2007; Castellanos-Martínez and Gestal 2013). For this reason, the fishermen's communities and even the consumers can't recognize the parasitic infections in octopuses easily.

According to fishermen, its observed octopuses are infected with some unknown agents that cause lesions on their bodies and arms. Biotic and abiotic factors can cause a lesion in the environment where octopuses live. Pascual et al. (2006) reported that injuries could be caused by parasites, bacterial or viral infections, or sometimes by any substance that can cause injury to the animal in its natural environment. Fishermen proposed that injuries in octopuses were mainly caused by predators like eel fish and other carnivores that hunt on an octopus. . Still, more studies are needed to determine the association between eel fish and octopus.

Table 6. Results for molecular analysis showing the positive and negative samples for the PCR reaction for 1F and 2R primers

S/N	Specimen name	Specimen no.	DNA Results
1	OP	3	+
2	OK	12	-
3	OKW	3	-
4	OP	9	-
5	OS	3	+
6	OS	1	+
7	OP	14	-
8	OK	10	-
9	OKW	5	+
10	OK	3	+
11	OK	15	+
12	OK	7	+
13	OKW	5	+
14	OS	12	+
15	OK	11	+
16	OP	13	+
17	OK	14	-
18	OS	8	+
19	OK	12	+
20	OP	6	+
21	OS	7	+
22	OKW	4	+
23	OP	15	+
24	OP	11	-
25	OK	2	-

Note: OKW: octopus from Kwale, OP: Octopus from Pangani, OS: octopus from Sahare, OK: octopus from Kigombe. (+) - DNA present, (-) - DNA absent

Table 7. GenBank reference sequences of *Aggregata* spp. used in the construction of phylogenetic trees

Field sequence	Name	Isolate	Accession no.	% Identity	Country	Host	Year
S1	*A. octopiana*	RV1	KC188342.1	86	Spain	*O. vulgaris*	2013
	A. eberthi	RV2	KC188343.1	83	Spain	*S.officinalis*	2013
	A. octopiana		DQ096837.1	82	Czech Republic	*O. vulgaris*	2006
	A. eberthi		DQ096838.1	81	Czech Republic	*S.officinalis*	2006
S2	*A. octopiana*	RV1	KC188342.1	86	Spain	*O. vulgaris*	2013
	A. eberthi	RV2	KC188343.1	84	Spain	*S.officinalis*	2013
	A. octopiana		DQ096837.1	82		*O. vulgaris*	2006
	C. ubiquitum	JFM10	AB697056.1	77	Japan	*A. speciosus*	2013
S3	*A. octopiana*	RV1	KC188342.1	86	Spain	*O. vulgaris*	2013
	A. eberthi	RV2	KC188343.1	84	Spain	*S.officinalis*	2013
	A. octopiana		DQ096837.1	82	Czech Republic	*O. vulgaris*	2006
	A. octopiana	H1	LC186909.1	89	Spain	*O. vulgaris*	2017
S4	*A. octopiana*	RV1	KC188342.1	85	Spain	*O. vulgaris*	2013
	A. eberthi	RV2	KC188343.1	83	Spain	*S.officinalis*	2013
	A. octopiana		DQ096837.1	82	Czech Republic	*O. vulgaris*	2006
	A. eberthi		DQ096838.1	81	Czech Republic	*S.officinalis*	2006
S5	*A. octopiana*	RV1	KC188342.1	87	Spain	*O. vulgaris*	2013
	A. eberthi	RV2	KC188343.1	84	Spain	*S.officinalis*	2013
	A. octopiana		DQ096837.1	83	Czech Republic	*O. vulgaris*	2006
	A. eberthi		DQ096838.1	82	Czech Republic	*O. vulgaris*	2006
S6	*A. octopiana*	RV1	KC188342.1	86	Spain	*O. vulgaris*	2013
	A. eberthi	RV2	KC188343.1	83	Spain	*S.officinalis*	2013
	A. octopiana		DQ096837.1	82	Czech Republic	*O. vulgaris*	2006
	A. eberthi		DQ096838.1	81	Czech Republic	*S.officinalis*	2006

Note: O: octopus; S: Sepia; A: Apodemus; A: aggregata; C: cryptosporidium; S: Consensus sequences

The fishermen's opinions of parasite existence in octopuses were observed by categorizing them by age groups. That was intended to get different opinions depending on the respondent's age and experience in octopus fishing. Age groups helped in gaining information concerning parasite infection in octopuses. Fishermen in the middle age group (31-53) were more knowledgeable about octopus parasite existence than other age groups. Most of the fishermen interviewed perceived that octopus had neither been infected by any parasite nor had any disease, while some knew nothing about parasites supporting that the knowledge of parasites and disease in octopuses is limited (Pascual and Guerra 2001).

It was possible to get some information about the effects of octopus consumption in the fishing community. In the survey, some fishermen confirmed the occurrence of allergies in some people after eating octopus resulting in rashes, stomachaches, and vomiting. According to previous reports on octopus diseases and parasites, these allergies could be associated with some worms reported in octopuses, such as nematodes, *Anisakis* sp. (Angelucci et al. 2011; Tsabouri et al. 2012). However, according to the experiences of the fishermen's community, they overcame the allergic reaction by cooking octopus meat or stopping the allergen victim from eating octopus.

The reported results for the histopathology study and fresh squash examinations of the liver, gills, kidney, and intestine, especially the cecum of *O. cyanea*, were likely like those reported by Licciardo et al. (2005) and Betancor et al. (2013) in the intestine of common octopus (*O. vulgaris*). This study found no association between parasite load and octopus weight, although host size has been reported to contribute to parasite infection (Catalano et al. 2014). The weight of the octopus influences parasite pathology, as described by Betancor et al. (2013). Octopuses with small body weights and sizes were observed to be no or slightly infected, as described by Storero and Narvarte (2013). The larger in size and weight of the octopus, the greater the possibility of harboring parasites or being heavily infected with protozoan parasites, as discussed by Storero and Narvarte (2013).

In this study, no ectoparasites were found, even though some were reported in octopuses, such as copepods *Pennella* spp. and *Octopicola* spp. located in the gills of common octopuses (Pascual et al. 1996). Nematode larvae or Monogenean trematodes like *Diphyllobothrium* sp. were also expected to be attached in gills, as Pascual et al. (1996) reported in common octopus. But the finding was different as the results revealed no ectoparasite observed in *O. cyanea* captured along the coast of Tanga. Although ectoparasites were not found, some octopuses had lesions on their skin, possibly due to parasites or any other mechanical damage that occurred during octopus catch.

Recent papers indicate that enteric coccidian infection in octopuses is the most important consequence since they have been causing an economic loss in reared cephalopods (Gestal et al. 1999). In this study, almost all the infected octopuses examined were found to have an enteric coccidian infection.

Compared with other *Aggregata* spp., the *Aggregata* sp. found in *O. cyanea* had sporocysts with more than eight sporozoites, as shown in Figure 5. Poynton et al. (1992) reported *Aggregata dobelli* in *Octopus dofleini* (Wülker, 1910) from the Northeast Pacific Ocean, with sporocysts having more than eight sporozoites as those observed in this study. The sporocysts of *Aggregata* sp. in octopuses from the coastal area of the Tanga region were found to have a cover resembling that of *A. octopiana* for 86% compared to other coccidian species but having 9-22 numbers of sporozoites.

The caecum is the usual site of infection with coccidian, as described by Estevez et al. (1996) and Gestal et al. (2002). Some pathological effects of the coccidian parasite noticed in histopathological study of the intestine, caecum, and gills were the same as those described by Gestal et al. (2000). In heavily infected octopus, the mucosal and epithelial cells were replaced by coccidian parasites (Pascual et al. 2007). Betancor et al. (2013) reported pathological reactions of *A. octopiana* in the caecum, intestine, and gills of wild and reared octopus, similar to the infected *O. cyanea* in this study.

The replacement and destruction of mucosal and epithelial cells with sporocysts can cause malabsorption syndrome and loss of intestinal and caecum epithelium (Baldascino et al. 2017). Pathology caused by these coccidian parasites is reported to weaken the host and increase the susceptibility to other pathogens (Pascual et al. 2007). The host and parasite (*Aggregata* sp.) have been reported to influence the pathological state (Gestal et al. 2002). This study noted that parasite pathology was not high in wild *O. cyanea* compared to that reported by Betancor et al. (2013) in reared and wild common octopuses.

Apart from *Aggregata* sp., Dicyemid mesozoans were also reported to be host specific and found in the Northern Indian Ocean with no evidence in the Eastern Indian Ocean by Castellanos-Martínez et al. (2011). None of the octopuses examined was found to be infected with these mesozoans. Most of the described mesozoan parasites have been reported to be host-specific (Castellanos-Martínez et al. 2011), so it could be possible to find one in *O. cyanea*, but only *Aggregata* sp. was observed in octopus. Therefore, it cannot be concluded that mesozoan parasites are completely absent in this specie of octopus; more work must be done to prove the reality of dicyemids' condition along the Indian Ocean.

The existence of visible cysts in the wall of the digestive tract, oocysts having a large number of sporocysts, the shape of oocysts and sporocysts used as evidence and key taxonomic features of identifying gastrointestinal protozoan as *Aggregata* sp. since they are only coccidian parasites infecting cephalopods including octopuses (Pascual et al. 2007; Baldascino et al. 2017). Pathology of the parasite in the host intestine was useful for parasite identification in this case since it is the well-known common feature of any coccidian parasite infection in their host gastrointestinal tissue (Pascual et al. 2007).

This report is the first of parasite existence in *O. cyanea* along the Indian Ocean of Tanzania coast. Many

studies have reported *A. octopiana* infections in European countries in common octopus, *O. vulgaris* (Gestal et al. 2005). Isolating *A. octopiana* in *O. cyanea* is a new finding. The task of this study was to determine parasites affecting *O. cyanea*, but only one parasite, *A. octopiana*, was identified and confirmed by molecular techniques.

The prevalence of coccidian parasites found in examined octopuses differed depending on the geographical location (site) where they were collected, as shown in Table 2. Geographical location has a great influence on the distribution of parasites, as reported by Castellanos-Martínez et al. (2011), Storero and Narvarte (2013), and Catalano et al. (2014). In this study, the location where the octopus is collected can be one factor leading to parasite abundance variation. González et al. (2003) documented that ecosystem structure has something to do with parasite richness, so this could be the reason for the variation of parasite prevalence among the octopuses collected from four study sites.

Molecular identification showed that the isolates coccidian parasite of *O. cyanea* was the *A. octopiana*. Morphological and molecular identification of the parasites were compared with other published findings. The results from this study confirmed the presence of coccidian parasites in *O. cyanea*. Combining morphological and molecular identification revealed that the enteric coccidian parasite was the *A. octopiana*, also found in the common octopus, *O. vulgaris* (Gestal et al. 2002). More studies should be done on the parasite of *O. cyanea* to prove the coccidian parasite inhabiting this cephalopod species.

From the confirmed 23 coccidian-infected octopuses, 17 microscopically positive animals were confirmed by PCR. The PCR was the most specific technique for diagnosing the parasite than using the microscope, which showed 23 animals were positive. The PCR results showed that four octopuses from Pangani, five from Sahare, three from Kwale, and five from Kigombe were positive, less than the positive number obtained from the microscopic examination. In most diseased cases, PCR has been used and reported to be the most sensitive technique of disease confirmation than the less sensitive microscopic method (Johnston et al. 2006).

In addition, the absence of morphological and structural information on helminths, crustaceans, and coccidian parasites affecting *O. cyanea* made it difficult to identify them. Therefore, there could be some confusion in identifying the parasite in *O. cyanea* due to the absence of detailed information on the parasite harboring them. Moreover, further investigation is needed in our coastal area.

In conclusion, like other octopus species, octopuses along the Indian Ocean coast of Tanzania have been infected by parasites. About 41% of the collected octopuses were infected with the coccidian parasite. The parasites are dangerous when it comes to octopus rearing as they can cause mortality to the infected host once exposed to other biotic or abiotic stressors. The parasite does not cause any disease to consumers, but it is advised the octopus must be well cooked to prevent any disease-causing agents. Combining this study with previous reports on octopus parasites, this must consider a serious problem to bring more awareness to East African countries on the existence of parasites in octopuses along the Indian Ocean. The expectation is that more research can be done on investigating more parasite species found in all *octopus* spp. in Tanzania, where the species is being exploited. Although the *A. octopiana* was found as the only parasite in the present study, more stains of coccidian or *Aggregata* spp. from *O. cyanea*, even in other cephalopod species, are expected to be discovered in the Indian Ocean as many species have been described in other countries. That is the first time octopuses have been investigated for parasites along the Tanzanian coast. Literature for references was difficult to assess, but this challenge will be reduced if more research is conducted on octopus to simplify future work.

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Heavy metal contamination in water, sediments, and fauna of selected areas along the Kenyan coastline

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Abstract. *Mwatsahu SH, Wanjau R, Tole M, Munga D. 2020. Heavy metal contamination in water, sediments, and fauna of selected areas along the Kenyan coastline. Ocean Life 4: 37-47.* Every year millions of tons of industrial waste and municipal sewage are dumped into the world's oceans as rivers carrying agricultural wastes discharge their waters into the ocean. The Mombasa inshore (Kilindini and Portreiz) waters and Sabaki/Malindi bay, Kenyan coastline, complex are characterized by rapid urbanization and industrial activities, including shipping, agriculture, and tourism. As a result, the coastal fauna, sediments, and seawater are polluted with heavy metals, raw sewage, and organochlorine pesticides. Heavy metals such as Cadmium (Cd), Copper (Cu), Chromium (Cr), Lead (Pb), Iron (Fe), Manganese (Mn), Mercury (Hg), and Zinc (Zn) have threshold limits (USEPA) above which they are toxic. The aim of this study was to assess the levels of heavy metals Cd, Cu, Cr, Fe, Mn, Hg, and Zn in selected areas along the Kenyan Coastline. The analysis was done during the dry and wet seasons. The levels of the heavy metals were determined using Atomic Absorption spectro-photometry flameless Atomic Absorption Spectrophotometry (Mercury cold vapor), and Energy Dispersive X-ray fluorescence. The levels of the metals were as follows: In sediments, Hg: nd, Cr: nd, Cu: 9.912-40.412 µg/g, Fe: 3,195.667-35,435.981 µg/g, Mn: 135.467-228.780 µg/g, Zn: 54.700 -181.725 µg/g. Biota: Hg: nd, Cr: nd, Cu: 43.963-229843 µg/g, Mn: 29.770-486.611 µg/g, Fe: 379.853-35,277.752 µg/g, Zn: 99.476-872.852 µg/g, Cd: nd, Pb: 135.407-833.111 µg/g. Sea water: Cr: 0.015-0.026 µg/mL, Fe: 0.062-16.524 µg/mL, Cu: 0.010-0.033 µg/mL, Zn: 0.028-0.066 µg/mL, Pb: 0.006-0.010 µg/mL. Cd and Hg levels were not detected in seawater, sediments, and fauna. In contrast, Cr was not detected in sediments and fauna but had levels above USEPA guidelines at Kilindini, Malindi Bay, and Sabaki estuary sites. Cu had levels above USEPA guidelines at Sabaki in the water and sediments at most sites. Pb levels were above USEPA guidelines at most sites of the Kenyan Coastline, while Zn levels indicated moderate pollution in seawater, sediments, and fauna. These findings indicated that seawater sediments and fauna from the Kenyan Coastline are moderately polluted with Cu, Pb, and Zn according to USEPA guidelines. It is recommended that regular assessments of pollution levels be done and measures put in place to prevent increased pollution of the Kenyan Coastline.

Keywords: Heavy metal, industrial, ocean, waste

INTRODUCTION

Human beings greatly impact the environment since they manipulate it for their benefit. The components of the ecosystem, namely air, water, and soil, are greatly affected by pollution. Therefore, pollution in the world is a major health and environmental concern. This concern is a priority for action since environmental pollution affects the well-being of all organisms in their habitats (Magothe 2009; Anderson et al. 2012; Vikas and Dwarakish 2015; Manisalidis et al. 2020). Marine pollution has been defined as the introduction of substances or energy into the marine environment resulting in such deleterious effects as harm to living resources, hazards to human health and hindrance to maritime activities, and reduction of amenities. Further point source pollution is contamination that enters the environment through discernible, confined, and discrete conveyance (Advameg Inc. 2011). Non-point sources, on the other side, are those that spread over a large area and have no specific outlet or discharge point (NOAA 2011).

Mombasa is the largest coastal city, an island surrounded by Kenyan coastal waters, with an estimated population of 939,370 people (Kenya National Bureau of Statistics 2009). That is a source of large quantities of domestic sewage, which contribute loads of Biological Oxygen Demand (BOD), nutrients, and microbial contaminants to surface and groundwater, including coastal waters (Mwaguni and Munga 1997). The city's water run-offs are a significant source of pollution to the marine environment. In addition, inefficient collection, inadequate treatment, and disposal of solid waste are potential sources of groundwater and coastal water pollution (Munga et al. 2005; Brand et al. 2017; Lestari and Trihadiningrum 2019).

However, to increase food productivity in farms, sewage sludge and agricultural chemicals such as inorganic fertilizers, herbicides, and pesticides have been used on crops and soils (Magothe 2009; Lestari et al. 2017; Mokaya et al. 2018). Commercial phosphate fertilizers and sewage sludge contain small amounts of heavy metals, which may accumulate in soil with repeated fertilizer applications, thus contaminating the soil. In addition, some elements such as Cu, Zn, and Mo are essential for plant growth (Greaney 2005; Tsonev and Lidon 2012; Hafeez et al. 2013; Sturikova et al. 2018). In the Kenyan coastal region

farming along Athi-Galana – Sabaki river basin and on steep slopes enhance soil erosion and result in the transportation of high loads of suspended sediments downriver which discharge into the Sabaki estuary and Malindi Bay. The heavy loads of sediments eventually discharge into the Indian Ocean and are a potential source of nutrients, pesticides, residues, and heavy metals (Kithiia 2006).

Tourism is also one of the factors that significantly contribute to marine pollution. The cruise ships carrying tourists produce hazardous waste (toxic) from several on-board activities and processes, including silver, mercury, lead, and cadmium through dry cleaning, photographic processing, print shops, painting activities, equipment cleaning, and other sources (Smart Guide to World Cruise Ship 2011). Kenya's Coastal tourism is estimated to account for about 60% of the national tourism industry, with some urban centers attributing their rapid development to tourism, namely Malindi, Watamu, and Diani. Tourism development and activities have impacted wetlands, contributing to beach erosion. In addition, they are directly responsible for the over-exploitation of marine resources. At the same time, poor waste management practices at tourist establishments and tourist-satellite centers are responsible for declining water quality and loss of aesthetic value of some destinations (Mwaguni and Munga 1997).

Urbanization, industrialization, tourism, shipping (harbor), and agricultural run-offs are key and feared to cause marine environment pollution. Sabaki estuary, Malindi Bay, and Kilindini harbor are some areas along the Kenyan Coastline that are at risk of high levels of heavy metal pollutants due to surrounding activities. Through the food chain-heavy metals, including Pb, Cd, Zn, Mn, Cr, Cu, and Hg in seawater, sediments, fish, and other marine fauna affect the health of human beings. There is, therefore, the need to assess these heavy metals in the Kenyan coastline.

This study aims to determine levels of heavy metals in water, sediments, and fauna in Mombasa inshore areas (Kilindini and PortReitz), the Sabaki estuary, and the Malindi Bay complex. Then, specific objectives are: (i) To determine the levels of Pb, Cd, Zn, Mn, Cr, Cu, Fe, and Hg in Mombasa inshore areas and Sabaki estuary/Malindi Bay complex during wet and dry seasons in seawater. (ii) To determine the levels of Pb, Cd, Zn, Mn, Cr, Cu, Fe, and Hg in Mombasa inshore areas and Sabaki estuary/Malindi Bay complex during wet and dry seasons in marine sediments. (iii) To determine the levels of Pb, Cd, Zn, Mn, Cr, Cu, Fe, and Hg in Mombasa inshore areas and Sabaki estuary/Malindi Bay complex during wet and dry seasons in marine fauna.

MATERIALS AND METHODS

Study area

The study area covered the Kenyan coastal area between Malindi Bay in the North and Funzi Bay on the South Coast, Kenyan coastline (Figure 1). Figure 1A shows the whole length of the study area while Figures 1B, C, and D shows the sampling sites of Sabaki estuary/Malindi Bay complex, Kilindini harbor/port Reitz creek, and Shirazi respectively. Figure 1C is an enlarged Kilindini/Port Reitz Creek map showing sampling sites.

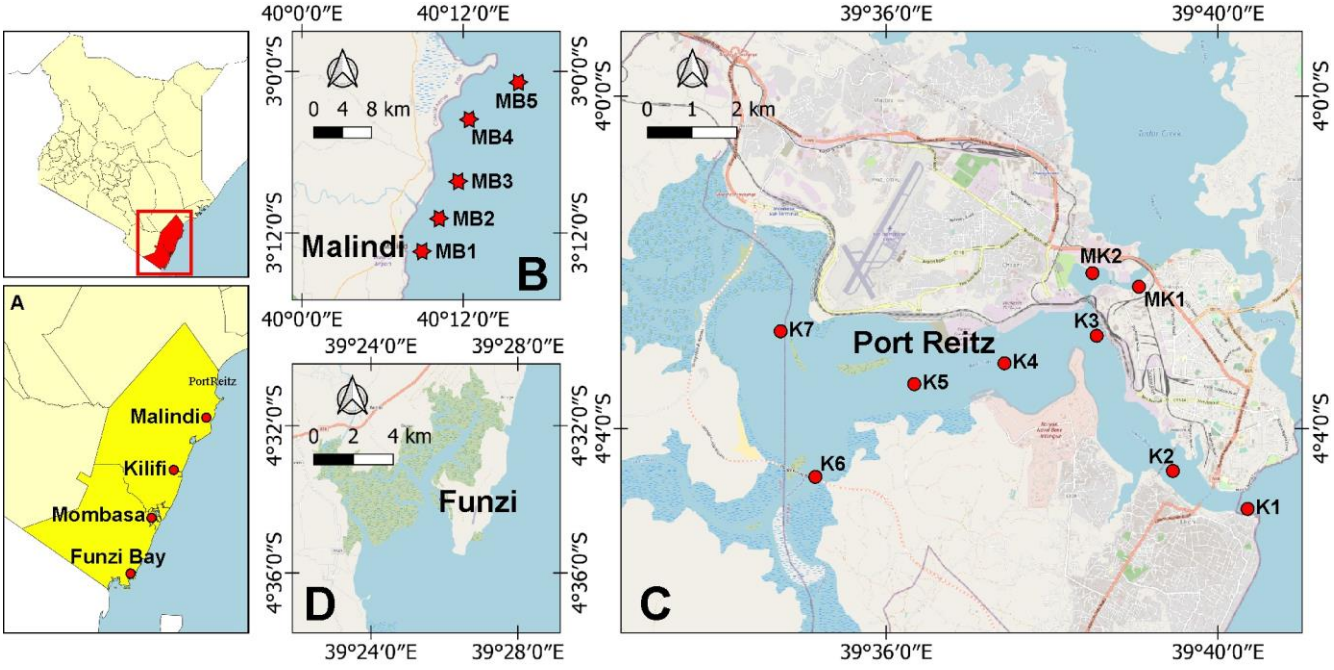

Figure 1. A-D. Maps of the Kenyan coastline showing sampling sites.

Research design

A quantitative research design was used in this study. Quantitative research design, where an environmental phenomenon, namely marine pollution, was accurately converted into numerical data. Samples of water, sediments, and fauna were collected from various points along the Kenyan coastline to analyze heavy metals (Cd, Cu, Mn, Fe, Cr, Pb, Zn, and Hg).

Sampling design

Sampling was done during wet and dry seasons. Samples of marine sediments, fauna, and ocean water were collected from three sites along the Kenyan coast: Sabaki estuary/Malindi bay complex, Kilindini, and Port Reitz creek system, and Funzi bay/Shirazi, which served as the reference site. The sampling and sample analyses were conducted as a scheme shown in Figure 2.

Description of sampling sites

Sampling points in the Sabaki estuary/Malindi bay complex were selected in the inshore/nearshore and 2 km from the shore. Four sampling points were selected within the bay and along the shoreline, and another 3 points were selected within the Sabaki estuary (Figures 1B, C and D).

Sampling points at Mombasa were on the Kilindini harbor and Port Reitz creek, where shipping activities, including bunkering and discharging crude oil, occur. There are also industries, the Kipevu oil terminal and the Kenya Naval base (Figure 1E). The reference site was located in Funzi bay at Shirazi, 70 Km south of Mombasa. The only activities were subsistence farming with low levels of agrochemicals used, artisanal fishing, and little tourist activities without heavy metal pollution.

Cleaning

Teflon vessels, glassware, and plastic ware (including sampling bottles) were soaked in a soap solution overnight in a plastic bucket. The apparatus was rinsed in tap water and then with distilled de-ionized water. They were put and left to stand for six days in a 10% (v/v) concentrated HNO_3 solution at room temperature. They were then rinsed thoroughly with distilled de-ionized water and dried in the open air. The dried apparatus were put in closed plastic polythene bags and stored.

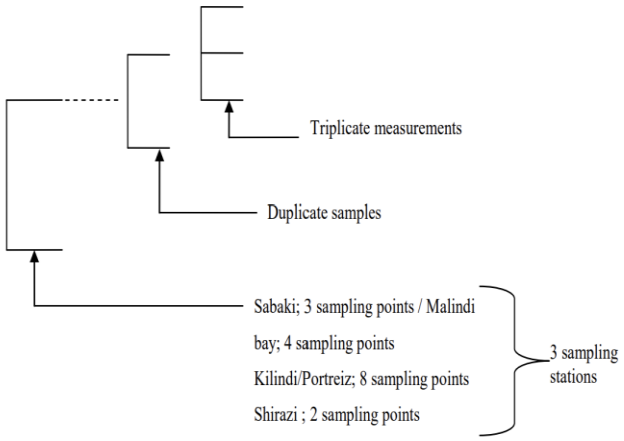

Figure 2. Sampling and sample analysis scheme used in this study

Collection of samples

Water samples were collected from the ocean surface directly into plastic bottles by sub-merging the sample bottle just below the water surface. Samples were immediately acidified with 1 mL concentrated HNO_3 and stored at a temperature of 4ºC. Surface sediments were collected using a Van Veen grab sampler and put into plastic bags, labeled, and transported to the laboratory. The sediments were dried at 50ºC and stored. Fauna was picked manually, put in plastic bags, labeled, kept in ice during transport, and stored below 0ºC. Then they were dried at 50ºC. The flesh inside the shell was used for the heavy metal determination.

Chemicals, reagents, and solvents

Chemicals, reagents, and solvents used were of Suprapur, analytical grade.

Sample preparation

Digestion of sediment for AAS analysis

A sum of 0.2000 g portion of the dry sample was weighed accurately (Adam Equipment Co. Ltd, Model AAA 160/L, AE04876108) in labeled Teflon vessels. Next, 1 mL aliquotof aqua regia (HNO_3: HCl, 1:3 v/v) was added slowly, followed by 6 mL of concentrated Hf, and left to stand at room temperature for 1 hour. Next, the Teflon vessels were closed, placed on a hot plate, and heated at 120ºC for 2 hours and 30 minutes. Samples were allowed to cool to room temperature then the Teflon vessels were opened. Separately, 2.7000 g portions of boric acid were weighed, put in labeled volumetric flasks, and dissolved in 20 mL distilled de-ionized water. The sediment samples above were then transferred into the flasks and put in a water bath for 30 minutes until all boric acid was dissolved. They were then allowed to cool to room temperature and put in plastic containers (Azenard et al. 2006), labeled, and stored.

Digestion of fauna for AAS analysis

Portions of 0.2000g of dry fauna samples were weighed accurately in the labeled Teflon vessel. A 5 mL aliquot of concentrated HNO_3 was added and left at room temperature for 1 hour. The vessel was then closed and heated on a hot plate at 90ºC for 3 hours. They were allowed to cool to room temperature and then transferred to a 50 mL volumetric flask, and the volume was adjusted to mark with distilled de-ionized water (Azenard et al. 2006).

Calculation of concentrations of sediment and fauna samples

The concentrations calculations were carried out using the calibration curvemethod and worked out as follows:

$$\mu g/g = \frac{\text{Measured concentration (ppm) x Dilution x Sample Volume (mL)}}{\text{Dry Weight of Sample (g)}}$$

Preparation of water samples for EDXRF analysis

Sea water samples were analyzed using by EDXRF technique. First, a preconcentration procedure with Ammonium Pyrrolidine Dithiocarbonate (APDC) and analysis was done as follows: The seawater samples (100 mL of sample) were acidified with 5 mL dilute HNO_3 and pH adjusted to between 3.5-4.0 using dilute ammonia

solution. Next, 10 mL of freshly prepared 1% APDC solution was added, followed by 0.2000 g of Cd solution added as a carrier before precipitation. Next, they were thoroughly mixed by manually agitating after every 10 minutes for 40 minutes at room temperature, forming a suspension. The suspension was then filtered through a Nucleopore filter (0.45 µm pore size). Finally, the Nucleopore filter was air-dried and irradiated to determine the elements (IAEA 1997).

Preparation of stock and standard solutions

Commercial standards of 1000 µg/g strength were diluted using the formula $V_1C_1=V_2\ C_2$, and standards of concentrations of 0-10.0 µg/g were prepared.

Preparation of reference standard and blank

Reference material IAEA- 433 was used. The reference material solutions were prepared using the same procedure as the sediment samples. The blank was prepared using distilled/de-ionized water instead of a sample.

Analysis of samples by FAAS

The FAAS instrument used was Buck Scientific (1996) 210VGP Atomic Absorption Spectrophotometer. The machine operational parameters for the analysis are given in Table 1. The standards were first aspirated, and the absorbance was recorded. A calibration curve was obtained for each element analyzed. The sediments, fauna samples, and reference materials were then aspirated, and the absorbances were recorded. The samples' concentrations were calculated by extrapolating the individual samples' absorbances on the elements' graphs.

Analysis of samples by HGAAS

The cold vapor technique was used for the analysis of Hg. First, a mercury hollow cathode lamp was placed in the holder and allowed to warm up for about 15 minutes. Then, the burner head of the AAS was removed, and the absorption cell and holder were positioned. Next, the lamp current (6.5 MA) and wavelength (253.6 nm) were optimized, 50 mL of sample solution was pipetted into the reaction flask, and 5 mL of mixed acid solution (3:1HCl:H_2SO_4) was added.

A 10.0000 g portion of analytical grade stannous chloride was put in an empty acid-cleaned 100 mL plastic bottle, then 20 mL concentrated HCl, 2 grams tin metal, and 75 mL distilled de-ionized water was added. The bottle was swirled gently to dissolve the solids. Next, 5 mL of the 10% stannous chloride reductant solution, prepared as above, was injected into the septum of the flask stopper assembly with a syringe.

The argon gas was switched on, and the highest signal reading on the machine was taken. The reading was allowed back to zero, the Argon bubbles flow was turned off, and the reaction flask was removed. The flask was rinsed with 5% HCl / 5% H_2O_2 solution, and also the bubbler tube was soaked in the same solution to remove traces of the $SnCl_2$. Finally, it was reassembled for the next run. The analysis started with the reagent blank run, the standards, and, lastly the samples.

Table 1. Operational parameters of the AAS

Operational parameters	Elements						
	Cu	**Cd**	**Cr**	**Ni**	**Pb**	**Zn**	**Mn**
Wavelength	324.7	324.8	357.9	232.0	283.3	213.9	279.5
Slit width (nm)	0.7	0.7	0.7	0.2	0.7	0.7	0.7
Lamp current (ma)	3-8	5-10	5-10	7-15	3-7.5	3-10	5 -15
Detection limit (µg/mL)	0.02	0.005	0.05	0.04	0.1	0.005	0.01
Flame	Air-acetylen						
Flame temp.	2300 ^oC						

Method validation

The methods used in this study, the AAS and EDXRF, were validated using reference materials and commercial standards.

Data analysis

The data were analyzed using the student t-test and the Standard Statistical Package for Social Sciences (SPSS) Version 17.

RESULTS AND DISCUSSION

Method validation

The EDXRF validation

The EDXRF level of accuracy and quality control comprised the measurement of ICP Multi-element standard solution 1 CertiPUR (Certified reference material). The results are presented in Table 2.

The AAS validation

The AAS level of accuracy and quality control was determined by certified reference material "IAEA 433" for trace elements and methyl mercury in marine sediments and certified reference material "IAEA 436" for Trace elements in the fauna. The comparison of means between the levels in the certified reference material and measured values is presented in Tables 2 and 3.

Levels of heavy metals in seawater

The levels of Pb, Cd, Zn, Mn, Cr, Cu, Fe, and Hg in seawater were determinedusing EDXR and are presented in Table 4 for the wet and dry seasons, respectively.

Lead (Pb)

During the dry season, Pb levels ranged from < dl at MB1 at the Malindi marine park to 0.014 µg/g at S1 at Sabaki river, 2 kilometers before the river water entered the sea. These levels were lower than the guide level set by USEPA, implying that the sea water is safe from Pb. However, Pb accumulation is known to be dangerous, resulting in the inhibition of enzyme-catalyzing formation of haem (UNEP 1984). Other studies of the Kenyan coastline also found levels of Pb in seawater to be lower than the USEPA guidelines (Oyugi et al. 2000), Mwashote (2003). Oyugi et al. *(*2000) reported a range from 0.012 to 0.06 µg/mL, while Mwashote (2003) reported Pb levels of 0.05 to 0.62 in seawater at Mombasa, concluding that there was a need for constant monitoring of the region. A comparison of Pb between the dry and the wet seasons showed a difference ($P < 0.05$).

Table 2. The EDXRF results for ICP Multi-element Standard Solution1CertiPUR

Element	Concentration in µg/g (*n* = 3) Certified (mean ± SE)	Measured (mean ± SE)	*t-test* tcal
Cr	25.3 ± 0.50	19.754 ± 6.03	0.92
Fe	15.2 ± 0.50	15.336 ± 3.85	0.03
Cu	20.3 ± 0.50	19.444 ± 3.25	0.26
Zn	20.0 ± 0.50	20.525 ± 3.48	0.15
Pb	203 ± 4.00	182.902 ± 31.76	0.63
Mn	5.1 ± 0.20	5.00 ± 0.20	0.50
Cd	20.8 ± 0.50	20.40 ± 0.50	0.80

Note: Measured values were not significantly different from certified values since *t* -calculated values were less than the *t* - critical value (*tcrit* = 4.303, df = 2, α = 0.05). Therefore the EDXRF method and hence the results presented in this study are accurate and reliable

Table 3. Comparison of mean levels of heavy metals in CRM IAEA433 and 436 and measured values

Element	Concentration in µg/g (*n* = 3) Certified (mean ± SE)	Measured (mean ± SE)	*t-test* tcal
CRM IAEA433			
Cu	30.8 ± 2.60	30.3 ± 2.60	0.19
Zn	26.0 ± 2.70	25.5 ± 2.50	0.20
Mn	101 ± 8.00	103.37 ± 8.20	0.28
MeHg	316 ± 16.00	315.5 ± 15.90	0.03
Cd	136 ± 10.00	135 ± 9.50	0.10
Cr	0.17 ± 0.07	0.14 ± 0.06	0.50
Cu	0.153 ± 0.03	0.140 ± 0.03	0.43
CRM IAEA 436			
Cu	1.73 ± 0.19	1.70 ± 0.18	0.17
Zn	19.0 ± 1.30	19.0 ± 1.40	< 0.001
Mn	0.238 ± 0.04	0.250 ± 0.04	0.30
MeHg	3.67 ± 0.42	3.55 ± 0.04	3.00
Cd	0.052 ± 0.01	0.050 ± 0.01	0.20
Cr	0.194 ± 0.06	0.193 ± 0.06	0.02
Cu	1.73 ± 0.19	1.70 ± 0.18	0.17

Note: tcal = t calculated. Measured values were not significantly different from certified values since *t* -calculated values were less than the *t* - critical value (*tcrit* = 4.303, df = 2, α = 0.05). The AAS results presented in this study are accurate and reliable

Table 4. Mean levels of heavy metals in seawater during the dry and wet season

SITE	Concentration µg/mL±SE (n=3) Pb	Zn	Mn	Cr	Fe	Cu
Dry season						
K1	0.007±0.001^{bc}	0.072±0.029^{b}	0.010±0.001^{abc}	0.014±0.000	0.174±0.013^{a}	0.006±0.001^{a}
K2	0.007±0.000^{bc}	0.044±0.014a^{b}	0.008±0.004^{ab}	0.015±0.003	0.260±0.068^{a}	0.015±0.004^{ab}
K3	0.008±0.001^{bc}	0.013±0.000^{a}	0.011±0.002^{abc}	0.013±0.002	0.171±0.056^{a}	0.011±0.001^{ab}
K4	0.010±0.002^{bc}	0.031±0.001^{ab}	0.013±0.000^{abc}	0.014±0.002	0.159±0.005^{a}	0.013±0.002^{ab}
K5	0.009±0.001^{bc}	0.013±0.002^{a}	0.011±0.001^{abc}	0.012±0.001	0.220± 0.013^{a}	0.014±0.002^{ab}
K6	0.012±0.001^{bc}	0.029±0.000^{ab}	0.011±0.000^{abc}	0.056±0.042	0.339±0.035^{a}	0.011±0.001^{ab}
K7	0.008±0.001^{bc}	0.024±0.002^{ab}	0.016±0.002^{bc}	0.013±0.002	0.459±0.017^{a}	0.016±0.001^{b}
K8	0.009±0.000^{bc}	0.023±0.000^{ab}	0.019±0.003^{c}	0.019±0.001^{b}	0.787±0.021^{b}	0.010±0.001^{ab}
MB1	0.000±0.000^{a}	0.025±0.004^{ab}	0.010±0.000^{abc}	0.060±0.049	0.079±0.013^{a}	0.012±0.002^{ab}
MB2	0.006±0.003^{bc}	0.034±0.009^{ab}	0.005±0.003^{a}	0.009±0.005	0.430±0.255^{a}	0.017±0.004^{b}
MB3	0.013±0.003^{c}	0.033±0.006^{ab}	0.008±0.004^{ab}	0.017±0.001	0.196±0.070^{a}	0.013±0.000^{ab}
MB4	0.005±0.002^{b}	0.021±0.001^{ab}	0.014±0.003^{abc}	0.008±0.004	0.343±0.293^{a}	0.015±0.003^{b}
S1	0.011±0.000^{bc}	0.072±0.000^{ab}	0.089±0.000^{d}	0.013±0.013	17.613±0.006^{d}	0.036±0.000^{ab}
S2	0.009±0.000^{bc}	0.070±0.000^{b}	0.036±0. 000^{e}	0.032±0.000	20.351±0.014^{e}	0.036±0.000^{d}
S3	0.010±0.001^{bc}	0.057±0.000^{b}	0.045±0.000^{abc}	0.033±0.001	11.609±0.003^{a}	0.027±0.001^{d}
SH1	0.007±0.000^{bc}	0.054±0.028^{ab}	0.013±0.001^{abc}	0.017±0.002	0.063±0.003^{a}	0.009±0.000^{ab}
SH2	0.009±0.001^{bc}	0.034±0.001^{ab}	0.015±0.001^{abbc}	0.013±0.002	0.061±0.004^{a}	0.011±0.001^{ab}
Wet season						
K1	0.005±0.001^{ab}	0.044±0.005^{abc}	0.012±0.001^{a}	0.012±0.000^{a}	0.068±0.007^{a}	0.011±0.001
K2	0.006±0.000^{ab}	0.040±0.002^{ab}	0.011±0.002^{a}	0.022±0.002^{a}	0.061±0.002^{a}	0.012±0.001
K3	0.006±0.000^{ab}	0.040±0.002^{ab}	0.011±0.002^{a}	0.022±0.002^{a}	0.061±0.002^{a}	0.012±0.001
K4	0.006±0.000^{a}	0.040±0.002^{ab}	0.011±0.002^{a}	0.022±0.002^{a}	0.061±0.002d^{e}	0.012±0.001
K5	0.005±0.001^{ab}	0.045±0.000^{abc}	0.009±0.000^{a}	0.011±0.001^{a}	0.094±0.006^{bcd}	0.008±0.000
K6	0.007±0.001^{ab}	0.042±0.001^{abc}	0.010±0.001^{a}	0.014±0.001^{a}	0.150±0.005^{f}	0.010±0.001
K7	0.008±0.001^{b}	0.023±0.002^{a}	0.009±0.001^{a}	0.019±0.002^{a}	0.098±0.005^{cd}	0.036±0.001
K8	0.006±0.000^{ab}	0.046±0.000^{abc}	0.009±0.000^{a}	0.015±0.000^{a}	0.307±0.000^{h}	0.009±0.000
MB1	0.005±0.000^{a}	0.038±0.000^{ab}	0.010±0.000^{a}	0.012±0.000^{a}	0.082±0.000^{abc}	0.008±0.000
MB2	0.008±0.001^{b}	0.032±0.002^{ab}	0.010±0.001^{a}	0.013±0.001^{a}	0.079±0.005^{abc}	0.010±0.000
MB3	0.005±0.000^{a}	0.041±0.001^{ab}	0.012±0.001^{a}	0.022±0.001^{a}	0.096±0.002^{cd}	0.012±0.000
MB4	0.007±0.001^{ab}	0.064±0.000^{c}	0.013±0.001^{a}	0.117±0.052^{b}	0.093±0.004b^{cd}	0.018±0.000
S1	0.014±0.001^{e}	0.158±0.020^{e}	0.024±0.003^{c}	0.024±0.000^{a}	4.326±0.007^{j}	0.019±0.005
S2	0.014±0.000^{e}	0.177±0.001^{f}	0.027±0.000^{d}	0.023±0.001^{a}	4.323±0.^{006j}	0.024±0.001
S3	0.010±0.000^{c}	0.086±0.000^{d}	0.065±0.001^{e}	0.053±0.001^{a}	15.610±0.002^{k}	0.037±0.000
SH1	0.006±0.000^{ab}	0.027±0.000^{ab}	0.011±0.000^{a}	0.016±0.000^{a}	0.071±0.000^{ab}	0.011±0.000
SH2	0.014±0.000^{a}	0.012±0.000^{d}	0.011±0.000^{a}	0.194±0.000^{g}	0.012±0.000	0.039±0.000^{ab}

Note: Mean values followed by the same letter(s) within the same column are not significantly different. (Oneway ANOVA, SNK-test, α = 0.05)

Zinc (Zn)

Zinc levels in seawater in the dry ranged from 0.013 µg/mL at sampling point k3 at oceanic sampling at Kilindini harbor to 0.072 µg/mL at K1 next Mombasa industrial area at Kilindini harbor. Zn was also detected at all other sampling points. These levels were lower than the guidelines of 81.0 µg/L set by USEPA. That implies that the results show that the seawater was polluted by zinc, except at Sabaki during the wet season.

Previous studies of the Kenyan coastline reported levels lower than the USEPA guidelines (Oyugi et al. 2000 Hashim 2001). Oyugi et al. (2000) reported a range of 0.03 µg/g at Mombasa to 0.07 at Vanga, while Hashim (2001) reported 0.57 µg/L to 8.10 µg/L at Mombasa. Other water bodies worldwide reported 0.5-27.6 µg/L in seawater at Port Elizabeth, South Africa. That indicated pollution attributed to industrial-runoff sources (Fatoki and Mathabatha 2001).In the wet season, Zn levels in seawater were higher, 0.177 µg/L at Sabaki. In a non-statistical comparison, Kenyan Coastline seawater's Zn levels are lower than the USEPA guidelines. A comparison between the Zn levels in the dry and wet seasons showed a significant difference ($P< 0.05$). The wet season had higher levels than the dry season.

Manganese (Mn)

Mn levels in seawater in the dry season ranged from 0.005 µg/mL at MB2 in front of the Malindi Jetty sampling point at Malindi Bay to 0.89 µg/mL at S1 at Sabaki river. Mn was also detected in all other sampling points. These levels are lower than the WHO recommended levels of 0.4 µg/L for drinking water (WHO 2000). The Mn levels indicated the Mn pollution of the seawater at Sabaki.In the wet season, seawater levels ranged from 0.009 µg/mL at K8 at Mwache to 0.065 µg/mL at the S3 sampling point at the Sabaki river. These levels are higher than the WHO-recommended level of 0.4 µg/L. Previous studies of the Kenyan Coastline, Hashim (2001) reported a range from 0.24-0.51 µg/mL at Mombasa and 0.12-0.19 µg/g at Malindi. The above levels were indicative of Mn pollution of the seawater of the Kenyan coastline.

Studies from other regions of the world, in the North sea, the northeast Atlantic Ocean, the English channeled, and the Indian Ocean, Mn levels were reported to range from 0.3 to 4.0 µg/L (Howe et al. 2004).

A comparison between the Mn levels in the dry and wet seasons showed no significant difference, $p > 0.05$.

Chromium (Cr)

Chromium levels in seawater in the dry season ranged from 0.008 µg/L at MB4 at Malindi Bay in front of Gongoni to 0.117 µg/mL at MB3 at Malindi Bay in front of Mambrui. The level at NB 3 is higher than the USEPA guidelines of 50 µg/L for Cr(vi); since the Cr level found was total Cr and not differentiated between Cr (III) and Cr(Vi), the pollution status of Cr pollution could not be inferred from above levels. Furthermore, Cr has not been reported in previous studies of the Kenyan Coast.

Iron (Fe)

Seawater's iron levels in the dry season ranged from 0.061 µg/mL at SH2 at Shirazi to 20.351 µg/mL at 52 at Sabaki. Previous studies of the Kenyan Coastline reported comparable Fe levels in seawater. Oyugi et al. (2000) reported levels ranging from 0.11 µg/mL at Mombasa Marine park to 13.5 µg/mL at English point Mombasa, while Hashim (2001) reported iron levels of 0.44-1.33 µg/mL at Mombasa, 6.34 - 44.21 µg/mL at Malindi and 0.67-8.53 µg/mL at Gazi. These two previous studies reported higher levels than this study could be due to the difference in sampling sites. In unpolluted oceanic waters, iron levels ranging between 2.8 to 29 µg/L and 224-1228 µg/L have been reported (UK Marine Special Areas of Conservation 2011). USEPA quality criteria do not exist for iron as it is considered a non-priority pollutant (UK Marine Special Areas of Conservation 2011).

Copper (Cu)

Copper levels in seawater in the dry season ranged from 0.006 µg/mL at K1 at the oceanic sampling point at Kilindini to 0.037 µg/mL at S1 and S2 at Sabaki river. In another study of the Kenyan coastline, Hashim (2001) reported Cu levels in seawater of 0.05-0.13 µg/mL at Mombasa, 0.05-0.11 µg/mL at Malindi and 0.04-0.08 µg/mL at Gazi. These figures are higher than those reported in this study, which could be due to the difference in sampling points. However, the levels also show a reduction in Cu indicative of a reduction in Cu pollution of the Kenyan coastline.

In the wet season, levels of Cu in seawater ranged from 0.011 µg/mL at KI atthe oceanic sampling station at Kilindini to 0.037 µg/mL at S1 at Sabaki river. There is no significant difference in the levels during the dry and wet seasons. The levels of Cu in this study in this study are higher than the USEPA guidelines of 3.1 µg/L in seawater, indicating Cu pollution in the Kenyan coastline seawater.

Levels of heavy metals in marine sediments

The Pb, Cd, Zn, Mn, Cr, Cu, and Fe levels in sediments were determined using AAS. The Hg levels in sediments were determined using the cold vapor method. The AAS calibration curve for Pb is shown in Figure 3. Calibration curves for other metals are in Appendix II. The results for dry and wet seasons are presented in Table 5. Site abbreviations are explained in the abbreviations and acronyms list. Cd, Cr, and Hg, however, were not detected in marine sediment samples.

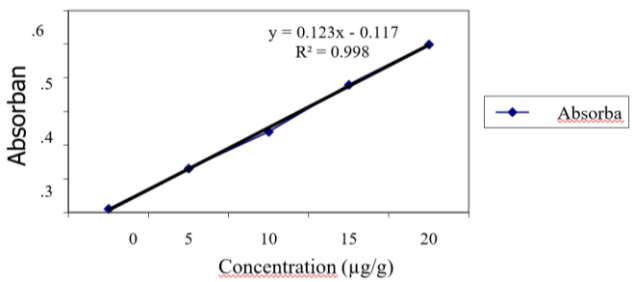

Figure 3. Calibration curve for lead

Table 5. Mean levels of heavy metals in sediments during the dry and wet season

Site	Concentration in µg/g ± SE (*n* = 3)				
	Pb	Zn	Mn	Fe	Cu
Dry season					
K1	170.769±0.116^{g}	140.859±0.335^{k}	460.674±0.163^{g}	46314.705±0.103^{l}	43.034±0.034^{g}
K2	170.769±0.116^{g}	141.093±0.052^{i}	460.840±0.160^{g}	46315.705±0.103^{m}	41.064±0.032^{f}
K3	133.400±0.100^{b}	145.169±0.037^{m}	400.917±0.220^{c}	40554.153±0.078^{j}	43.034±0.034^{g}
K4	133.400±0.100^{b}	145.093±0.001^{m}	400.917±0.220^{c}	40554.153±0.078^{h}	43.034±0.034^{g}
K5	134.417±0.209^{c}	137.288±0.006^{j}	450.075±0.038^{e}	35129.710±0.095^{k}	44.077±0.033^{i}
K6	160.673±0.163^{e}	145.077±0.038^{m}	455.717±0.148^{f}	41500.185±0.094^{i}	32.902±0.004^{e}
K7	230.556±0.067^{j}	332.507±0.004^{o}	420.979±0.226^{d}	35203.435±0.219^{f}	28.027±0.002^{d}
K8	185.700±0.153^{h}	137.281±0.000^{j}	450.323±0.007^{e}	33351.784±0.045^{c}	28.027±0.001^{d}
MB1	290.800±0.115^{k}	83.592±0.001^{g}	130.117±0.060^{a}	9351.835±0.017^{c}	10.133±0.002^{b}
MB2	290.833±0.120^{k}	83.104±0.058^{f}	130.450±0.275^{ab}	9351.835±0.017^{g}	10.134±0.001^{b}
MB3	230.667±0.176^{j}	101.495±0.005^{h}	750.547±0.227^{k}	33425.595±0.298	27.018±0.009^{c}
MB4	165.700±0.153^{f}	101.495±0.005^{h}	1050.853±0.07^{4i}	83425.609±0.304	94.595±0.003^{k}
MB5	165.900±0.208^{f}	102.496±0.004^{i}	1051.187±0.29^{2i}	83425.275±0.327^{p}	94.597±0.007^{k}
MK	220.509±0.065^{i}	311.154±0.001^{n}	460.261±0.069^{g}	52520.900±0.153^{o}	60.510±0.006^{j}
S1	131.167±0.083^{a}	52.343±0.001^{a}	520.670±0.165^{i}	12231.387±0.202^{d}	43.916±0.003^{h}
S2	135.445±0.028^{d}	58.208±0.004^{e}	540.677±0.162^{j}	14907.500±0.323^{e}	43.903±0.009^{h}
S3	135.445±0.028^{d}	56.308±0.00a4^{d}	480.393±0.018^{h}	48240.730±0.015^{n}	43.916±0.003^{h}
SH1	135.497±0.088^{d}	55.250±0.000^{c}	130.677±0.162^{ab}	3250.533±0.291^{b}	9.912±0.006^{a}
SH2	135.467±0.067^{d}	54.147±0.013b	131.038±0.221^{b}	3140.800±0.416^{a}	9.912±0.006^{a}
Wet season					
K1	187.767±0.145^{c}	142.192±0.004^{j}	471.813±0.094^{h}	47314.705±0.103^{l}	43.913±0.006i
K2	187.667±0.167^{c}	142.164±0.033^{j}	471.810±0.146^{h}	47315.705±0.103^{m}	40.810±0.005^{h}
K3	135.646±0.180^{b}	146.093±0.001^{k}	442.718±0.034^{d}	41754.153±0.078^{i}	43.913±0.006^{i}
K4	135.646±0.180^{b}	146.093±0.001^{k}	443.051±0.351^{d}	41754.153±0.078^{i}	43.913±0.006^{i}
K5	135.639±0.182^{b}	138.288±0.006^{i}	464.664±0.168^{g}	37129.710±0.095^{g}	43.913±0.006^{i}
K6	187.820±0.160^{c}	146.077±0.038^{k}	462.756±0.172^{f}	41759.185±0.094^{j}	33.309±0.004^{g}
K7	239.789±0.120^{e}	333.507±0.004^{m}	428.177±0.089^{c}	36203.435±0.219^{f}	27.028±0.001^{d}
K8	239.789±0.120^{e}	138.281±0.000^{i}	457.197±0.103^{e}	34351.784±0.045^{e}	28.024±0.002^{e}
MB1	291.778±0.111^{f}	84.110±0.006^{f}	138.269±0.134^{a}	19400.407±0.348^{c}	11.215±0.010^{c}
MB2	291.778±0.111^{f}	84.110±0.006^{f}	139.035±0.201^{b}	19500.407±0.348^{d}	10.896±0.017^{b}
MB3	231.721±0.140^{d}	100.497±0.004^{g}	725.254±0.127^{i}	53300.373±0.190^{o}	30.167±0.017^{f}
MB4	187.700±0.153^{c}	101.495±0.005^{h}	1210.890±0.059^{k}	95420.100±0.058^{q}	95.204±0.004^{k}
MB5	187.667±0.167^{c}	101.495±0.005^{h}	1210.890±0.059^{k}	95422.100±0.058^{r}	95.204±0.004^{k}
MK	239.563±0.054^{e}	310.153±0.002l	464.698±0.201^{g}	53796.092±0.099^{p}	60.808±0.004^{j}
S1	239.589±0.006^{e}	65.700±0.005^{c}	1710.890±0.059^{l}	40833.144±0.099^{h}	43.912±0.006^{i}
S2	187.671±0.165^{c}	67.972±0.008^{e}	1783.198±0.117^{l}	45462.908±0.030^{k}	43.613±0.306^{i}
S3	187.873±0.127^{c}	66.937±0.022^{d}	1015.216±0.009^{j}	51943.400±1.224^{n}	43.870±0.035^{i}
SH1	130.493±0.194^{a}	56.250±0.000^{b}	138.272±0.136^{a}	6582.777±0.400^{b}	10.134±0.001^{a}
SH2	130.340±0.070^{a}	54.240±0.020^{a}	138.185±0.093^{a}	6500.252±0.144^{a}	10.134±0.001^{a}

Note: Mean values followed by the same letter(s) within the same column are not significantly different. (One-way ANOVA, SNK-test, α = 0.05)

Cadmium, Chromium and Mercury (Cd, Cr, Hg)

Cd, Cr, and Hg concentrations in the marine sediments were below detection limits in all stations sampled during both the dry and the wet seasons. Oyugi et al. (2000) had the same observations. However, Kamau (2002) and Mwashote (2003) reported concentrations of 1.0 µg/g and 0.08 µg/g of Cd in Makupa and Kilindini harbors, respectively. The difference could be due to sampling points, sampling time, and activities taking place. For chromium and mercury, no reports are available. However, cadmium levels are lower than those reported earlier by Oyugi (2000) and Kamau (2002), and Mwashote (2003), who studied cadmium in Kilindini Creek.

A study in Algiers in the Mediterranean region (Soualili et al. 2008) reported sediment cadmium levels of 0.76µg/g. USEPA guidelines (PEL Indicators) forcd is 4.21 µg/g, for Cr is 160 µg/g and for Fg is 0.7 µg/g (Gidarakos and Hahladakis 2012).

Lead (Pb)

The results for Pb levels in marine sediments are given in Table 5. In the dry season, Pb levels in marine sediments ranged from 135.47 µg/g at S2at Shirazi to 290.83 µg/g at MB 2 at Malindi Bay. In the wet season, Pb levels ranged from 13.4 µg/g at S2 at Shirazi to 291.78 µg/g at MB1 in Malindi Bay. There was a significant difference in the Pb levels during the dry and wet seasons (P-value > 0.05).

In earlier studies on Pb pollution in the Kenyan Coastline, Oyugi et al. (2000) and Hashim (2001) reported Pb levels of 7.76±0.96 and 52.22±7.92 µg/g, respectively. In a study to the south, Sheikh et al. (2007) reported lead levels of 228-1150 µg/g for Zanzibar port. These high lead levels may be due to the discharge of effluents from a tannery in the city. USEPA guidelines (PEL Indicators) for Pb is 8.1 µg/g (Gidarakos and Hahladakis 2012).

Within the Sabaki estuary/Malindi bay complex, the lead concentrations increased from the Sabaki River (S1) towards the ocean at S3. At Malindi bay, there was an increase from MB1 at Malindi marine park toward M4, where the Sabaki River enters the ocean. The high concentrations could be due to pelagic activities bringing some pollutants from the open sea. In Kilindini, the high concentrations of lead could be due to the shipping activities at the port and the domestic and industrial wastes from the municipal treatment works, the Kipevu oil terminal, and other industries. Concentrations at Shirazi are due to the terrigenous activities of the region. Due to their geographical separation, there was no relationship between the concentrations at the Malindi/Sabaki complex and Kilindini.

Zinc

In the dry season, the Zn levels in sediments ranged from 52.34 µg/g at SH2 at Shirazi to 332.51 µg/g at K7 at Kiindini harbor. In the wet season, the Zn levels ranged from 54.24 µg/g at SH2 at Shirazi to 333.51 µg/g at K7 at Kilindini harbor.

There was no significant difference in concentrations between the dry and wet seasons, p-value >0.000. The high concentrations could have been due to industrial activities around Kilindini creek. These levels were comparable with Oyugi et al. (2000), at 27.68 µg/g at English Point Mombasa and 347.73 µg/g levels at Makupa creek. Another study by Hashim (2001) reported levels ranging from 60.97-5.38 µg/g at Malindi to 320.17±76.31 µg/g at Mombasa. In a study to the south, Sheikh et al. (2007) reported Zn levels of 339-3600 µg/g for Zanzibar port and 107-3190 µg/g for Dar es Salaam port. Zinc concentrations in the Sabaki river had no variations from S1 to S3. However, there was an increase in the Malindi Bay from 83.59±0.01 µg/g at the Malindi marine park (MB1) to 102.50±0.04 µg/g at the mouth of the Sabaki river (MB5). This rise could be due to pelagic activities at MB5. At Kilindini, the significantly high level was at K7 with a concentration of 332.51±0.04 µg/g. This concentration could be due to the local geochemical background.

USEPA guidelines (PEL indicators) for Zn is 81.0 µg/g (Gidatakos and Hahladakis 2012); hence the Zn levels of the Kenyan coastline are indicative of Zn pollution.

Manganese (Mn)

The Mn levels in sediments in the dry season ranged from 130.68 µg/g at SH1 at Shirazi to 1051.19 µg/g at MB5 in Malindi Bay. In the wet season, Mn levels ranged from 138.19 µg/g at SH2 at Shirazi to 1783.20 µg/g at S2 at Sabaki river.

In earlier studies, Oyugi et al. (2000) reported Mn levels that were lower than those of this study, ranging from nd at Diani Beach to 1,100.02 µg/g at Vanga. Hashim (2001) found even lower Mn levels, from 97.54±19.33 µg/gat Gazi to 550.83-52.55 µg/g at Malindi. In their study, Sheikh et al. (2007) reported 537-812 µg/g for Zanzibar harbor and 277-725 µg/g levels - for Dar es Salaam port.

No trend in the sediment manganese concentrations was observed along the Sabaki and at Kilindini. But at Malindi Bay, there was an increase from MB5. That could be due to marine activities in the open sea showing the highest levels at MB5 since the monsoons were strongest at that point. In addition, there was a significant difference in sediment manganese concentrations between the dry and wet seasons (P value > 0.05), possibly due to siltation.

According to USEPA guidelines, sediment Mn levels> 500 µg/g indicate heavy Mn pollution (Ahdy and Khaled 2009). Therefore, the Kenyan coastline could be considered heavily polluted with Mn.

Iron (Fe)

In the dry season, the Fe levels in sediments ranged from 3,14.80 µg/g at SH2 at Shirazi to 83,425.61 µg/g MB4 in Malin di Bay. In the wet season, Fe levels ranged from 6,500.25 µg/g at SH2 at Shirazi to 95,422.1 µg/g at MB 5 in Malindi Bay. Fe levels at Kilindini harbor ranged from 34,351.78 at K8 to 47,314.70 µg/g at K1. In earlier studies, Oyugi et al. (2000) reported iron levels ranging from 800.19 µg/g to 19,312.05 µg/g along the south coast to the Mombasa area. Kamau (2002) reported iron concentrations ranging from 4,268 µg/g to 42,660 µg/g at Port-Reiz creek, Mombasa.

A none-statistical comparison shows lower levels in Oyugi et al. (2000) and Kamau's (2002) studies than in this study. That could be due to differences in time and sampling points. Studies done in Zanzibar and Dar es Salaam ports by Sheikh et al. (2007) reported levels in the range of 67,500-12,400 µg/g and 43,300-7,040 µg/g, respectively. There was a trend in the Fe levels, as seen in Table 5, that the iron sediment concentrations increased from 12,231.39±0.20 µg/g at S1 to 48,240.73±0.02 µg/g. Also, at Malindi Bay, the Fe concentrations increased from 9,351.84±0.0.02 µg/g at MB1 at the Malindi Marine Park to 83,425.28±0.33 µg/g at MB5. That could be the effects of maritime activities at the open sea. Pollution is being transported towards the East African Coastal zone by the very strong currents of which the South East monsoon is the driving force (Everaats and Nieuwenhuize 1995). No trend was observed in the iron concentrations at Kilindini.

Copper (Cu)

Table 5 indicate Cu levels in Marine sediments in the dry season ranged from 9.91 µg/g at SH1 and SH2 at Shirazi to 94.6 µg/g at MB4 and MB5 in Malindi Bay. In the wet season, Cu levels ranged from 10.13 µg/g at SH1 and SH2 at Shirazi to 95.2 µg/g at MB4 and MB5 at Malindi Bay. These figures are comparable to those of an earlier study by Hashim (2001), with levels of 47.68±4.83 µg/g for the Mombasa area and 13.96±1.47 µg/g for the Gazi area, which is near Shirazi. Oyugiet al (2000) also reported concentrations ranging from 18.70 µg/g at Mombasa Marine Park to 49.97 µg/g at Diani. Kamau (2002) reported concentrations ranging from 2.30 µg/g to 32.30 µg/g at Kilindini creek. Individual differences could be due to differences in sampling points. In studies for other water bodies, Sheikh et al. (2007) reported levels of 4-75 µg/g for Zanzibar port and 36 µg/g for Dar es Salaam harbor.

The sediment copper concentrations were the same along the Sabaki (43.92±0.03 µg/g). However, in Malindi

Bay, an increasing trend from 10.13±0.02 µg/g at MB1 to 94.60±0.07 µg/g at MB5, which could be due to the effects of the strong currents of the South East monsoon. There was no trend of sediment copper concentrations at Kilindini. USEPA guidelines (PEL indicators 0 for Cu sediments is µg/g) (Gidarakos and Haliladakis 2012). Therefore the Kenyan coastline is free of sediments and Cu pollution.

Levels of heavy metals in marine fauna

The levels of Pb, Cd, Zn, Mn, Cr, and Cu analyzed in the fauna are shown in Table 6.

Cadmium, mercury, and chromium (Cd, Hg, Cr)

Cd, Hg, and Cr levels in the fauna samples were below the detection level in both wet and dry seasons. These levels are lower than those found by Bor (2000), who reported levels at Mombasa ranging from 0.29±0.01 µg/g to 2.36±0.70 µg/g for cadmium chromium and mercury levels in fauna had not been reported in previous studies of the Kenyan coastline.

Lead (Pb)

Table 6 show lead concentration levels for *Cerithidea decollata* (Nyambua) ranged from 135.41±0.07 µg/g at Shirazi during the dry season to 236.62±3.21 µg/g at Kilindini during the wet season. For *Saccostrea cucullata* (rock oyster), the levels ranged from 135.507±0.058 µg/g to 833.11±0.68 µg/g at Malindi Bay during the wet season. These levels are significantly higher than those found in another study (Bor 2000), which recorded levels for pearl oysters at Mombasa ranging from 2.35±0.11 µg/g to 121±0.02 µg/g.

The present study was done ten years after that of Bor, so it can be suggested that lead pollution has taken place in the subsequent period and that the sea animals have accumulated lead over time. However, t-statistics for temporal variations showed a *p*-value > 0.05, meaning there was no significant difference in leadconcentrations of fauna between dry and wet seasons at the Kenyan coastline.

A study in the Middle East (Fowler et al. 1993) recorded levels for rock oysters ranging from 0.08 µg/g to 2.1 µg/g. Mostafa et al. (2009) reported lead concentrations of rock oysters in Yemen in the range of 2.6 – 15.4 µg/g.

Zinc (Zn)

Zinc levels for C. decollata ranged from 99.48±0.26 µg/g at Sabaki to 381.48±0.01 µg/g at Shirazi during the wet season. Zinc levels for *S. cucullata* ranged from 446.88±0.09 µg/g at Malindi during the dry season to 873.07±0.19 µg/g at Shirazi during the wet season (Table 6). Bor (2000) reported zinc levels at Mombasa ranging from 30.1±1.81 µg/g to 119±1.4 µg/g. These are much lower levels of zinc than those found in this study. The high lead levels could be due to boating activities at Malindi, a busy tourist resort, and shipping activities at Kilindini harbor. The high zinc concentrations in oysters at Shirazi could be due to the biogeochemical environment. The zinc concentrations on the Kenyan Coast are high. The Australian National Health and Medical Research Council recommended 1,000 µg/g (Brown and McPherson 2003).

Zinc tends to be bioaccumulated by bivalves. Oysters especially contain large amounts of zinc. For example, the highly contaminated English Restrongnet Creek oysters contain zinc concentrations above 10,000 µg/g dry weight. However, like most heavy metals, zinc does not tend to biomagnify, so it causes little harm to sea birds or marine mammals (Kennish 1996).

Manganese (Mn)

Manganese levels for *C. decollata* ranged from 29.77±0.12 µg/g at Sabaki during the wet season to 486.61±0.22 µg/g at Kilindini during the dry season. For *S. cucullata*, the manganese levels ranged from 210.92±0.04 µg/g at Shirazi during the dry season to 334.29±0.16 µg/g at Malindi during the dry season.

Iron (Fe)

Iron levels for *C. decollata* ranged from 379.85±0.18 µg/g at Sabaki during the dry season to 35,278.09-0.35 µg/g at Kilindini during the wet season. For *S. cucullata*, the levels ranged from 564.91±0.05 µg/g at Shirazi during the dry season to 8343.03±0.32 µg/g at Malindi during the wet season (Table 6). Bor (2000) recorded much lower levels ranging from 25.60±0.90 µg/g to 242.00±14.50 µg/g at Mombasa. Elsewhere in the Middle East, Fowler et al. (1993) recorded Fe levels for rock oysters, ranging from 11 to 266 µg/g.

Table 6. Levels of heavy metals in fauna during the dry and wet season

		Concentration (µg/g)± SE (*n* = 3)				
Site	**Sample**	**Mn**	**Fe**	**Cu**	**Zn**	**Pb**
Dry season						
Shirazi	*Cerithium decollata*	37.002±0.049^{b}	565.576±0.289^{c}	145.323±0.039^{c}	380.480±0.010^{c}	135.407±0.007^{a}
Shirazi	*Saccostrea cucullata*	210.920±0.040^{c}	564.909±0.053^{b}	43.963±0.024^{a}	872.852±0.174^{e}	135.412±0.006^{a}
malindi	*Saccostrea cucullata*	334.286±0.162^{d}	8342.698±0.154^{d}	229.843±0.081^{d}	446.882±0.009^{d}	831.778±0.969^{c}
Sabaki	*Cerithium decollata*	29.770±0.119^{a}	379.853±0.176^{a}	128.382±0.009^{ab}	99.476±0.262^{a}	135.663±0.169^{a}
Kilindini	*Cerithium decollata*	486.611±0.222^{e}	35277.753±0.037^{e}	43.970±0.025^{a}	157.909±0.054^{b}	236.485±3.143^{b}
Wet season						
Shirazi	Cerithium decollata	35.669±0.285^{b}	565.909±0.524^{b}	145.474±0.074^{c}	381.480±0.010^{c}	135.507±0.058^{a}
Shirazi	Saccostrea cucullata	211.413±0.322^{c}	565.242±0.287^{b}	43.990±0.010^{a}	873.067±0.186^{e}	135.407±0.007^{a}
malindi	Saccostrea cucullata	333.200±1.604^{d}	8343.031±0.316^{c}	230.177±0.283^{d}	446.948±0.040^{d}	833.111±0.676^{c}
Sabaki	Cerithium decollata	29.867±0.133^{a}	380.187±0.260^{a}	128.546±0.023^{b}	99.737±0.263^{a}	135.997±0.274^{a}
Kilindini	Cerithium decollata	485.611±0.812^{e}	35278.086±0.348^{d}	44.037±0.042^{a}	158.038±0.085^{b}	236.624±3.214^{b}

Note: Mean values followed by the same letter(s) within the same column are not significantly different. (One-way ANOVA, SNK-test, $\alpha = 0.05$)

The high iron concentrations could be due to the regional geochemical environment. Marine organisms accumulate iron. Normally, tissue concentrations of iron are related to water and sediment concentrations, but there can be considerable variability (UK Marine Special Areas of Conservation 2011).

Copper (Cu)

Copper levels for *C. decollata* ranged from 43.97±0.03 µg/g at Kilindini during the dry season to 145.47±0.07 µg/g at Shirazi during the wet season. Copper levels of *S. cucullata* ranged from 43.96±0.02 µg/g at Shirazi duringthe dry season to 230.18±0.28 µg/g at Malindi during the wet season (Table 6).The study by Bor (2000) reported a much lower Cu level for pearl oysters of 4.01±0.01 µg/g at Mombasa.

Copper is essential for marine animals, especially decapods; gastropods and cephalopods need copper in their respiratory pigment hemocyanin. Hemocyanin is a protein that (like hemoglobin) binds oxygen to transport it to the tissues (Perton et al. 1997). Oysters appear to accumulate large amounts of copper in their leucocytes (a type of blood cell), where they do little harm. However, like most other metals, copper does not show biomagnifying characteristics (Kennish 1996).

To conclude, from the findings of this study, the following conclusions were made: (i) The Kenyan coastline e is polluted with heavy metals. In particular, Cu, Pb, Zn, and Mn levels were higher than the USEPA guidelines. (ii) Marine fauna had the highest levels of heavy metals due to the bioaccumulation of the metals. However, exceptions were found in Fe and Mn, which were higher in sediments attributed to the local biogeochemical environment. There were no significant differences in the levels of heavy metals in the wet and dry seasons, sediments, and marine fauna.

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