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Physicochemical influence on the spatial distribution of faecal bacteria and polychaetes in the Densu Estuary, Ghana

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Coastal ecosystems are increasingly impacted by man-made disturbances including pollution from agriculture, aquaculture and municipal waste. This study employed multiple ecological indicators to assess environmental quality of the Densu Estuary and understanding of environmental controls on the spatial distribution of organisms. Physicochemical parameters were measured *in situ*. Water and sediment samples were collected from ten stations and analysed for nutrients, total suspended solids and organisms using standard methods. The water quality index for the Densu Estuary ranged from 359.5 to 484.4, suggesting an unhealthy ecosystem. The abundance of indicator species, e.g. faecal bacteria (*Escherichia coli*, *Enterococcus* species) and polychaetes (*Capitella* and *Nereis* species) varied significantly (p<0.05) among stations. Contaminated sites are located landwards with high human impacts. Faecal bacteria and polychaete abundance correlated significantly (p<0.05) with the respective physicochemical parameters. Canonical analysis (74.11%) showed the physicochemical influence on the spatial distribution of species. The pH significantly (p<0.05) controlled the spatial distribution of faecal bacteria and polychaetes in the Densu Estuary. The results suggest environmental pollution in the Densu Estuary, useful baseline information for effective legislation towards its sustainable management.

Key words: Biological indicators, water quality index, pollution, estuarine ecology, Densu Estuary.

INTRODUCTION

Estuaries contain mixed fresh and marine water which include productive wetlands and most productive biogenic zones of nearshore waters (Twilley et al., 1992; Armah, 1993; McLusky and Elliot, 2010; Mahu et al., 2016). They are habitats for benthic invertebrates, feeding ground for nektonic, migratory birds and nursing grounds of fishes (Lamptey and Armah, 2008; Aggrey-Fynn et al., 2011; Greene et al., 2015). Growing human

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populations, urbanization and industrial activities increasingly affect coastal ecosystems (Monney et al., 2013; Nyarko et al., 2015; Yeleliere et al., 2018). The management of solid and liquid waste is a major problem in Ghana, especially in urban areas and cities due to inadequate waste treatment facilities and management (Aglanu and Appiah, 2017). The waste is mostly transported by rivers and streams into estuaries and eventually into the sea (Mahu et al., 2015; Klubi et al., 2018). Access to clean and safe drinking water in cities is inadequate in supply and many people die of water-borne diseases (Shuval, 2003; Cabral, 2010; Odonkor and Ampofo, 2013).

The coastal environments are mostly impacted through pollution, land use and hydrological changes (Shuval, 2005; Stewart et al., 2008; Lamptey et al., 2013; Klubi et al., 2019). Estuaries are complex dynamic environments that are susceptible to anthropogenic alterations (Bucci et al., 2012; Greene et al., 2015; Klubi et al., 2018). Intensive industrialization and population growth exists around urban coastal areas of Ghana. The human activities (e.g., agrochemical inland runoff, mining, industrial and domestic waste discharges) can alter the environmental characteristics of the coastal water bodies including estuaries, lagoons, rivers among others (Lamptey and Armah, 2008; Okyere et al., 2011; Nyarko et al., 2015; Yeleliere et al., 2018).

The Densu Estuary is located in the dry equatorial climate region of Ghana with the climate governed by the monsoon, the harmattan and the equatorial air masses (Armah and Amalalo, 1998; Teley, 2001; Karikari and Ansa-Asare, 2006). The primary aim of this study was to assess the human impacts on the environmental quality of the Densu Estuary through multiple ecological indicators. The specific objective was to gain knowledge on how physicochemical factors influence the spatial distribution of organisms in the Densu Estuary. We hypothesize that (i) physicochemical parameters vary in the estuarine system and (ii) physicochemical parameters are drivers for the spatial distribution of organisms within the system. The preliminary results established hydrochemical dynamic coupled with the ecological conditions of the Densu Estuary. The spatial pattern of organism reflects not only physicochemical characteristics of the water body, but also the state of sediment quality and further insight into estuarine ecology.

MATERIALS AND METHODS

Study site

The Densu Estuary is located between 5°30'N and 5°31'N and 0°17'W and 0°18'W (Figure 1). The river basin has a catchment area of 2,565 km^2 and is 116 km long (Debrah, 1999). The Densu River has its source in the Atewa-Atwiredu mountain range near Kibi in the East Akyem District of the Eastern Region of Ghana (Hagan et al., 2011). The Densu River Basin is one of the most important river basins of Ghana. It encompasses the northwestern suburbs of Accra, the capital of Ghana and is densely populated. The basin is endowed with rivers and streams, mostly ephemeral, but few perennial known to be polluted (WRI, 2003; Fianko et al., 2009). The major tributaries include Adeiso (Adaiso), Nsakyi (Nsaki), Dobro, Mame and Kuia. The Densu River enters the Weija Reservoir and discharges into the Densu Estuary, which drains into the Gulf of Guinea, Ghana.

The Densu Estuary is surrounded by a wetland (also known as “Densu Delta”, “Densu Wetland”, “Densu Ramsar”), recognized as the Ramsar site due to its ecological biodiversity. It is surrounded by mangroves (e.g., *Avicennia africana*), which serve as nursery grounds for migratory fish species (Koranteng, 1995), habitat for birds (especially long-distance migratory birds along the East Atlantic Flyway); it supports approximately 57 species of seashore birds (population ~35,000 specimens). The estuarine waters support about 15 species of finfish (14 genera and 9 families, most common of which are *Sarotherodon melanotheron* and *Tilapia zilli*). The beachfront is also a nursing ground for marine turtles (e.g., *Lepidohelys olivacea, Chelonia mydas* and *Dermochelys coriacea*). The estuary is also used for crab fisheries and oyster farming. Furthermore, the wetland serves as floodplain. The availability and quality of water in the estuary and wetland play an important role in defining not only where people can live, but also their quality of life (Solley et al., 1998).

Field sampling

At ten stations (S1 to S10) water and sediment samples were taken to assess the ecological integrity of the Densu Estuary (Figure 1 and Table 1). Sampling line transects started landwards and ended seawards (Figure 1). Sampling was carried out on the 19th of May 2017 during mid-tide (11:15 to 13:30). Samples were taken from water depths ranging from 0.10 to 0.70 m. Coordinates for each station are recorded using the Global Position System (GPS), Garmin etreX Model (www.garmin.com).

Physicochemical parameters such as temperature, salinity, specific electrical conductivity, redox potential, dissolved oxygen concentration and saturation were measured *in situ* using a Horiba Digital Water Quality Multi-parameter instrument (Horiba Probe, Model U-52G 30M, Horiba Company Limited, Japan). Sample bottles were washed three times with estuary water before filling. Water samples (N = 10) were collected from 10 cm depth in 500 ml bottles for phosphate and nitrates and suspended particles analyses. Additionally, water samples (N = 10) were collected in 500 ml plastic bottles covered with black polythene bags for chlorophyll-*a* concentration estimates. Furthermore, water samples (N = 10) were collected in 200 ml sterilized water bottles for microbiological analyses.

Surface sediments were collected at the sampling stations from 20 to 30 cm depths using an Ekman Grab (area: 0.04 m^2) (Mudroch and Azcue, 1995). The sediment was put into interim storage in a bowl and immediately scooped into labelled polythene bags (bacteria and benthos, N=10 each) for further analyses.

The samples for nutrients and bacterial analyses were kept on ice cubes stored in ice-coolers to reduce biological activity. The sediment sample for macrobenthic analysis was preserved in 10% buffered formaldehyde and stained with Rose Bengal. All samples were then transported to the laboratory and kept in a refrigerator at 4°C for 30 min before analysis. The microbiological analyses were carried out within 24 h at the Environmental Biology Laboratory, Council for Scientific and Industrial Research (CSIR)-Water Research Institute (WRI), Accra, Ghana.

Determination of chemical parameters

The chemical parameters were determined according to procedures

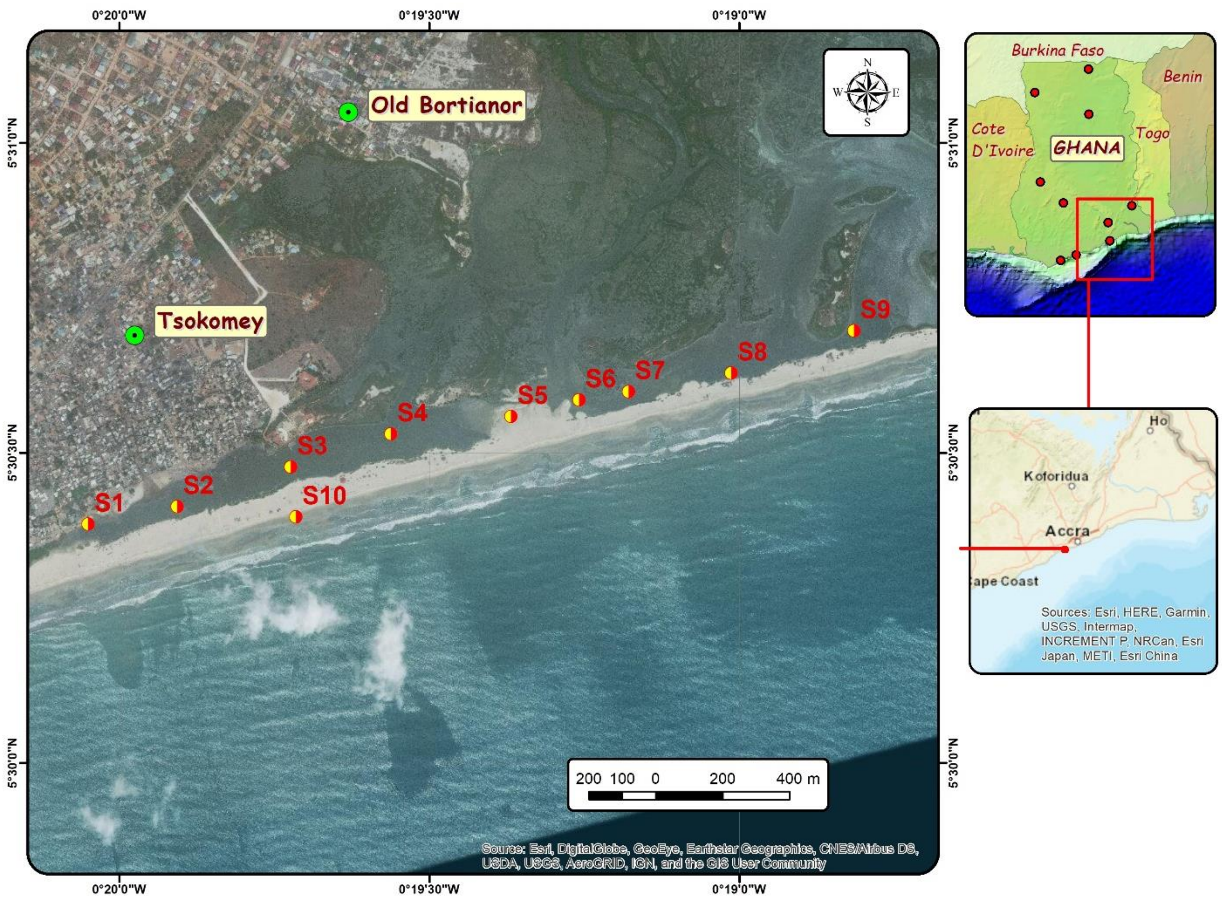


Figure 1. Map showing the ten sampling stations in the Densu Estuary, Ghana.

Table 1. Description of ten sampling stations at the Densu Estuary, Ghana.

Station	Site description of stations	Coordinates (Latitude N, Longitude W)
S1	Separated from the sea by sand, landfill site, Bortianor	5°30'23.1", 0°20'03.0"
S2	Fishing boat landing site, Bortianor	5°30'24.8", 0°19'54.4"
S3	Middle stream, river flow into estuarine, Bojo-Tsokomey	5°30'28.6", 0°19'43.4"
S4	Mixing of fresh and sea water	5°30'31.8", 0°19'33.7"
S5	Mixed fresh and sea water, wetland zone, Shore birds, crab farming	5°30'33.5", 0°19'22.1"
S6	Mixing zone, oysters farming, Faana village	5°30'35.1", 0°19'15.5"
S7	Mixing zone, Mangroves, Panbros village	5°30'35.9", 0°19'10.7"
S8	Tidal influence, mangrove rich zone	5°30'37.7", 0°19'00.8"
S9	Downstream, open to sea, intrusion of saline water	5°30'41.8", 0°18'48.9"
S10	Surf zone sea water	5°30'23.8", 0°19'42.9"

outlined in the Standard Methods for the Examination of Water and Wastewater (APHA, 1998, 2012). Nitrate (NO_3^-), phosphate (PO_4^{3-}) and sulfates (SO_4^{2-}) were measured using a HACH 2010 Spectrophotometer (Model DR/2010) with a precision of ± 0.10 v mg/L (HACH Company, Loveland, Colorado, USA) (www.hach.com; HACH, 2012). Total dissolved solids (TDS) are determined by filtering, weighing the sampled water and measuring it gravimetrically after drying it in an oven to a constant weight at 105°C (APHA 2012). To measure total suspended solids (TSS), 100 ml of the water sample was filtered through a pre-weighed filter that was dried in an oven at the temperature of 104°C to constant weight and repeated for 3 steps, then the total suspended solids is calculated (thus the TSS, mg/L is equal to average weight from step 3 in g minus average initial weight from step 1 in g multiple by 1000 mg/L divided by sample volume in L) (APHA, 2012).

Chlorophyll-*a* concentration was extracted from the water

Table 2. Average values of selected physicochemical parameters measured and computed water quality index for the Densu Estuary, Ghana.

Parameter	Average values	USEPA (2009) standards	Assigned Weight (AW)	Relative weight (RW)
pH	8.35	6.5-8.5	4	0.22
Alkalinity [mg/L]	3.66	100	2	0.11
Dissolved oxygen [mg/L]	10.48	5	4	0.22
Electrical conductivity [µScm^{-1}]	43850	2500	3	0.17
Nitrates [mg/L]	2.98	10	4	0.22
Phosphates [mg/L]	0.33	0.1-0.3	1	0.06
Σ(SI)			18	1.00
			WQI	416.50 ± 44.07
Classification			> 300-unsuitable water for drinking	

samples using 96% ethanol and measured with a UV/Vis spectrophotometer at 665 and 649 nm (APHA, 2012). Chlorophyll-*a* concentration was estimated as a proxy for phytoplankton concentration and thus as an indicator of the trophic state (Monbet, 1992; Hinga et al., 1995; Boyer et al., 2009). The trophic status of the ecosystem was determined based on the estimated chlorophyll-*a* concentrations and classified using the following scheme: <1.0 µg/L = ultra-oligotrophic; 1.0 - 3.0 µg/L = oligotrophic; > 3.0 - 8.0 µg/L = mesotrophic; > 8.0 - 30.0 µg/L = eutrophic; > 30.0 µg/L = hypertrophic (da Silveira Fiori et al., 2013).

Water quality index

The Water Quality Index (WQI) is defined as a rating, reflecting the composite influence of different water quality parameters on the overall quality of water (Mophin-Kani and Murugesan, 2011; Tirkey et al., 2015; Fathi et al., 2016). It was first proposed in 1965 (Horton, 1965). The WQI was computed using recommended water quality standards (Sanchez et al., 2007; USEPA, 2009; Nyarko et al., 2015) and four steps followed. In the first step, each of the six environmental parameters (Table 2) has been assigned a weight (AW) according to its relative importance in the overall quality of water for drinking purposes. The maximum weight of 4 has been assigned to parameters such as pH, dissolved oxygen concentration and nitrate due to their major importance in water quality assessment (Ramakrishnaiah et al., 2009; Mophin-Kani and Murugesan, 2011; Nguyen and Sevando, 2019). Phosphate is given the weight of just 1 as it plays a minor role in the water quality assessment. Other parameters were electrical conductivity (assigned 3) and alkalinity (assigned 2) (Table 2). For the second step, the relative weight (RW) was computed using a weighted arithmetic index (Equation 1):

$$RW = \frac{AW_i}{\sum_{i=1}^{n} AW_i} \quad (1)$$

where RW is the relative weight, AW is the assigned weight of each parameter and n is the number of parameters.

In the third step, a quality rating scale (Q_i) for each parameter was assigned by dividing its measured value of the water quality parameter in each water sample by its respective standard according to the guidelines of USEPA (2009) and then multiplied by 100:

$$Q_i = \left[\frac{C_i}{S_i}\right] \times 100 \quad (2)$$

where Q_i is the quality rating, C_i is the measured value of the water quality parameter in each water sample in mg/L, and S_i is the WHO drinking water standard for each chemical parameter in mg/L according to the guidelines of USEPA (2009).

In the fourth step, the sub-indices (S_i) were first determined for each chemical parameter (Equation 3),

$$SI_i = RW \times Q_i \quad (3)$$

where SI_i is the sub-index of the i^{th} parameter and Q_i is the rating based on the concentration of i^{th} parameter.

The overall WQI was then calculated by adding the sub-index values of each water sample as follows:

$$WQI = \sum_{i=1}^{n} SI_i \quad (4)$$

Computed WQI values were categorized based on the water quality classification scheme: < 50-exellent; 50-100, good water; 200-300, very poor water and > 300, unsuitable water (Sahu and Sikdar, 2008; Ramakrishnaiah et al., 2009; Lamptey et al., 2013; Duncan, 2018).

Biological analyses

Bacteriological examination of water and sediment samples was conducted using standard methods (Horan, 2003; Cabral, 2010; Odonkor and Ampofo, 2013). Total coliforms and faecal bacteria were determined by the membrane filtration method using M-Endo-Agar Les (Difco) at 37°C and on MFC Agar at 45°C, respectively (Cabral, 2010). In total 20 ml of each water sample were separately filtered through 0.45 µm pore size membrane filter paper, mounted on a filtration pump, whereas 5 g of each sediment sample was given into a sterile 50 ml tube. Thereafter, 45 ml of PBS was added the sample vortexed for 30 s to homogenize it. The pH was slowly adjusted to 9.0 by adding drops of 0.1 N NaOH. The prepared sample was vigorously mixed with the help of a shaker for 30 min at room temperature. The sample was left to stand for 15 min and 1 ml of the supernatant diluted with 10 ml sterile distilled water before membrane filtration.

Determination of total coliforms and *Escherichia coli* were undertaken by aseptically placing filters on poured and solidified Cromocult Agar Media in Petri dishes and incubated at 37 ± 0.5°C for 18 to 24 h. Similarly, for the enumeration of *Enterococcus* species, the filters were aseptically plated on Slanetz and Bartley medium and incubated at 45°C. Typical presumptive colonies were identified as total coliform (purple-blue colonies), *E. coli* (only blue colonies) and *Enterococcus* spp. (pinkish to red colonies); these were counted with the aid of a colony counter and expressed in CFU/100 ml and CFU/g for water and sediment samples, respectively.

For macrofauna analysis, the fixed sediment samples were washed with tape water through a 0.2 mm mesh sieve to remove the fixative. Thereafter, the samples were sorted and animal groups identified and quantified under a dissecting microscope. Their abundance was determined by counting their head, the identification followed taxonomical keys (Day, 1967a, b).

Statistical analysis

Physicochemical parameters and biological data that were normally distributed were subjected to a One-way analysis of variance (ANOVA) to test for spatial variation. A significance level of 5% was adopted. The physicochemical parameters were standardized, while the biological data were log (X+1) transformed.

Principal component analysis (PCA) was used to identify the relationship between the species composition and the effects of the physicochemical parameters (Šmilauer and Lepš, 2014). This allows separation of effects of space and environmental variables on the oribatid community structure (Šmilauer and Lepš, 2014). The PCA and Redundancy analysis (RDA) are both linear quantitative ordination methods, which are closely related to linear regression. However, PCA is an indirect gradient analysis, while RDA is a direct gradient analysis (Šmilauer and Lepš, 2014). The ordination scores can be used to model the distribution of taxa along physicochemical gradients and to estimate values of preferred environments, environmental tolerance and peak abundance (Patzkowsky and Holland, 2012). The PAST software was used to two-way constrained cluster analysis (Hammer et al., 2001). The Canoco software was employed for the multivariate statistical analyses (e.g., PCA, CA and RDA) (Šmilauer and Lepš, 2014). Descriptive statistics were calculated using PAST and Excel spreadsheets. Furthermore, the PRIMER 6 package was used to run cluster analyses (Clarke and Gorley, 2006) to identify groups of similar associations (e.g., taxa and environmental variables) and to display the relationships among them (Patzkowsky and Holland, 2012).

Pearson's correlation coefficient (r) was used to test the strength of linear associations between the biological data and physicochemical parameters (Khamis, 2008), using a statistical package for social sciences (SPSS 21.0). The correlation coefficient (r) was determined to estimate the degree of the relationships (Khamis, 2008; Yadav, 2018). The dimensionless quantity value of the coefficient of correlation (r) can range from -1 (perfect negative correlation) through 0 (no correlation) to +1 (perfect positive correlation) (Möller and Scharf, 1986; Nagelkerke, 1991; Yadav, 2018). Significance levels of 0.05 and 0.01% were adopted.

RESULTS

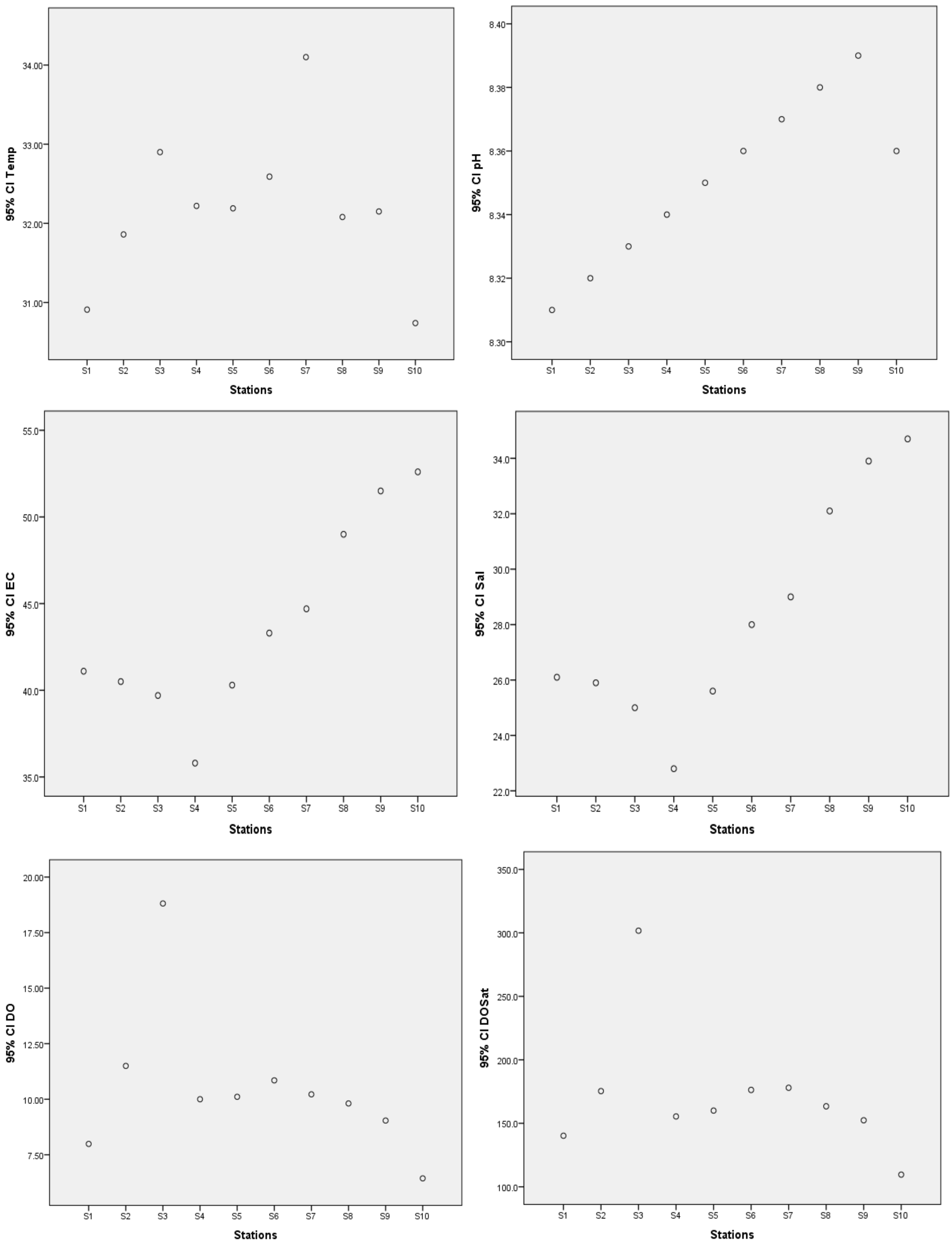
Physicochemical parameters

The spatial distribution of physicochemical and microbiological characteristics of the Densu Estuary is expressed in Figure 2, providing the 95% confidence interval. The mean values are summarized in Table 3. There was a significant variation for some physicochemical parameters (mainly dissolved oxygen concentration and saturation, total dissolved solids, alkalinity, nitrate, phosphate and chlorophyll-*a* concentration) and microbiological organisms (total coliforms, *E. coli* and *Enterococcus* spp., in water and sediment) among the sampling stations (One-way ANOVA $F_{12, 117} = 3.50$; $p < 0.05$).

Surface water temperature ranged from 30.7 to 34.1°C. The lowest temperature was recorded at S1 and the highest at S7. S1 is located landwards, without any freshwater influence, separated from the sea by a sand bar and is characterized by shallow water depth, while S7 is characterized by an estuarine environment, more subject to marine water intrusions. The pH ranged from 8.31 to 8.39. The lowest pH was recorded at S9 and the highest at S1. S9 is influenced by near-shore oceanic waters with its typical pH. The electrical conductivity ranged from 35.8 to 52.6 mScm^{-1}, the salinity between 22.8 and 34.7 (PSU scale). Lowest values were recorded at S4 and highest at S10. S4 is characterized by a mixture of fresh and seawater, while S10 is surf zone, oceanic water hence high salinity.

The dissolved oxygen concentration ranged between 6.44 and 18.81 mg/L, and the water was in all cases oversaturated with oxygen. The highest dissolved oxygen concentration and saturation was recorded at S3. Redox potential ranged from 92 to 200 mV. The lowest redox potential was recorded at S1 and the highest at S8 (detritus zone). Total dissolved solids ranged from 21.9 to 31.6 mg/L. The lowest total dissolved solids were recorded at S4 (estuarine zone) and the highest at S10 (surf zone seawater). Total suspended solids ranged from 18 to 38 mg/L. The lowest concentration of total suspended solids was recorded at S1 and the highest at S9, which is influenced by the ocean and tidal influence, hence with loaded suspended materials from the near-shore environment.

Alkalinity ranged from 195.52 to 237.97 mg/L. The lowest alkalinity was recorded at S10 and the highest at S3 (more freshwater mixes with sea water). Phosphate concentrations ranged from 0.05 to 1.10 mg/L. The lowest phosphate was recorded at S1 and the highest at S9. Nitrates ranged from 1.7 to 7.5 mg/L. The lowest nitrates were recorded at S7 and S8 (intermediate zone) and the highest at S9. The liquid waste discharges directly into near-shore waters and swimming activities at the Densu beach may have contributed to an increase in nutrient load in seaward direction. Furthermore, a small village with cage cultures for crabs, fish farming and farming activities is located at the seaward direction. This may result in a higher nutrient load in downstream direction of the estuary. Sulfate ranged from 15 to 37 mg/L. The lowest sulfate concentration was recorded at S4 (estuarine zone) and the highest at S1 (landwards). Chlorophyll-*a* concentration ranged from 0.96 to 4.38 µg/L. The lowest concentration was recorded at S3 (mixing zone) and the highest at S1 (landwards). Areas, with intense freshwater discharge into the estuary system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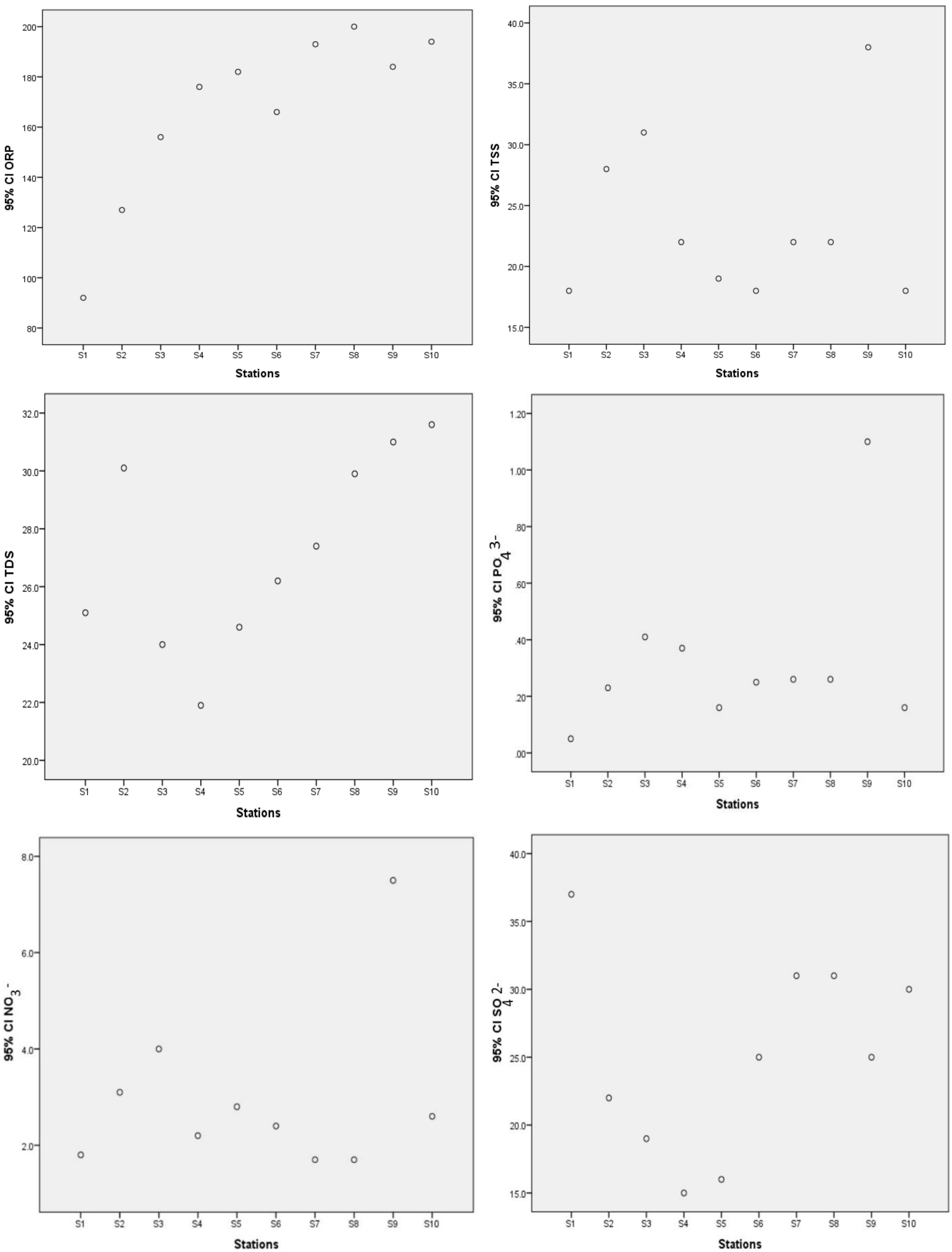
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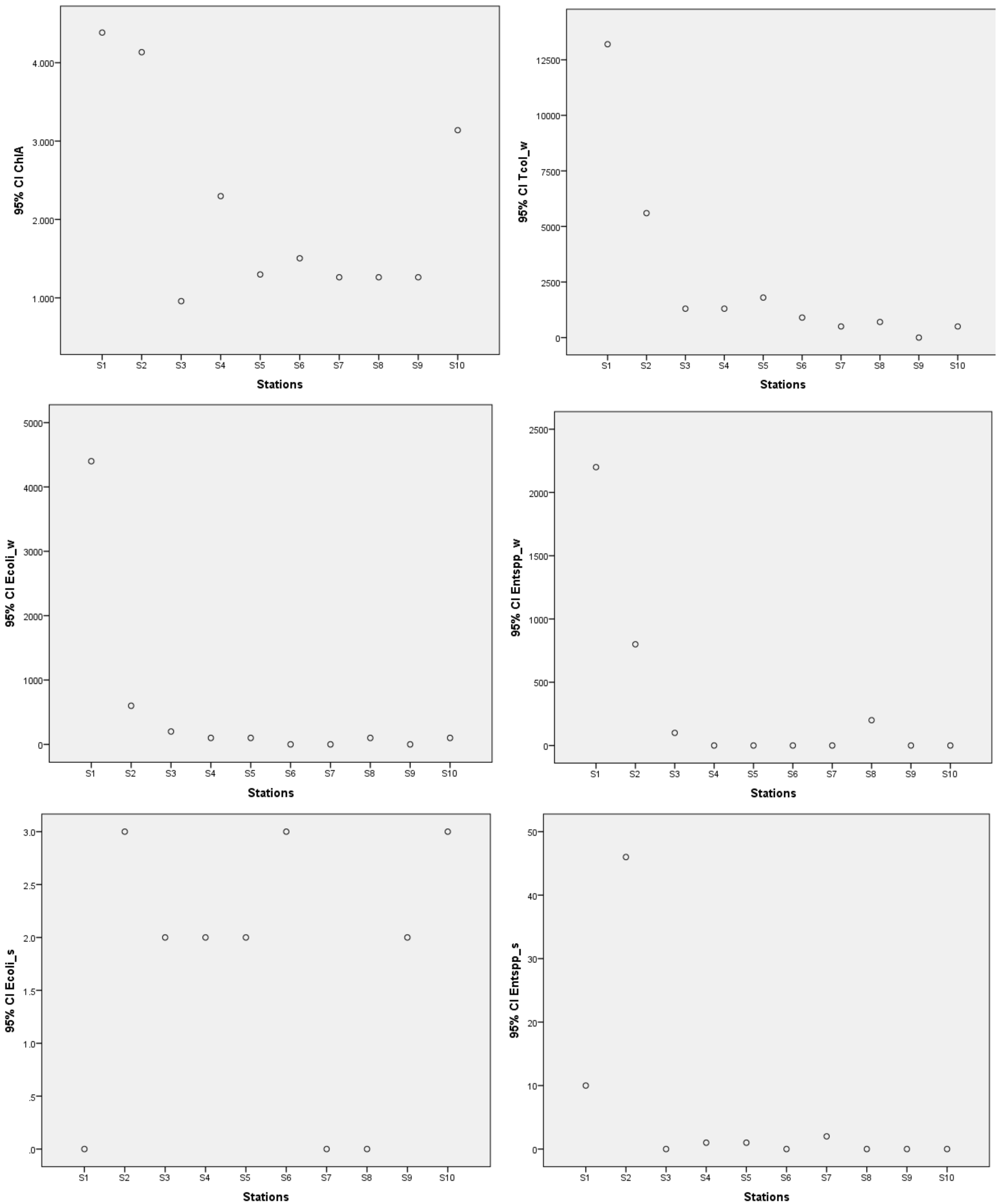

Figure 2. Spatial distribution of physicochemical and biological characteristics (95% Confident interval-CI) of the Densu Estuary, Ghana.

Table 3. Mean (±SD) of physicochemical and biological characteristics of the Densu Estuary, Ghana.

Parameter	Symbol [units]	Mean ± standard deviation (SD)
Temperature	Temp [°C]	32.17 ± 0.95
pH	pH	8.35 ± 0.03
Electrical conductivity	EC [mS/cm]	43.85 ± 5.54
Salinity	Sal	28.31 ± 4.03
Dissolved oxygen concentration	DO [mg/L]	10.48 ± 3.27
Dissolved oxygen saturation	DO Sat [%]	171.24 ± 50.18
Total dissolved solids	TDS [g/L]	27.18 ± 3.33
Total suspended solids	TSS [mg/L]	23.60 ± 6.70
Alkalinity	Alk [mmol/L]	3.66 ± 0.24
Phosphate	PO_4^{3-} [mg/L]	0.33 ± 0.29
Nitrate	NO_3^- [mg/L]	2.98 ± 1.74
Sulphate	SO_4^2 [mg/L]	25.10 ± 7.20
Chlorophyll-*a*	Chl-a [µg/L]	2.25 ± 1.32
Escherichia coli in water	*E*. *coli*_w [CFU/100 ml]	560.00 ± 1360.72
Enterococcus spp. in water	*Ent*. spp_w [CFU/100 ml]	330.00 ± 702.46
Escherichia coli in sediment	*E*. *coli*_s [CFU/1g]	1.67 ± 1.32
Enterococcus spp. in sediment	*Ent*. spp_s [CFU/1g]	6.56 ± 15.14
Polychaetes	POLY	27.5 ± 9.24

(mixing zone), show high turbidity, leading to limited photosynthesis and hence low chlorophyll-*a* concentration at S3, whereas at S1 nutrient inputs from terrestrial land sources may facilitate increased primary production. The WQI ranged from 359.49 to 484.62, minimum values were recorded at S4 (estuarine zone) and maximum values at S9 (seaward zone).

Biological analyses

The abundance of bacteria (*E. coli* and *Enterococcus* spp.) significantly varied in the water of distinct stations (One-way ANOVA $F_{9,\ 10}$ = 8.52; p < 0.05). However, the abundance of bacteria in the sediment showed no significant variation among the stations (One-way ANOVA $F_{9,\ 10}$ = 1.09; p>0.05). Bacterial (*E. coli* and *Enterococcus* spp.) loads in the water did not differ significantly (p>0.05) from the counts in sediment (p>0.05 both, at one tail and two tails). In water, total coliforms ranged from 0 to 13,200 CFU/100 ml; *E. coli* ranged from 0 to 4,400 CFU/100 ml and *Enterococcus* spp. ranged from 0 to 2,200 CFU/100 ml. In sediment, the total coliforms ranged from 136 to 558 CFU/1 g; *E. coli* ranged from 0 to 3 CFU/g and *Enterococci* spp. ranged from 0 to 46 CFU/g. High bacteria (total coliforms, *E. coli*, *Enterococci* spp.) counts from water and sediment samples were found at S1 and S2, closer to a landfill site and waste disposal, while low abundance was evident seawards (S9). *Escherichia coli* is the numerically dominant bacteria in water (Figure 3a), while *Enterococcus* spp. is more abundant in the sediment (Figure 3b).

Polychaetes were the most dominant living macrofauna. In total, 275 individuals of polychaetes were counted. The relative abundance of polychaetes ranged from 5.09 to 14.55%, mainly *Capitella* and *Nereis* species were found. Lowest abundance (14 individuals per 0.04 m^2) was recorded at S1 and the highest abundance (40 individuals per 0.04 m^2) at S8. S1 is a shallow area with coarse grains without much vegetation cover and high human impact, whereas station S8 is characterized by mangroves with rich detritus-debris, muddy soft bottom and possibly with high organic matter, favourable conditions for polychaetes.

Multivariate statistics

Cluster analyses

The cluster analyses revealed two main groups with similar physicochemical parameters (Figure 4a). There was a significant association between alkalinity, temperature and pH (Figure 4a). The dendrograms for the organisms, namely total coliforms, faecal bacteria (*E. coli* and *Enterococcus* spp.) and polychaetes showed two similar associations (Figure 4b), (i) total coliform in water and sediment, (ii) *E. coli* and *Enterococcus* spp. in water, and (iii) *E. coli*, *Enterococcus* spp. and polychaetes in sediment (Figure 4b). The combined biological data and physicochemical parameters (Figure 5a) were grouped into three distinct clusters, namely landwards (S1 and S2), mixing zone (S3-7) and seawards (S8-10) (Figure 5b).

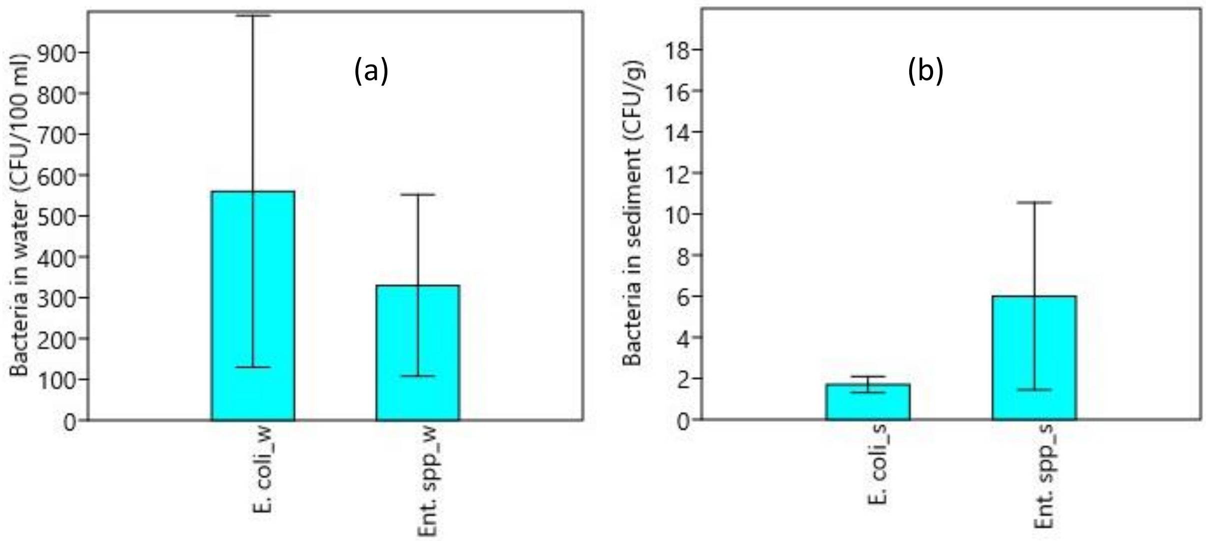

Figure 3. (a) Bacteria counts in water (CFU/100 ml) and (b) sediment (CFU/g). *Escherichia coli* is dominant in the water while *Enterococcus* spp. is more abundant in sediment. *E. coli*_w = *Escherichia coli* in water (CFU/100 ml), *Ent*. spp_w = *Enterococcus* spp. in water (CFU/100 ml), *E. coli*_s = *Escherichia coli* in sediment (CFU/g), *Ent*. spp_s = *Enterococcus* spp. in sediment (CFU/g).

Principal component analysis

The canonical analysis triplot diagram displays the spatial distribution of biological data and physicochemical parameters at the studied stations of the Densu Estuary (Figure 6). It shows the influence of physicochemical parameters on the spatial distribution of organisms at the ten stations. The first axis contributes 59.96% and the second axis 14.15% to the total variation (Figure 6). The long arrows (chlorophyll-*a*, pH, phosphate, nitrates, total dissolved solids and redox potential) show physicochemical parameters that significantly influence the spatial distribution of the species. The first axis is correlated with chlorophyll-*a*, alkalinity and temperature. The second axis reflects a gradient related to pH, phosphate, nitrates, total dissolved solids, redox potential, water depth, salinity, electrical conductivity, sulphate, total dissolved solids, dissolved oxygen concentration and saturation (Figure 6). The redundancy analysis (RDA) revealed that the first axis contributes 51.09% and the second axis 28.49% to the abundance of organisms at the ten stations (Figure 7). The pH is the primary environmental factor influencing the faecal bacteria and polychaete distribution in the Densu Estuary. The pH significantly (p = 0.002) explained 51.1% of the variation in the species abundance (Figure 7).

Pearson's correlation

The Pearson correlation coefficient (r) (Table 4) indicated that salinity significantly correlates positively with specific electrical conductivity (r = 0.999) and pH (r = 0.735). Specific electrical conductivity significantly correlates positively with pH (r = 0.720). Furthermore, the redox potential significantly correlated positively with pH (r = 0.850). Total dissolved solids also correlated positively with two variables: specific electrical conductivity (r = 0.863) and salinity (r = 0.864). In contrast, chlorophyll-*a* significantly correlated negatively with three variables: temperature (r = -0.691), pH (r = -0.675) and dissolved oxygen saturation (r = -0.725). Alkalinity significantly correlated negatively with three parameters: specific electrical conductivity (r = -0.891), salinity (r = -0.896) and total dissolved solids (r = -0.757). The concentrations of phosphate and nitrate also correlated significantly (r = 0.914), as did the nutrients with total suspended solids (phosphate, r = 0.862, nitrates, r = 0.870). Sulfate significantly correlated positively with water depth (r = 0.679). The WQI significantly correlated positively with pH (r = 0.727), specific electrical conductivity (r = 0.991, strong positive linear relationship) and total dissolved solids (r = 0.855) and negatively with alkalinity (r = -0.902, strong negative linear relationship) (Table 4). However, a significant ($p < 0.005$) linear association exists between physicochemical variables only (Table 4) and among biological data only (Table 5).

The abundance of the organisms correlated significantly among each other. Total coliforms in sediment significantly correlated positively with total coliforms in water (r = 0.641, p = 0.046) and chlorophyll-*a* (r = 0.815, p = 0.004), and negatively with redox potential (r = -0.656, p = 0.039) and pH (r = -0.832, p = 0.003). *E. coli* in sediment significantly correlated positively with water depth (r = 0.684, p = 0.029). *Enterococcus* spp. in sediment significantly correlated positively with total coliforms in sediment (r = 0.684, p = 0.029) and

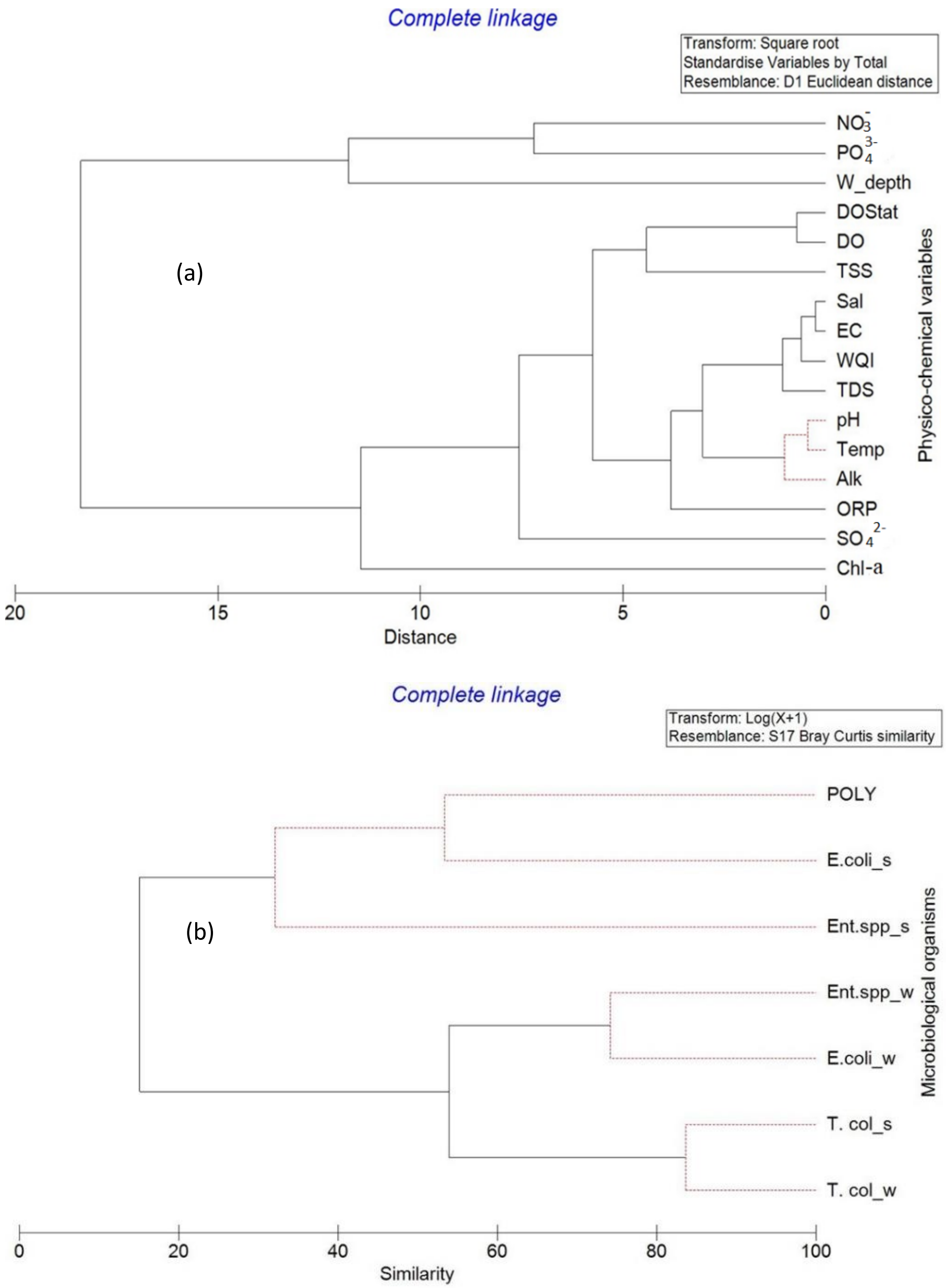

Figure 4. Hierarchical cluster of (a) physicochemical and (b) biological characteristics of the Densu Estuary. A significant similarity of associations exists between alkalinity, temperature and pH. A Significant similarity between organisms in the water and sediment. The thin red dotted lines indicate the significant structure of similarity (SIMPROF Test p<0.05). The black thick lines indicate no significant structure.

chlorophyll-*a* (r = 0.668, p = 0.035). Polychaetes significantly correlated positively with temperature (r = 0.639, p = 0.047), pH (r = 0.816, p = 0.004), redox potential (r = 0.778, p = 0.008) and negatively with chlorophyll-*a* (r = -0.857, p = 0.002), total coliforms in water (r = -0.684, p = 0.029), total coliforms in sediment (r = -0.833, p = 0.003), and *Enterococcus* spp. in water (r = -0.643, p = 0.045).

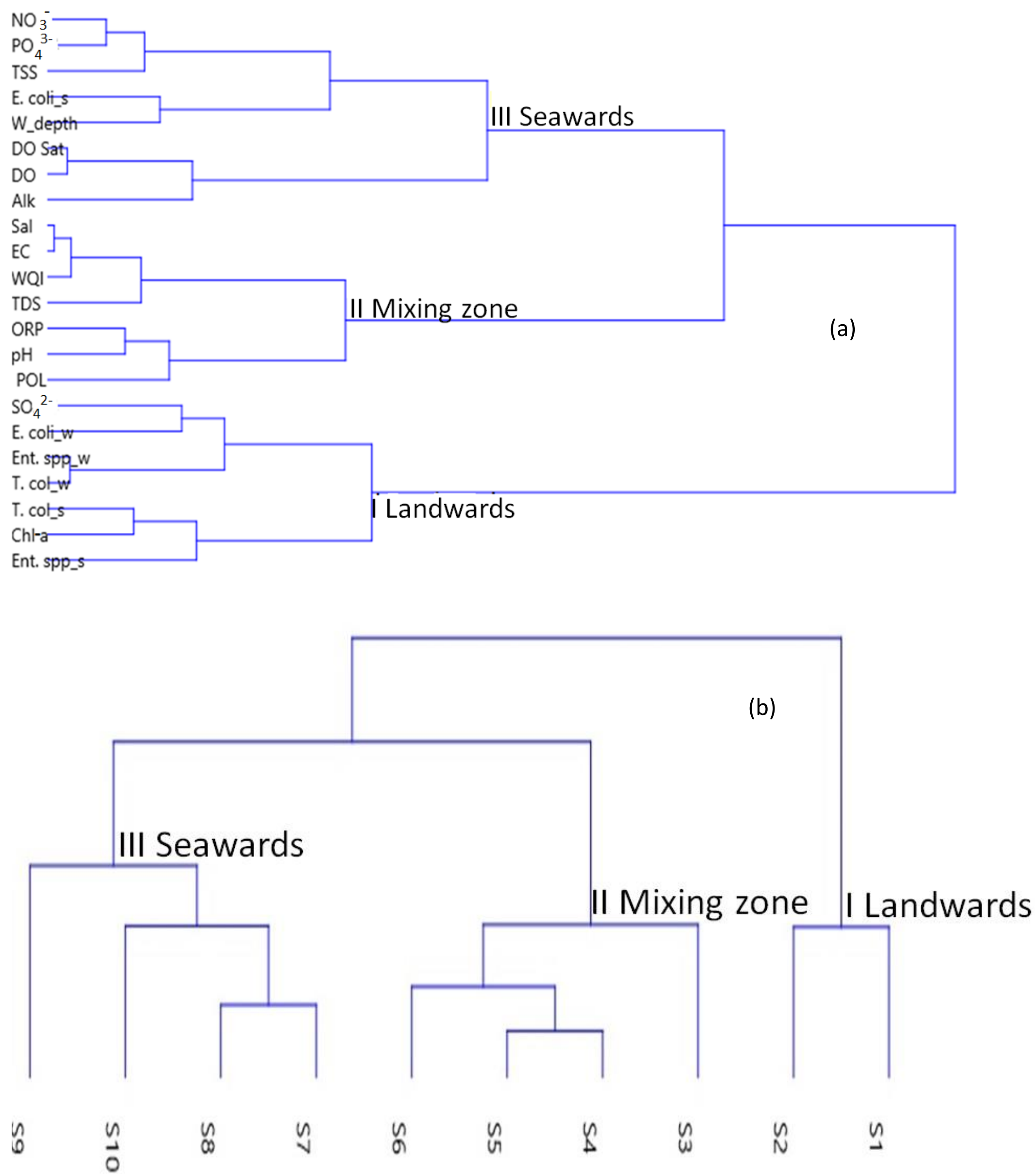

Figure 5. Cluster analysis (After Ward's method two way-constrained) of combined physicochemical and microbiological data (a) with the corresponding cluster of stations (b). The species-physicochemical data were group into three distinct clusters; (i) landward sources of pollution; (ii) mixing zone–estuarine condition (a mixture of river water and seawater); (iii) seaward sources of contamination. The corresponding stations (three discernable clusters) are landwards (S1-2), mixing zone (S3-6) and seawards (S7-10).

DISCUSSION

Physicochemical parameters

Estuaries are highly variable environments and controlled by estuarine flushing times (Cloern and Jassby, 2010; Day Jr. et al., 2012). Some physicochemical parameters (Figure 2 and Table 3) varied among the stations, demonstrating the heterogeneous condition of the Densu Estuary. The physicochemical nature of this estuary can be classified into three zones; the landward zone (without any freshwater input), the intermediate zone, (characterised by a mixture of fresh and oceanic water) and the end of the estuary (characterised by the intrusion of seawater).

The mean temperature (32.17 ± 0.95°C) (Table 3) recorded in April reflects the water temperature condition for coastal waters in the dry season (31 to 33°C) (Biney, 1982; Biney, 1993; Karikari and Ansa-Asare, 2006). The coastal waters of Ghana are situated in the tropical and

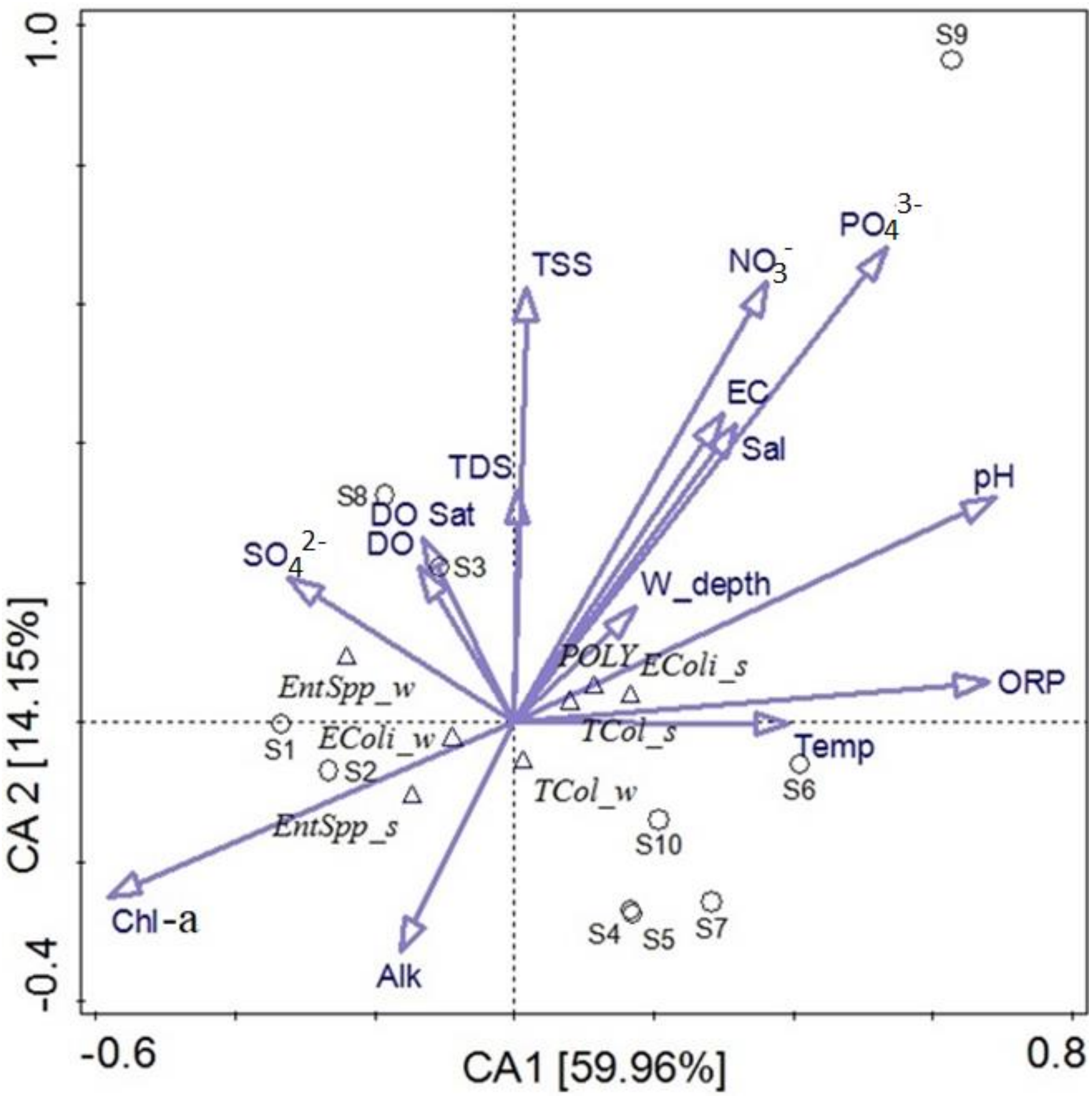

Figure 6. Canonical analysis (CA) of physicochemical (blue arrows) at the ten sampling stations (S1-10). The first and second axes contribute 74.11% of the total variation of the influence of physicochemical factors on the abundance of microbiological organisms among the stations. The orientations of these arrows indicate the correlation of these variables in CA ordination axes. The longer arrows indicate the most significant physicochemical variables on species distribution.

equatorial climate belt and annual mean temperatures range between 25 and 36°C with little variation throughout the year (Biney, 1982, 1993; Karikari and Ansa-Asare, 2006). The two climatic zones in the coastal areas of Ghana are both characterized by homogeneous temperatures between 23 and 32°C with a mean annual value of 27°C (Karikari and Ansa-Asare, 2006). Highest temperature (32°C) occurs in March-April and the lowest (23°C) in August (Dickson et al., 1988). A pH range from 7 to 9 is suitable for estuarine life (Anzecc, 2000). The pH values for the Densu Estuary were between 8.31 and 8.39 and thus within the range for natural waters not acidified yet (Stumn and Morgan, 1981; Biney and Asmah, 2010; Lamptey et al., 2013). The pH may be modified by biological activity, photosynthesis, temperature, oxygen content, ocean acidification, cation and anion composition (Doney et al., 2015; Abdel-Halim and Aly-Eldeen, 2016; Apriani et al., 2018; Tanjung et al., 2019). Furthermore, increasing carbon dioxide in the atmosphere can cause acidification of coastal marine

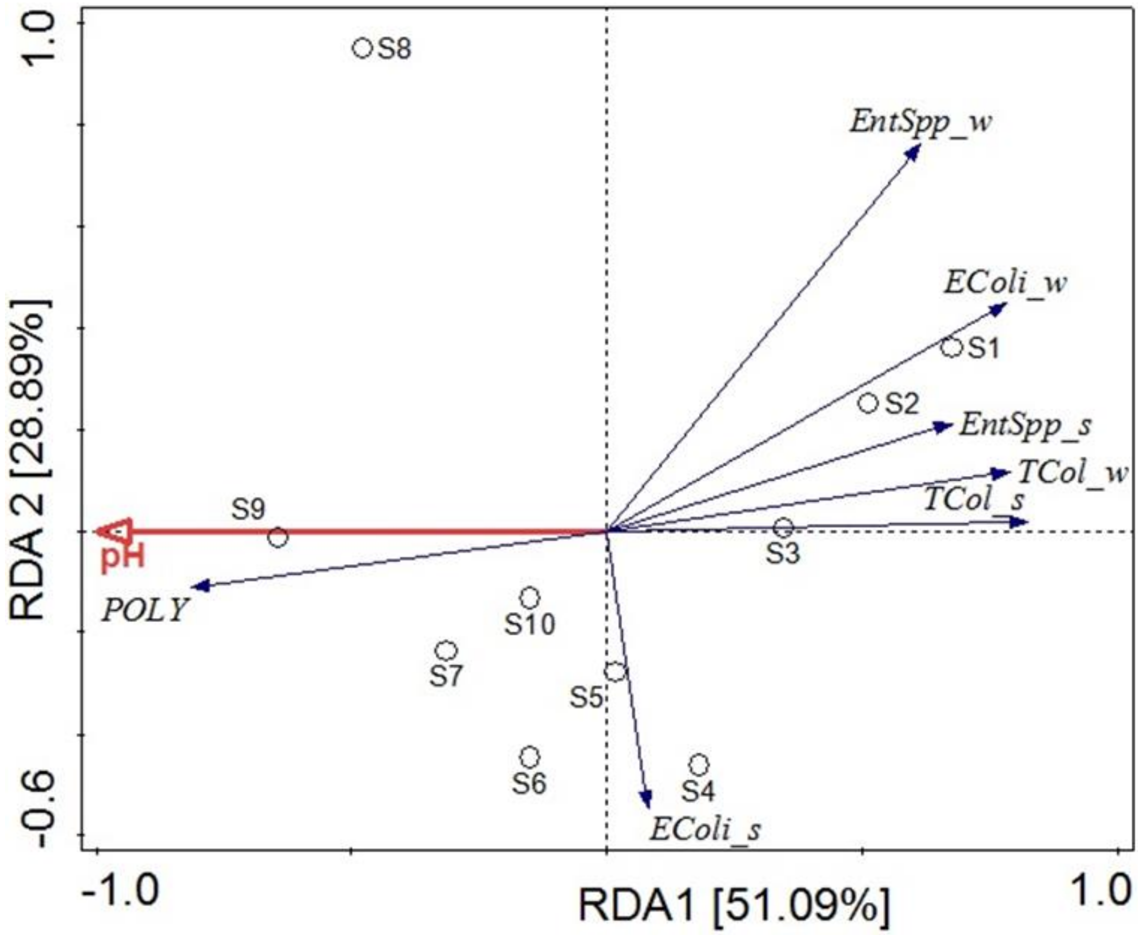

Figure 7. Redundancy analysis (RDA) diagram with best environmental variable (red arrow) selected by the forward selection procedure influencing the organisms (blue arrows) at the ten stations. The ordination diagram shows the first axis (horizontal) and the second (vertical) axis of distance-based constrained RDA. The first axis and second axis explained 79.58% of the total variation. The pH significantly (p = 0.002) explained 51.1% of the total variation in the faecal bacteria and polychaete distribution.

waters affecting its organisms (flora and fauna), function and ecosystem processes (Fabry et al., 2009; Doney et al., 2015; Curry, 2020).

The conductivity ranged from 35.8 to 52.5 $mScm^{-1}$, which is typical for estuarine waters (Biney and Asmah, 2010). Values for estuaries are typically from 20 to 40 $mScm^{-1}$, marine waters have much higher values (that is, 51.5 $mScm^{-1}$). Increased conductivity is directly related to increased concentrations of salinity and total dissolved solids. Thus, high conductivity levels are often associated with sewage discharge and leaching of inorganic contaminants (Harrison, 1999).

The salinity ranged from 22.8 to 34.7, which is categorized as polyhaline (18 - 30) at landward stations and mixoeuhaline (30 - 40) conditions at seaward stations (Vernice System, 1959). Salinity increases with increase specific electrical conductivity; this was also established for Songor Wetland (Klubi et al., 2019). Salinity significantly (r = 0.999) correlated positively with conductivity, suggesting a strong linear association.

The mean dissolved oxygen concentration (6.44 - 18.81 mg/L) was above the natural background (7.0 mg/L) (Biney, 1993; Clark, 2000). Dissolved oxygen concentrations of unpolluted water bodies range from 8.0 to 10.0 mg/L at 25°C (Pearce et al., 1999). The low level of total dissolved solids at S4 could be due to a less turbulent environment, whereas the high total dissolved solids in marine oceanic waters due to accumulated solutes in suspension and tidal influence. The highest dissolved oxygen concentration and saturation were recorded at S3, which is influenced by freshwater inflow from the Densu River with high hydrodynamics condition.

The redox potential ranged from 92 to 200 mV, which is below the redox potential for natural waters (between 500 and 600 mV) (McLusky and Elliot, 2004, 2010). The alkalinity levels (195.52 - 237.97 mg/L) was also below

Table 4. Pearson correlation among physicochemical parameters measured at the Densu Estuary, Ghana.

Parameter	Temp	pH	EC	Sal	DO	DOSat	ORP	W_depth	TDS	TSS	Alk	PO_4^{3-}	NO_3^-	SO_4^{2-}	Chl-a	WQI
Temp	1															
pH	0.312	1														
EC	-0.226	0.720*	1													
Sal	-0.220	0.735*	0.999**	1												
DO	0.514	-0.278	-0.468	-0.486	1											
DOSat	0.549	-0.234	-0.409	-0.429	0.991**	1										
ORP	0.368	0.850**	0.483	0.501	-0.118	-0.108	1									
W_depth	-0.218	0.098	0.026	0.032	0.305	0.210	0.311	1								
TDS	-0.264	0.494	0.863**	0.864**	-0.413	-0.395	0.263	0.108	1							
TSS	0.232	0.219	0.161	0.156	0.425	0.423	0.041	0.353	0.260	1						
Alk	0.545	-0.552	-0.891**	-0.896**	0.625	0.597	-0.411	-0.179	-0.757*	-0.032	1					
PO_4^{3-}	0.203	0.544	0.350	0.356	0.107	0.119	0.296	0.295	0.266	0.862**	-0.240	1				
NO_3^-	0.000	0.339	0.339	0.333	0.156	0.154	0.102	0.382	0.317	0.870**	-0.229	0.914**	1			
SO_4^{2-}	-0.249	0.106	0.541	0.531	-0.475	-0.376	-0.204	-0.679*	0.468	-0.264	-0.460	-0.217	-0.252	1		
Chl-a	-0.691*	-0.675*	-0.142	-0.144	-0.407	-0.441	-0.725*	-0.204	0.139	-0.268	-0.082	-0.429	-0.264	.342	1	
WQI	-0.264	0.727*	0.989**	0.991**	-0.566	-0.512	0.467	-0.001	0.855**	0.151	-0.902**	0.382	0.366	.528	-.099	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). Temp = Temperature (°C), EC = Electrical conductivity (mS/cm), Sal = Salinity, DO = Dissolved oxygen concentration (mg/L), DO Sat = saturation of dissolved oxygen (%), ORP = Redox potential (mV), TDS = Total dissolved solids (mg/L), TSS = Total suspended solids (g/L), Alk = Alkalinity (mg/L), PO_4^{3-} = Phosphate (mg/L), NO_3^- = Nitrates (mg/L), SO_4^{2-} = Sulphate (mg/L), Chl-a = Chlorophyll-*a* (µg/L) and WQI = Water quality index.

Table 5. Pearson correlation analysis between abundance of organisms found in water and sediment at the Densu Estuary, Ghana.

Correlation	T. col_w	*E. coli*_w	*Ent*. spp_w	T. col_s	*E. coli*_s	*Ent.* spp_s	POLY
T. col_w	1						
*E. coli*_w	0.962**	1					
Ent. spp_w	0.988**	0.971**	1				
T. col_s	0.641*	0.478	0.564	1			
*E. coli*_s	-0.308	-0.432	-0.380	0.256	1		
Ent. spp_s	0.456	0.217	0.429	0.684*	0.247	1	
POLY	-0.684*	-0.589	-0.643*	-0.833**	-0.360	-0.571	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). Bacteria in water (T. col_w = Total coliforms, *E. coli*_w = *Escherichia coli*, *Ent.* spp_w = *Enterococcus* spp.), Bacteria in sediment (T. col_s = Total coliforms, *E. coli*_s = *Escherichia coli*, *Ent*. spp_s = *Enterococcus* spp.) and POLY = Polychaetes.

the maximum contaminant levels of 500 mg/L suggested by the WHO, alkalinity ranging from 300 to 400 mg/L has been recommended for drinking water (WHO, 1999, 2011).

Nutrients (mainly phosphate and nutrients) are important chemical compounds in water quality monitoring (Conley et al., 2009). In the Densu Estuary, the mean phosphate concentration ranged between 0.05 and 1.10 mg/L, which is higher than the typical value for coastal marine waters of 0.02 mg/L (Biney, 1993; Oduro, 2003; Ouffoué et al., 2013). In most natural waters, phosphate ranges from 0.005 to 0.020 mg/L (Chapman, 1992), in some pristine waters, the phosphate concentration may even be as low as 0.001 mg/L (Chapman, 1992). The phosphate concentration can be used to categorized ecosystem: (i), <0.02 mg/L = healthy ecosystem, (ii) 0.02 to 0.3 mg/L = fair ecosystem, and (iii) > 0.3 mg/L = poor ecosystem. Thus, the Densu Estuary is classified as a poor estuarine ecosystem.

The nitrate concentration ranged from 1.7 to 7.5 mg/L and are thus at four of the five stations (S2, S3, S5 and S10) higher than the recommended 0.25 mg/L for coastal waters. Large quantities of nitrate and phosphate lead to eutrophication with high primary productivity (algal blooms) (Conley, 2000; De Jonge et al., 2002; Saad and Younes, 2006; Cook et al., 2018). However, in turbid estuaries, the light may limit phytoplankton blooms (Ambasht and Ambasht, 2005; Bucci et al., 2012; Green et al., 2015). The trophic state (chlorophyll-*a* concentration ranged between 0.96 and 4.38 µg/L) is interpreted as ultra-oligotrophic (<1.0) to mesotrophic (3.0-8.0) condition. Chlorophyll-*a* concentration is an estimate of phytoplankton biomass (Tripathy et al., 2005) and thus a key component of food availability for benthic and filter-feeding animals (Fujii, 2007). A positive relationship was found between the chlorophyll-*a* concentration and the abundance of macrofaunal species (Lamptey and Armah, 2008; Musale and Desai, 2011).

The WQI ranged between 359.49 and 484.62 and was thus >300, which is categorized as unsuitable water for drinking and indicates a deteriorating ecosystem (Ramakrishnaiah et al., 2009; Lamptey et al., 2013; Nguyen and Sevando, 2019). However, a good water quality is necessary to sustain the living resources of the ecosystem (Nyarko et al., 2015; Duncan, 2018; Tanjung et al., 2019).

Biological indicators of environmental quality

The dominance organisms reflect the sediment quality, which is also reflection of the water quality status. High bacteria load (total coliforms, *E. coli* and *Enterococcus* spp.) (Figures 2 and 3 and Table 3) especially at S1 and S2 reflect high anthropogenic activities (e.g., landfill sites, domestic and animal waste discharges) in the landward direction of the estuary. Faecal bacteria enter surface waters by direct deposition of human and animal waste discharges and indirectly through land runoff and leach into the Densu Estuary. Microbial contamination of water and sediment is a growing concern for the ecosystem and human health (Odonkor and Ampofo, 2013; Walker et al., 2015). Faecal indicator organisms are often used to detect and quantify aquatic pollution (Horan, 2003; Odonkor and Ampofo, 2013). The faecal bacteria (total coliforms, *E. coli* and *Enterococcus* spp.) are used to indicate pathogens of faecal origin in surface and coastal water bodies (Medema et al., 2003; Pandey et al., 2014). Faecal bacteria (*E. coli* and *Enterococcus* spp.) are characteristic intestinal bacteria of warm-blooded animals (Medema et al., 2003; Shuval, 2005). *Escherichia coli* (also known as faecal coliform) is the best bacterial indicator of faecal pollution (Stewart et al., 2008; Walker et al., 2015). Faecal enterococci are also used as complementary microbiological water quality indicator (Byamukama et al., 2000). The presence of coliform bacteria suggests a potential for waterborne related pathogens to be present (Shuval, 2003; Jain, 2013). The presence of faecal bacteria (*E. coli* and *Enterococci* spp.) indicates sources of human and animal pollution in the Densu Estuary.

The dominance of polychaetes (*Capitella* and *Nereis* spp.) also suggests environmental pollution in the Densu Estuary. Polychaete worms were the most dominant living macrofauna. These polychaete species are most abundant in organic matter enriched sediments (Saleh, 2012; Aqilah et al., 2016). *Capitella* spp. are sedentary deposit feeders (Levinton and Kelaher, 2004; Lamptey and Armah, 2008; Musco et al., 2009). Polychaetes may be used as sensitive indicators of anthropogenic disturbances, such as organic pollution (Cai et al., 2001; Elias et al., 2006; Metcalfe and Glasby, 2008). Several polychaetes are opportunistic species capable of reproducing after an increase in organic matter (Giangrande et al., 2005; Musale and Desai, 2011). In an estuary and mangrove environment, polychaetes provide food for shorebirds, for instance, the Bar-tailed Godwit *Limosa lapponica* feeds on *Nereis* spp. (McLusky and Elliot, 2010). The polychaetes, *Capitella* and *Nereis* spp. often dominate soft bottoms of polluted and organic enrichment waters (Alongi, 1990; McLusky and Elliot, 2004; Wada et al., 2008; Cai et al., 2013). Under anoxic conditions, most of the macrofaunal can become extinct (McLusky and Elliot, 2010). A small number of empty shells of molluscs and gastropods was observed.

Spatial similarity and variations of stations

The hierarchical clusters (Figures 4a-b and 5a-b) indicated spatial similarity among physicochemical factors only, biological data only and combined effects (Mac Nally, 1996). Cluster and Pearson's correlation analyses revealed a significant ($p < 0.05$) association of microbiological organisms in water and sediment (Figures 4a-b and 5a-b); (i) total coliforms in water and sediment, (ii) *E. coli* and *Enterococcus* spp. in water, and (iii) *E. coli*, *Enterococcus* spp. and polychaetes in sediment. There was a clear evidence of ecological interactions. Temperature, pH and alkalinity were significant factors in the estuary system. Any change of these variables may change the condition of the estuary. The contamination of stations (S1 and S2) originates from land sources, apparently anthropogenically influenced (e.g., domestic and animal waste disposal). The mixing zone (S3-7) is characterized by catchments of the river mixing with marine water. The marine zone is located downstream of the estuary with the intrusion of saline, oceanic waters into the Densu Estuary (S8-10). This shows the interaction of water and sediment characteristics of eco-hydrochemical estuarine conditions. Principal component analyses (PCA), in harmony with the cluster analyses (CA) (Figures 5 to 7), indicated landward sources of major pollution, hydrodynamic condition of freshwater and seawater mixing and marine intrusion into the estuarine system. Thus, the Densu Estuary is characterized by distinct hydrochemical dynamic pathways as observed in Songor Wetland (Klubi et al., 2019).

There is a strong interaction of biotic and abiotic component of the ecosystem and among each other (Tables 4 and 5 and Figures 5 to 7), as expected (Borja et al., 2012). The state of water quality is reflected also in the sediment quality, an insight into the ecological integrity of the Densu Estuary. The integration of physico-chemical and biological assessment of coastal ecosystems pollution status is critical for the broader understanding of various pathways of environmental contamination and sustainable management of coastal waters (Ambasht and Ambasht, 2005; Ouffoué et al., 2013; Larbi et al., 2018).

Conclusion

The knowledge of ecological integrity of estuaries along the coast of Ghana is still scarce. Because of its fisheries resources the Densu Estuary is of high socio-economic importance for the coastal communities. The deforestation of the Densu Delta wetland (a Ramsar site) and poor sanitation in the vicinity will not only affect the regulation of the local hydrological cycles, loss of habitats and introduce flood-related risks, but also water-borne diseases. The environmental quality of the estuary was assessed using multiple ecological indicators. The study emphasized on physicochemical drivers of organisms in the Densu Estuary.

Significant variation ($p < 0.05$) of some physicochemical parameters occurred among the stations. There is a clear salinity gradient from 22.8 to 34.7. Nutrient (nitrate and phosphate) concentrations exceeded recommended levels for natural coastal waters, indicating a degraded ecosystem. Phosphate concentrations ranged from 0.05 to 1.10 mg/L. Nitrate ranged from 1.7 to 7.5 mg/L. Dissolved oxygen concentrations ranged from 6.44 to 18.81 mg/L. The computed WQI ranged from 359.49 to 484.62 and thus indicated a deteriorating system.

The abundance of organisms significantly varied between the sampling stations. The presences of faecal bacteria (total coliforms, *E. coli* and *Enterococcus* spp.) suggest faecal contamination. The dominance of key macrofaunal (e.g., *Capitella* and *Nereis* spp.) suggests organic pollution. The results indicate an impacted environment of the Densu Estuary, which can impose ecosystem and human health risks, a cause for further investigation.

The cluster analyses helped to classify the stations into three major groups; landward zone, intermediate zone and seaward zone. The physicochemical parameters coupled with biological data were also grouped into three distinct clusters, mainly landward, mixed zone and marine sources. The significantly contaminated stations (S1 and S2), may be influenced by domestic and animal waste disposal, the intermediate zone (S3-7), characterized by mixing of freshwater and marine water and the seaward zone (S8-10) is characterized by seawater intrusions into the estuary. The PCA performed on physicochemical and biological data helped to identify natural and anthropogenic sources of the contamination. The first two axes explained 74.11% of the variation in the abundance data. The pH is the most influential ecological factor that explained the spatial distribution of faecal bacteria and polychaetes in the Densu Estuary.

The faecal bacteria (*E. coli* and *Enterococcus* spp.) showed significant ($p < 0.05$) positive correlation with chlorophyll-*a* concentration, but negatively correlated with redox potential and pH, whereas polychaetes displayed a significant positive correlation with temperature ($r = 0.64$), pH ($r = 0.82$) and redox potential ($r = 0.78$), but negative with chlorophyll-*a* concentration ($r = -0.86$).

The findings provide an ecological baseline for environmental monitoring and for effective policy formulation to control discharges of waste into coastal waters. The study contributes to an ecological perspective on the environmental quality of the Densu Estuary using multiple indicators to identify the different sources of environmental pollution.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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