



**Mitigation of Dinoflagellate Biofilms using *Dendrobranchiata Sp.* along Iko River Estuary,  
South Eastern Nigeria**

**Ini-Ibehe Nabuk Etim<sup>a,b,\*</sup>, Emmanuel Asuquo Akpan<sup>a,\*</sup>, Ini-Abasi Cornelius Akpan<sup>a</sup>, Paul  
Sunday Udom<sup>a</sup>**

Received: March 13, 2024 / Accepted: May 18, 2024

© REJOST 2024

**Abstract**

Biofilms are complex surface-attached communities of bacteria held together by self-produced polymer matrix, and primarily composed of extracellular polymeric complexes. Biofilms are known to adhere to any material surface due to the growth and adhesion of microorganisms and are a vital component in the microbial corrosion of concrete. One of the peculiar types of bacteria that produce biofilm is the *Dinoflagellates sp.* This study aims to adopt chitosan component derived from *Dendrobranchiata sp.* (Prawn) to mitigate the biofilm growth of *Dendrobranchiata sp.* The sampling was carried out at the Iko River Estuary, Eastern Obolo, South Eastern Nigeria. Samples were taken from 5 different stations; station 1 (Iko Jetty), station 2 (Otowaji), station 3 (Ayalekpong), station 4 (Estuarine mouth), and station 5 (Akasa). The characterization methods adopted for the study were surface morphology using a microscope, UV-spectrophotometric technique for the determination of the concentration of *Dinoflagellates sp.*, and physicochemical parameters determination of the turbidity, salinity, temperature, pH, dissolved oxygen (DO), and conductivity. Chitosan samples obtained from *Dendrobranchiata sp.* (Prawn) undergo three stages of reactions; demineralization, deproteinization, and deacetylation. The surface morphology characterization showed the presence of various dinoflagellate species such as *Dinophysis caudata*, *Cochlodinium pirum*, *Phalacroma cuneus*, and *Dinophysis hastata*. A higher pH value of about 9.0 was observed at the mouth of the estuary due to the influx of seawater. The 5-day results showed an increase in the biofilm growth as observed in the concentration results in the five stations. The highest concentration value of about 0.75 mg/L was observed on day 1, and the lowest value of about 0.40 mg/L was observed on day 5. The concentration value for media with chitosan was about 0.20 mg/L, while the media with *Dinoflagellates sp.* had about 0.40 mg/L. The reduction could be attributed to the decrease in the availability of nutrients for bacteria growth when chitosan was applied. The mitigation approach indicates that the chitosan acted as an excellent antimicrobial agent for the mitigation of *dinoflagellates sp.* biofilm growth.

✉ Ini-Ibehe Nabuk Etim' [ini-ibeheetim@aksu.edu.ng](mailto:ini-ibeheetim@aksu.edu.ng); Tel.: +2348064028238

<sup>a</sup> Marine Chemistry and Corrosion Research Group, Department of Marine Science, Akwa Ibom State University, P. M. B. 1167, Nigeria

<sup>b</sup> Africa Centre of Excellence in Future Energies and Electrochemical Systems (ACE-FUELS), Federal University of Technology, PMB 1526, Owerri, Nigeria

\* The manuscript writing was equally contributed

**Keywords:** Biofilm, *Dinoflagellates sp.*, *Dendrobranchiata sp.*, Chitosan, Iko River Estuary

## 1.0 Introduction

They are embedded in polymeric structural scaffolds and serve as a dynamic milieu for intercellular communication through physical and chemical signaling. The marine ecosystem has distinct microorganisms that produce biofilms that adhere to the environment. It is relatively known that biofilms are those extracellular polymeric substances produced from microbial secretion. Biofilm has also been reported in some literature due to its importance in biocorrosion and environmental studies (Etim *et al.*, 2018; Li & Ning, 2020). A biofilm secretion is understood to be an assembly of sessile microbes with a distinctive organized form and features such as projections and channels (Das *et al.*, 2023). Clear observations have revealed that biofilm microorganisms prevail, metabolically and numerically, in practically all nutrient-tolerable marine ecosystems. Thus, these biofilm microorganisms dominate many of the environmental, medical, and industrial issues and sectors of benefit to researchers (Costerton *et al.*, 2003). Deciphering the microbial ecology of biofilms in various natural or engineered settings has revealed the coexistence of microorganisms like *dinoflagellates sp.* from all domains of life, including eukarya, bacteria and archaea (Sadiq *et al.*, 2022).

The dinoflagellates belonging to the class (Dinophyceae) and division (Pyrrhophyta) are the essential part of phytoplankton in marine environment (Hackett *et al.*, 2004). They are part of the main groups of phytoplankton existing in freshwater and marine habitats,

exceeding 2000 species and over 200 genera (Taylor *et al.*, 2008; Vernal *et al.*, 2013). A dinoflagellate is characterized by its two flagella of unequal length. One of the flagella is lying in the groove around the body and the other is extending from the center. It also has an armor-like shell or pellicle, a dinokaryon, and a dinoflagellate toxin. Many of them are photosynthetic. The pigments present are chlorophyll a and c, carotenoids, and xanthophylls. Considering the vast span within the freshwater and sea environment of the globe, dinoflagellates exhibit a key part in the water ecology and trophic chain (Carlsson *et al.*, 1999). They form an essential cluster of unicellular eukaryotic microorganisms and could exist as autotrophic, some parts are photosynthetic, parasitic, heterotrophic, and/or symbiotic, or mixotrophic. The majority of the autotrophic dinoflagellates have a unique carotenoid peridinin, pigment, in connotation with chlorophylls c and a and various pigments like dinoxanthin, carotene, and diadinoxanthin. Dinoflagellates are also diverse in their modes of nutrition. Autotrophic, auxotrophic, mixotrophic, and heterotrophic forms have been documented (Canion, 2004). Based on the aforementioned adverse effect of *dinoflagellates sp.*, it is pertinent to seek mitigating ways of reducing its harmful influence on the marine ecosystem. Many researchers have presented useful tools to mitigate the influence of bacteria on the marine ecosystem such as the use of antibiotics (Castaño-Ortiz *et al.*, 2023), physical cleaning methods, the application of chitosan (Etim *et al.*, 2015a; Etim *et al.*, 2015b) and many others.

Interestingly, the common method often used is the use of biologically synthesized chitosan from microorganisms because it is environmentally friendly.

Chitosan is a natural biodegradable, and non-toxic biopolysaccharide that is derived from chitin and has the potential to be used as a natural antimicrobial agent. Chitosan is a  $\beta$ -1, 4-D-glucosamine polymer derived from alkaline chitin deacetylation (James *et al.*, 2019; Kou *et al.*, 2021). Chitin is a structural component of fungi, insects, and crustaceans insoluble in many solvents, but soluble in organic acids (Iqbal *et al.*, 2019). Chitosan exhibits high antimicrobial activity against a wide variety of spoilage and pathogenic microbes. The antimicrobial activities of the derived chitosan were influenced by the variety of chitosan, the extent of polymerization, the type of microbes, environmental conditions, and the occurrence of the other components. The adoption of chitosan via food systems should be on the knowledge of the mechanisms of the antimicrobial approach of function (James *et al.*, 2019). A typical chitosan is derived from the shells of *Dendrobranchiata sp.* commonly found in the sediment and other parts of the marine environment (Chowdary *et al.*, 2022; Hartoko & Rudiyananti, 2010; Ubulom *et al.*, 2022). The *Dendrobranchiata sp.* otherwise known as prawns are marine crustaceans relating to the decapoda family and are regularly characterised based on their body habitat and structure (Gbenebor & Sewanu, 2021). The prawn shell of *Dendrobranchiata sp.* is characterized by overlapping side plates which distinguish their body structure from other crustaceans like shrimps. Their  $\text{CaCO}_3$  presence in the *Dendrobranchiata sp.* makes it useful as a chitosan.

Marine ecosystems are governed by microbes, single-celled organisms, including autotrophic and heterotrophic prokaryotes, autotrophic and heterotrophic eukaryotes, and viruses (Ekeocha

*et al.*, 2023; Gómez, 2019; Umoh *et al.*, 2014). In the case of prokaryotes, they are characterized by their small size (0.3-1.0 $\mu\text{m}$ ), large numbers (*i.e.* bacteria 10<sup>6</sup> cells per litre), fast growth, and capability to occupy all types of environments (soil, fresh and salty waters), even under extreme conditions of pH, pressure, or temperature. Moreover, these organisms exhibit an essential function within the biogeochemical cycles of nutrients, *e.g.*, carbon, nitrogen, phosphorus and sulphur that affect the global climate. Thus microbes, in general, and algae and bacteria in particular, are important components of the global ecosystem and key players in its functioning (Gómez, 2019; Udemé *et al.*, 2022). In several marine ecosystems, a great number of microorganisms thrive in polymeric-enveloped biofilms and provide significant nutrient cycling and energy flow (Battin *et al.*, 2003). Multiple organisms and material-attached biofilms usually control microbial life in marine environments such as rivers and streams, contributing considerably to biogeochemical mechanisms. The microorganism's variance in naturally existing biofilms in marine ecosystems is much and may have essential consequences for the functioning of the ecosystem services and marine environments they produce (Besemer, 2015). The microbial habitat represents a phenomenal diversity of microorganisms from different kingdoms of life that occupy an impressive set of ecological niches. Most, if not all, microorganisms that once colonize a surface develop architecturally complex surface-adhered communities, which we refer to as biofilms (Etim *et al.*, 2020; Uzoma *et al.*, 2023).

Biofilm produced by *Dinoflagellates sp.* is one of the most important components of the marine ecosystem due to its adverse effects. The removal of this microbial species from the environment to limit marine ecosystem deterioration is paramount to researchers and industrial personnel. This peculiar study has

gained less attention, especially in southern Nigeria's coastal environment. Thus, it became imperative to investigate and outline the reduction of this biofilm produced by *Dinoflagellates sp* from the marine environment. In order to achieve this objective, chitosan made from *Dendrobranchiata sp.* was adopted to mitigate the biofilm growth of the microbes in south-eastern Nigeria.

## 2.0 Experimental

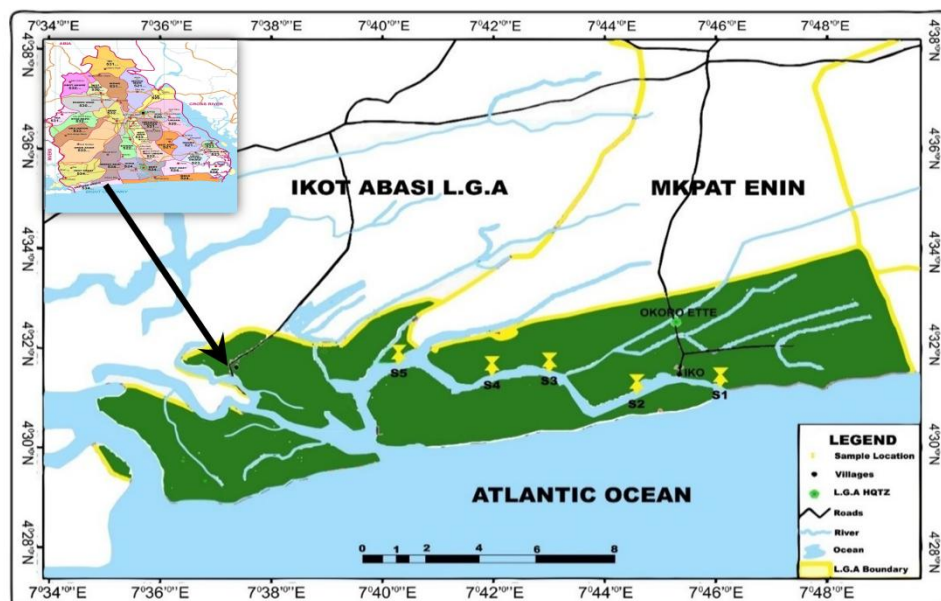
### 2.1. Sample location, Materials and methods

In this study, five different sampling stations were selected; station 1 (Iko Jetty), station 2 (Otowaji), station 3 (Ayalekpong), station 4

(Estuarine Mouth), and station 5 (Akasa) as shown in Table 1 and Fig.1. At each station, a series of samples were collected and brought to the laboratory in sterile polythene bottles for investigation and analysis. Qualitative dinoflagellate samples were collected by towing a 55  $\mu\text{m}$  mesh plankton net just below the water surface for 5 minutes at each sampling station. The samples were immediately fixed in an ice-block cooler (Miyakawa et al., 2024; Schuler et al., 2023), and moved for analysis in the Department of Marine Science, Akwa Ibom State University, Nigeria, for analysis and identification. The samples were preserved in sample bottles.

**Table 1:** The sample locations and its coordinates

Station	Name of station	Longitude	Latitude
1	Iko Jetty	7°46'' E	4°31'' N
2	Otowaji	7°45'' E	4°31'' N
3	Ayalekpong	7°43'' E	4°31'' N
4	Estuarine Mouth	7°44'' E	4°31'' N
5	Akasa	7°40'' E	4°31'' N



**Figure 1:** Plot of the sample location

## 2.2 Adoption of physicochemical parameters

### 2.2.1 Turbidity

Turbidity measurement was carried out using a “secchi disk”. This disk represents an 8-inch-long disk that has alternating white and black quadrants often immersed within the water column for a period of time until it disappears from the surface (Bowers et al., 2020). The region where the secchi disk disappears represents the water turbidity. The calibrated line was adopted as a substitute for immersing the disk within the water body. The depth where the disk sides vanish in the water body was marked on the line and taken as the turbidity depth.

### 2.2.2 Salinity

The measurement of salinity was carried out adopting refractometer equipment. Refractometer works by measuring how much light bends as it passes through the water (refraction) (Talley & Talley, 2008). To use the refractometer, a few drops of water were placed on the glass surface and then the clear cover was placed over the sample. The refractometer was pointed at a bright light source, preferably in the direction of the sun. Then the eyepiece was viewed through the back to get the measurement. The salinity measurement was taken wherever the coloured half of the viewfinder stopped.

### 2.2.3 Surface water temperature

A thermometer was adopted to check the temperature of the samples collected. The thermometer was cleaned before use to aid proper reading of the device. The tip of the thermometer was then placed carefully in the water bottle containing the water samples and held in there for about 2 -3 minutes before being removed to take the reading.

### 2.2.4 pH

The pH meter was used for this purpose. The equipment was calibrated/standardized before use with known buffers such as pH 4 (acidity),

pH 7 (neutral), and pH 10 (alkalinity). Before calibration, the pH meter was turned on and allowed to warm up for about 30 minutes, and then the probes/electrodes were washed with distilled water and kept clean for the tests. The buffers for calibration were then prepared starting from neutral to alkaline. After the buffer was chosen, it was allowed to reach the same temperature as the pH meter because pH readings are temperature-dependent. The buffers were then poured into individual beakers for calibration. The electrodes were then placed in the buffer zone after the other until the desired pH value was reached. The pH meter was then set up after the reading had stabilized. The water sample was filtered to remove impurities with filter paper, and then the electrodes were placed in the filtered water sample for about 1-2 minutes and allowed to be read.

### 2.2.5 Dissolved Oxygen (DO)/Conductivity

A portable conductivity meter was used to measure the DO and conductivity. The device was used for in situ and ex situ analysis. The device consists of a probe connected to a meter/analyzer and is similar in construction to pH meters. When the sensor is submerged in a liquid, oxygen from the liquid crosses the membrane and reacts with the cathode, causing a measurable current change; this change was converted into a millivolt output and was finally displayed by the meter.

## 2.3 Preparation of chitosan

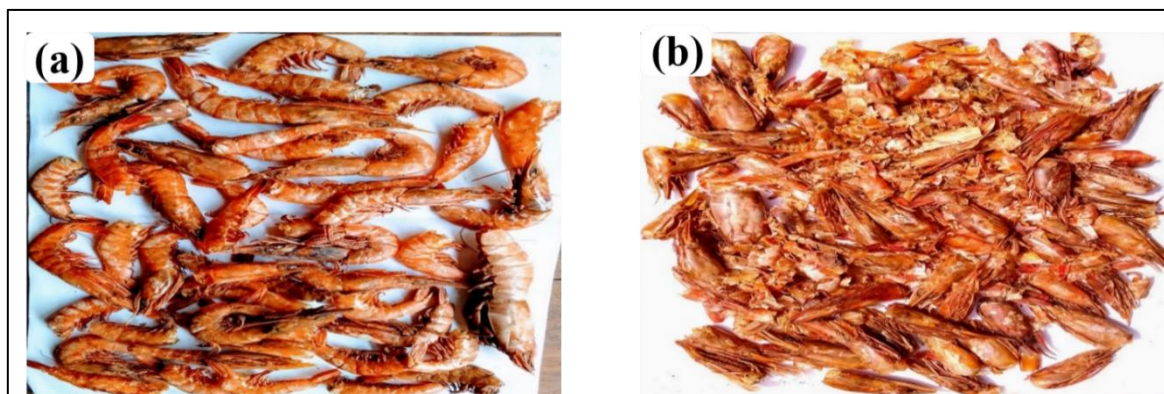
Chitosan was prepared from crustacean shell waste (Etim et al., 2015; Kou et al., 2021) as shown in Figure 2. Figure 2a shows the *Dendrobranchiata sp* after purchase from a local market while Figure 2b is the selected shell from *Dendrobranchiata sp*. The chitosan was weighed to know the percentage yield as calculated in equations 1 and 2. The percentage yield was calculated to compare the yield (quantity) of the product obtained with what could have been obtained in theory, if all of the

reactants were converted with no loss or waste. The three detailed processes - demineralization, deproteinization and deacetylation are reported in Table 2.

$$\text{Percentage yield (\% yield)} = \frac{\text{Weight of Chitosan} \times 100}{\text{Weight of Sample}} \quad (1)$$

$$\begin{aligned} \text{Percentage yield (\% yield)} &= \frac{3.50 \times 100}{10} \\ &= 35\% \end{aligned} \quad (2)$$

Figure 3 shows the chitosan sample derived from *Dendrobranchiata sp.* (Prawn) which underwent three stages of reactions; demineralization, deproteinization and deacetylation.



**Figure 2:** (a) Shows the *Dendrobranchiata sp.* used for the preparation of the chitosan and (b) shows the shell waste obtained from the *Dendrobranchiata sp.*

## 2.4 Characterization of chitosan using UV-visible spectroscopy

The chitosan sample for the analysis was prepared by dissolving 0.1 g of chitosan in 50 ml of a 1% (w/v) HCl solution. After complete dissolution, the solution was centrifuged at 3000 rpm for 10 minutes to eliminate the insoluble material. The UV-visible absorption result was achieved by adopting a UV1000 spectrophotometer (Tech-comp Ltd., China) in the range of 200 - 500 nm. Thus, the data can be analyzed to check the spectroscopic properties of the chitosan powder. For the determination of the concentration of dinoflagellates, a wavelength – 474 nm was used (Besemer, 2015; Wang & Liu, 2017).

## 3.0 Results and discussion

### 3.1 Surface morphology characterization

Figure 4 shows the surface morphologies of

different dinoflagellate species observed using a light microscope in dinoflagellate samples obtained from the five stations, starting from station 1 (Iko Jetty) to station 5 (Akasa) respectively.

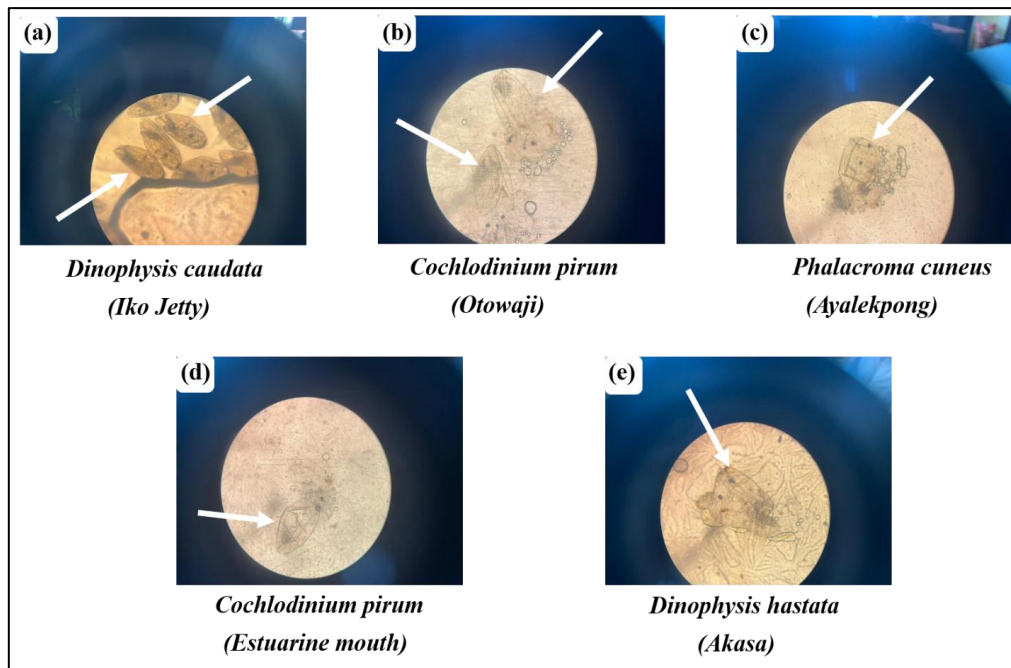
### 3.2. In situ physiochemical analysis

Figure 5 represents the salinity and temperature at every sampling site. Salinity was found to be higher in stations 5 (Estuary Mouth) and station 5 (Akasa). This is because these stations were located at the river mouth and influenced by seawater. Salinity gradually decreased at station 3 to 1. Largely, fluctuations within the salinity of brackish water environments like estuaries are prone to the incursion of freshwater through land runoff, initiated by tidal fluctuations or monsoons. Temperature measurement was moderate in all the stations.

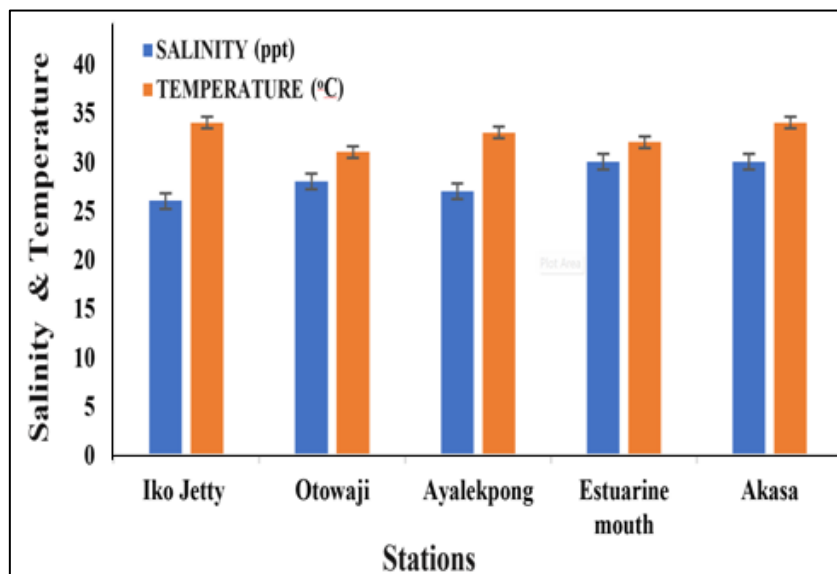
**Table 2:** Preparation of chitosan from *Dendrobranchiata sp.* (Prawn) shell

DEMINERALIZATION	STEPS
	<ol style="list-style-type: none"> <li>I. 5 ml of hydrochloric acid (HCl) was weighed and filled into the volumetric flask.</li> <li>II. 50 ml of H<sub>2</sub>O was added into the 1000 ml measuring cylinder.</li> <li>III. HCl was added to 50ml of H<sub>2</sub>O.</li> <li>IV. 50 ml of H<sub>2</sub>O was added into the solution to make it 100 ml of distilled water.</li> <li>V. About 10 grams of the material from prawn was added to the conical flask on the weighing balance.</li> <li>VI. The medium was then included to the raw materials in the conical flask to remove the CaCO<sub>3</sub> in the shell by reacting for 2 hours with agitation.</li> <li>VII. After stirring, it was filtered through the filter paper to get the CaCO<sub>3</sub> from the shell of the prawn (Kou et al. 2021).</li> </ol>
DEPROTEINIZATION	<ol style="list-style-type: none"> <li>I. 7 grams of Sodium hydroxide (NaOH) was weighed and added into 1000 ml measuring cylinder.</li> <li>II. 100 ml of distilled H<sub>2</sub>O was added and stirred completely till it dissolved.</li> <li>III. 50 ml of NaOH was added to the sieved residues and heated for 6 hours at the rate of 85 °C.</li> <li>IV. The solution was removed and filtered after it was cooled (Kou et al. 2021).</li> </ol>
DEACETYLATION	<ol style="list-style-type: none"> <li>I. 40 % of NaOH was added to the prawn particles and heated at the temperature of 140 °C.</li> <li>II. Distilled water was used in washing the particles to obtain the normal pH.</li> <li>III. The chitosan was obtained by drying at 60 °C for 24 hours in the oven (Kou et al. 2021).</li> </ol>

**Figure 2:** Sample of prepared chitosan



**Figure 4:** Surface morphologies of dinoflagellate species



**Figure 5:** Column chart representing temperature and salinity at each station

Figure 6 indicates that the turbidity (having lees or sediment disturbed) was found to be higher at station 2 (Otowaji) and lower at stations 1, 3, 4, and 5. The results could be attributed to rural and commercial fishing activities, sand dredging, and high tides going on in the environment.

### 3.3 Ex situ physiochemical analysis

Figure 7-8 shows the results of ex situ physiochemical parameters taken from each of the stations. These parameters were carried out to understand some of the processes occurring in the Iko River estuary.



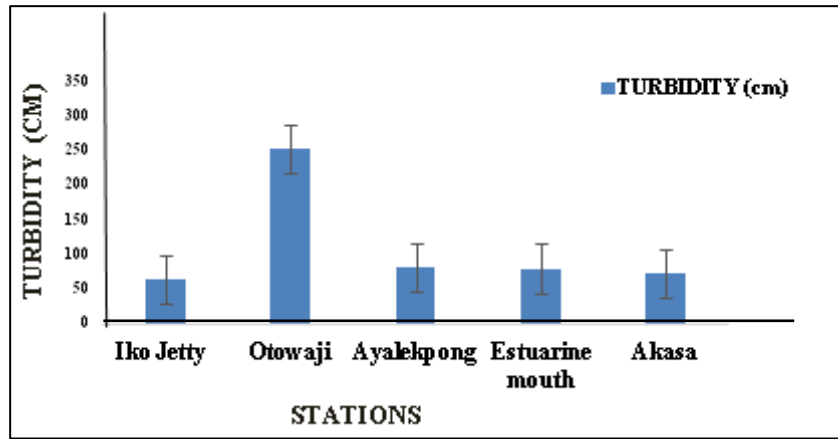


Figure 6: Column chart showing turbidity at each station

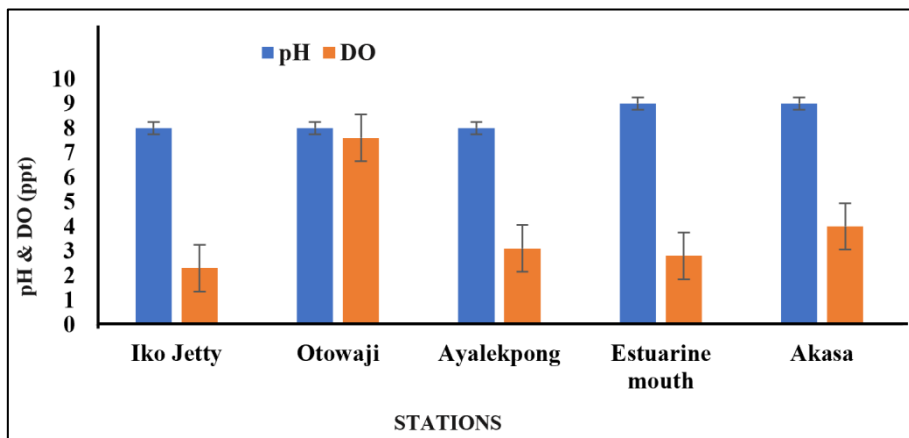


Figure 7: Column chart representing pH and DO at each station

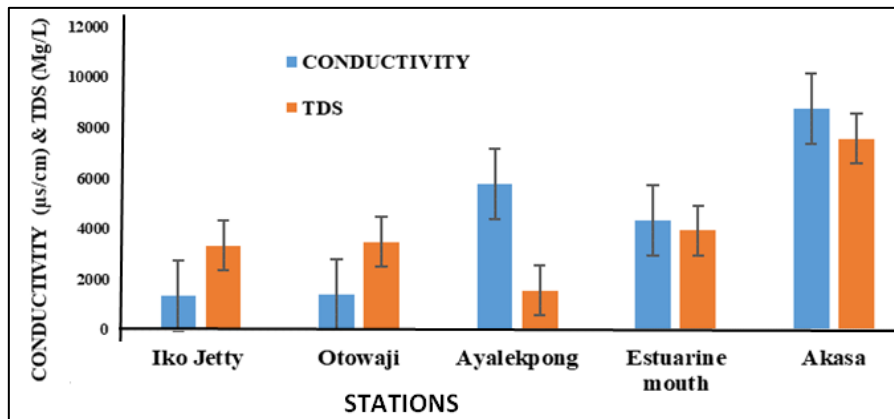


Figure 8: Column chart representing Conductivity and TDS at each station

Ex situ pH, DO, TDS, and conductivity measurements were obtained from the different sample stations. The pH values within the surface waters in Iko River estuary were found ranged from 8.0 to 9.0. In general, a higher pH value was observed at the mouth of the estuary as a result of the dominance and influx of

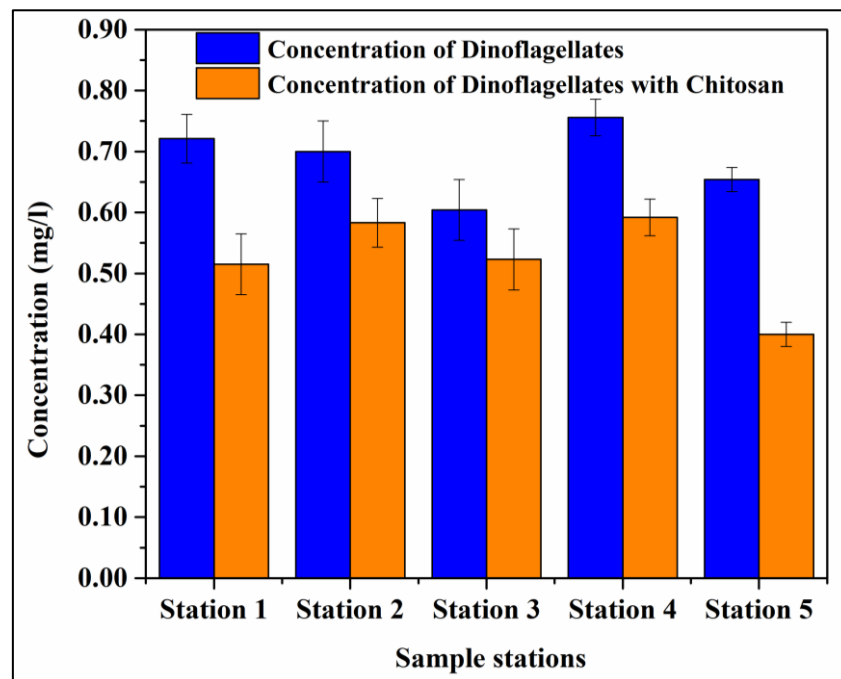
seawater. Also, fluctuations in the pH values can be attributed to factors like the elimination of CO<sub>2</sub> by photosynthesis (Bach *et al.*, 2013; Ji & Gao, 2023), reduction of salinity and decomposition of organic matter (Canavan *et al.*, 2006). The differences in the DO within sample stations were due to weather which has

a significant influence on water quality within the marine environment. For instance, rainfall could affect sediment runoff by increasing the flow rate, thus influencing temperature, turbidity, DO and pH. The differences in the conductivity of the stations are the concentrations of ions, the variety of ions, and the temperature of the stations (Dong et al., 2022; Etesin et al., 2021; Udo et al., 2024).

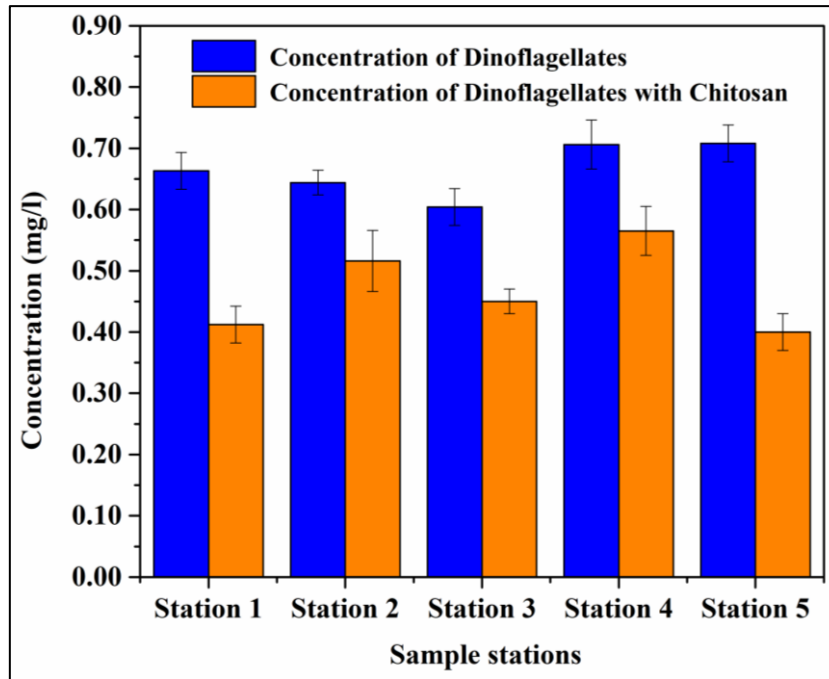
### 3.4 Determination of dinoflagellates concentration in the dinoflagellate's samples collected at each sampling station using a UV-Spectrophotometer

Figure 9-13 show the concentration of dinoflagellates in the samples collected at each sampling station and the differences seen when chitosan samples were added within five days. The results showed that dinoflagellates concentration was higher in station 5 (Akasa)

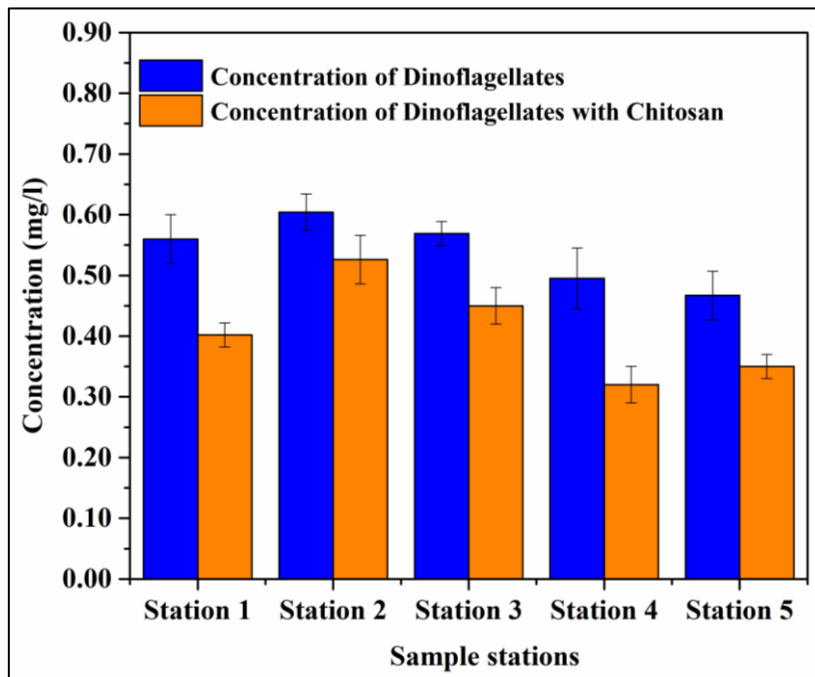
and station 4 (Estuarine mouth) which could be attributed to excessive availability of nutrients (nitrogen and phosphorous) there and gradually decreased at stations 3 to 1 because nitrogen and phosphorous were limited in those stations. The observance of the bacteria occurrence using the UV spectroscopic technique is in line with previous literature (Larios-Muñiz et al., 2024; Zakariah et al., 2024). When monitoring the differences in the concentration of dinoflagellates and the concentration of dinoflagellates after the antimicrobial agent (chitosan) was added to the samples, it was observed that dinoflagellate concentrations reduced within the 5-day test period. This was a result of the chitosan added to the dinoflagellate sample, reducing the excessive increase in the number of microbial growths related to dinoflagellate cells.



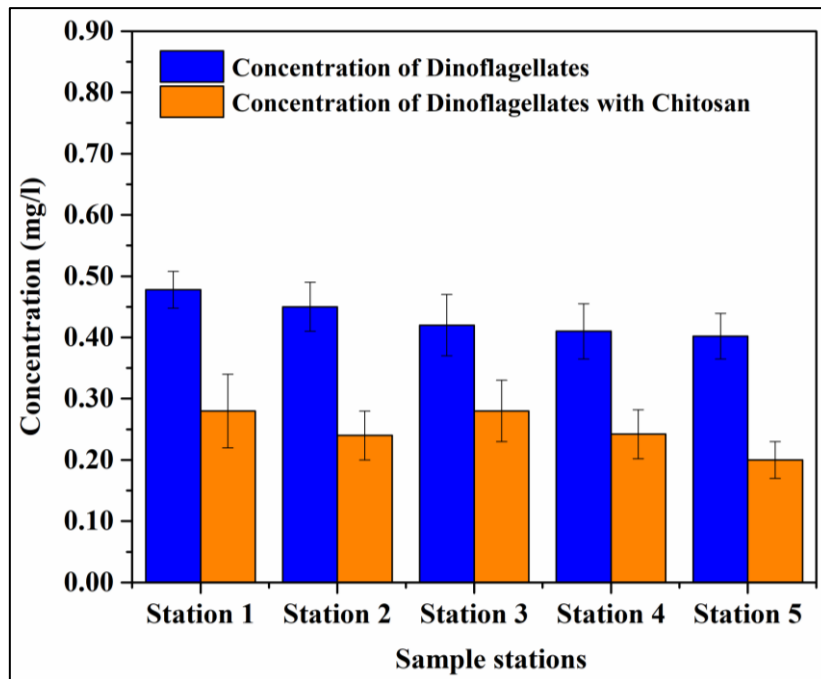
**Figure 9:** Column chart representing concentration of dinoflagellates and concentration of dinoflagellates with chitosan for day 1



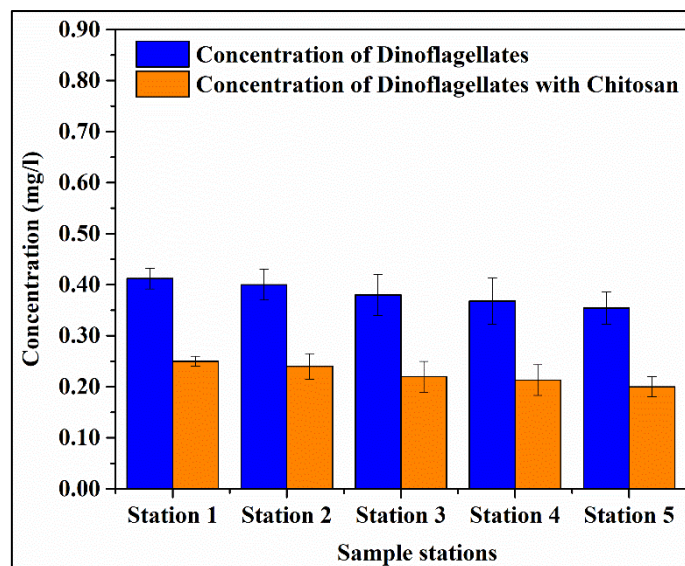
**Figure 10:** Column chart representing concentration of dinoflagellates and concentration of dinoflagellates with chitosan for day 2



**Figure 11:** Column chart representing concentration of dinoflagellates and concentration of dinoflagellates with chitosan for day 3



**Figure 12:** Column chart representing concentration of dinoflagellates and concentration of dinoflagellates with chitosan for day 4



**Figure 13:** Column chart representing concentration of dinoflagellates and concentration of dinoflagellates with chitosan for day 5

#### 4. Conclusion

The mitigation of dinoflagellates biofilm using *dendrobranchiata sp.* within the Iko River estuary, South Eastern Nigeria, Gulf of Guinea, presents detailed data showing the existence of microbiologically influenced activities in the study area. The biofilm growth was noted in all the study areas with concentration values

ranging from 0.30 to 0.75 mg/L. When the chitosan was applied, the biofilm growth reduced to the lowest value of about 0.20 mg/L. The surface characterization showed the presence of some forms of dinoflagellate organisms like *Dinophysis hastata*, *Dinophysis caudata*, *Phalacrocoma cuneus*, and *Cochlodinium pirum*. Notably, the growth of

dinoflagellates species was seen in all the study locations, and the application of chitosan components derived from *Dendrobranchiata sp.* (prawn) tended to mitigate the dinoflagellate biofilms in the coastal waters.

## References

- Bach, L. T., MacKinder, L. C. M., Schulz, K. G., Wheeler, G., Schroeder, D. C., Brownlee, C. & Riebesell, U. (2013). Dissecting the impact of CO<sub>2</sub> and pH on the mechanisms of photosynthesis and calcification in the coccolithophore *Emiliana huxleyi*. *New Phytologist*, 199(1), 121–134. <https://doi.org/10.1111/NPH.12225>
- Battin, T. J., Kaplan, L. A., Newbold, J. D. & Hansen, C. M. E. (2003). Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* 2003 426:6965, 426(6965), 439–442. <https://doi.org/10.1038/nature02152>
- Besemer, K. (2015). Biodiversity, community structure and function of biofilms in stream ecosystems. *Research in Microbiology*, 166(10), 774–781. <https://doi.org/10.1016/J.RESMIC.2015.05.006>
- Bowers, D. G., Roberts, E. M., Hogue, A. M., Fall, K. A., Massey, G. M. & Friedrichs, C. T. (2020). Secchi Disk Measurements in Turbid Water. *Journal of Geophysical Research: Oceans*, 125(5), 5. <https://doi.org/10.1029/2020JC016172>
- Canavan, R. W., Slomp, C. P., Jourabchi, P., Van Cappellen, P., Laverman, A. M. & van den Berg, G. A. (2006). Organic matter mineralization in sediment of a coastal freshwater lake and response to salinization. *Geochimica et Cosmochimica Acta*, 70(11), 2836–2855. <https://doi.org/10.1016/J.GCA.2006.03.012>
- Canon, A. (2004). Comparative Population Dynamics of Freshwater Dinoflagellates Comparative Population Dynamics of Freshwater Dinoflagellates of the Genera *Peridinium* and *Peridiniopsis* of the Genera *Peridinium* and *Peridiniopsis*. *Honors Theses*, 68, 1–2. [https://egrove.olemiss.edu/hon\\_thesis/68](https://egrove.olemiss.edu/hon_thesis/68)
- Carlsson, P., Granéli, E., & Segatto, A. Z. (1999). Cycling of biologically available nitrogen in riverine humic substances between marine bacteria, a heterotrophic nanoflagellate and a photosynthetic dinoflagellate. *Aquatic Microbial Ecology*, 18(1), 23–36. <https://doi.org/10.3354/ame018023>
- Castaño-Ortiz, J. M., Serra-Compte, A., Álvarez-Muñoz, D., Barceló, D. & Rodríguez-Mozaz, S. (2023). Antibiotics in the marine environment: occurrence and impact. *Contaminants of Emerging Concern in the Marine Environment: Current Challenges in Marine Pollution*, 73–104. <https://doi.org/10.1016/B978-0-323-90297-7.00001-9>
- Chowdary, V. T., Roshan Baba, S. K., Divya, J., & Varma, P. K. (2022). Role of chitosan in eco-friendly management of plant diseases for sustainable agriculture. *New and Future Developments in Microbial Biotechnology and Bioengineering: Sustainable Agriculture: Revisiting Green Chemicals*, 219–244. <https://doi.org/10.1016/B978-0-323-85581-5.00019-7>
- Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., & Lappin-Scott, H. M. (2003). MICROBIAL BIOFILMS. <https://doi.org/10.1146/Annurev.Mi.49.100195.003431>, 49, 711–745. <https://doi.org/10.1146/ANNUREV.MI.49.100195.003431>
- Das, A., Patro, S., Simnani, F. Z., Singh, D., Sinha, A., Kumari, K., Rao, P. V., Singh,

- S., Kaushik, N. K., Panda, P. K., Suar, M., & Verma, S. K. (2023). Biofilm modifiers: The disparity in paradigm of oral biofilm ecosystem. *Biomedicine & Pharmacotherapy*, 164, 114966. <https://doi.org/10.1016/J.BIOPHA.2023.114966>
- Dong, M., Liu, L., Wang, D., Li, M., Yang, J., & Chen, J. (2022). Synthesis and Properties of Self-Polishing Antifouling Coatings Based on BIT-Acrylate Resins. *Coatings* 2022, Vol. 12, Page 891, 12(7), 891. <https://doi.org/10.3390/COATINGS12070891>
- Ekeocha, C. I., Onyeachu, B. I., Etim, I.-I. N., & Oguzie, E. E. (2023). Review of forms of corrosion and mitigation techniques: A visual guide. *African Scientific Reports*, 2, 117. <https://doi.org/10.46481/asr.2023.2.3.117>
- Etesin, U. M., Harry, T. A., & Isotuk, U. G. (2021). Assessment of the Quality of water samples from Private Boreholes in Eket Local Government Area, Akwa Ibom State, Nigeria. *Researchers Journal of Science and Technology*, 1(1), 72–88. *Researchers Journal of Science and Technology*, 1, 72–88. <https://rejost.com.ng/index.php/home/article/view/8>
- Etim, I. N., Etiuma, R. A., Okafor, P. C., Obadimu, C. O. & Chemistry, A. (2015). Degradation of Phenol in Aqueous System by Solar Photocatalysis, Photolysis and Adsorption Processes. *Chemical and Process Engineering Research*, 34, 56–63.
- Etim, I. N., Okafor, P. C., Etiuma, R. A., & Obadimu, C. O. (2015). Solar Photocatalytic Degradation of Phenol Using Cocos Nucifera (Coconut) Shells as Adsorbent. *Journal of Chemistry and Biochemistry*, 3(1), 35–45. <https://doi.org/10.15640/jcb.v3n1a3>
- Etim, I., Dong, J., Wei, J., Nan, C., Pokharel, D., Umoh, A., Xu, D., Su, M., & Ke, W. (2020). Effect of organic silicon quaternary ammonium salts on mitigating corrosion of reinforced steel induced by SRB in mild alkaline simulated concrete pore solution. *Journal of Materials Science and Technology*. <https://doi.org/10.1016/j.jmst.2019.10.006>
- Etim, I. N., Dong, J., Wei, J., Nan, C., Felix Daniel, E., Babu Subedi, D., Xu, D., Prasad Yadav, A., Su, M. & Ke, W. (2020). Mitigation of sulphate-reducing bacteria attack on the corrosion of 20SiMn steel rebar in sulphoaluminate concrete using organic silicon quaternary ammonium salt. *Construction and Building Materials*, 257. <https://doi.org/10.1016/j.conbuildmat.2020.119047>
- Etim, I. N., Njoku, D. I., Uzoma, P. C., Kolawole, S. K., Olanrele, O. S., Ekarenem, O. O., Okonkwo, B. O., Ikeuba, A. I., Udoh, I. I., Njoku, C. N., Etim, I. P., & Emori, W. (2023). Microbiologically Influenced Corrosion: A Concern for Oil and Gas Sector in Africa. *Chemistry Africa*, 6(2). <https://doi.org/10.1007/s42250-022-00550-x>
- Gbenebor, O.P., Omoera, M.J., Faton, S. and Adeosun, S.O. (2020). Characterizing Chitin from Dendrobranchiata and Caridea Decapod Crustaceans. *Nigerian Research Journal of Engineering and Environmental Sciences*, 5(1), 105-115.
- Gómez, M. T. C. (2019). Diatoms, dinoflagellates and their distinct effects on the structure and function of the bacterioplankton. *Ecosystem and Environment Research Program*, 10, 74. <https://doi.org/10.1101/513606>.
- Hackett, J. D., Anderson, D. M., Erdner, D. L. & Bhattacharya, D. (2004). Dinoflagellates: A remarkable evolutionary experiment. *American Journal of Botany*, 91(10), 1523–1534. <https://doi.org/10.3732/ajb.91.10.1523>

- Hartoko, A., & Rudiyanti, S. (2010). Application of Chitosan for Water Quality and Macro-benthic Fauna Rehabilitation in Vannamei Shrimps (*Litopenaeus Vannamei*) Ponds, North Coast of Semarang, Central Java - Indonesia. *Journal of Coastal Development*, 14(1), 1410–5217.
- Iqbal, A., Ahmad, N., Noor, N. M., Wilson, L. D., & Ibrahim, N. H. (2019). Mitigation of toxic alexandrium tamiyavanichii using chitosan-silica composite. *Malaysian Journal of Analytical Sciences*, 23(1), 31–39. <https://doi.org/10.17576/mjas-2019-2301-04>
- James, D. O., Maaji, S. P., Kevin, E. U., Joseph, E. T. & Gimba, G. C. (2019). Antimicrobial Potential Of Chitosan And Moringa Oleifera Leaf Powder. *Nigerian Research Journal of Chemical Sciences*, 7, 2682–6054.
- Ji, Y., & Gao, K. (2023). Responses of Marine Macroalgae to Climate Change Drivers. *Climate Change and Estuaries*, 335–354. <https://doi.org/10.1201/9781003126096-20>
- Kou, S. (Gabriel), Peters, L. M., & Mucalo, M. R. (2021). Chitosan: A review of sources and preparation methods. *International Journal of Biological Macromolecules*, 169, 85–94. <https://doi.org/10.1016/j.ijbiomac.2020.12.005>
- Larios-Muñiz, M., Gonzalez-Silvera, A., Santamaria-del-Ángel, E., Guzman-Hernandez, M. E., Betancur-Turizo, S., Torres-Beltrán, M., & López-Calderón, J. (2024). Light absorption properties of dinoflagellate blooms in Todos Santos Bay, Mexico (northeast Pacific Ocean). *Regional Studies in Marine Science*, 72, 103438. <https://doi.org/10.1016/J.RSMA.-2024.103438>
- Li, Y., & Ning, C. (2020). Latest research progress of marine microbiological corrosion and bio-fouling, and new approaches of marine anti-corrosion and anti-fouling. <https://doi.org/10.1016/j.-bioactmat.2019.04.003>
- Miyakawa, Y., Otsuka, M., Shibata, C., Seimiya, T., Yamamoto, K., Ishibashi, R., Kishikawa, T., Tanaka, E., Isagawa, T., Takeda, N., Kamio, N., Imai, K., & Fujishiro, M. (2024). Gut Bacteria-derived Membrane Vesicles Induce Colonic Dysplasia by Inducing DNA Damage in Colon Epithelial Cells. *Cellular and Molecular Gastroenterology and Hepatology*, 17(5), 745–767. <https://doi.org/10.1016/J.JCMGH.2024.01.010>
- Sadiq, F. A., Hansen, M. F., Burmølle, M., Heyndrickx, M., Flint, S., Lu, W., Chen, W., & Zhang, H. (2022). Trans-kingdom interactions in mixed biofilm communities. *FEMS Microbiology Reviews*, 46(5), fuac024–fuac024. <https://doi.org/10.1093/FEMSRE/FUAC024>
- Schuler, C. G., Winebrenner, D. P., Elam, W. T., Burnett, J., Boles, B. W., & Mikucki, J. A. (2023). Microbial Transport by a Descending Ice Melting Probe: Implications for Subglacial and Ocean World Exploration. *Https://Home.Liebert-pub.Com/Ast*, 23(11), 1153–1164. <https://doi.org/10.1089/AST.2021.0106>
- Talley, D. M., & Talley, T. S. (2008). Salinity. *Encyclopedia of Ecology, Five-Volume Set*, 3127–3132. <https://doi.org/10.1016/B978-008045405-4.00541-3>
- Taylor, F. J. R., Hoppenrath, M., & Saldarriaga, J. F. (2008). Dinoflagellate diversity and distribution. *Biodiversity and Conservation*, 17(2), 407–418. <https://doi.org/10.1007/s10531-007-9258-3>
- Ubulom, S. R., Ekanim, M. P., Etim, I.-I. N.,

- Joseph, O. & Ewa-oboho, I. (2022). Assessment of Contamination Status of Heavy Metals in Sediment due to Gas Flaring at Esit Eket , South Eastern Nigeria. *Researchers Journal of Science and Technology*, 2(1), 50–63.
- Udeme, M., Etim, I., Udom, P., Ewa-oboho, I., Udo, R. & Koffi, U. (2022). Evaluation of the Impact of Crude and Purified Biosurfactants Synthesized by *Pseudomonas aeruginosa* in the Marine Environment. *Journal of Academia and Industrial Research*, 11(2), 26–32.
- Udo, G. J., Uwanta, E. J., Awaka-ama, J. J., Uwem U. Ubong, & Igwe, R. (2024). Implications of Crude Oil Exploration and Exploitation on Seasonal Variability of pH and Electrical Conductivity of Arable Soil, Water and Sediment of Ibeno Coastal Area, Niger Delta, Nigeria. *Researchers Journal of Science and Technology*, 4(3 SE-Articles), 51–68. <https://rejost.com.ng/index.php/home/article/view/111>
- Umoh, U., Dan, S., & Etim, I. (2014). Mineral Iron Content of *Commelina benghalensis*, *Paspalum vaginatum*, *Ipomoea pes-caprae* and *Philoxerus vermiculeris* found along Ibeno Coastline, Nigeria. *Journal of Academia and Industrial Research ...*, 3(4), 4–7. <http://www.jairjp.com/-SEPTEMBER 2014/10 UMOH.pdf>
- Uzoma, P. C., Etim, I. N., Okonkwo, B. O., Olanrele, O. S., Njoku, D. I., Kolawole, S. K., Emori, W., Ikeuba, A. I., Njoku, C. N., Ekerenam, O. O., Etim, I. P., Daniel, E. F., & Udoh, I. (2023). Recent design approaches , adhesion mechanisms , and applications of antibacterial surfaces. *Chemical Engineering Journal Advances*, 16(September), 100563. <https://doi.org/10.1016/j.ceja.2023.100563>
- Vernal, A., Rochon, A., & Radi, T. (2013). Dinoflagellate. *The Encyclopedia of Quaternary Science*, 2, 800–815.
- Wang, M. Y., & Liu, Y. J. (2017). Theoretical Study of Dinoflagellate Bioluminescence. *Photochemistry and Photobiology*, 93(2), 511–518. <https://doi.org/10.1111/PHP.-12657>
- Zakariah, E. I., Ariffin, E. Y., Nokarajoo, D., Mohamed Akbar, M. A., Lee, Y. H., & Hasbullah, S. A. (2024). Highly sensitive of an electrochemical DNA biosensor detection towards toxic dinoflagellates *Alexandrium minutum* (A. minutum). *Microchemical Journal*, 199, 109997. <https://doi.org/10.1016/J.MICROC.2024.109997>