

Assessment of Saxitoxin in Water and Shellfishes within Lagos Lagoon, South Western Nigeria

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Abstract

Saxitoxin is a potent neurotoxin produced by microalgae responsible for paralytic shellfish poisoning. The present study aims to ascertain the safety of shellfishes obtained from Lagos lagoon. Water and shellfishes samples were collected from six points during the wet and dry season of 2016. Identification of microalgae in water samples was done using standard laboratory procedures. Saxitoxin concentrations in both water and shell fishes were determined by spectrophotometry method. *Melosira sphaerica*, *Asterionellopsis glacialis*, *Corethroncriophilum*, *Guinardia striata*, *Oscillatoria*, *Microcystis*, *Coscinodiscus*, *Rhizosolenia* and *Scenedesmus* were the identified microalgae. Saxitoxin concentrations in water samples for both wet and dry seasons were 2.1 - 2.4 and 3.0 - 3.04 mg/ml respectively. Saxitoxin concentrations in shellfishes in µg/g for both wet and dry seasons were: carapace of *Callinectes sapidus* 69.93 ± 0.90 and 114.71 ± 0.02, pancreas of *Callinectes sapidus* 8.18 ± 0.02 and 9.01 ± 0.03, flesh of *Pomadasys jubelini* 12.46 ± 0.09 and 17.86 ± 0.02, head of *Pomadasys ubelini* 52.21 ± 0.06 and 62.11 ± 0.04, shell of *Parapeneopsis atlantica* 97.83 ± 0.45 and 151.33 ± 0.15, flesh of *Parapeneopsis atlantica* 8.27 ± 0.00 and 9.95 ± 0.00, *Mytilus edulis* flesh 13.69 ± 0.02 and 17.13 ± 0.02, flesh of *Littorina littorea* 6.29 ± 0.01 and 11.39 ± 0.04 and *Crassostrea gigas* flesh 8.04 ± 0.08 and 12.38 ± 0.03 respectively. Consumption of some of these shellfishes may pose a potential health risk to humans depending on the frequency, season and species of sea foods consumed. Monitoring programmes are urgently needed to identify human exposures and to safeguard human health.

Keywords: Saxitoxin; Microalgae; Shellfishes; Wet And Dry Seasons; Lagos Lagoon; Human Exposure

Abbreviation

NIOMR: Nigeria Institute for Oceanography and Marine Research

Introduction

Some micro algae are known to produce phycotoxins that cause various poisoning in humans. Examples of such poisoning include Amnesic Shellfish Poisoning (ASP), Diarrhetic Shellfish Poisoning (DSP) and Paralytic Shellfish Poisoning (PSP) [1]. Phycotoxins can enter the food chain when they contaminate and accumulate in various marine species such as fish, crabs or filter feeding bivalves (shellfish) such as mussels, oysters, scallops and clams. These toxins can be toxic for the marine fauna or for consumers via shellfish or fish. Usually these toxins are classified by their clinical signs observed during intoxication. Paralytic Shellfish Toxin (PST) poses a great threat to public health because no antidote has been found.

Saxitoxins (STXs) are highly polar, non-volatile tricyclic perhydropurine alkaloids derived from Imidazoline guanidinium that belong to the class of organic marine dinoflagellates and cyanobacteria. Saxitoxin is a neurotoxin, a sodium channel blocker and a marine metabolite with a structure based on a 2,6-di amino-4-methyl-pyrrolo (1,2-C) purin-10-01 skeleton [2]. This toxin cause paralytic shellfish poisoning, a potentially fatal malady associated with the consumption of shellfish containing STX [3]. These toxins are mainly produced by marine dinoflagellates, in particular, *Alexandrium* species, *Gymnodinium catenatum* and *Pyrodinium bahamense* [4], but have also been found to be produced by freshwater cyanobacteria [5]. Shellfish feeding on these algal species can accumulate the toxins without exhibiting adverse effects on themselves. Shellfish contaminated with these toxins pose severe risks to human consumers and numerous accounts of intoxications leading to illness or death have been recorded from around the world [6].

There is a considerable concern about the human health implications of saxitoxin in polluted coastal and inland waters that are in proximity to large population centers. Researchers have established presence of saxitoxin in various lagoons having negative effect on human health through the ingestion of contaminated water, shellfishes or fishes in western countries [1,3,6-8]. Lagos lagoon is continuously exposed to industrial effluent and domestic waste and in recent times, these have raised natural concentrations of nutrients causing serious environmental problems such as algal blooms. Since coastal people rely heavily on seafood as a chief source of protein, the present study therefore assessed saxitoxins in water and shell fishes in Lagos lagoon during the wet and dry seasons of 2016/2017.

Study sites

A litre of water sample was collected from each of six study sites located in Lagos lagoon, South-Western Nigeria (Figure 1). The sites were chosen based on distinct differences in their environmental conditions. The studied sites with their longitude and latitude are presented in table 1. The first site is Lekki toll gate also known as Shoprite low tide, nearby lekki sand fill, very close to the town of Maroko. The second site is Law school very close to Falomo. The third site is Mekuen near Obalende. The Fourth is CMS near Lagos harbor. The fifth site is Federal palace very close to Ogogoro village. The sixth site is Nigeria Institute of Oceanography and Marine Research (NIOMR) jetty close to Eko Atlantic city.

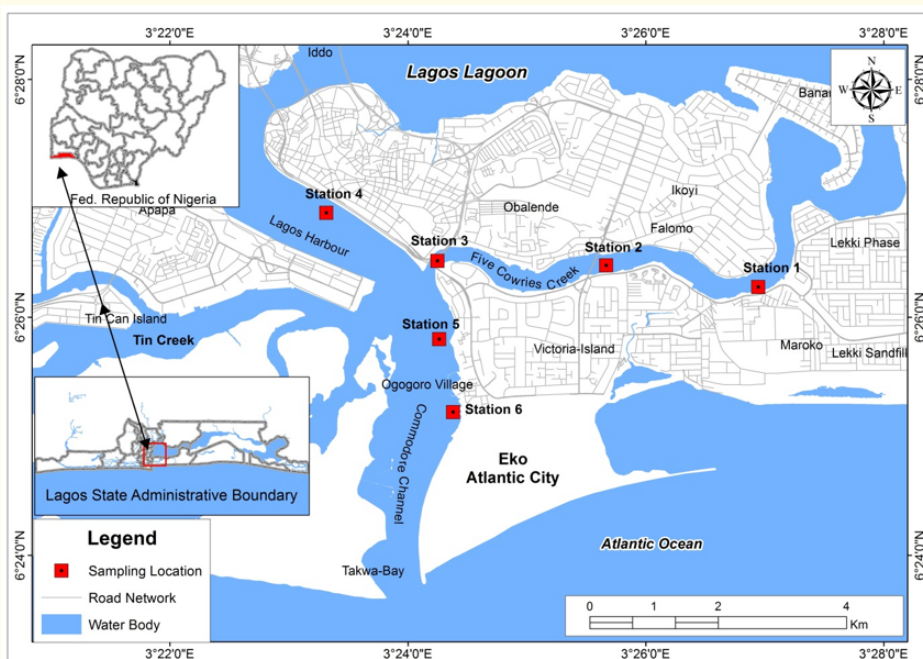


Figure 1: Sampling locations in the south western part of Nigeria (Lagos Lagoon).

Station number	Station name	Longitude	Latitude
1	Lekki Toll gate	3° 26' 56.76"	6° 26' 16.08"
2	Law school	3° 25' 39.36"	6° 26' 25.8"
3	Mekuen	3° 24' 14.76"	6° 26' 27.96"
4	CMS	3° 23' 18.24"	6° 26' 52.44"
5	Federal palace	3° 24' 14.76"	6° 25' 49.08"
6	NIOMR Jetty	3° 24' 23.4"	6° 25' 11.64"

Table 1: Geographical location of studied sites.

Materials and Methods

Sample collection

Water samples

Six samples per site totaling thirty-six (36) water samples were collected from each of the six studied sites (five Cowries creeks of Lagos and Lagos harbor) at about 0.45 kilometers (± 0.1) from each point of collection. The water samples were placed into 1litre bottles which had been degreased in dilute sodium hydroxide (NaOH), soaked in dilute hydrochloric acid (HCL) and then soaked in deionized water for 24 hours before use [9]. The bottles were treated so as to reduce adsorption of saxitoxin from the water into the plastic containers and vice versa. All samples were stored at 4°C in the dark.

Phytoplankton samples

Sampling was carried out using 20 μ m mesh phytoplankton net. The net was lowered to a depth of 5 meters and then pulled up slowly to the surface several times. The collection chamber was emptied into a clean opaque glass bottle (500 ml volume) and samples were stored at 4°C [4].

Shellfishes samples

A total of 328 Shellfishes samples which included *Crassostrea gigas* (Oysters, 80), *Mytilus edulis* (Mussels, 80), *Pomadasy jubelini* (Mullet, 14), *Parapeneopsis atlantica* (Shrimps, 80), *Callinectes sapidus* (Crab, 14), and *Littorina littorea* (Snails, 60) were obtained from Fishermen fishing on Lagos lagoon, Nigeria during the wet and dry seasons of 2016. Samples were collected into an iced chest and transported to Nigeria Institute for Oceanography and Marine Research (NIOMR) Victoria Island, Lagos for identification and then transported to Cherish Environmental Consultancy Limited, Ibadan, Oyo State, Nigeria where they were stored at - 20°C.

Analysis

Phytoplankton samples

Phytoplankton samples were inoculated on BG11 medium and then incubated using fluorescent incubator at 22°C for three to four weeks under 12 hours light and 12 hours darkness [10]. Sub culturing was carried out severally until pure isolates were obtained. Small drop of fresh phytoplankton sample was placed on a glass slide and covered with a glass cover slip. A drop of immersion oil was placed over the cover slip and the slide observed at x10, x40 and x100 magnification under dissecting microscope lighting system by using a computer based program (available with microscope) and pictures taken by a digital sight camera. Identification of microalgae was done based on detailed morphological, physiological and cellular characterization of the isolates [11-13].

Extraction of saxitoxin from water samples

Ten milliliters of water sample was measured into a 250 ml beaker and 50 ml of dichloromethane was added to the sample. The mixture was homogenized for 30 minutes. The uniform homogenate was separated into organic and aqueous layer by means of 100 ml sepa-

rating funnel with the organic layer containing the analyte which was removed into a 30 ml test tube while the aqueous layer was left in the separating funnel. The aqueous layer was washed twice into the filtrate until there was no trace of the analyte using 5 ml 0.1M acetic acid. The combined filtrate was used for spectrophotometric determination [14].

Extraction of saxitoxin from shellfishes

The shellfish samples were thawed and prepared by dissecting and removing the whole flesh from their shell. Residual fragments of the shell were removed from the flesh prior to being homogenized with antibiotics (0.02% of each ethoxyquin, ampicillin, erythromycin, and oxytetracycline) using a warring blender or laboratory mortar and pestle (for shell) as the case may be and dispensed into sterile glass test tubes. Ten grammes of homogenized shellfishes were mixed with 20 mL of dichloromethane and was homogenized for 30 minutes. The uniform homogenate was filtered into a clean labeled 30 mL test tube using Whatman No. 9 filter paper, pH was adjusted to 7 with 0.2 M NaOH. The residue was washed twice into the filtrate until there is no trace of the analyte using 5 ml of 0.1M acetic acid. The combined filtrate was kept in tubes purged with nitrogen and hermetically sealed with aluminum seal and stored at 5°C [14]. This procedure was repeated for fish shells and tissues.

Spectrophotometry determination

Saxitoxin working standard of 0 to 5 part per million (ppm) was obtained from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). One millilitre of each working standard was pipetted into a 30 mL test tube and appropriately labeled. One millilitre of acetyl choline red reagent was added to each working standard to develop a red colour upon standing for 2 hours. The extracts from shellfish samples were treated similarly with acetyl choline red reagent to develop colour for 2 hours before reading their absorbance on the spectrophotometer at a wavelength of 420 nm [5]. Saxitoxin concentration was determined from absorbance using the formula below:

Gradient (working standard) = 1/Absorbance

Average Gradient (working standard) = Summation (Σ) of all gradient/number of samples

Saxitoxin concentration ($\mu\text{g/g}$) = Absorbance of each sample x average gradient/Weight of sample x 1000.

Data obtained were subjected to Analysis of Variance (ANOVA) test using Statistical Package for Social Science (SPSS) version 17.0.

Results

Microalgae identified were *Coscinodiscus* species (Plate 1), *Melosira sphaerica* (Plate 2), *Microcystis* species (Plate 3), *Rhizosolenia* species (Plate 4), *Asterionellopsis glacialis* (Plate 5), *Corethroncriophilum* (Plate 6), *Oscillatoria* species (Plate 7), *Guinardia striata* (Plate 8) and *Scenedesmus* species (Plate 9).

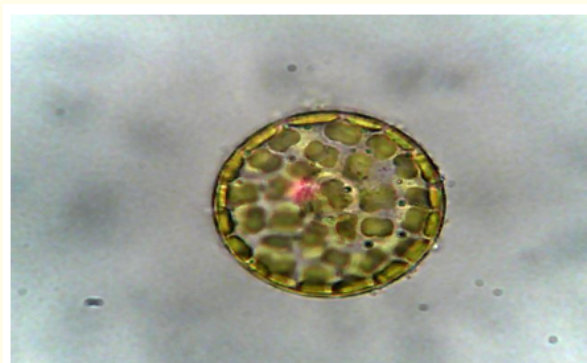


Plate 1: *Coscinodiscus*.



Plate 2: Melosira sphaerica.

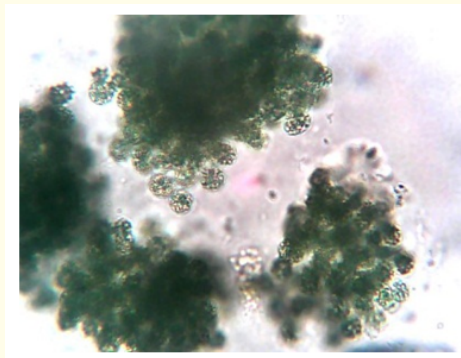


Plate 3: Microcystis.

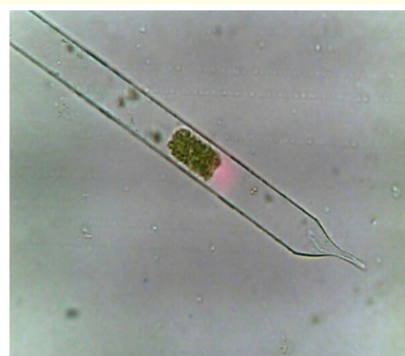


Plate 4: Rhizosolenia.

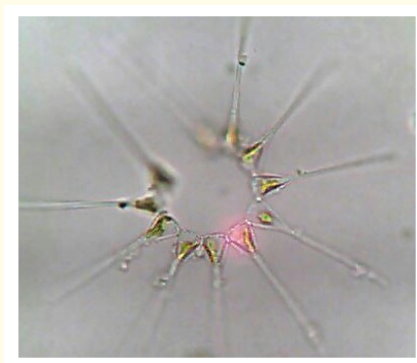


Plate 5: *Asterionellopsis glacialis*.

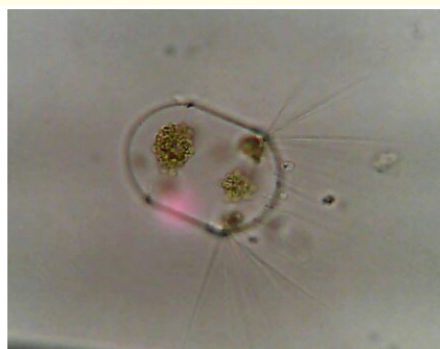


Plate 6: *Corethroncriophilum*.

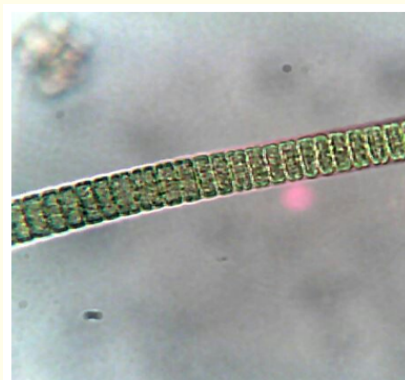


Plate 7: *Oscillatoria* species.

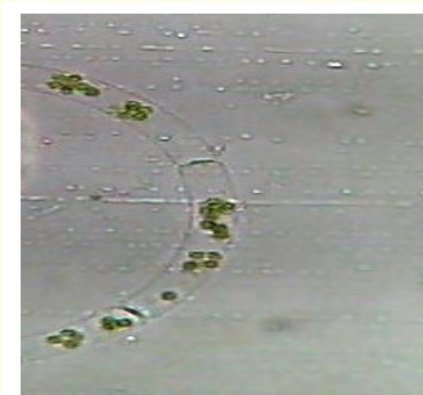


Plate 8: *Guinardia striata*.



Plate 9: *Scenedesmus species*.

Coscinodiscus species: The cells were in disc, cylindrical shaped and solitary with distinct rosette of large areolae in the center of the valve.

Melosira sphaerica: The cells were connected to form long chain, looking like a string of beads with several plate-like chromatophores and the nucleus at the center.

Microcystis species: Colonies form spherical clusters with extended reticulate mass, suspended colonies appears as small blue-green clots, with small individual cells that are very small (3 - 5 millimeters in diameter) with conspicuous, highly refractive pseudo vacuoles that cause the colonies to be buoyant, cells of a colony are held together by a transparent, gelatinous matrix.

Rhizosolenia species: The cells are long, cylindrical, slightly curved cells with a spine - like process on one end. Cells are in short chains. The sub conical segmented girdle bands are in longitudinal columns roughly parallel to the perivolar axis. Cells have numerous small chloroplasts.

Asterionellopsis glacialis: The cells are rod - like with distinctly dissimilar ends, one end enlarged, larger ends united into star like spiral colonies.

Corethroncriophilum: The cells were solitary and cylindrical, values were strong convex, and furnished with a marginal crown of well-developed slender spines directed outwards at an angle with numerous plate - like chromatophores.

Oscillatoria species: The organism has cylindrical, unbranched trichomes that are slightly wavy and very long. Cells of the trichome are discoid and shorter than broad.

Saxitoxin concentration of water samples collected from Lagos lagoon during wet and dry seasons of 2016

The values of saxitoxin concentration in water samples collected from Lagos Lagoon during the wet and dry seasons of 2016 are presented in table 2. The total values for both wet and dry seasons were 2.26 ± 0.11 mg/ml and 3.02 ± 0.02 mg/ml respectively with significant difference at P < 0.05.

	Wet	Season	Dry	Season
Station	Absorbance	STX conc.mg/ml	Absorbance	STX conc.mg/ml
1	0.57 ± 0.00 ^d	2.14 ± 0.00 ^d	0.79 ± 0.00 ^e	2.99 ± 0.00 ^e
2	0.61 ± 0.00 ^b	2.31 ± 0.01 ^b	0.81 ± 0.00 ^a	3.04 ± 0.00 ^a
3	0.58 ± 0.02 ^c	2.19 ± 0.06 ^c	0.80 ± 0.00 ^c	3.02 ± 0.00 ^c
4	0.57 ± 0.00 ^{cd}	2.15 ± 0.01 ^{cd}	0.80 ± 0.00 ^d	3.00 ± 0.00 ^d
5	0.63 ± 0.00 ^a	2.39 ± 0.00 ^a	0.80 ± 0.00 ^b	3.04 ± 0.00 ^b
6	0.64 ± 0.00 ^a	2.40 ± 0.00 ^a	0.80 ± 0.00 ^b	3.04 ± 0.01 ^b
Mean		2.26	0.8	3.02
WHO	0.6	1.6 - 3 mg/L		1.6 - 3 mg/L

Table 2: Saxitoxin concentrations in water samples collected from selected sites in Lagos lagoon during the wet and dry seasons of 2016/2017.

Values with the same letters in a column are not significantly different from each other.

1= Lekki toll gate, 2= Law school, 3= Mekeun, 4= CMS, 5= Federal palace, 6= NIOMR (Nigeria Institute for Oceanography and Marine Research) jetty. Values with the same letters in a column are not significantly different from each other. STX = Saxitoxin.

Saxitoxin quantification in shell fishes collected from Lagos lagoon during the wet and dry seasons of 2016

The concentration of saxitoxin in shell fishes from Lagos lagoon for both seasons is presented on table 3. The accumulated saxitoxin in sampled shell fishes ranged from 6.29 to 97.83 µg/g in wet season and 9.01 to 151.33 µg/g in dry season.

Discussion

All over the world microalgae has been known to cause water blooms in eutrophic water. They also produce diverse range of toxins including neurotoxins (for example anatoxin-a, anatoxin a(s), and saxitoxins) and hepatotoxins (for example microcystins, nodularins and cylindrospermopsins) which cause a variety of human illness and are responsible for deaths in native and domestic animals [15]. Micro algae identified in this study were *Coscinodiscus* species, *Melosira sphaerica*, *Microcystis* species, *Rhizosolenia* species, *Asterionellopsis glacialis*, *Corethroncriophilum*, *Oscillatoria* species, *Guinardia striata*, and *Scenedesmus* species.

Among the micro algae *Microcystis* is the most representative genus of toxic bloom - forming cyanobacteria. It produces a cyclic heptapeptide hepatotoxin, termed microcystin and a depsipeptide chymotrypsin - inhibitor called cyanopeptolin [16]. Some species of *Oscillatoria* has been recorded as the cause of blue - green algal toxicosis due to pronounced growth ‘water bloom’ especially during eutrophication.

Sample	Wet	Season	Dry	Season
	Absorbance	STX conc. µg/g	Absorbance	STX conc. µg/g
RC	0.20 ± 0.03 ^b	69.93 ± 0.90 ^b	0.40 ± 0.001 ^b	114.71 ± 0.02 ^b
RF	0.03 ± 0.00 ^c	12.46 ± 0.09 ^d	0.05 ± 0.001 ^d	17.86 ± 0.02 ^d
RH	0.29 ± 0.05 ^a	97.83 ± 0.45 ^a	0.50 ± 0.01 ^a	151.33 ± 0.15 ^a
RM	0.04 ± 0.001 ^c	13.69 ± 0.02 ^d	0.05 ± 0.001 ^d	17.13 ± 0.02 ^d
RN	0.02 ± 0.003 ^c	6.29 ± 0.01 ^f	0.04 ± 0.003 ^e	11.39 ± 0.04 ^e
RP	0.03 ± 0.004 ^c	8.18 ± 0.02 ^e	0.03 ± 0.002 ^f	9.01 ± 0.03 ^f
RR	0.02 ± 0.001 ^c	8.27 ± 0.00 ^e	0.03 ± 0.001 ^f	9.95 ± 0.00 ^f
RU	0.18 ± 0.002 ^b	52.21 ± 0.06 ^c	0.23 ± 0.001 ^c	62.11 ± 0.04 ^c
RY	0.03 ± 0.002 ^c	8.04 ± 0.08 ^e	0.04 ± 0.002 ^f	12.38 ± 0.03 ^e

Table 3: Saxitoxin concentration (µg/g) in shell fishes collected from Lagos lagoon during the wet and dry seasons of 2016/ 2017.

Values with the same letters in a column are not significantly different from each other.

STX: Saxitoxin, RC: *Callinectes sapidus* Carapace, RF: *Pomadasy jubelini* flesh, RH: *Parapeneopsis atlantica* Shell, RM: *Mytilus edulis* flesh, RN: *Littorina littorea* tissue, RP: *Callinectes sapidus* Pancreas, RR: *Parapeneopsis atlantica* Flesh, RU: *Pomadasy jubelini* Head, RY: *Crassostrea gigas* flesh.

cation and the release of hepatotoxins or neurotoxins [1]. The increase of some species of *Rhizosolenia* can also kill fish by clogging the gills with their hard silica exterior and from post bloom anoxia. *Melosira sphaerica* can form prolific blooms in unshaded stony streams or rivers, commonly the subject of pollution complaints [17,18].

The genus *Coscinodiscus* with phylum name Bacillariophyta is a diatom that was first reported in the late 1970’s along the Belgian coast, attached to various marine life [19]. It is considered a potentially harmful alga due to its production of insoluble mucilage which adversely affects fishing activities [20].

Nutrient depletion, adversely affecting mussel and causes consequent economic loss It is likely that the organism was introduced by the discharge of ballast water [21].

As invasive organism *Coscinodiscus* replaces the native phytoplankton by competition, decreasing biodiversity [20]. The success is related to its resistance to different temperatures and salinities, higher tolerance to heavy metals [22]. Some species could be toxic with negative consequences on public health and to commercial fishery and aquaculture [21].

The major route of exposure of cyanotoxins (saxitoxin) to humans is the recreational use of lakes and rivers (oral and dermal route) and the consumption of drinking water and algal health food tablets (oral route). One of the earliest effects is within 15 to 30 minutes. Death may preceded by coma, muscle tremors, paddling and laboring breathing, watery or bloody diarrhea, skin and eye irritation, hay fever, dizziness, fatigue, acute gastroenteritis, generally it has not been possible to determine which of these groups (hepatotoxins and neurotoxins) is responsible for the observed symptoms [23].

Eutrophication which is as a result of nutrient enrichment of water bodies is the main driver for cyanobacteria abundance [24,25] and persistence [26]. Saxitoxin concentration showed significant variations among Stations 1, 2, 3 (Lekki toll gate, Law school and Mekuen respectively) with a slight variation in Station 4 (CMS) in wet season. In dry season, saxitoxin concentration showed significant variation among Stations 1, 2, 3 and 4 while no significant variation observed between Stations 5 and 6 (Federal palace and NIOMR jetty respectively) for both seasons. The concentrations of saxitoxin in water samples were higher in the eutrophic Stations. All the values recorded

in the Stations exceeded the provisional guideline value of 1.0 mg/L adopted by WHO for microcystin- LR [27] excluding other known cyanotoxins since there was insufficient data to derive guideline values for these toxins.

The presence of saxitoxin in water affects all other organisms present in the water including fishes which in turn affect the health of humans after ingestion of contaminated water or fishes, most especially shellfishes. The poisoning produced is called Paralytic Shellfish Poisoning (PSP). PSP has been recorded in humans who consumed contaminated Mussels, Oysters, Crab, Clam that contain high levels of saxitoxin, a natural substance produced by certain types of plankton [1,3]. The result showed a significant variation in both seasons among *Parapeneopsis atlantica* shell (RH), *Callinectes sapidus* carapace (RC) and *Pomadasys jubelini* head (RU) likewise in tissues of *Mytilus edulis* (RM) and *Pomadasys jubelini* (RF) while Pancreas of *Callinectes sapidus* (RP) and Tissue of *Parapeneopsis atlantica* (RR) were not significantly different from each other during both seasons.

Tissues of *Littorina littorea* (RN) and Oysters significantly differ from each other during wet season but with no significant difference observed during the dry season. Apart from Oysters flesh, Crab (*Callinectes sapidus*) pancreas and *Littorina littorea* which had a close or equal value with WHO limit during the wet season, all other shellfishes analyzed had concentrations above WHO limit of 0.8 mg/100g saxitoxin equivalent. All examined shellfishes during dry season had higher concentrations of saxitoxin which were above WHO limit. These emphasizes the speed with which saxitoxin can accumulate in shellfish tissue [28]. Concentration of saxitoxin in *Parapeneopsis atlantica* (Shrimps) shell was found to be the highest among studied shellfishes.

The concentrations in studied shellfishes followed the accumulation order *Parapeneopsis atlantica* (Shrimps) shell > *Callinectes sapidus* (Crab) carapace > *Pomadasys jubelini* (Mullet) head > *Pomadasys jubelini* flesh > *Mytilus edulis* (Mussels) flesh > *Crassostrea gigas* (Oysters) flesh > *Littorina littorea* (Snail) flesh > *Parapeneopsis atlantica* flesh > *Callinectes sapidus* pancreas. From this study, the concentrations of saxitoxin were highest in shell of *Parapeneopsis atlantica* (RH), *Callinectes sapidus* (RC) carapace and *Pomadasys jubelini* (RU) head compared to their flesh and pancreas this result showed that shell of fishes has the tendency to accumulate saxitoxin than in tissues.

Conclusion

This study revealed the presence of saxitoxin in Lagos lagoon, Nigeria and in shellfishes, therefore showing the importance of establishing a water quality monitoring of the studied areas. The results also showed that some values of saxitoxin concentration in shellfishes were above the limit of 80 µg/100g proposed by WHO. The accumulation of saxitoxin in humans that consume such fishes can lead to chronic exposures that may pose health hazard. Monitoring programmes are urgently needed to identify human exposures and to protect human health.

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