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Harmful Marine Phytoplankton Community in Shirazi Creek, Kenya

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ABSTRACT

Globally, coastal creek waters represent important aquaculture zones for shellfish culture, commercially important fin-fishes larvae and crustaceans due to their richness in phytoplankton community. The harmful phytoplankton community in Kenyan coastal waters causes mortalities to aquatic organisms both in the wild and culture areas. Therefore, as a result of the high economic values attached to these resources and taking into consideration that Shirazi creek waters has been used for the culture of bivalve oysters as early as 1990s; its investigation for the presence of harmful phytoplankton community became necessary. Surface water samples were monthly collected from September, 2013 to August, 2014 at five fixed different stations. The harmful marine phytoplankton species were microscopically identified and counted. Sixteen potentially harmful marine phytoplankton species, with mean cell concentration of abundance ranged from 10±1-210±11 cells L^{-1}, were found in the creek. Ten out of the total identified species were those capable of producing potent toxins to humans. Three were the main cause for the indiscriminate killing of fish and invertebrates during blooms as a result of oxygen depletion. The remaining three species were harmful to fish and invertebrates through damaging or clogging of gills. The present study registered increase in the number of these potentially harmful phytoplankton species compared to the previous investigations. However, the study highlights on possible threat from future bloom occurrences as a result of increased eutrophication owing to climate change.

Key words: Phytoplankton, harmful, creek waters, microalgae, species

INTRODUCTION

The planktonic microalgae in the world's Oceans are critical food for filter feeding shellfishes (oysters, mussels, scallops and clams), larvae of commercially important crustaceans and fin-fishes. However, in some situations, they cause severe economic loss to aquaculture, fisheries and tourism operations resulting into major environmental and human health impacts (Geraci *et al*., 1989; Hallegraeff, 1993; Maclean, 1993; Yuki, 1994). A specific Kenyan case that decreased the economy was observed when a Microcystis species bloom seriously hampered salt production in 1992 and 1993 due to lack of public awareness (Munga *et al*., 1992).

There is also growing evidence that sites of aquaculture deployments eventually exhibit harmful algal bloom events (Hallegraeff, 1993) of global dimensions. This is because the bloom events affect the environment, fisheries, aquaculture, public health, tourism and drinking water quality. Reports associated with human illnesses or damage to aquaculture operations as a result of harmful algal events have thus continued to receive increased attention globally in newspapers, electronic media and the scientific literature (Hallegraeff, 1993). As a result of the high economic importance attached to these natural phytoplankton resources, most researchers are now surveying their local waters for the presence of these harmful microscopic causative planktonic organisms.

Harmful marine microalgae information of the Kenyan coastal waters is scarce with only the works of Wawiye *et al*. (1999), who studied the potentially harmful marine microalgae in the Kenyan waters, Mwaluma *et al*. (2003) investigating the composition, abundance and seasonality of zooplankton and microalgae in Mida Creek, Kenya. Munga *et al*. (1992) used Artemia to control algal blooms in the Kenyan salt ponds. Recently, Kiteresi *et al*. (2012) studied the influence of land based activities on the phytoplankton communities of Shimoni-Vanga. The purpose of this study was to present a baseline screening survey on the Shirazi aquaculture creek waters in order to characterize the potentially harmful marine phytoplankton communities.

MATERIALS AND METHODS

Study site: Shirazi creek is located on the Kenyan South-Coast waters at 4°32'0"South, 39°25'0" East. Its importance resulted from the culture of oysters under the "Shirazi Oyster Project" in the early 1990s by the Shaza women group as a source of alternative livelihood through the Belgian funded micro-intervention programme to boost their income. The creek (Fig. 1) receives its freshwater input from the perennial river Ramisi that has been previously having no great industrial activity within its environs, apart from sport and artisanal fishing. However, presently, Ramisi environs are being utilized for sugarcane production and have a danger of possible nutrient load addition to the waters thus enhancing the proliferation of the creeks microalgae communities.

Phytoplankton sample collection procedures: A boat was used to access the sites and samples were collected during the first week of every month for a year beginning from September, 2013 to August, 2014 at five stations within the creek (Fig. 1). However, due to the relatively shallow depths involved in most stations, samples were taken from the surface. Water samples were collected in triplicates for quantitative analysis using a 20 L bucket and filtered through 20 μm mesh-size plankton net for concentration to 100 mL. This concentrate was then transferred to sample bottles, labeled and immediately preserved with Lugol's iodine and transported to the laboratory for identification and enumeration.

Water quality parameter and nutrient water samples collection procedures: Water quality parameters (pH, temperature, salinity and turbidity) were measured in situ at each station. pH was measured using an electronic pH probe (pH Testr 2; Aquatic Eco-Systems, Inc., USA), temperature using an YSI Model 550A and salinity using a hand-held refractometer (all from Yellow Springs Instruments, Yellow Springs, OH, USA). Turbidity was measured using a locally made Secchii disc. Nutrient water samples for dissolved inorganic nitrates (NO_3), phosphates (PO_4) and ammonia (NH_3) were aseptically collected from each station and stored in a pre-cooled ice-box and transported to the laboratory for analysis using Parsons *et al*. (1984) methods.

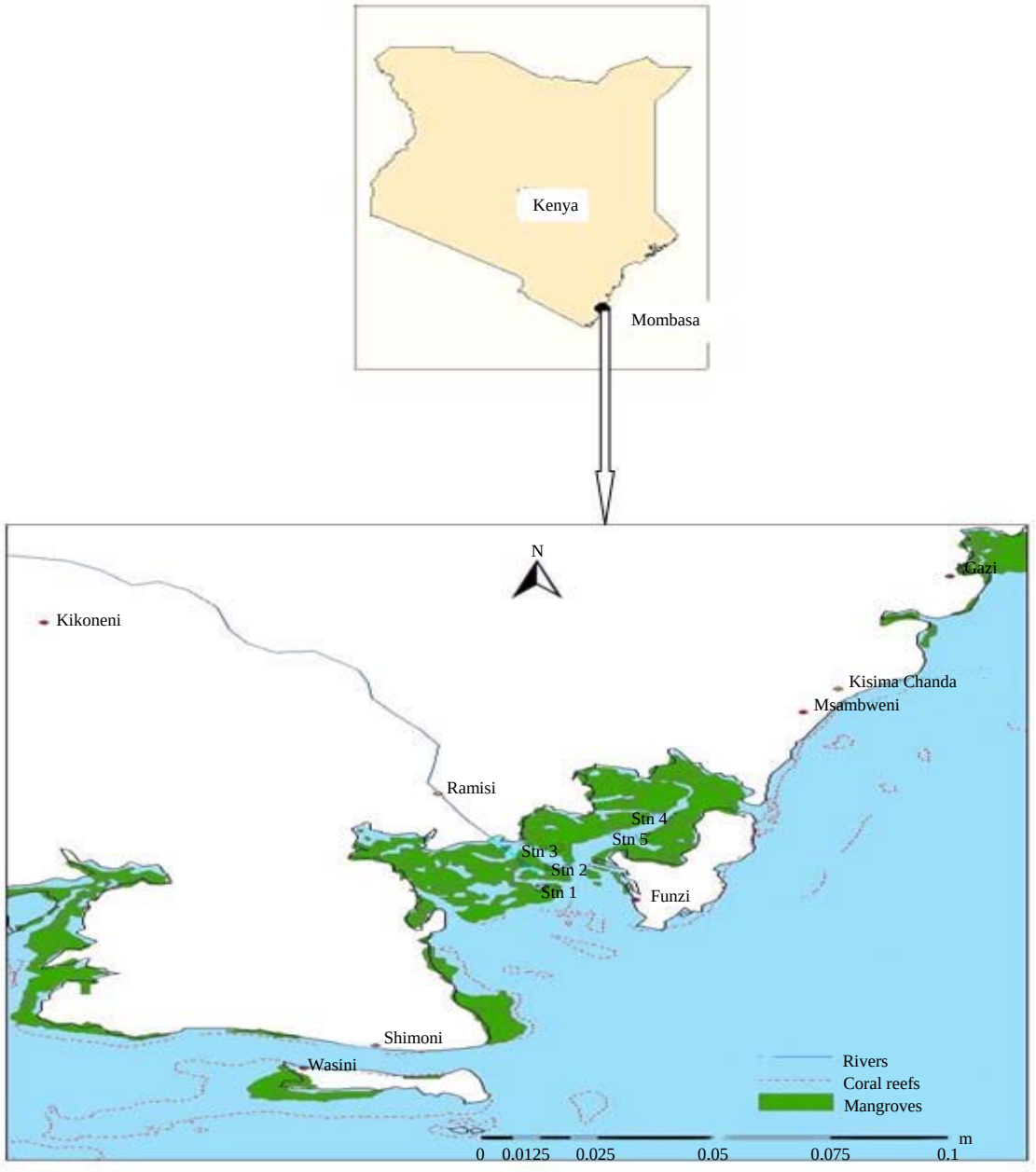

Fig. 1: Map showing the sampling stations (stn 1-5)

Phytoplankton sample analysis procedures: One milliliter triplicate aliquots of the samples preserved in Lugol's iodine solution was used to determine cell density using an inverted microscope (Leica DMIL) and a Sedgewick Rafter Chamber and cell counts were recorded. The phytoplankton were identified using a manual of "Identifying Marine Phytoplankton" book edited by Tomas (1997), an IOC manual and guides no. 41 on "Potentially Harmful Microalgae of the Western Indian Ocean" (Hansen *et al*., 2001a, b), a "Phytoplankton Identification Catalogue of Saldanha Bay, South Africa" (Botes, 2003). For numerical analysis and species identification, 250 mL of water samples were fixed in 5% Lugol's solution and kept undisturbed for three to four days till complete sedimentation was achieved. The samples were further concentrated to a volume

of about 100 mL after which 1 mL (in triplicate) of the concentrated sample was drawn and used for enumeration. The results were expressed as 'number of cells per litre'. The cell counts were used to compute cell density using Stirling (1985) formula in which plankton density is estimated by:

$$N = \frac{A \times 1000 \times C}{V \times F \times L}$$

Where:
N = No of plankton cell per litre of original water
A = Total No. of plankton counted
C = Volume of final concentrate of the sample in mL
V = Volume of a field in mm^3
F = No. of fields counted
L = Volume of water filtered in litre

Determination of the relative abundance of the harmful phytoplankton species: The quantity of harmful phytoplankton in the samples was evaluated using an inverted microscope and Utermohl (1958) sedimentation chamber method useful for counting algae in low concentrations of less than 10^2-10^4 cells L^{-1}. The 95% confidence limit of the cells L^{-1} was then calculated using Poisson distribution method:

$$\pm(2\times\sqrt{n}\times100\%)/n = \pm200\%/\sqrt{n}$$

where, n is the counted number of the phytoplankton species having the distribution shown in Table 1. The concentrations (cells L^{-1}) of all the species observed in the creek were then calculated using:

$$C = N \times \frac{Ba/Bc}{V}$$

where, V is the volume of the counting chamber in mill, Ba is the area of the counting chamber bottom in mm^2, Bc the area of the counted part of the chamber bottom in mm^2 and N the number of cells scored for the species viewed under the inverted microscope magnification window of 20x as contained in Hallegraeff *et al*. (2004). Relative absolute percentage confidence limits of expectation (±) were also calculated to satisfy Poisson distribution criterion (Table 1).

Table 1: Relationship between counted number of cells and 95% significance confidence limit levels, adopted from Hallegraeff *et al*. (2004)

Counts per mills	Confidence limits± (%)
1	200
2	140
4	100
5	90
10	63
20	45
40	32
50	28
100	20
200	14
400	10
500	9
1000	6

Data analyses: All data analyses were performed using MINITAB version 14 software. Data was first tested for normality and homogeneity of variances for parametric statistics application and where the criterion was not met, data was log transformed. Mean abundance of the observed phytoplankton species between stations in the creek were tested using one-way ANOVA at a significance level of $\alpha = 0.05$ whereas their distribution abundances within stations tested using a two-way analysis of variance. The relationship between the physico-chemical parameters and the observed phytoplankton species numbers was also tested using the General Linear Model (GLM).

RESULTS

Composition and abundance of harmful phytoplankton species in the creek: The harmful phytoplankton community distribution within the creek revealed the presence of sixteen species (Fig. 2). These were 9 dinoflagellates (*Alexandrium cohorticula*, *Prorocentrum lima*, *Prorocentrum mexicanum*, *Gymnodium catenatum*, *Ostreopsis lenticularis*, *Gonyaulax polygramma*, *Ceratium furca*, *Ceratium fusus* and *Scrippsiela trochoidea*), 4 cyanobacteria; (*Trichodesmium erythraeum*, *Anabaena spiroides*, *Microcystis aeroginosa* and *Oscillatoria limosa*), 2 diatoms (*Pseudonitzschia pungens*, *Chaetoceros curvisetus*) and 1 flagellate (*Dictyocha fibula*). Their mean concentration of abundance ranged from 10±1-210±11 cells L^{-1} (Table 2).

The species *Dictyocha fibula*, *Pseudonitzschia pungens*, *Trichodesmium erythraeum*, *Anabaena spiroides*, *Microcystis aeroginosa*, *Ceratium fusus*, *Scippsiela trochoidea*, *Ceratium furca*, *Chaetoceros curvisetus* and *Oscillatoria limosa* were found present in all the stations within the creek (Table 2). The most abundant species (cells L^{-1} ±5% value limits) in station 1 were *Chaetoceros curvisetus*, *Ceratium furca*, *Oscillatoria limosa* and *Dictyocha fibula*. Whereas, *Oscillatoria limosa*, *Ceratium furca*, *Dictyocha fibula*, *Chaetoceros curvisetus*, *Prorocentrum lima* were abundant in station 2. Those in station 3 were C*haetoceros curvisetus*, *Anabaena spiroides*, *Dictyocha fibula*, *Oscillatoria limosa* and *Pseudonitzschia pungens*. Station 4 showed *Chaetoceros curvisetus*, *Ceratium furca*, *Ceratium fusus* and *Pseudonitzschia pungens*. The species found in station 5 with high abundances of cells were *Ceratium furca*, *Oscillatoria limosa*, *Chaetoceos curvisetus*, *Pseudonitzschia pungens*. The most common species occurring in all stations were *Chaetoceros curvisetus*, followed by *Oscillatoria limosa*, *Ceratium furca*, *Dictyocha fibula*, *Scrippsiela trochoidea* and finally *Trichodesmium erythraeum*.

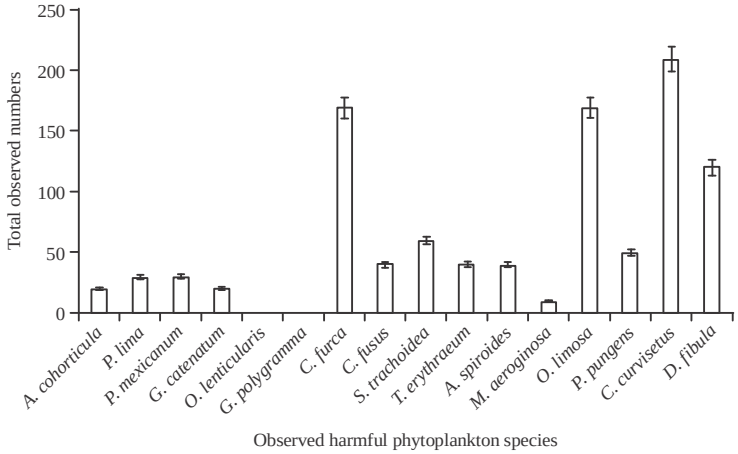

Fig. 2: Overall composition and abundance of the harmful phytoplankton species within the creek (error bars represent ±5% value limits)

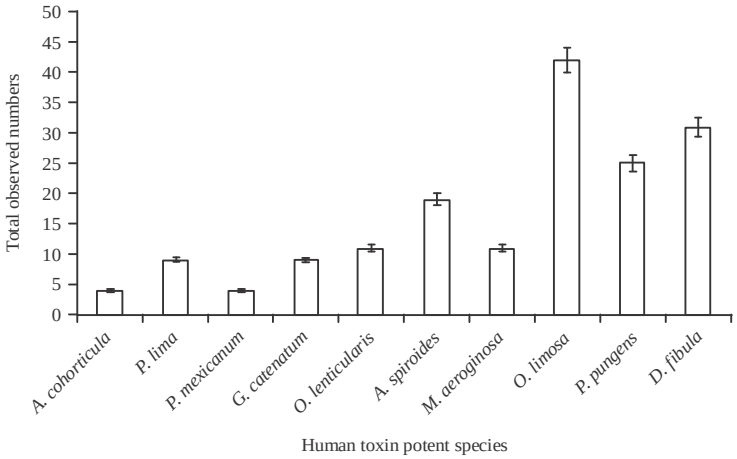

Fig. 3: Relative composition and abundance of the harmful phytoplankton species that produce potent toxins to humans found in the creek (error bars represent ±5% value limits)

Table 2: Mean concentration abundance (cell numbers±5% limit value of expectation) of all harmful phytoplankton species analyzed within the five stations of Shirazi creek

	Mean concentration abundance					ANOVA	
Phytoplankton species	Station 1	Station 2	Station 3	Station 4	Station 5	F	P
Alexandrium cohorticula	20±1	0	0	20±1	0	4.08	0.078
Prorocentrum lima	30±2	60±3	0	0	0	5.57	0.046
Prorocentrum mexicanum	30±2	0	0	0	10±1	0.00	1.000
Gymnodium catenatum	20±1	0	10±1	0	30±2	0.20	0.665
Ostreopsis lenticularis	0	40±2	20±1	30±2	20±1	8.32	0.014
Gonyaulax polygramma	0	0	0	0	20±1	10.98	0.011
Ceratium furca	170±9	70±4	40±2	60±3	110±6	10.79	0.011
Ceratium fusus	40±2	10±1	30±2	60±3	30±2	4.76	0.061
Scrippsiela trochoidea	60±3	10±1	10±1	10±1	10±1	11.56	0.009
Trichodesmium erythraeum	40±2	30±2	10±1	30±2	30±2	0.93	0.364
Anabaena spiroides	40±2	20±1	70±4	20±1	40±2	2.23	0.178
Microcystis aeroginosa	10±1	20±1	40±2	20±1	20±1	0.75	0.412
Oscillatoria limosa	170±9	80±4	50±3	40±2	80±4	0.64	0.448
Pseudonitzschia pungens	50±3	30±2	50±3	50±3	70±4	2.04	0.191
Chaetoceros curvisetus	210±11	60±3	80±4	70±4	70±4	6.81	0.031
Dictyocha fibula	120±6	60±3	50±3	30±2	50±3	10.88	0.011

Six of the population species including *Ceratium furca*, *Chaetoceros curvisetus*, *Dictyocha fibula*, *Gonyaulax polygramma*, *Ostreopsis lenticularis* and *Scrippsiela trochoidea* were found to have good evidence of significant variation in their overall mean abundance among the species ($p<0.05$). This implied they are likely to bloom within the stations ($F = 7.01$) and ($p = 0.000$) when tested using a two-way ANOVA.

Human toxin producing phytoplankton species within the creek: The total number of cells encountered and counted for the various species within the creek was, 42 (*Oscillatoria limosa*), 31 (*Dictyocha fibula)*, 25 (*Pseudonitzschia pungens*), 19 (*Anabaena spiroides*), 11 (*Microcystis aeroginosa*), 11 (*Ostreopsis lenticularis*), 9 (*Prorocentrum lima*), *Gymnodium catenatum* and 4 cells for both *Prorocentrum mexicanum* and *Alexandrium cohorticula* (Fig. 3). Microscopic analysis of the samples revealed the presence of ten potent human toxin producing phytoplankton organisms; *Prorocentrum lima*, *Prorocentrum mexicanum*,

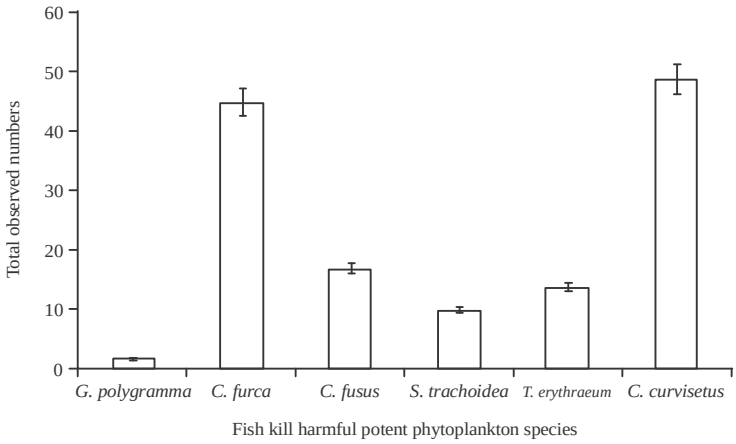

Fig. 4: Relative composition and abundance of the harmful phytoplankton species, which cause indiscriminate kills of fish and invertebrates in the creek (error bars represent ±5% value limits)

Table 3: Observed mean values of physico-chemical parameter factors

	Observed mean values per station						ANOVA	
Parameters	Stn 1	Stn 2	Stn 3	Stn 4	Stn 5	Min-Max	F	P
NO_3 (mg L^{-1})	0.02±0.05	0.01±0.05	0.03±0.05	0.01±0.05	0.02±0.05	0.01-0.03	0.82	0.628
PO_4 (mg L^{-1})	0.02±0.05	0.02±0.05	0.02±0.05	0.03±0.05	0.02±0.05	0.02-0.03	0.33	0.982
NH_3 (mg L^{-1})	0.08±0.05	0.08±0.05	0.10±0.05	0.06±0.05	0.08±0.05	0.06-0.10	0.57	0.859
Salinity (%)	36.20±0.5	38.10±0.5	39.30±0.5	37.90±0.5	40.10±0.5	36.2-40.1	1.64	0.103
Temperature (°C)	31.10±0.5	29.90±0.5	28.60±0.5	30.60±0.5	32.80±0.5	28.6-32.8	0.59	0.845
pH	8.50±0.05	8.10±0.05	7.90±0.05	8.50±0.05	8.70±0.05	7.9-8.7	0.65	0.792
Turbidity (m)	0.90±0.1	0.60±0.1	0.50±0.1	0.80±0.1	2.00±0.1	0.5-2.0	0.64	0.804

Min: Minimum, Max: Maximum, Stn: Station

Alexandrium cohorticula, *Gymnodinium catenatum*, *Microcystis aeroginosa*, *Ostreopsis lenticularis*, *Anabaena spiroides*, *Pseudonitzschia pungens*, *Dictyocha fibula* and *Oscillatoria limosa*. The mean concentration abundance ranged from 20±1-170±9 cells L^{-1} (Table 2).

Harmful species to fish and invertebrates within the creek: Six major blooming phytoplankton species (*Gonyaulax polygramma*, *Scrippsiela trochoidea*, *Trichodesmium erythraeum*, *Ceratium fusus*, *Ceratium furca* and *Chaetoceros curvisetus*) were recorded capable of harming fish and invertebrates by damaging or clogging of gills (Fig. 4). Their counted numbers varied from 49 cells for *Chaetoceros curvisetus*, 45 for *Ceratium furca*, 17 for *Ceratium fusus*, 14 for *Trichodesmium erythraeum*, 10 for *Scrippsiela trochoides*, 2 for *Gonyaulax polygramma* within the creek (Fig. 4).

Water quality parameters: Nitrate concentrations ranged from 0.01±0.05-0.03±0.05; phosphates from 0.02±0.05-0.03±0.05 and ammonia from 0.06±0.05-0.10±0.05 (mg L^{-1}) (Table 3). Salinity values were nearly equal between the stations ranging from 36.2±0.5-40.1±0.5 (‰). Temperature (°C) varied between 28.6±0.5 and 32.8±0.5, whereas pH between 7.9±0.05 and 8.7±0.05. Turbidity measurements (m) ranged between 0.5±0.1 and 2.0±0.1. The mean water quality parameters were registered in Table 3 during the study period. The mean water quality parameters were tested by

using the General Linear Model (GLM) multi-way ANOVA. They revealed a possible non-significant influence on the abundance and distribution of the species within the creek (all had p-values>0.05) (Table 3).

DISCUSSION

Tropical waters are generally oligotrophic and development of blooms is usually reduced in frequency as was seen in the low cell densities of harmful algal species in this study. The bloom extent may therefore have toxic localized impacts with mosaic formations in close areas. In such ecosystems, the toxins are transferred through the food chain by the organisms feeding on algae through bioaccumulations (Bourdeau *et al*., 2001). Most of the potential harmful algae encountered in this study have the potential of forming blooms while in high densities brought about by other physicochemical characteristic of the aquatic systems. For example, *Gymnodinium* sp., is known to cause fish kills and benthic fauna at cell densities (>10^7 cells L^{-1}) (Andersen, 1996). However, some species such as *Alexandrium* sp. are also known to be highly toxic at low cell densities, when having no visible discolorations in the water.

The observed low cell densities of these harmful algal species could be attributed to the creek's altered photochemistry brought about by the high turbidity levels caused by the Ramisi river inflow and tidal movements that resulting into sediment flocculation, flushing out and sinking of attached algal cells (Pierce *et al*., 2004). The wide distribution of the observed harmful algal species in this study (but in low cell densities) could be explained by the fact that these species are less affected by coastal nutrient enrichments (Hansen *et al*. 2001b). On the other hand, the high number of *Pseudo-nitzschia* sp. described as cryptic bloomers (Trainer *et al*., 2009), could be attributed to its ability to grow over a wide range of salinities (Thessen *et al*., 2005) and the ability of its blooms to arise from shifts in population rather than appearance of fundamentally different assemblage. Some encountered species in this study such as *Alexandrium* sp., *Ostreopsis* sp. and *Prorocentrum* sp. produce different categories of toxins which accumulate in the food chain leading to specific syndromes in the predators. For instance, Paralytic Shellfish Poisoning (PSP) which is causative organisms are the dinoflagellates (*Alexandrium* sp. and *Gymnodinium* sp.) that produce saxitoxins characterized by gastrointestinal and neurological symptoms, nausea, vomiting, diarrhea, tingling or numbness around lips with gradual and more severe paralysis, respiratory difficulty and death through respiratory paralysis.

The presence of *Prorocentrum* sp. in this study is indicative of the potential possibility of Diarrhetic Shellfish Poisoning (DSP) caused by a group of toxins. It may be represented by okadaic acid and characterized by gastrointestinal symptoms (nausea, diarrhea, abdominal pain and vomiting) following chronic exposure. This may evolve into digestive system tumors from continuous consumption of contaminated seafood by these microalgae. Unfortunately, local communities living along the Kenyan coast depends on coastal fisheries as a source of livelihood through mariculture, artisanal fishing and offshore fishing. This increases the possibility of exposure to the potential harmful algae that is also expected to lead to massive fish deaths and destruction to most fishing grounds. Alternatively, closure of fishing areas with harmful algae must subject fish to depurate. Hence, Kenya as a country is bound to suffer losses resulting from rejection of fish by the international market. That is because fish toxin levels in fish products must be reported as a requirement in some export destination countries. In such cases, Aquaculture can also be greatly impacted by harmful algae through massive fish deaths leading to serious economic losses. Blooms that cause water discolorations also reduce the visibility of these areas making snorkeling and diving difficult. Beaches can also be rendered inhabitable from foul smell of

hydrogen sulphide resulting from bacterial activity on dead organisms. The bacteria act on dead organisms resulting from the anoxic conditions caused by the massive harmful algal bloom cells to produce hydrogen sulphide foul smell. Alternatively, this foul smell may lead to reduced tourist visit influx to these areas resulting into reduced economic activities leading to low revenue.

CONCLUSION

The study revealed the presence of some potential toxin producing phytoplankton community species that have been documented elsewhere in the world as causative agents for algal toxic event outbreaks. Therefore, there is a real danger for future algal toxic event outbreaks in Kenya and future economic loss to the Kenyan fishery in addition to human health impacts that cannot be underestimated. Hence, a need to set up regular monitoring programs for their detection is urgent. Also, effective education, dissemination and communication of the available information are necessary to ease regulation, harvesting and use of the fishery resources.

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