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Full Length Research Paper

Heavy metals pollution index of surface water from Commodore channel, Lagos, Nigeria

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Surface water pollution poses a major risk to all biotic entities dependent on the water body. This study assessed the surface water quality of commodore channel, Lagos lagoon with respect to its heavy metals pollution level using indexing approach to determine the sources of heavy metal and its associated pollution risk. Samples were collected at a depth of 10-15 cm within a distance of 4 km from the shore line. Metal concentrations were evaluated using Atomic Absorption Spectrometry. The Concentrations of Pb (0.203-2.601 mg/L), Fe (0.253-1.049 mg/L), Cd (0.017-0.133 mg/L), and Co (0.000-0.226 mg/L) exceeded the recommended limits set by the World Health Organization, while Zn concentration (0.007-0.319 mg/L), was within acceptable limits for all locations investigated. Heavy metal pollution index (HPI) of the study area was found to be 3532.1, exceeding the critical pollution index value of 100. Similarly, the Metal Index (MI) of 150.5 of the study area was above threshold limit value of 1, suggesting that the area is seriously polluted with heavy metals. Sample t-test, confirms that there is a significant statistical different (with p value at $p<0.05$) between samples collected from the industrial areas and non-industrial area. The study revealed the sources of the heavy metals are primarily from anthropogenic source attributed to the untreated industrial discharge, and municipal solid waste disposed in the area. Prompt enforcement of environmental protection laws is needed to prevent continuous pollution of the area.

Key words: Water quality, heavy metals, atomic absorption spectrometry, surface water, heavy metal pollution index, metal index.

INTRODUCTION

Water is exposed to numerous anthropogenic effects in the form of pollutants including toxic metals such as lead,

cadmium and chromium. The impairment of water quality due to introduction of these pollutants is regarded as a

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Major problem faced by most industrial cities around the world. The uncontrolled discharge of waste effluents to large water bodies has and is still adversely impacting on both water quality and aquatic life (Das and Acharya, 2003).

Heavy metals are bioaccumulated in several compartments across the food webs (Oyewo (1998). Metal bioaccumulation can be hazardous at all trophic levels, especially for human at the end of the food chain. Heavy metals are inorganic pollutants of great environmental concern as they are non-biodegradable, toxic and persistent with serious negative ecological ramifications on aquatic ecology (Jumbe and Nandini, 2009). Poorly planned urbanization and industrialization in some developing countries has been attributed to continuous pollution of the environment (Bhagure and Mirgane, 2010; Varalakshmi and Ganeshamurthy, 2010).

Poor enforcement of environmental protection laws by government agencies in Nigeria has aided the discharge of untreated effluent and municipal waste into the water bodies (Oludayo (2012). Various industries and refineries discharge their effluent without treating it and these unhealthy practices have the tendency of deteriorating water quality.

Tsai et al. (2003) had established that the distribution of heavy metals in surface water can provide an evidence of the anthropogenic impact on aquatic ecosystems and therefore aid in the assessment of the risks associated with the discharged waste. Heavy metal pollution index (HPI) and Metal Index (MI) had been widely deployed as an effective tool for the assessment of heavy metal risk in surface water bodies (Ameh and Akpah 2011; Goher et al., 2014, Ojekunle et al., 2016) and ground water (Kumar et al., 2012, Tiwari et al., 2016)

Philips et al. (2012) has identified Commodore channel as an essential part of Lagos lagoon intricate system which connects the Lagos lagoon to the Atlantic Ocean. Abiodun and Oyeleke (2016) assessment of sediment in the Lagos lagoon had observed discharge of industrial and domestic waste within the Commodore channel. To the best of the authors knowledge there is no published report on the heavy metal pollution of surface water of this area.

Hence, the overall objectives of this research work are to:

1. Evaluate the heavy metals concentrations of the surface water within Commodore channel;
2. Assess the associated pollution risk using indexing approach;
3. Determined the possible source of contamination.

MATERIALS AND METHODS

Study area

The study area is Commodore channel, Lagos State. It is situated within the Lagos lagoon (Figure 1). The brackish coastal lagoon lies within latitude 6°26'20.7" N and longitude 3°21'32.7" E. Lagos Lagoon empties into the Atlantic Ocean through Lagos harbour. The study area covers four kilometres (4 km) from the shore, and 500 m width. It is a tidal estuary with an average depth of 10 m. The area has a tropical climate and average annual rainfall is 1693 mm, the average temperature is 27.0°C. The fauna is composed of marine and brackish water species; depending on the season, among the fauna exploited for commercial purposes are finfish and shellfish. The channel is bounded by commercial offices, industries, ports and shipping companies (Table 1). The industries are mainly engaged in sugar refinery and paint production. There is visible discharge of sewage into the channel from the industries and shipping companies.

Sampling and sample treatment

Surface water were sampled in April, 2016 from ten sampling stations determined using the Global Position System (GPS); nine samples were collected from areas of industrial activities and one was taken from a non-industrious area (P6) Table 1. The samples were collected at 10-15 cm depth using decontaminated polypropylene bottles. Collected samples were acidified with concentrated nitric acid to a pH below 2.0 to minimize precipitation and adsorption on container walls. The samples were kept at 4°C in an ice-container and transported to the laboratory for analysis. The samples of water were digested and then transferred into plastic bottles, labeled for analysis. They were analyzed for Pb, Zn, Fe, Cd and Co using atomic absorption spectrophotometer. The selected heavy metals are some of the major toxic metals as identified by USEPA (2002) in waste and water bodies. The analysis was carried out in accordance with the standard procedures specified in APHA 2005 and USEPA 3005 (USEPA 1987).

Digestion of sample and quality assurance

50 mL of each sample was digested with HNO_3 as described by USEPA SW Method 3005 (USEPA 1987) procedure for the digestion of water sample: 50 ml HNO_3 was added to sample in the beaker, covered and heated using hot plate placed in a fume cupboard until the volume has been reduced to 15-20 mL. Samples were allowed to cool and filtered using Whatman No. 42 filter paper. It was then transferred quantitatively to a 50 mL volumetric flask and made up to the mark with distilled water.

Quality control measures and blanks were utilized in the course of the analysis. Sample blanks and replicate samples were analyzed along with samples to ensure precision and accuracy of analyses.

Data evaluation

Heavy metal pollution index (HPI)

Heavy metal pollution index (HPI) was applied for the assessment of water quality on the basis of heavy metal concentration. Heavy metal pollution index (HPI) according to Mohan et al. (1996) is determined as thus:

$$HPI = \sum_{i=1}^{n} \frac{Wi*Qi}{Wi} \qquad (1)$$

Where Qi represent Sub index of the ith parameter, Wi denote unit weight of the ith parameter and n is the number of parameters determined. The sub index (Qi) of each parameter is calculated as:

$$Qi = \sum_{i}^{n} 100 \, X \left(\frac{Mi-Li}{Si-Li}\right) \qquad (2)$$
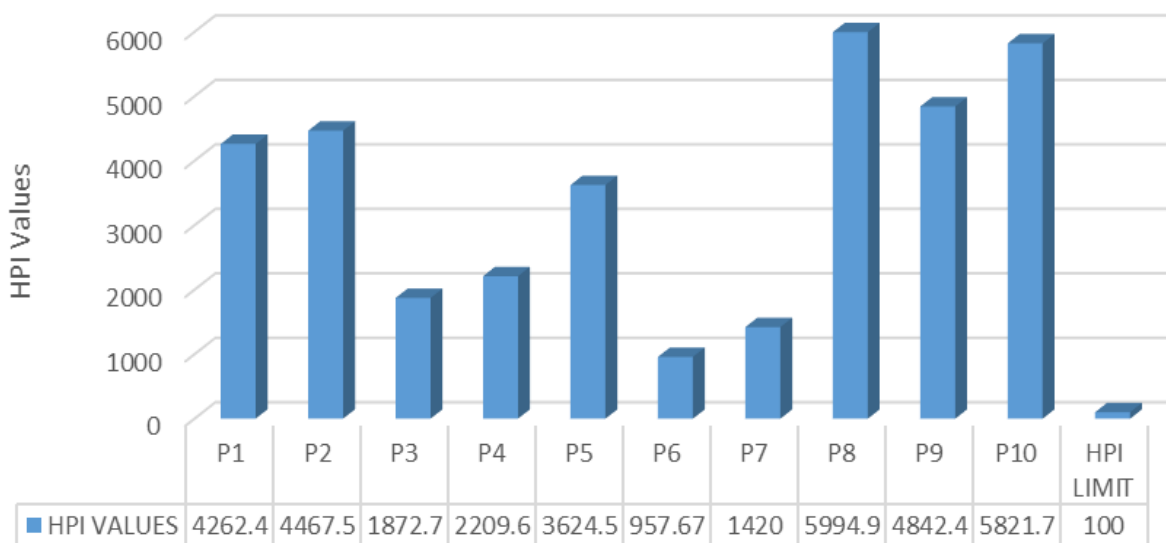
Figure 1. HPI values at various sampling points.

Table 1. Sample location and brief location description.

Sample location	Location description
P1	Industries present, port facilities
P2	Industrial activities, sewage discharge
P3	Ports and shipping anchorage
P4	Pathway, industrial presence
P5	Dangote refinery, jetty
P6	Beach, fishing activities, nearby rural settlement
P7	Energy industry, Sewage discharge
P8	Paint manufacturing industry, Jetty
P9	Sewage discharge, nearby dumpsite
P10	Bua sugar refinery, sewage discharge

Where M_i is the evaluated value of heavy metal of the ith parameter, I_i denoted the ideal value of the ith parameter, S_i represent standard value of ith parameter.

Metal index (MI)

The metal index (MI) was defined by Tamasi and Cini (2004) as:

$$MI = \sum_{i=1}^{n} \frac{C_i}{(MAC)i} \qquad (3)$$

Where MI is the metal index, C is the concentration of each element in solution, MAC is the maximum allowed concentration for each element, and the subscript i is the ith sample.

RESULT AND DISCUSSION

Heavy metal concentrations

Heavy metals concentrations (mg/L) of samples investigated is shown in Table 2. The ranges of the heavy metal concentrations varied widely: Pb (0.203-2.601), Zn (0.007-0.319), Fe (0.253-1.049), Cd (0.017-0.133), and Co (0.0000-0.226). The mean concentrations of Pb, Zn, Fe, Cd, and Co are 1.232, 0.193, 0.629, 0.053 and 0.075 mg/l, respectively. Mean concentrations of Pb, Fe, Cd, and Co exceeded the permissible limit for portable water set by World Health Organization (WHO, 2006) While Zn was within the desirable limit. This could be attributed to the poor content of zinc in the discharge effluent or municipal waste and its low content within the sampling area. The relative abundance of the heavy metals were in the order Pb>Fe>Zn>Co>Cd. The elevated heavy metal concentrations of the study area could pose serious health challenges to most dwellers within the areas that largely depend on the water for agricultural and domestic uses. The possible effects of these heavy metals in humans and animals are presented in Table 3.

Table 2. Chemical analysis of waters in the study area.

Location	Longitude	Latitude	Heavy metal concentrations(Mg/L)				
			Pb	Zn	Fe	Cd	Co
P1	6.441326N	3.391041E	1.422	0.011	0.886	0.078	ND
P2	6.438612N	3.395637E	1.75	0.29	0.61	0.056	0.026
P3	6.433634N	3.393293E	0.61	0.317	0.45	0.028	0.084
P4	6.433720N	3.389129E	0.86	0.221	0.511	0.026	0.037
P5	6.435582N	3.384398E	1.016	0.317	0.591	0.077	0.074
P6	6.433870N	3.378352E	0.325	0.007	1.049	0.017	ND
P7	6.432827N	3.368201E	0.203	0.094	0.565	0.044	0.07
P8	6.441326N	3.363216E	2.601	0.319	0.634	0.039	0.184
P9	6.437801N	3.359049E	2.113	0.243	0.75	0.039	0.053
P10	6.437987N	3.358626E	1.422	0.119	0.253	0.133	0.226
Minimum			0.203	0.007	0.253	0.017	ND
Maximum			2.601	0.319	1.049	0.133	0.226
Mean			1.232	0.193	0.629	0.053	0.075
WHO (2006)			0.01	3	0.3	0.003	0.01

Table 3. Evaluation of heavy metals in waters of the study area for drinking/domestic purposes and possible health effects (Modified after WHO, 2006; Levinson, 1980).

Heavy metals (mg/L)	WHO (2006) guideline		Values from study area		Evaluation for drinking and/possible health effects
	Desirable limit	Permissible limit	Range	Mean	
Cd	<0.01	<0.01	0.017-0.133	0.053	Very high. May be carcinogenic; May cause kidney damage, lung cancer, ostemalcia or osteoporosis, anaemia, teeth discolouration.
Co	<0.01	<0.01	0.000-0.226	0.075	Very high. May cause vomiting, dermatitis, asthma, skin rashes, vision and heart problem.
Zn	<1.0	<1.0	0.007-0.319	0.193	Excellent to good. High levels may cause stomach cramps, nausea and vomiting.
Fe	<0.1	<0.3	0.253-1.049	0.629	Very high. May cause vomiting and conjunctivitis, stains and taste.
Pb	<0.001	<0.003	0.203-2.601	1.232	Very high. May cause kidney damage, anorexia, encephalopathy (brain swelling), dizziness, digestive disorder, lung cancer, coma and death.

Assessment of metal contamination

Two quantitative methods were used in assessing the risk level of heavy metal concentrations contamination in the samples: Heavy metal pollution index (HPI) and metal index (MI).

The heavy metal pollution index for the study area was calculated using the mean concentration values of the selected metals (Pb, Zn, Fe, Cd and Co); the mean HPI was found to be 3532.1 (Table 4) which exceeds the critical pollution index value of 100. This implies that the study area (commodore channel), is critically polluted

with very high concentrations of heavy metals. HPI for all sampling point were found to be greater than acceptable (HPI> 950) with the highest value (5994.92) recorded at P8 and the lowest value (957.67) recorded at P6 (Figure 2 and Table 5). Although P6 is characterized by non-industrial activities in the area, this outcome could be linked to dilution effect from discharge point towards the area (Kithiia, 2006). This shows that concentration of metals decreased with increasing distance from the pollutant emission sources. Metal index for the study area revealed very poor water quality with MI value of 150.5 (Table 6) which is above the threshold limit of MI value >1

Table 4. HPI recorded at different sampling locations.

Sampling location	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
HPI	4262.4	4467.5	1872.7	2209.57	3624.5	957.67	1420	5994.92	4842.37	5821.74

Σ HPI = 3544.
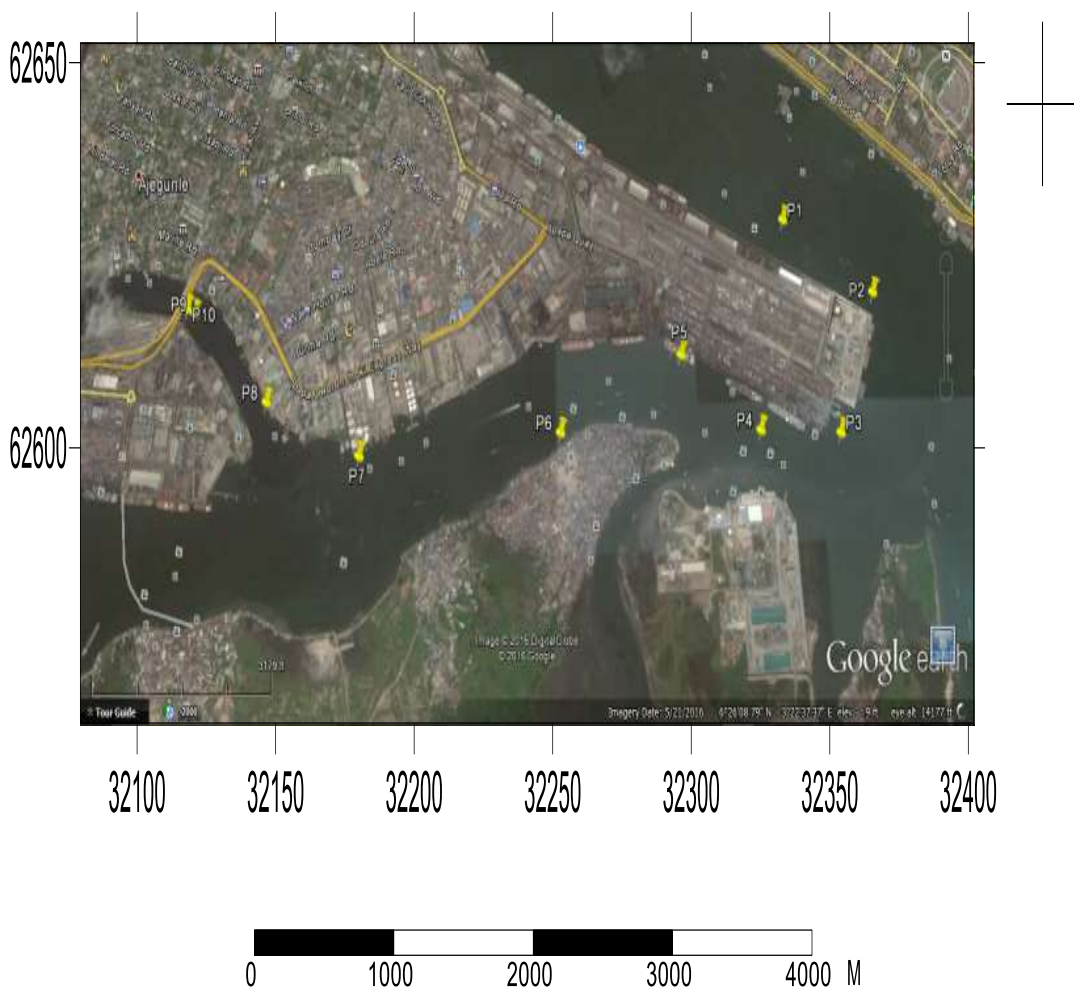
Figure 2. Google Earth image showing the sampling points in study area.

(Table 7). This observation buttress the initial observation that the channel has high burden of heavy metals concentration (Lyulko et al., 2001; Caerio et al., 2005).

This findings is higher than index values reported by Manoj et al. (2012) for Subarnarekha River (India) in which HPI value is 49.12, Reza and Singh (2010) for river water Angul-Talcher region, India in which the HPI value is 36.19 in summer and 32.37 in winter seasons. The findings are in agreement with research by Kumar et al. (2012), in which heavy metal pollution index was utilized to evaluate contamination in Chennai city, India. His result showed the sources of contamination were primarily anthropogenic had a common origin.

To determine if the presence of industrial activities played any role in concentration of heavy metal in the study area, a statistical tool (Sample t-test) was utilized, mean concentration of each heavy metal from areas of industrial activities were tested against each heavy metals from an area of non-industrial activity (P6); results showed that there is significant difference in concentration of heavy metals between the industrial areas and the non-industrial area with P value ($p<0.05$), using a 95% confidence level for a 2-tailed test and degree of freedom (n-1) 8. The location of non-industrial activity (P6) recorded fairly low concentrations of all investigated heavy metals except for Fe concentration which was

Table 5. Mean HPI calculation for surface water sample

Heavy metal (mg/L)	Mean concentrations (Vi)	Highest permitted value (Si)*	Unit weightage (Wi)	Subindex (Qi)	Wi x Qi
Pb	1.232	0.01	100	12320	1232000
Zn	0.193	3	0.333	6.43	2.14
Fe	0.629	0.3	3.3	209.67	691.91
Cd	0.053	0.003	333.33	1766.67	588884.11
Co	0.075	0.01	100	750	75000
Σ			536.96		1896578.16

HPI = 3532.1. * World health organization drinking water guideline, 2006.

Table 6. Mean MI of commodore channel.

Heavy metal (mg/L)	Mean concentration (C_i)	Highest permitted value (MAC_i)	MI
Pb	1.232	0.01	123.22
Zn	0.193	3	0.064
Fe	0.629	0.3	2.096
Cd	0.053	0.003	17.66
Co	0.075	0.01	7.5

Σ MI = 150.5.

Table 7. Water quality classification using MI (Lyulko et al., 2001; Caerio et al., 2005).

MI	Characteristic	Class
<0.3	Very pure	I
0.3 - 1.0	Pure	II
1.0 - 2.0	Slightly affected	III
2.0 - 4.0	Moderately affected	IV
4.0 - 6.0	Strongly affected	V
>6.0	Seriously affected	VI

found to be very high (1.049 mg/L) compared to other sampled locations This could be associated to iron mobility in water bodies. According to Kabata-pendias (2001), iron is relatively immobile under most environ-mental conditions mainly due to the very low solubility of iron (III) hydroxide in its various form. Its solubility is strongly influenced by redox conditions; his findings recorded highest concentration of iron in regions of base-poor buffering capacity and he further suggested that the non-degradable nature and possible slow rate of dispersion may be responsible for higher levels observed near the shore region.

Conclusion

The study revealed that Zn concentration was fairly low and within recommended limit, while levels of Pb, Fe, Cd and Co in water samples exceeded the World Health Organization (WHO, 2006), standard limits portable water.

Heavy metal indexing approach is a very useful tool in evaluating overall pollution of water bodies with respect to concentrations of heavy metals. The HPI and MI models indicated a high degree of heavy metal pollution in the study area which could be traced to primarily anthropogenic sources from untreated industrial discharge, refuse dumping, municipal waste and processing activities in the area. Using the sample t-test, it was confirmed that the industrial activities around the study area could play a major role in the increased level of heavy metal concentration observed. Prompt enforcement of environmental protection laws is needed to prevent continuous pollution of the area. These findings represent the first reported assessment of the study area.

Recommendation

Companies discharging effluent into the commodore

channel should be made to put in place waste water treatment plant capable of effectively trapping potential heavy metals in the generated effluents.   Further research is needed to assess the direct impact of the pollution on human and plant health within the area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Assessment of water quality in the lower Nyong estuary (Cameroon, Atlantic Coast) from environmental variables and phytoplankton communities' composition

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The aim of this study was to provide a first-hand water quality assessment of the River Nyong estuary, Southern Atlantic coast of Cameroon. Environmental variables and phytoplankton communities were monitored at four surface stations in the estuary for 2 season cycles from 2014 to 2015. A total of 208 species of phytoplankton, belonging to five (5) groups of microalgae, were identified. The Shannon-Weaver diversity index showed a qualitative seasonal difference in composition of the phytoplankton community. Dissolved nutrients (nitrogen and phosphorus) values showed seasonal fluctuations throughout the sampling period. A ratio appeared high (42.78) in the rainy season and low (5.89) in the dry season, two values corresponding to the change in specific richness (high and low respectively during the major rainy season and the small dry season). The Water Quality Index (WQI) showed that water quality fluctuates from good to bad, in conjunction with biological indicators (Shannon-Weaver, Abundance and Specific richness).

Key words: Quality assessment, Nyong estuary, environmental variables, phytoplankton, water quality index.

INTRODUCTION

Marine and coastal zone of Cameroon are subjected to huge economic, social and environmental issues, which, like elsewhere in the world, make it a highly sensitive area. This coastal area of Cameroon is increasingly being sought by a number of economic activities, mainly fishing and tourism. There are also numerous development projects, industries (Kribi deep-sea port, gas plant, Douala autonomous port, cement plant, agro-industries, fisheries) and the future Kribi marine protected area that aims at sheltering several patrimonial species (Turtles,

Manatees, Sharks and pelagic species) (Folack, 2011).

The overall environmental concerns of this zone are potentially linked to oil, industrial, agro-industrial, port, urban and cross-border pollution, as well as fishing, navigation and maritime transport. Since the middle of the last century, estuaries are being profoundly modified regarding not only their geometry but also the natural hydrological and sedimentological processes. In addition, waste and pollutant releases resulting from uncontrolled urbanization and industrialization often exceed estuaries assimilative capacity, modifying the ecological balance of estuaries (Boto and Bunt, 1981; Poulin, 2008). It is therefore appropriate to propose models of balanced management of these sensitive hydrosystems. The major challenge is to find methods to evaluate the potential of these ecosystems, to diagnose their disturbances through basic research works and to predict their evolution at lower cost.

Water quality is generally assessed by the coupled use of physico-chemical and biological parameters collected from the field. Monitoring based solely on physicochemical analysis is often insufficient, and reliable results rely mainly on investigations close to the contamination sources (Samocha and Lawrence, 1995; Casé et al., 2008). However, although physico-chemical analyzes provide information on water quality during sampling, living organisms that fulfill all or part of their life cycle in the aquatic environment also provides information on the conditions of their evolution/growth (Beyene et al., 2009). A more robust investigation of water quality then requires the combination of both physicochemical variables and biological indicators (Jones et al., 2001; Casé et al., 2008; Gharib et al., 2011). Among water quality bioindicators, phytoplankton organisms are recognized as good indicators of the health status of aquatic environments due to their sensitivity to human activities and climate change at different time scales (Fathy et al., 2001; Zeng et al., 2004; Pongsarun et al., 2007; Fonge et al., 2013). In addition, the composition of the phytoplankton stand reflects previous conditions that would have characterized the environment before the study (Gharib et al., 2011).

The presence of some algal groups in a particular environment is related to specific levels of pollution. The group of Chlorophyta, Cyanophyta and some spcecies of Chrysophyta which are found in tropical estuaries are common species with tolerance of pollution. Some species are indicators of moderate pollution and other are indicators of organic pollution (Luan and Sun, 2010; Ganai and Parveen, 2013). *Closterium aciculare* and *Nitszchia sp* were used as the best single indicator of pollution and were associated with the highest degree of civic pollution at Yangtze River Estuary and the adjacent east China sea in summer, 2004 (Nandan and Aher, 2005).

In Cameroon, very few studies have been done on phytoplankton of estuaries with existing data relates mainly to the Wouri estuary (Fonge et al., 2013). Data on coastal and port-industrial city of Kribi are disconnected and refer only to aquatic insects (Tchakonté et al., 2014a; 2014b) or to marine and freshwater shrimps (Makombu et al., 2014). However, due to their nutrient enrichment by continental waters, estuaries constitute precious nursery and breeding habitats for many living species.

The Nyong River is one of the most important rivers in South Cameroon. Its downstream runs through the Douala-Edéa Forest Reserve, where active fisheries develop in the mangrove zone. Intensive farming is settled on the banks of its middle course, whereas offshore oil and gas activities are developed in the surrounding area. On the upriver Nyong, several hydrological and geochemical studies have been performed (Ndam et al., 2007). Research studies on the downstream part which is influenced by tidal dynamics are recent, and revealed a high phytoplanktonic diversity consisting in marine and freshwater species, as reported from several other estuaries in the tropical zone (Okosisi et al., 2012; Mama et al., 2016).

The present study was designed in an attempt to investigate water quality of the Nyong estuary, by assessing its physicochemical status through/using the Water Quality Index (WQI) as well as the phytoplankton community structure, diversity and distribution.

MATERIALS AND METHODS

Study site and sampling stations

The Nyong estuary is part of the Campo-Nyong estuary hydrosystem of the Atlantic meridional Coast of Cameroon. The Campo-Nyong falls within the equato-guinean climatic zone characterized by four seasons known as the short rainy season (SRS), the major rainy season (MRS), the small dry season (SDS) and the large dry season (LDS) (Dzana et al., 2011). climate is influenced by the Southwest monsoon winds, limited in average at 10km/h because of the highly developed rainforest at the Southern plateau of Cameroon, and associated strong yearly rainfall (about2919mm/an) (Olivry, 1986). Four sampling stations N1, N2, N3 and N4 were chosen between latitudes 2°48' and 4°32'N, and longitudes 9°54' and 13°30'E (Figure 1). Point N1, a marine station, was fixed at 1 km offshore the mouth. The point N2 was chosen in the middle of the river mouth. N3 and N4 were located upstream respectively at 1.5 km and 4 km from the point N2. Stations were chosen according to their positions to give a global characteristic of the estuary. N1 was a marine station, while N2 was inside the mouth, behind the sandbar giving the mouth a lagoon aspect. The N3 and N4 stations were positioned respectively in the mangrove creeks and at the entrance of the mangrove islands upstream (Figure 1).

Water and phytoplankton sampling

All the seasons were covered during the survey: the major dry
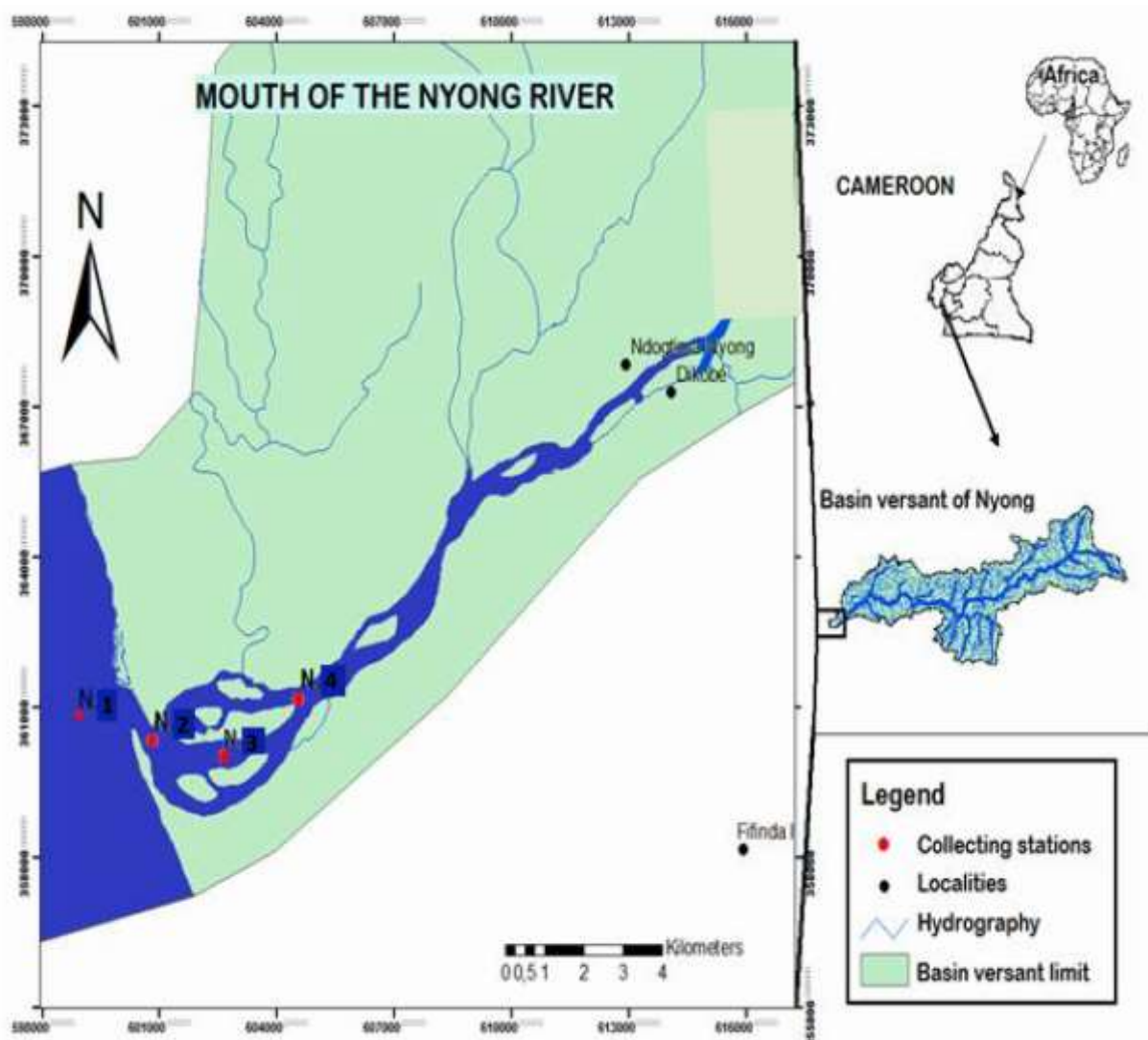
Figure 1. Map of the study area with the sampling stations (adapted from Mama et al., 2016).

season (MDS) in December 2014 and December 2015, the short dry season (SDS) in June 2014 and July 2015, the large rainy season (LDS) in September 2014 and September 2015 and the small rainy season (SRS) in March 2014 and April 2015. For each season, two samplings were carried out at each station during low tide and high tide respectively. Surface water (50cm) was sampled using a 1.7 L Niskin bottle. Water samples for analysis of physicochemical parameters were stored in 1.5 L double-capped polyethylene bottles and transported to the laboratory in an adiabatic enclosure at 4°C (APHA, 1998; Kaniz et al., 2014).

Physicochemical parameters (listed here) were determined according to the Aminot and Chaussepied (1998) method coupled with the standard methods of APHA (2005). Temperature and pH were measured using field pH-meter (Hanna model HI 98130, equipped with a SENTIX 4 electrode). Conductivity and salinity were recorded using a WTW series 3310 set2 connected to a tetracon electrode. Dissolved oxygen was recorded using the EXTECH oxymeter model Exstik II DO 600. Soluble reactive inorganic phosphorous (PO_4^{3-}), nitrites (NO_2^-), nitrates (NO_3^-),

ammonium(NH_4^+), and total suspended solid (TSS), turbidity and total disolved solid (TDS) were determined using a spectrophotometer (HACH DR/2800), following APHA (1998) and Rodier et al. (2009) standard methods. BOD was measured following the standard protocol of Rodier (1996).

Identification and counting of phytoplankton

A total of 64 water samples were collected for phytoplankton analysis, 32 samples per tides. Surface water (50cm) was sampled using a 1.5 L bottle. These samples were immediately fixed by lugol's iodine solution (15 ml) containing 10% acetic acid, and left to stand for 24 h, to allow phytoplankton settle (Sournia, 1978). Then the lower layer (25 ml) containing the sedimented algae was used for identification and counting of algae, using Malassez's cell, under an upright Olympus optical microscope at 60x magnification. Identification of the phytoplankton species followed relevant textbooks and publications, including Iltis (1980), Botes (2001),

Gopinathan et al. (2007), de Ba et al. (2006), Carmelor (1997), Verlencar (2004), and Karlson (2010).

Data analysis

Water quality index

The water quality index (WQI) is a mathematical tool used to transform several water characterization data into a single number that express the water quality level according to Mishra and Patel (2001) scale (0 to 25 for excellent; 26 to 50 for good; 51 to 75 for bad; 76 to 100 for very bad; above 100 for unfit) (Sanchez et al., 2007; Gharib et al., 2011). In this study, seven physico-chemical parameters (pH, dissolved oxygen, nitrate, nitrite, ammonia, phosphate and silicate) were used as recommended by Wepener et al. (2006) for estuarine water quality assessment. Standard values for bathing water given by the World Health Organization were used to obtain the WQI, following the protocol described by Khwakaram et al. (2012) and Behnmanesh et al. (2013). Then, a quality value (Q value) from 0 to 100, based on the normal data range, was assigned to each parameter. Each Q value was multiplied by a weighing factor based on the importance of the parameter, and summation of the weighed Q values yielded the WQI. The determination of the WQI was made by the method of the arithmetic weight index, according to the following steps:

(1) The quality rating scale (q$_n$): Let n be water quality parameters, quality rating level (q$_n$) corresponding to the nth parameter is a number representing the relative contribution of this parameter in polluted water with respect to its permissible standard value

$$q_n = \frac{V_n - V_{i0}}{S_n - V_{i0}} \cdot 100 \tag{1}$$

With, V_n = estimated value of the nth parameter at a given sampling station;
S_n = the standard permissible value of the nth parameter
V_{io} = the ideal value of the nth parameter in pure water. All ideal values are taken equal to zero, except that of pH = 7 and that of dissolved oxygen OD = 14.6 mg / l.

(2) Unit weight (Wn): The unit weight (Wn) was calculated by a value inversely proportional to the recommended standard value **S$_n$** of the corresponding parameter

$$W_n = K/S_n \tag{2}$$

Where, K is a constant of proportionality with $K = 1/\sum 1/S_n$
S_n is a standard value for n[th] parameters
The combination of all the aforementioned equations gives the formula of WQI that follows:

$$WQI = \frac{\sum(W_n \cdot q_n)}{\sum W_n} \tag{3}$$

Specific richness

Specific richness (S) is defined as the total number of identified species in a sample. This parameter can well be a distinctive criterion of ecosystems or stations studied within a given

ecosystem.

Shannon-Weaver diversity (H')

Shannon-Weaver diversity index is a quantitative measure that reflects how many species there are in a sample (specific richness), and simultaneously takes into account how evenly the basic entities (such as individuals) are distributed among those types. The counts were expressed in relative abundances of the species or taxa of the different phytoplankton groups ($P_i = N_i/N$).

$$H' = -\sum_{i=1}^{s} P_i log_2 P_i \tag{4}$$

Where, $P_i = N_i/N$, N_i the number of individuals of one particular species found and N the total number of individuals

Statistical analysis

Simultaneous comparison of seasonal mean values of WQI was performed using the one-factor analysis of variance (ANOVA) test (Fisher's F test) when the conditions of normality and equality of variances are verified. The relationship between physicochemical and biological parameters were verified by the Pearson correlation at a significance level α = 0.05. Species abundance calculations were carried out and the structure of stands was studied for diversity by the Shannon index (Shannon and Weaver, 1963).Rapid variation in nutrient concentrations from one season to another or between two stations required the calculation of the ratio N / P (deviation from the mean redfield ratio N / P = 16. The Spearman correlation test was performed between the Redfield ratio and the specific diversity index. These analyzes were carried out in the STATSTICA 7.1 software.

RESULTS

Physico-chemical characteristics

Seasonal mean values of the physicochemical parameters measured at the Nyong River Estuary are shown in Table 1.
Temperature on the Nyong River varied between 23.57 and 28.70°C. Mean conductivities were higher during the dry season on Nyong (21337µS/cm), than in the major rainy season (12242.9µS/cm). Average dissolved oxygen values range from 5.88 mg/l to 2.58 mg/l. The highest value of the seasonal mean TSS was observed in the LDS (17.04 mg/l), and the lowest in the MRS (6.18 mg/l). In general, the Nyong estuary waters were almost neutral with the mean values of pH varying between 6.63 and 7.4. Nitrogen and phosphate nutrient values show seasonal fluctuations throughout the sampling period. The highest average ammonium concentration (1.24 mg/l) was observed in the large dry season. The average nitrate values ranged from 0.27 mg/l to1.42 mg/l. Nitrites showed a fluctuation between 0.0034 mg/l (major rainy season) and 2.20 mg/l (small rainy season). The highest

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Table 1. The average seasonal physicochemical parameters of the Nyong estuary during the study period.

Seasons	LDS/DJF	SRS/MAS	SDS/JJO	MRS/SON
T (°C)	27.95±2.99	28.70±0.67	23.57±0.35	25.58±0.78
DO (MG/L)	4.69±0.70	4.72±0.80	4.2±1.03	2.58±0.64
CONDUCTIVITY (µS/CM)	21337±30205	12563±32741	14449±12031	12243±26372
TSS (MG/L)	17.04±8.90	8.47±7.00	7.91±5.94	6.18±5.12
PH	7.4±0.69	6.63±0.36	7.3±0.46	7.01±0.31
NH_4^+ (MG/L)	1.24±1.10	0.74±0.61	1.16±1.01	1.18±1.50
NO_3^-(MG/L)	1.08±10	1.38±20	1.42±1.24	0.27±0.36
NO_2^- (MG/L)	0.25±0.50	2.20±2.10	0.53±0.83	0.0034±0.004
PO_4^{2-} (MG/L)	0.36±0.23	0.67±0.69	1.9±0.59	0.80±0.85
BOD (MG/L)	45.88±29.19	14.65±9.53	12.6±25.63	24.68±22.22
N/P	42.78±69.4	18.7±1168	5.89±2.05	18.55±34.6
WQI	43.47±16.08	84.01±20.23	85.80±40.33	68.05±25.22

Table 2. Taxonomic composition and representation of phytoplankton groups in the Nyong estuary.

Division	Class	Order	Family	Genus	Species	Percentage (%)
Chrysophyta	4	14	19	49	77	37.01
Chlorophyta	4	12	22	49	68	32.69
Cyanophyta	2	4	9	22	35	16.90
Pyrrophyta	2	7	11	13	16	7.6
Euglenophyta	1	2	2	7	12	5.80
Total	13	39	63	140	208	100

average seasonal concentration of orthophosphate ions (1.9 mg/l) was observed in the small dry season and lower (0.36 mg/l) during the major dry season. The ratio (N/P = 16) was the highest in the main dry season (42.78) and the lowest in the short dry season (5.58). The seasonal mean of biological oxygen demand (BOD5) varied from 12.6 mg/l to 45.88 mg/l. Water quality index (WQI) values on the estuary indicate good water quality during the main dry season (43.47), and bad water quality during the short dry season and the small rainy season (85,80 and 84.01 respectively). During the major rainy season, Nyong estuarine water presents a moderate status with the mean WQI of 68.05. Significant difference was observed among WQI between seasons (ANOVA 1, P < 0.05). Pearson correlation revealed that some physicochemical parameters of Nyong were correlated: BOD and suspended matter (r = 0.95, P <0.05) and WQI and Nitrites (r = - 0.97, p <0.05).

Phytoplankton stand structure

Specific richness

Of the 64 samples collected, a total of 208 species of

phytoplankton were identified and distributed in 5 groups of microalgae (Mama et al., 2016): Chrysophyta, Chlorophyta, Cyanophyta, Pyrrophyta and Euglenophyta. Chrysophyta made up the highest number with 49 genera and 77 species; while Euglenophyta ranked low with 7 genera and 12 species identified (Table 2).

Seasonal variation of phytoplankton

Specific diversity

The evolution of diversity index on the Nyong estuary by box-whiskers shows a seasonal variation (Figure 2). During the MRS and the LDS, interquartile ranges were less spread than in the other two seasons, showing an appreciable level of homogeneity of observed species. Moreover, the distribution was symmetrical in all seasons. Shannon index values ranged from 3.3 to 5.4, with the greatest value obtained during the SDS and the smallest during the SRS. The diversity index values (H') were generally high at all stations on the estuary. The maximum (H'= 4.81) was observed at station N4, and the minimum (H'= 3.81) at station N3, during the small dry season / SDS (Figure 3). Shannon index (H') variances
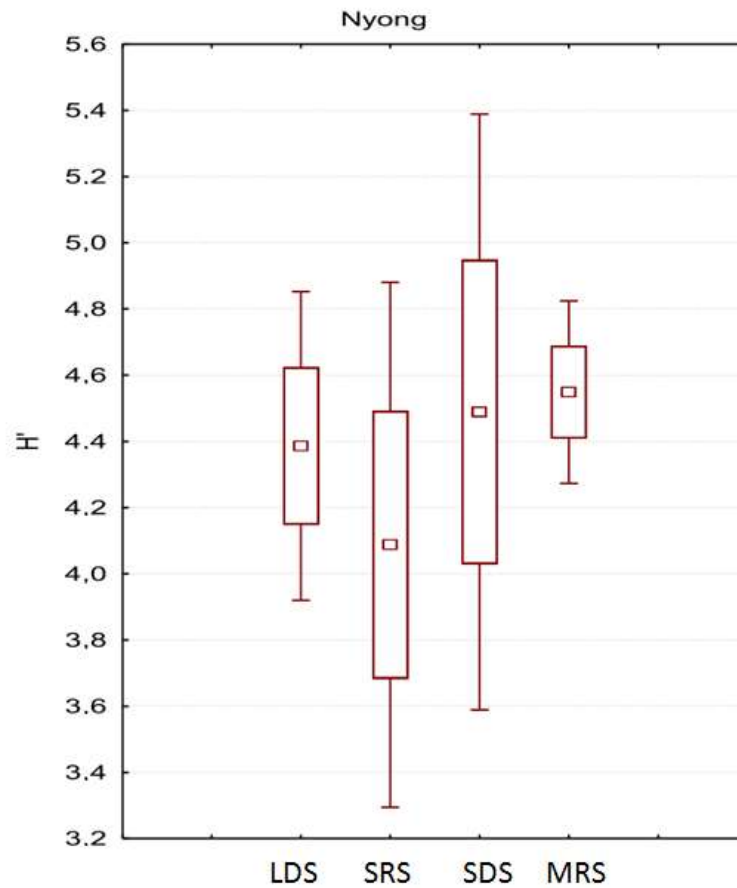
Figure 2. Seasonal variability of the Shannon-Weaver Diversity Index on the Nyong Estuary.

between seasons indicated a significant difference (ANOVA 1, P <0.05) (Figure 2).

During the study period, Chrysophyta and Chlorophyta groups were found to be the dominant microalgae on the Nyong estuary during the whole study period. These two groups constituted respectively 38.50% and 29.70% of the total cells counted (351.7×10⁵ cells/l) during the study. Their specific abundances and compositions varied from one station to another depending on the season (Figure 3). Chrysophyta division rank first in all seasons, except in the LDS where they contributed only 12.22% of the total counts. During this season the Chlorophyta were dominant (51.15%) followed by Cyanophyta (23.53%). A seasonal fluctuation of phytoplankton abundance was observed, from 113.50×105 cell/l during the MRS to 93.90×105 cell/l in the SRS/short rainy season. The minimum was observed during the SDS with 71.64×105 cell/l. The contribution of Euglenophyta and Pyrrophyta were the least important at all seasons, with a percentage abundance of 6.20 and 4.08% respectively.

The total mean of microalgae abundance was 18.78×10⁵±12.14×10⁵ cell/l. Chlorophyta was the most abundant phytoplankton group (31.33×10⁵ cell/l), followed by Chrysophyta (27.71×10⁵ cell/l). Phytoplankton groups were present at all stations in different proportions. At the N1 marine station, Chlorophyta dominated in quantity on the other groups with 15.16×10⁵ cell/l. From a spatial point of view, Chlorophyta of the genus *Closterium* was more abundant (2.75×10⁵ cell/l) at station N1. At station N2, *Calothrix brevissima* (Cyanophyta) was the dominant species. Station N3 was dominated in terms of cell abundance by the genus *Ophiocytium* of the Chrysophyta group (1.67×10⁵ cell/l); and the N4 station by 1.75×10⁵ cell/l of *Synedraulna*.

The average phytoplankton abundance during the LDS was 14.53×10⁵± 9.53×10⁵cell/l. The highest percentage of abundance was Chlorophyta group (38%), followed by Cyanophyta (27.62%). Chlorophyta was dominant at all stations except at station N3 where the Cyanophyta group predominated with 5.65×105 cell/l. *Botryococcus braunii* species (Chlorophyta) dominated at stations N1
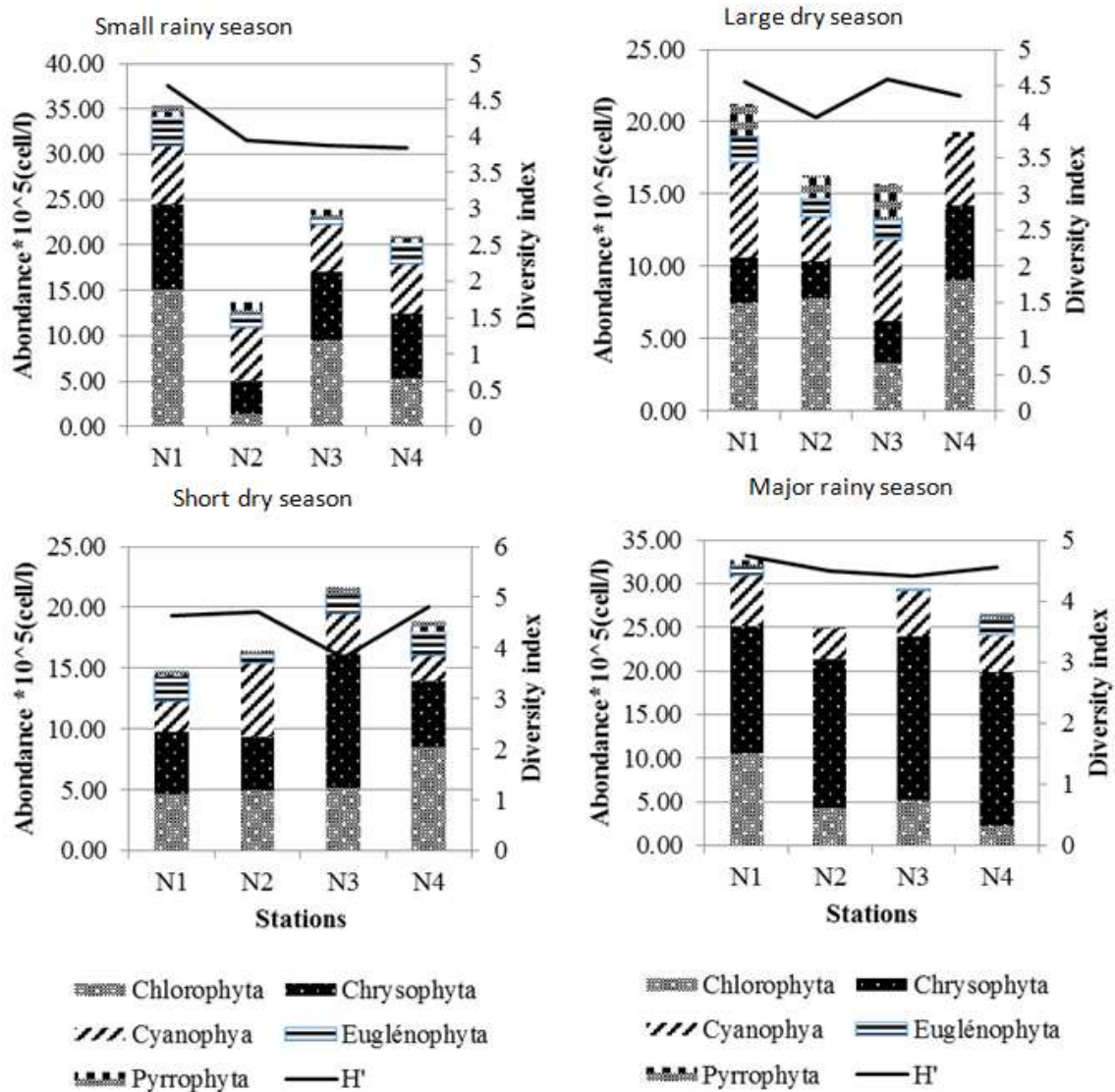
Figure 3. Seasonal variation in the abundance of phytoplankton groups and specific diversity index in the Nyong estuary During SRS,

and N2 with 3.98×105 cell/l and 1.66×10⁵ cell/l respectively. However at station N4, it was the *Closterium aciculare* species (Chlorophyta) that was dominated with 1.66×10⁵ cell/l. During this season, the Pyrrophyta group (6.72×10⁵ cell/l) was more abundant than the Euglenophyta group (4.53×10⁵ cell/l)which was absent at station N4.

During the SDS, the seasonal mean of the total phytoplankton cell abundance was 14.33×10⁵±10.32×10⁵cell/l. The groups of chrysophyta and chlorophyta were the most abundant with 36.04% and 32.51% respectively. Chrysophyta was dominant at station N3 with 10.94×105 cell/l, while Chlorophyta dominated at station N4. At station N2, Cyanophyta

dominated with 6.08×105 cell/l, with the majority of species of the genus *Gomphosphaeria*. At station N3, *Tetraspora gelatinosa* species (Chlorophyta) ranked first with 1.50×105 cell/l. The proportions of Euglenophyta ranged from 0.78×105 cell/l to 2.08×105 cell/l and they were present at all stations. This last group dominated in N4 with the genus *Trachelomonas* (2.25×105 cell/l).

The highest phytoplankton abundance was observed during the MRS (22.70×10⁵ ± 27.01×10⁵ cell/l). Chrysophyta group appears to be dominant at all stations with a percentage abundance of 60.03%, followed by Cyanophyta (18.63×10⁵ cell/l) and Chlorophyta (22.25×10⁵ cell/l) divisions. The most widespread genus of chrysophyta in all stations was the *Nitszchia* genus.

The group of Euglenophyta (3.13×10^5 cell/l), were absent in N2, and Pyrrophyta (1.38×10^5 cell/l) and were the least represented in N3

Correlation analysis

A strong positive correlation was obtained between the abundance and dissolved oxygen concentration (r = 0.95, P <0.001), between the Shannon-Weaver index and dissolved oxygen (r =0.69, P <0.05), which would reflect the high oxygen production (respiration) linked to the abundance and the specific diversity of the estuary. The strong negative correlation observed between nutrients and abundance confirms this strong microalgae production, which is accompanied by a decline in nitrate (NO_3^-, r = -0.72, P <0.05) and nitrite (NO_2^-, r = -0.56, P <0.05) concentrations. Between ammonium and abundance a positive correlation was observed (r = 0.41, P <0.05).

DISCUSSION

A permanent monitoring of physicochemical parameters in aquatic ecosystems provides information on the health status of the environment (Sargaonkar and Deshpande, 2003). In this study, the combination of these variables with phytoplankton made it possible to diagnose the quality of Nyong estuarine waters. Water temperature at the Nyong estuary was high, and follows the atmospheric evolution. It is high in the dry season and low during the rainy seasons.

The average value of pH higher or equal to 7 during the dry seasons or the small rainy season indicates that water bodies of the estuary are made up mainly of basic marine water. These estuarine water bodies have a pH of less than 7 but close to neutrality in the rainy season due to the influence of freshwater from rivers. The increase in conductivity in the dry season is explained by the mineralization of these hydro-systems when the tidal flow prevails over the flow of freshwater.

Nutrients concentrations value obtained during this study were low compared to those of the Douala, Wouri and Dibamba estuaries. For instance, in the study area, and during the rainy and dry seasons respectively, ammonium, nitrate and phosphate concentrations respectively varied in the ranges (0,74 ; 1,18) mg/l, (0,27 ; 1,38) mg/l and (0,67 ; 0,80) mg/l on one hand, and (1,16 ; 1,24) mg/l, (1,08 ; 1,42) mg/land (0,36 ; 1,90) mg/l on the other hand, respectively during the rainy and the dry seasons. Whereas especially in the Douala estuary, previous ammonium, nitrate and phosphate concentrations respectively varied in the ranges (108,8 ; 118,6) mg/l, (107,5 ; 129,9) mg/l and (0,04 ; 0,38) mg/l during the wet seasons, and (42 ; 57,5) mg/l, (31 ; 44) mg/l and (0,03 ; 0,04) mg/l during the dry seasons (Fonge et al., 2013).

Unlike the Douala estuary where ammonium concentrations were high in the rainy season due to urban drainage load in the effluent, it is during the dry seasons that the highest values were observed in the Nyong. In the Nyong estuary, nutrients are both from agro-industrial effluents and organic matters decomposition. During rainy seasons, significant freshwater inputs would dilute chemical concentrations at thresholds considered limiting for the growth of some microalgae (Akoma et al., 2008).

Guildford and Hecky (2000) reported that, the ratio N/P in marine and continental area covert the same class of values, and could allow to predict any modification in phytoplankton composition. Ratio is influenced by the morphology of the environment. Actually, the residence time and depth of the estuary affects the availability of nitrogen and phosphorus, which would become limiting nutrient for the production of phytoplankton (Le Gall, 2012). N/P ratio value obtained in the course of this study presented intra-seasonal (during the Short and the Large Dry Seasons) and inter-seasonal variations. But globally, results indicated that the Nyong Estuarine waters were phosphorus-deficient during the LDS (N/P>33) (McDowell et al., 2009) and nitrogen-deficient during the SDS. This contrasts with observations made during the rainy seasons, where nitrogen- and phosphorus were both deficient. However, rainy seasons were the richest in terms of abundance and diversity of phytoplankton.

As observed by Jiyalalram (1991) in the Mahi estuary in India and Nwankwo (1998) in the Epe lagoon in Nigeria, highest phytoplankton abundance (H'= 4.55) and diversity (113.50×10^5 cell/l) were recorded during the Major Rainy Season (MRS) on the Nyong estuary. The large population of phytoplankton corresponding to low nutrient values in the rainy seasons could also indicate high consumption rate. These seasons were marked by low dispersal of species (homogeneity), which means that enrichment would favor the development of some species at particular stations.

Distribution, composition and diversity of phytoplankton organisms in the estuary are influenced by nutrient concentrations that fluctuate from season to season, depending on location and physical nature of the environment. This has an impact on the quality of the ecosystem, as biodiversity differs from changes in ecosystems. Microalgae growth depends on the ability of the organism to adapt to ecological changes and interactions between variables (Reid, 1961; Fonge et al., 2012).

The Chlorophyta group was the most abundant during the short rainy season at the Nyong estuary. This abundance (31.33×10^5 cell/l) compared to other groups of micro-algae was in line with other works which showed that rivers of Cameroon (Nyong included) are richer in Chlorophyta species (Nguetsop et al., 2009; Mama et al.,

2016).The limnohalin character of the estuary (mean salinity<5‰) justifies that fresh waters prevail over marine waters (Ayissi, 2014).The dominance of the genus *Closterium* (Chlorophyta) with 2.75×10^5 cell/l, and species *Botryococcus braunii* (Chlorophyta) with 3.98×10^5 cell/l and *Calothrix brevissima* (1.88×10^5 cell/l) and the presence of Cyanophyta group indicates an excess of nutrients in the environment which is the major characteristic of eutrophic ecosystems.

These findings correlate with those of Hans et al. (2001) which state that in tropical waters the excessive presence of Chlorophyta accompanied by Cyanophyta occurs when the environment has important nutritional conditions. The values of the Redfield ratio (greater than 16) obtained during the rainy seasons and the large dry season reflect the enrichment of the medium in nitrogen ion to the detriment of the phosphate ion. The average temperature of 27°C and the basic pH of the estuarine water confirm this eutrophic character of the River Nyong mouth. These results were similar to the findings of Ryding and Rast (1989) which state that Cyanophyta grows particularly well in eutrophic, alkaline environments and in lukewarm water.

The Chrysophyta group was dominant in quantity on Nyong (38.50%), mainly the class of Diatomophyceae of the genus *Nitszchia* and also the genus *Ophiocytium* of the class Xanthophyceae. This group is mainly found in freshwater, but some species have developed abilities to live in brackish and marine waters. Several species of Diatoms have been identified in Cameroon estuaries such as *Chaetoceros testissimus*, *Nitzschia closterium*, *Diatomavulgare* sp., *Trachyneis* sp. and *Coscinodiscus* sp. (Folack et al., 1991).

The adaptability of some of these species to brackish water makes them toxic (Yupeng et al., 2006). This constitutes a constraint for the practice of aquaculture activities in estuaries (Hans, 2001). The alkaline environment and temperature of the Nyong estuarine waters favor the presence of these species in the environment. This is in agreement with the work of Wetzel (1983) which showed that *Nitszchia* sp. are oligotrophic species. Similar results were found in the estuaries of Dibamba and Douala (Fonge et al., 2012).

Given the revealed importance of the Campo-Nyong estuarine system, particularly the Nyong estuary for its useful nursery role in fisheries and aquaculture development, a permanent monitoring of the evolution of physico-chemical and biological parameters, would allow a better management of this mangrove estuary. Moreover, as the Nyong estuary is part of the Douala-Edea reserve, these findings could be the baseline for the development of aquaculture activities and ecotourism. The WQI calculated on the basis of a greater number of variables would give better information on the health of the environment. Combined with biological indicators, this would allow a good management of the estuarine hydrosystem.

CONCLUSION

Fluctuations in the composition and abundance of phytoplankton groups are significant in the Nyong estuary. From the combination of environmental variables to abundance and phytoplankton diversity, the Nyong estuary contains, according to seasons, phytoplankton species indicating an oligotrophic and eutrophic state. The WQI used here indicates that Nyong water fluctuates from good to bad. These different states correspond to periods when the biological index indicates polluted or moderately polluted water. In conclusion, the combination of environmental variables and biological indicators allows complementarity in assessment of the quality of aquatic ecosystems. This knowledge is fundamentally important to understand the estuary dynamic and predict future changes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Bacterial community changes during composting of municipal crop waste using low technology methods as revealed by 16S rRNA

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Bacterial communities are actively involved in composting process but the environment within the compost influences their diversity, abundance and succession. In this study, the authors investigated the bacterial communities in tropical crop waste compost using pyrosequencing. Municipal crop wastes from the tropics (Uganda) were composted under four different low-technology methods. Samples were collected from the early thermophilic, late thermophilic, and mesophilic phases, and from mature compost. Pyrosequencing of the amplified variable V4 region of the 16s rDNA generated over 110 000 sequences. Chao1 and cluster analysis at 3% dissimilarity showed that bacterial community richness declined during the composting process. The community was dominated by a few bacterial taxa during the thermophilic phases. Species evenness increased as compost progressed to maturity despite a decline in the number of taxa over the successional progression. Bacterial community diversity, abundance and succession changed with the composting method. This pattern of diversity may be attributed to competition and selection during the microbial succession. A total of 22 phyla and 513 genera were identified from all the methods in the entire composting process. The most abundant phyla were *Proteobacteria, Firmicutes, Bacteriodes* and *Actinobacteria*. Pyrosequencing provided more information on compost bacterial community diversity and abundance than previously used molecular methods. Several novel bacteria existing in tropical crop waste compost remained unclassified.

Key words: 16S rRNA, succession, compost, bacterial community, pyrosequencing.

INTRODUCTION

The composting process is primarily driven by micro-organisms and understanding their succession, ecology, characteristics, diversity and abundance is important for its optimization as well as the understanding of nutrient cycling. Microorganisms involved in composting are primarily bacteria and fungi (Riddech et al., 2002), which

have variable roles, functions and ability. Therefore, revealing the identity, nature and functions of the organisms is a prerequisite for determining pathways of the composting process and the quality of mature compost. Composting has been widely used in management of organic wastes. In Uganda, about 5000 metric tons of municipal solid waste (MSW) was generated in Kampala city alone per month, which can be composted. MSW compost has a long residual effect in soil than only animal waste compost (Zing et al., 2017). The quantity of waste generated in the urban is increasing with population. Han et al. (2018) reported a positive linear relationship between waste generation amount and population size ($R^2 = 0.9405$). Most of the MSW composting studies in the tropics have concentrated on physicochemical properties of the process and product but there is relatively little knowledge available on microbial characteristics.

A high throughput method like pyrosequencing would be more suitable to elucidate the microbial community during MSW composting. Pyrosequencing provides massive parallel sequence based taxon identification compared with previously used molecular techniques (Shendure and Ji, 2009; MacLean et al., 2009; Fullwood et al., 2009). It has revealed more bacterial diversity in soil (Roesch et al., 2007; Acosta-Martínez et al., 2008; Jones et al., 2009) and during composting (de Gannets et al., 2013). Therefore, it is plausible that higher bacterial richness in compost than currently known will be revealed through pyrosequencing. The diversity, abundance and succession of bacteria community in the tropical mixed wastes such as municipal crop wastes composting ecology is currently to a large extent unexplored.

The composting process can be divided into four phases: initial mesophilic phase (<40°C), followed by a thermophilic phase (>40°C) before dropping to a second mesophilic phase and finally the compost maturing phase. Bacterial and fungal communities were noted to dynamically relate to the functional composting progress phases (Xi et al., 2016; Zhao et al., 2016). Each of these phases is characterized by different compost ecological conditions such as temperature, pH and nutrient concentrations, which may affect microbial community structure. Bacteria use a broad range of enzymes to degrade available organic substrates such as soluble carbohydrates, proteins and complex lignin, chitin, polyphenols and cellulose. Although, there have been studies on bacteria community in composting elsewhere, few of these studies have traced and described the microbial community during an entire composting process. For example, Takaku et al. (2006) used DGGE and PCR amplified 16S rDNA to study microbial community during composting of food garbage and rice hulls in Japan. Results showed change in microbial community between composting stages. A total of 33 DGGE bands which were identified belong to phyla

Proteobacteria, Firmicutes, Bacteroidetes and Actinomycetes. In a related study, high diversity of Formicates and Bacteroidetes was observed during thermophilic phased during composting of solid agricultural wastes (Song et al., 2014). Culture-method was noted to underestimate the diversity of microorganisms during the composting cycles as compared to sequencing of 16S rRNA (Chandna et al., 2013). Earlier, Horisawa et al. (2008) determined bacteria succession during decomposition of garbage but in a batch-fed garbage decomposer and showed that microbial community in compost were similar at each stage of composting irrespective of the environmental conditions.

Taiwo and Oso (2004) and Rebollido et al. (2008) traced microbial succession during composting of organic municipal solid wastes in Nigeria and Cuba, respectively but used the culture methods. There was a higher community of bacteria followed by Actinobacteria and fungi during composting dominated by *Bacillus, Streptomyces, Actinomyces, Pseudomonasi* and *Azospirillum* isolates (Rebollido et al., 2008). *Bacillus, Streptomycetes* and *Pseudomonas* in addition to faecal coliforms*, Seratia* and *Proteus* were the dominant microbial community isolates from pit method composted urban organic wastes (Taiwo and Oso, 2004). Other studies to establish bacteria community during composting process used kitchen waste, sewage sludge and source-separated municipal solid or household wastes mainly in temperate climate (Kowalchuk et al., 1999; Peters et al., 2000; Ryckeboer et al., 2003; Connon et al., 2005; Neher et al., 2013; Song et al., 2014). Some studies have concentrated on microbial succession within one phase of the compost process. For example, Danon et al. (2008) studies bacterial community succession in curing compost of sewage sludge and yard wastes from a commercial composting facility in Israel and reported microbial community changes but the dominant phyla were *Proteobacteria, Bacteroidetes* and *Actinobacteria*. The objectives of this study were to explore the bacterial community diversity, abundance and succession during composting of tropical MSW in four low-technology composting methods using pyrosequencing of the 16S rDNA V4 region.

MATERIALS AND METHODS

MSW were obtained from a fresh food market in Kampala city, Uganda and composted at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK). MUARIK is about 17 km north of Kampala City. The final composition of the MSW after removal of non-biodegradable materials and non-plant materials, included banana residues, fruits (e.g. oranges, mangoes, avocadoes, papaws, water melon and jackfruits). Other constituents were vegetables (cabbages, tomatoes and egg plant), sweet potato vines, maize residues, beans and peas haulms. The crop wastes were manually homogenized before composting.

Study design

Composting was carried out in open field experiments where four low technology methods (treatments) were arranged in a randomised complete block design, each with three replicates. The methods were: (i) pit-open (PO); (ii) pit-covered (PC); (iii) above ground-open (AO); (iv) above ground-covered (AC). Each of the composting structures had dimensions of 2 x 1.5 x 1.6 m (length x width x depth/height). The composting materials were turned manually with hands and forked hoes twice each week for the first three weeks, then once a week for another two weeks and once a fortnight for another four weeks in order to increase aeration and subject all the material to high temperatures, as well as mix the materials during the composting process. Moisture was monitored gravimetrically each week from a composite sample of compost and moisture content was maintained between 40 and 60% for the first 35 days.

Laboratory analysis

Compost samples were collected on each day of turning. A compost sample for each replicate comprised of samples picked randomly from ten spots during turning. The samples were packed in 200-ml plastic vials, transported under frozen condition and stored at -80°C until used for analysis. Microbial molecular studies were done at the Swedish University of Agricultural Science (SLU) in Sweden. Genomic DNA from the frozen compost was extracted following procedures using 3% hexadecyltrimethylammonium bromide (CTAB) buffer incubated at 65°C. Thereafter, centrifuge was done at 10000 round per minutes (rpm) for 5 min and the top 200 µl was extracted and mixed with chloroform and centrifuge was done again at 10000 rpm. This was followed by extraction of the top 150 µl and mixed with cold isopropanol and kept on ice for 30 min and later centrifuge at 13000 rpm for 13 min. Taking care not to dislodge the DNA pellets, the supernatant was discarded. The DNA pellets were washed with 200 µl of cold ethanol (70%) and centrifuge at 6500 rpm for 5 min. The supernatant was again discarded and pellets dried on the bench and eluted with 200 µl milliQ water and stored at -26°C until used. The quality of the DNA was very low and could not be used for PCR steps. Therefore, its quality was further enhanced by purification using a Jet Quick kit (Genomed GmbH, Löhne, Germany) following the manufacturer's instructions and eluted in 50 µl of preheated TE buffer. The concentration and quality of DNA was measured on the NanoDrop Spectrophotometer (ND1000). Purified DNA was kept at -26°C until used. Nested PCR was used for pyrosequencing of V4 16S rDNA. Universal bacteria primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 907R (5-CCGTCAATTCMTTTRAGTTT-3) were used for the first PCR step. A PCR reaction volume of 20 µl contained: 1x buffer, 0.2 µM dNTPs, 0.2 µM 27f and 907R each, 0.75 mM of $MgCl_2$, 0.02 U $µl^{-1}$ red Taq polymerase and 100 $ngµl^{-1}$ DNA was used. Amplification was performed on Applied Biosystems 2720 Cycler with initial denaturing at 94°C for 3 min, followed by 25 cycles of denaturation (92°C for 45 s), annealing (50°C for 30 s) and extension (72°C for 30 s). There was a final extension for 7 min at 72°C and then maintained at 4°C. The PCR product integrity was checked on 1% gel. In the second PCR, primers V4R1-4 with pyrosequencing adaptor-A added in the 5' end and V4F with tag and pyrosequencing adaptor B added in the 5' end were used (Cole et al., 2009). For the second PCR, 1 µl of the first PCR products were used as templates with the following: 1x buffer, 0.08 µM dNTPs, 0.38 mM $MgCl_2$, 0.15 µM AV4R, 0.08 U/µl red taq polymerase and 0.15 µM V4F primer, different tag for each sample in a total PCR reaction volume of 50 µl. The samples were gel purified using QIA Quick gel extraction kit, was concentration

measured with a spectrophotometer before pyrosequencing on a 454 GS FLX instrument at a concentration of 10 ng/µl. The sequences have been deposited in the Short Reads Archives (SRA) at NCBI (under accession number SRA 009487.3).

Alignment and clustering of 16S rDNA fragment

The sequences were processed using Ribosomal database project (RDP), release 10 pyrosequencing pipeline (Cole et al., 2008). Using the initial pipeline process, raw reads were sorted for each sample using the used tagged. This was followed by trimming off tags and eliminating poor and shorter sequences less than 150 bp. The same procedures were followed to eliminate sequences shorter than 200 bp. The libraries with sequences > 200 bp were used for classification studies and diversity studies. RDP using the naive Bayesian classifier classified more accurately sequences ≥ 200 bp (Wang et al., 2007). The tag-trimmed sequences were aligned by (i) methods of composting and (ii) composting phases and then clustered by complete linkage at 20 with a 5% increment and 3%with 1% increment. Clusters at 3% dissimilarity were used to estimate the number of OTUs in previous studies with pyrosequencing (Roesch et al., 2007; Acosta-Martínez et al., 2008). The clusters were therefore used at 3% dissimilarity to estimate bacterial community richness and diversity with the parametric indicator rarefaction and non-parametric indices (Shannon and Chao1) using the RDP pipeline. The rarefaction curves were drawn in MS excel software and curve fitting was performed with GraphPad Prism 5.0 (GraphPad Software) at 95% confidence interval using two phase association model. From previous studies, Roesch et al. (2007) observed that it is necessary to model and extrapolate rarefactions curves or use non-parametric methods to estimate OTU richness taking into account the community structure.

The RDP classifier tool was used to assign the sequences to phylotypes based on Naive Bayesian classifier of 16S rRNA. The RDP library was used to compare library sequences between methods of composting.

Non-metric multi-dimensional scaling (NMDS) was used for ordination of clusters at the 3% level as cluster data are categorical and not normally distributed. Data were square-root transformed and the Bray-Curtis dissimilarity index was used as input as implemented in Vegan. Environmental variables (pH, temperature, total nitrogen, total carbon, NO_3^-, NH_4^+) were overlaid to the ordination by means of vector-fitting. Significance was achieved by means of permuting data 999 times.

RESULTS

Bacteria community succession between phases of composting

A total of 110051 sequences were obtained and when filtered for sequences > 200 bp, the number reduced to 109728 of which 38243, 37372, 25641 and 8474 were from early thermophilic, late thermophilic, second mesophilic phases and mature compost respectively. RDP classified 99.7% of the sequences as bacteria at 80% bootstrap. Reducing the confidence level of 70%, RDP classified 99.9% of the sequences as bacteria leaving 40 sequences belonging to *Archaea*. Thirty five of the *Archaea* sequences were from thermophilic phase while four sequences and one sequence were from
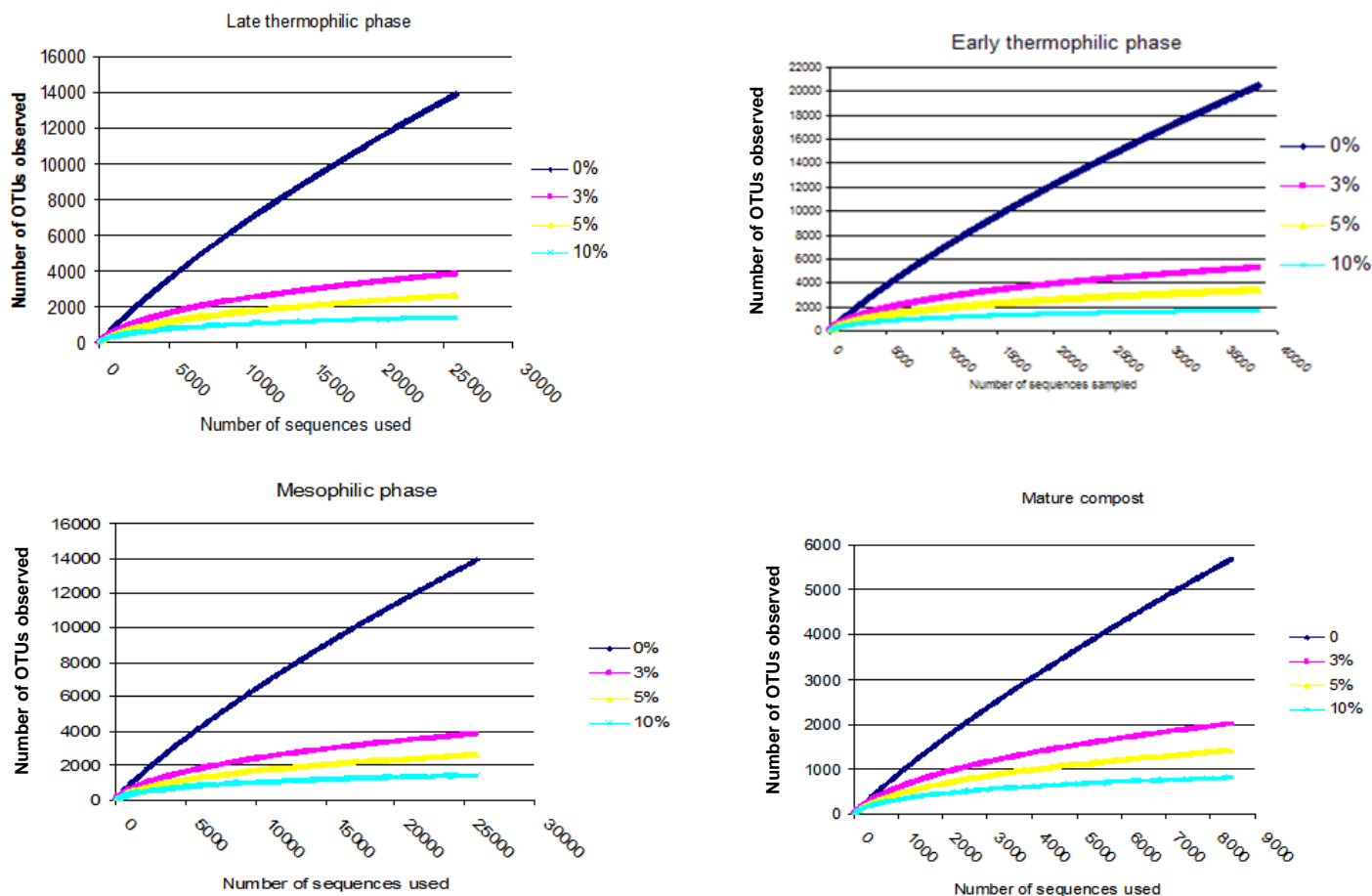
Figure 1. Rarefaction curves depicting the effect of % dissimilarity on the number of OTUs identified.

second mesophilic phase and mature compost respectively. Rarefaction curves showed variations in the number of OTUs at different phases of composting (Figure 1). From curve-fitting of rarefaction curves, 6919 OTUs (6884 and 6948 lower and upper 95% confidence interval respectively) were estimated during early thermophilic phase. The late thermophilic had 5141 OTUs (5118 to 5164 lower and upper 95%), while the mesophilic phase had 5118 OTUs (5093 to 5142 lower and upper 95% confidence interval) and further declined in mature compost to 2895 OTUs (2881 to 2909 lower and upper 95% confidence interval). This trend was similar to the cluster and observed OTUs and Chao 1 estimations at 3% dissimilarity. Shannon index showed a decrease in species richness between early thermophilic phase and mature compost phases (Table 1).

The pit methods generally had the highest richness. The PC method had the highest bacteria diversity as shown by OTUs estimated by rarefaction extrapolation in the early and mesophilic phases. It was followed by the PO method, which had the highest rare faction estimated

OTUs in mature compost. On the other hand, AO method had the least bacteria community diversity in the entire composting process except in the late thermophilic phase (Table 1). The clusters and Chao 1 values followed similar pattern except in the early thermophilic phase. Species richness decreased as the evenness increased during composting in all methods as shown by the H' index (Table 1). The AO method had successively increased bacterial diversity as compared to the other methods as compost progressed through maturity.

Proteobacteria, *Firmicutes*, *Bacteriodetes*, *Actinobacteria* and *Planctomycetes* were the most abundant bacterial phyla throughout the composting process (Table 2). *Proteobacteria* community abundance was highest during early thermophilic phase, decreased in late thermophilic and mesophilic phases but later increased in mature compost. A similar pattern was observed for *Cyanobacteria, Nitrospira, Spirochaetes* and *Acidobacteria*. The abundance of *Bacteriodetes* increased from the early thermophilic through the mesophilic phases but decreased in mature compost. There was

Table 1. Bacteria OTUs diversity at 3% dissimilarity for different composting method during each phase.

Composting method (Treatment)	Phase of composting	Number of OTUs observed	Expected OTUs by rarefaction extrapolation	Clusters	Chao	LCI 95	UCI95	Shannon index (H')
Above-ground covered	Early thermophilic	7269	2354	1674	2758.9	2574.2	2981.5	6.3863
Above-ground open	Early thermophilic	6645	1730	1194	2125.6	1942.6	2353.4	5.7051
Pit-covered	Early thermophilic	14181	2997	2216	3658.7	3434.6	3923.9	6.1753
Pit open	Early thermophilic	10192	1745	1409	2074.3	1939.9	2242.8	6.1931
Above-ground covered	Late thermophilic	392	406	192	435.3	342.1	586.4	4.7555
Above-ground open	Late thermophilic	11756	2370	1867	2780.2	2623.6	2969.2	6.1822
Pit-covered	Late thermophilic	7077	1803	1353	2075.6	1936.5	2247.9	6.1031
Pit open	Late thermophilic	9731	1869	1396	2386.9	2195.5	2624.2	5.9925
Above-ground covered	Second mesophilic	2945	564	415	696.0	606.9	826.5	4.5995
Above-ground open	Second mesophilic	63	313	43	97.4	64.0	183.8	3.6395
Pit-covered	Second mesophilic	16464	2878	2183	3562.6	3345.2	3820.8	6.2694
Pit open	Second mesophilic	6346	2681	1780	3163.2	2939.0	3430.8	6.6832
Above-ground covered	Mature compost	910	916	441	1022.2	843.3	1275.0	5.5087
Above-ground open	Mature compost	1554	392	318	447.5	399.3	524.2	5.0252
Pit-covered	Mature compost	1067	1061	515	1265.7	1058.6	1551.7	5.8884
Pit open	Mature compost	4945	1627	1120	2046.7	1856.7	2285.6	6.0952

a similar pattern for TM7, *Gemmitimonadates, Deinococcus-Thermus, Chlamydiae* and *Chloroflexi*. The *Actinobacteria* community increased abundance from 5.1% during thermophilic phase to 8.0% in late thermophilic phase but thereafter decreased successively. *Planctomycetes* community abundance was nearly consistent during early and late thermophilic phases but later successively increased through compost maturity. The minor phyla (*Chlamydiae, Deferribacteres, Chloroflexi, Acidobacteria, Lentisphaerae, Tenericutes,* TM7, BRC1, OP1, OD1, *Verrucomicrobia, Gemmatimonadates, Deinococcus-Thermus* and *Spirochaetes*) contributed less than 10% to the phyla abundance during all phases of composting. Several sequences remained unclassified with the highest

proportions at 38.5 and 26.8% of the sequences from second mesophilic and late thermophilic phases, respectively (Table 2).

At class level, α-*proteobacteria* and γ-*proteobacteria* had the highest abundance during the composting process followed by *Bacteroidetes* except in mature compost (Table 3). *Planctomycetacia* successively increased in abundance and dominance during composting until mature compost. *Chloroflexi, Lentisphaerae* and *Deferribacteres* were eliminated before compost matured (Table 3). At genus level, *Aquicella, Petrimonas, Alkaliflexus, Brevibacterium, Phenylobacterium* were the most abundant genera from early thermophilic to mesophilic phases but were succeeded by *Roseomonas, Streptococcus* and *Brevundimonas*

in mature compost (Table 4). Genera diversity successively decreased during composting. There were 246 genera during early thermophilic, which decreased to 201 in late thermophilic phase, further decreased to 197 and 150 for second mesophilic and mature compost respectively from all composting methods combined. There were some genera which appeared only in one composting phase. There were 44, 23, 21 and 22 unique genera that appeared only in the early thermophilic, late thermophilic, second mesophilic phases and mature compost, respectively. However, from the 20 most abundant genera (Table 4), *Crypanaerobacter* was detected only in early thermophilic phase, *Pirellula* was eliminated after the second mesophilic phases, while *Plastopirellula* and *Veilonella* were present only

Table 2. Relative abundance of phylum (%) during the different composting phases

Phylum	Early thermophilic	Late thermophilic	Second mesophilic	Mature compost
Acidobacteria	0.9	0.2	0.6	1.5
Actinobacteria	5.1	8.0	3.4	3.2
Bacteroidetes	10.6	14.1	16.2	4.7
BRC1	0.0	0.0	<0.1	<0.1
Chlamydiae	<0.1	0.2	0.1	0.1
Chloroflexi	0.3	0.4	0.4	0.1
Cyanobacteria	0.1	<0.1	0.0	0.1
Deferribacteres	0.1	0.1	<0.1	0.0
Deinococcus-Thermus	0.6	1.5	1.3	0.5
Firmicutes	9.5	18.3	4.7	12.7
Gemmatimonadates	0.3	0.2	1.5	0.1
Lentisphaerae	<0.1	<0.1	<0.1	0.0
Nitrospira	<0.1	0.0	0.0	<0.1
OD1	0.0	<0.1	<0.1	0.0
OP10	0.0	0.0	<0.1	0.0
Planctomycetes	2.6	2.5	3.1	8.8
Proteobacteria	42.3	26.0	26.5	45.2
Spirochaetes	0.2	<0.1	0.1	0.1
SR1	<0.1	0.0	0.0	0.0
Tenericutes	0.1	<0.1	<0.1	<0.1
TM7	1.2	1.2	2.5	0.4
Verrucomicrobia	1.8	0.5	1.1	1.5
Unclassified bacteria	24.3	26.8	38.4	21.0
Phyla diversity	19	18	19	17
Number of sequences	38243	37374	25641	8472

in mature compost. There was a decreasing successional trend in the number classes and genera for all the composting methods. More bacterial classes and genera were observed during early thermophilic and richness decreased in mature compost. In the early thermophilic phase, the AC method was significantly richer in the number of classes than all the other methods. Progressing to late thermophilic phase, the AO method had the highest number of classes while the AC method declined considerably. In the second mesophilic phase, the PC method had the highest class richness while the AC method had the lowest number of classes observed in the entire composting process. In mature compost, the most and least number of classed were from PO and PC methods, respectively. A similar pattern of succession was observed even at genera level (Figure 2).

There were differences in class or genera diversity and abundance among different composting methods even within the same composting phase. For example, during early thermophilic phase, the genus *Aquicella* was the most dominant in open systems (AO and PO methods) while *Alkaliflexus* and *Corynebacterium* were the most abundant in AC and PC methods, respectively. In the late thermophilic phase, the most abundant genera were *Brevibacterium* and *Bacillus* for the AC method, *Petrimonas* and *Hydrogenophaga* in the AO method, *Petrimonas* and *Pirellula* in the PC method, *Aquicella* and *Petrimonas* in the PO method. For the second mesophilic phase, the most abundant genera under different composting methods were *Petrimonas* and *Corynebacterium* in the AC method, *Petrimonas* and *Alkaliflexus* for AO method, *Aquicella* for the PC method, and *Phenylobacterium* and *Gemmatimonas* in the PO method. In the mature compost, the genera *Terrimonas* and *Phenylobacterium* were the most abundant in the AC method, *Streptococcus*, *Stenotrophomonas* and *Corynbacterium* in the AO method, *Roseomonas*, *Phenylobacterium* and *Pirellula* in the PC method and *Roseomonas*, *Brevundimonas* and *Devosia* in the PO method.

Bacteria succession in different composting methods

Bacterial succession patterns differed significantly depending on the composting methods and phases

Table 3. Bacteria classes occurring during each composting phase.

S/N	Early thermophilic	%	Late thermophilic	%	Second mesophilic	%	Mature compost	%
1	α-proteobacteria	25.08	α-proteobacteria	16.79	γ-probeobacteria	18.62	α-proteobacteria	27.39
2	γ-probeobacteria	17.16	Bacteriodetes	14.94	Bacteroidetes	16.48	γ-probeobacteria	15.85
3	Bacteroidetes	8.30	Clostridia	14.94	α-proteobacteria	13.69	Planctomycetacia	9.68
4	Clostridia	7.75	γ-probeobacteria	11.47	Planctomycetacia	4.76	Bacilli	7.48
5	Actinobacteria	6.09	Actinobacteria	10.39	Clostridia	5.10	Clostridia	5.70
6	β-proteobacteria	4.70	Bacilli	7.19	Actinobacteria	5.18	Actinobacteria	3.49
7	Planctomycetacia	3.18	β-proteobacteria	4.39	β-proteobacteria	2.83	β-proteobacteria	2.51
8	Bacilli	2.55	Planctomycetaca	3.27	Gemmatimonadetes	2.23	Bacteroidetes	2.48
9	Verrucomicrobiae	2.20	Deinococci	1.91	Deinococci	2.03	Acidobacteria	1.65
10	Sphingobacteria	1.17	Sphingobacteria	0.63	Sphingobacteria	1.83	Verrucomicrobiae	1.63
11	Acidobacteria	1.07	Verrucomicrobiae	0.59	Verrucomicrobiae	1.63	Sphingobacteria	0.70
12	Deinococci	0.74	Anaerolineae	0.43	Bacilli	1.23	Deinococci	0.52
13	Anaeolineae	0.32	Acidobacteria	0.32	Acidobacteria	0.93	Gemmatimonadates	0.14
14	Flavobacteria	0.38	Chlamydiae	0.28	δ-proteobacteria	0.73	Flavobacteria	0.14
15	Gemmatimonadetes	0.33	Gemmatimonadetes	0.22	Flavobacteria	0.85	Erysipelotrichi	0.14
16	δ-proteobacteria	0.24	Flavobacteria	0.20	Anaerolineae	0.64	δ-proteobacteria	0.13
17	Spirochaetes	0.20	δ-proteobacteria	0.15	Erysipelotrichi	0.20	Spirochaetes	0.12
18	Deferribacteres	0.11	Deferribacteres	0.08	Spirochaetes	0.18	Anaerolineae	0.12
19	Erysipelotrichi	0.10	Erysipelotrichi	0.07	Chlamydiae	0.10	Cyanobacteria	0.10
20	Cyanobacteria	0.08	Cyanobacteria	0.05	Mollicutes	0.05	Chlamydiae	0.06
21	Mollicutes	0.08	Spirochaetes	0.05	Lentisphaerae	0.04	Mollicultes	0.03
22	Chlamydiae	0.05	Lentisphaerae	0.01	ε-proteobacteria	0.02	Nitrospira	0.01
23	ε-proteobacteria	0.01	Mollicutes	<0.01	Deferribacteres	0.02	ε-proteobacteria	0.01
24	Lentisphaerae	0.01	ε-proteobacteria	<0.01	Unclassified	20.62	Unclassified	19.92
25	Nitrospira	<0.01	Unclassified	11.64				
26	Chloroflexi	<0.01						
	Unclassified	18.09						

(p<0.01). For example, in the AC method (Table 2), there was a decrease in abundance of *Firmicutes, Proteobacteria, Bacteroidetes* and *Planctomycetes* between early thermophilic phase and mature compost. However, *Proteobacteria* dominated the early thermophilic phase and mature compost while *Firmicutes* and *Bacteroidetes* were most abundant during late thermophilic and second mesophilic phases, respectively. The minor phyla abundance also successively increased during composting. The minor phyla included *Cyanobacteria, Nitrospira, Deferribacteres, Chloroflexi, Chlamydiae, Acinobacteria, Lentisphaerae, Tenericutes*, TM7, SR1, BRC1, *Verrucomicrobia, Gemmatimonadates, Spirochaetes* and *Deinococcus-Thermus*. For the AO method (Table 2), the abundance increased for *Firmicutes* and

Table 4. Predominant genera occurring during each composting phase.

S/N	Early thermophilic	%	Late thermophilic	%	Second mesophilic	%	Mature compost	%
1	*Aquicella*	21.71	*Petrimonas*	16.09	*Aquicella*	20.87	*Roseomonas*	12.57
2	*Petrimonas*	7.64	*Aquicella*	10.00	*Petrimonas*	9.71	*Streptococcus*	9.54
3	*Alkaliflexus*	6.65	*Brevibacterium*	8.66	*Alkaliflexus*	9.11	*Brevundimonas*	7.86
4	*Corynebacterium*	5.54	*Bacillus a*	4.64	*Phenylobacterium*	6.28	*Phenylobacterium*	6.11
5	*Chelatococcus*	4.88	*Lactobacillus*	4.55	*Gemmatimonas*	6.09	*Devosia*	5.49
6	*Stenotrophomonas*	4.53	*Truepera*	4.10	*Truepera*	5.46	*Stenotrophomonas*	4.63
7	*Symbiobacterium*	3.01	*Odyssella*	4.02	*Corynebacterium*	3.82	*Aquicella*	4.20
8	*Proteiniphilum*	2.45	*Hydrogenophaga*	3.67	*Pirellula*	3.53	*Petrimonas*	2.88
9	*Hydrogenophaga*	2.35	*Alkaliflexus*	3.54	*Stenotrophomonas*	1.53	Gp4	2.72
10	*Pirellula*	1.69	*Corynebacterium*	3.23	Gp6	1.42	*Alkaliflexus*	2.57
11	Gp6	1.67	*Proteiniphilum*	2.83	*Hydrogenophaga*	1.42	*Corynebacterium*	2.53
12	*Sedimentibacter*	1.63	*Chelatococcus*	2.36	*Proteiniphilum*	1.40	*Plastopirellula*	2.34
13	*Phenylobacterium*	1.57	*Propionibacterium*	1.71	*Planctomyces*	1.26	*Propionibacterium*	1.75
14	*Pseudoxanthomonas*	1.54	*Acinetobacter*	1.57	*Chitinophaga*	1.24	*Veilonella*	1.75
15	*Brevundimonas*	1.17	*Sedimentibacter*	1.53	GP4	1.13	Gp6	1.67
16	*Gemmatimonas*	0.94	*Staphylococcus*	1.48	*Chelatococcus*	1.05	*Truepera*	1.52
17	*Cryptanaerobacter*	0.90	*Pirellula*	1.24	*Legionella*	0.98	*Hyphomicrobium*	1.40
18	Gp4	0.90	*Clostridium*	1.10	*Propionibacterium*	0.95	*Peptoniphilus*	1.28
19	*Truepera*	0.86	*Planctomyces*	1.07	*Sedimentibacter*	0.85	*Lactobacillus*	1.25
20	*Anaerococcus*	0.78	*Syntrophomonas*	0.98	*Nitrosomonas*	0.79	*Clostridium*	1.13

Actinobacteria while it decreased for *Proteobacteria, Bacteroidetes* and *Planctomycetes*. For the PC method (Table 2), *Proteobacteria Bacteroidetes* and *Actinobacteria* had a successive decline in abundance but it increased for *Firmicutes* and *Planctomyces*. There were notable changes in the minor phyla; *Acidobacteria* and *Verrucomicrobia* successively increased from 0.1 and 0.4 to 4.2 and 3.4% between early thermophilic phase and mature compost, respectively.

For the bacterial succession under the PO method; there was a significant increase in abundance of *Proteobacteria*, *Firmicutes* and *Planctomycetes* and a decrease for *Actinonacteria*. The minor phyla *Verrucomicrobia*, TM7 and *Acidobacteria* were more abundant than *Cyanobacteria*, *Chlamydiae*, *Chloroflexi, Lentisphaerae*, *Tenericutes* and *Gemmatimonadetes*.

NMDS analysis gave two convergent solutions after 13 trials, stress value 20.2 and non-linear fit R^2 was 0.94 (Figure 3).

Temperature, total organic carbon, NH_4^+ and NO^{3-} explained most of the community variation but pH and total nitrogen were significant factors as well when vector-fitting was permuted 999 times (Table 5).

DISCUSSION

Pyrosequencing of 16S rDNA revealed successional patterns, decreased diversity of bacteria community and increased abundance of some taxa during composting than has been reported by any previously used molecular methods. Earlier, de Gannets et al. (2013) used 454 pyrosequencing and noted that diversity increased as composting progressed and matured compost was dominated by Archaea, Planctomycetes, Chloroflexi, α- and γ-protebacteria. A similar pattern was reported when source separated wastes were subjected to
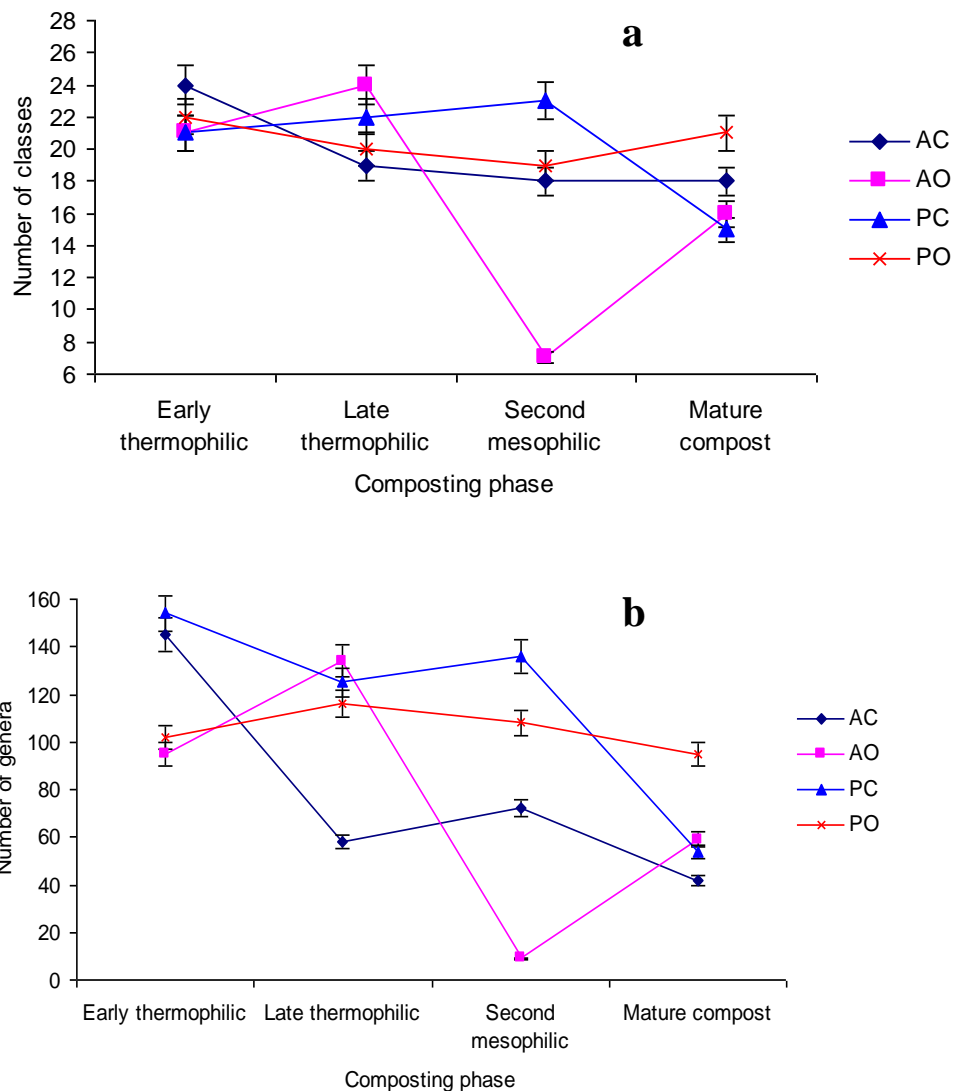
Figure 2. Bacterial community successional diversity at (A) class and (B) genus levels.

static aerobic composting where abundance of γ-protebacteria, actinobacteria and Bacillus was observed durng the composting cycle (Sundberg et al., 2011). The main microbial succession selection factors during composting include temperature, aeration, pH, moisture and nutrients. In this study, temperature, total organic carbon, NH_4^+ and NO^{3-} explained most of the community variation (Table 5). The NMDS analysis separated samples after successional stages rather than according to methods (Figure 3). This is mainly an effect of temperature. Carbon was significantly lost during early stages of composting, here, it is shown that the loss of carbon is as well a significant driver of community shifts during the succession. The significant effect of NH_4^+ and NO^{3-} on the community composition may show that nitrification is an important energy resource when the

easily degraded carbon is depleted. The general trend of a decreasing number of taxa with an increasing evenness may be explained by shifts in microbial ecological strategies during the compost succession. In plant ecology, patterns of community distribution have been explained by competition, dispersal and resource utilization. Dispersal is important in the early succession after a disturbance whereas competitive species dominate in rich environments and stress tolerant species inhabit the environment when resources are depleted. At the initial compost phases, the substrates are rich in nutrients and are inhabited by a large number of microbial taxa. As succession proceeds, the taxa show a more even distribution which is consistent with a shift to a community with many stress tolerant, less competitive species occurring in the late stages. Dispersal may be of
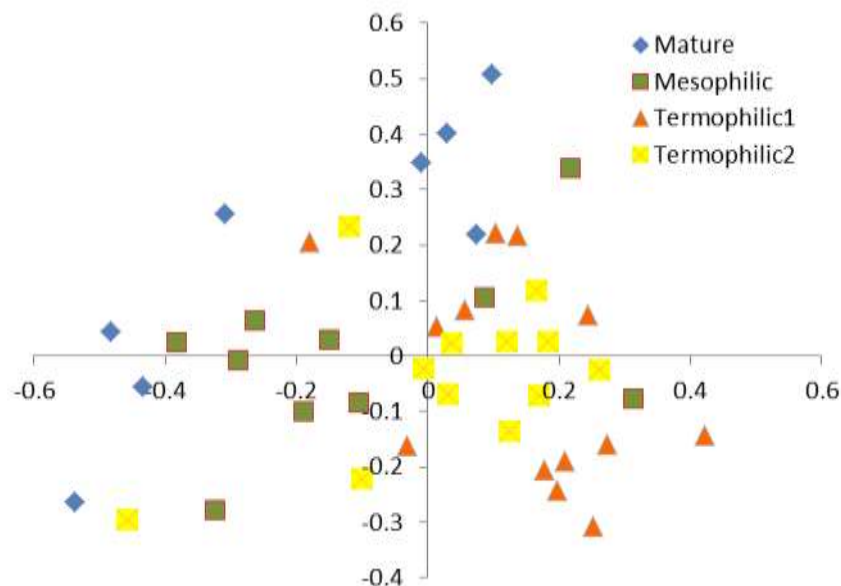
Figure 3. Non-metric multidimensional scaling plot showing diversity pattern of bacteria during different phases of composting municipal solid wastes.

Table 5. Non-metric multidimensional scaling analysis showing determinants of bacterial diversity during composting.

Variables	Dimension 1	Dimension 2	R^2	Probability
pH	0.479	-0.877	0.152	0.029
Temperature	-0.426	0.044	0.254	0.003
Total organic carbon	-0.414	0.909	0.278	0.002
Total nitrogen	-0.388	0.9215	0.314	0.001
Nitrate	0.426	-0.904	0.254	0.003
Ammonium	-0.414	0.909	0.278	0.002

minor importance in the compost environment since the compost material already is colonized with a large number of micro-organisms. The observed dominance of a few bacterial taxa (Table 2) during the thermophilic phases may be the result of strong selective factors in combination with strong competitive ability (Song et al., 2014). In the early stage of composting, temperatures are high, thereby eliminating non-thermophilic micro-organisms. Nutrient availability or pH are unlikely to have restricted the number of taxa in the composts since a broad range of nutrients at high concentration were present in the initial compost and initial pH of the compost substrate was around neutral. It is therefore tempting to suggest that high temperature could be the main selection pressure for the dominance of the three genera: *Aquicella, Petrimonas* and *Alkaliflexus* in the early, late and early mesophilic phases. In an earlier

study, Rebollido et al. (2008) observed that temperature was the most environmental factor which influenced microbial succession during composting process. Additionally, it is noted in the authors' previous study (Tumuhairwe et al., 2009), that there were no significant differences in pH and nutrients among the composting methods except differences between composting phases. Those three genera are not well represented in GenBank with less than 50 sequences archived in the database. Furthermore, none of these genera has been reported to dominate compost communities before or specifically as thermophilic genera. In the future, it would be interesting to isolate representatives of these three genera to test their competitive ability against other isolates and their heat sensitivity in order to better understand why they dominate the initial phases of tropical municipal waste compost communities. In this context, it is interesting to

add that most *Archaea* which include many well-known thermophiles (Lebedinsky et al., 2007; de Gannets et al., 2013) were found during the early thermophilic phase. The lowest observed number of classes and genera from the AO method during second mesophilic phase (Figure 2) could be attributed to fewer numbers of good sequences obtained but would probably not have changed the observed decreasing community richness as shown by the rarefaction estimates. The successive increase in diversity of OTUs in the PO method as compared to the other methods may be because PO method provides most favorable condition for the bacteria community in contrast to the AO method. The most plausible cause of decreased bacteria diversity under the AO method could be moisture constraint. Temperatures are usually high in the tropics, which increases evaporation of moisture from compost when it is not covered.

Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria and *Planctomycetes* which were the dominant phyla have been reported in other composting studies (Takaku et al., 2006; Danon et al., 2008; Székely et al., 2008; Vivas et al., 2009; Neher et al. 2013; Karadag et al., 2013; Song et al., 2014; Manyl-loh et al.. 2016). The high abundance and diversity of *Proteobacteria* was expected because this phylotype contains mainly chemo-organotrophs, which derive their energy from decomposition and oxidation of organic matter. The observation that *Proteobacteria* were most dominant during composting is consistent with composting studies elsewhere (Ntougias et al., 2006; Takaku et al., 2006; Danon et al., 2008; Vivas et al., 2009). However, the current results differ from the some previous studies by having *α-proteobacteria* as the most abundant class. Takaku et al. (2006) and Vivas et al. (2009) reported *β-proteobacteria* while Ntougias et al. (2006) observed *γ-proteobacteria* as the dominating classes in the composting process. The reason for this disparity is not clear but it may be attributed to differences in locality, composting substrate and methods used. The high abundance of *Bacteroidetes* is not surprising as they are known to degrade macromolecules (Michel et al., 2002) which are prevalent in municipal waste compost. The decline in abundance of *Bacteroidetes* could be associated with the compost progressing to maturity when the macromolecules have been degraded. The genus, *Stenotrophomonas* which was ubiquitous in all composting phase together as well as genera *Pseudoxanthomonas* and *Nitrospira* contain denitrifying bacteria (Lipski and Altendorf, 1997; Chen et al., 2002) and could also have contributed to the significant loss of N during composting of municipal crop wastes.

Planctomycetes have been reported in diverse ecosystems including compost (Buckley et al., 2006; Takaku et al., 2006; de Gannets et al. 2013). In compost,

Planctomycetes have currently been detected during the early composting stages (Takaku et al., 2006). However, in this study, *Planctomycetacia* steadily increased from the seventh to third most abundant community between early thermophilic phase and mature compost (Table 2) as also reported by de Gannets et al. (2013). This contrasting observation could be due to the paucity of information on functional activity of *Planctomycetes* during composting. However, *Planctomycetes* have been reported to show chemo-litho-autotrophic growth through oxidation of ammonium (Strous et al., 1999) and could also contribute to nitrification.

For the phylum *Firmicutes*, *Clostridia* dominated from early thermophilic through second mesophilic phases while *Bacilli* was more dominant in mature compost. *Firmicutes* have been reported during different stages of composting (Ntougias et al., 2006; Takaku et al., 2006; Karadag et al., 2013) and were favored by alkaline medium. Therefore, increase in abundance of *Bacillus* and *Clostridia* could be attributed to favorable alkaline pH during the composting. Characteristically, *Bacilli* and *Clostridia* are facultative and strict anaerobes, respectively. Therefore, their presence suggests incomplete aeration during composting. Complete aeration may be difficult to achieve under the low-technology composting methods used in this study. However, there are reports of *Bacillus* and *Clostridia* from other composting studies (Ntougias et al., 2006; Takaku et al., 2006; Sundberg et al., 2011; Karadag et al., 2013; Fui et al., 2017) indicating that it is difficult to achieve complete aeration during composting. The most abundant genus within *Firmicutes* was *Symbiobacterium* which was detected among the predominant 20 genera during early thermophilic phase (Tables 3 and 4). The successional pattern of *Symbiobacterium* could be explained by high temperature during early thermophilic phase since it has previously been isolated from thermophilic compost (Ueda et al., 2001).

Another abundant bacterial group was *Actinobacteria* (Table 3) predominated by *Corynebacterium* at the genus level (Table 4). *Actinobacteria* effectively degrade organic matter including complex substances such as cellulose and chitin and are favored by temperature between 45 and 55°C. Therefore, the successional pattern of *Actinobacteria* community during composting could be explained by the temperature gradient. The highest *Actinobacteria* community was observed during the late thermophilic phase when the temperatures were most favorable

The dominance of the genera *Streptococcus, Plastopirellula* and *Veilonella* communities in mature compost (Table 4) could be used as an indicator for compost maturity. *Streptococcus* had earlier been isolated from maturing compost from a mixed municipal solid waste and poultry manure in Nigeria (Taiwo and Oso, 2004). This could be the first study to identify the

predominance of genus *Devosia* in mature compost, previously isolated from aquatic and soil environments (Nakagawa et al., 1996; Rivas et al., 2003). The presence of phyto-beneficial bacteria in mature compost improves its value for soil fertility management and such examples include *Rhizobia* and *Bradyrhizobia* for symbiotic nitrogen fixation and *Roseomonas,* a genus that includes plant-growth promoting bacteria.

The current high number of taxa identified from compost than previous studies (de Gannets et al., 2013) further demonstrates the high throughput capacity of pyrosequencing in molecular microbial ecology studies even when several sequences remained unclassified at phylum and class levels. This study has also demonstrated the ability of pyrosequencing to detect both the dominant and minor bacterial population occurring during the composting process unlike PCR-DGGE or TRFLP which only detect the dominant bacteria taxa (Takaku et al., 2006). The high number of unclassified bacteria sequences at each stage of composting, most of which belonged to either unknown or uncultured bacteria suggests that pyrosequencing is a powerful technique that provides details on microbial diversity within compost. It also highlights the presence of novel groups of unknown bacteria that may exists when composting MSW in the sub-Saharan tropical environment. This study revealed the presence of bacterial taxa belonging to OD1, OP10, BRC1, SR1, *Deferribacteres, Lentisphaerae, Spirochaetes* and TM7 phyla within a compost environment. To the authors' knowledge, this is the first study to identify such high diversity and abundance of phyla, classes and genera existing in compost ecology in a single study. The significant variation in diversity and abundance of these minority phylotypes at different composting stages could provide insight into unknown functional groups that exist during the composting process. Although, all these genera were identified, their origin and functions during composting remain unknown. It could be that they were originally part of the municipal waste compost, introduced in water used for maintaining the moisture content during the composting process or drifted from soil and surrounding to composting material. Furthermore, it is also unknown whether they were inactive or in dormant state and this is one of the setbacks of DNA-based molecular techniques. However, the change in genotype abundance and diversity, and OTUs observed at different phases during composting period suggests an active community contributing to successional changes.

In the future, it would be interesting to study functional aspects of microbial communities. This may be pursued via the study of functional genes that are involved in ammonium oxidation and the cultivation of representative taxa to be used in functional studies of plant pathogen antagonism. Several novel multifunctional plant growth promoting bacterial strains were earlier isolated from compost (Fui et al., 2017), indicating multifunctional value of compost to plants when applied on the soil.

CONFLICT OF INTERESTS

ACKNOWLEDGEMENTS

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