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Evaluation of bacterioplankton species in calabar coastal water and ship ballast water

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Abstract
This study revealed that Bacterioplankton in Coastal water was dominated by *Bacillus spp* (54,277 cfu/ml), *Corynebacterium spp* (38,688 cfu/ml), *Pseudomonas spp* (38,381 cfu/ml) and *Enterobacteriaceae* (33,936 cfu/ml). In Ballast water bacterioplankton was dominated by *Bacillus spp* (96,588 cfu/ml), *Vibrio spp* (93,929 cfu/ml0, *Pseudomonas spp* (70,158 cfu/ml) and *Corynebacterium spp* (39,863 cfu/ml). Out of the 14 species, 8 species were observed in both coastal water and ballast water, 4 species were observed only in coastal water and 2 species were observed only in ballast water. Most of these species are harmful to plants and animals. Thus both waters are not suitable for use by man, which clearly suggests that both ballasting and deballasting be avoided within the study area.

Keywords: Bacterioplankton, Calabar coastal, Ship ballast, Water.

1. Introduction
Bacterioplankton are the bacterial component of the plankton that drifts in the water column [38]. They live in the open waters of the ocean. They are microscopic in size but often very abundant.
According to International Ballast Water Management Convention (IBWMC 2005, any organism that established and spread with the potential to cause harm to the local environment, economy, or human health are called Harmful Aquatic Organisms and Pathogens (HAOP). Invasive alien species are now generally recognized as one of the greatest threats to biodiversity globally. They also have serious economic, environmental and health impacts and, as a result, place major constraints on development.
Pathogenic effects are caused by microorganisms, where bacteria, virus and fungi are present in sufficient numbers to cause health hazard [11]. Bacteria are one of the main indicators of biological pollution. High faecal coliform count is mainly due to domestic waste [8], as well as animal waste (Choo, 1994; Law *et al*., 2001a) discharges.
Survey on the microbiology quality of shellfish has shown shellfishes to harbour pathogenic organisms [39]. These pathogenic organisms have been implicated in outbreaks of food-borne diseases in many parts of the world; these illnesses which include typhoid fever, hepatitis and disorders of the digestive system [10, 27] are due to the pollution of the waters in which the shellfish grow [13, 17] Since shellfishes are found in bodies of water containing untreated human and industrial waste, there is a tendency that they may concentrate and accumulate high levels of pathogens and toxic contaminants which can pose a significant health hazard to consumers [26, 28].
The aim of this study was to estimate the numbers of bacteria and find out what sort of bacteria they are, to enable possible inferences to be drawn on the suitability of the waters for use by plants and animals.

2. Materials and Methods
2.1 Study Area
The study area for the research work is the Calabar port situated in the eastern coastal zone of Nigeria in West Africa. Within this coastal stretch is the Calabar port (Figure 1), situated along the Cross river estuaries on the Bight of Biafra Bay. The Calabar port is located along 05 01’ 00’’

Latitude and 08 19’ 00’’ Longitude. The fairway buoy lies in 04 18’ 30.5’’ Latitude, and 08 14’ 48’’ Longitude. The port is accessible by water channels from Mbo, Oron, Uruan all in Akwa Ibom State and from Cameroon through Bakassi in Cross River State. The water depth at the port is between 6.4 – 7.6 metres, with a total length of the navigable channel of about 85 kilometres, approximately 45 Nautical miles. The width of the navigable channel is 150 metres. For the tidal cycle, high water is 3.3 metres, low water is 0.8 metres above the chart datum, tidal range is 2.5 metres average. It has an estimated annual capacity of about 1.5 million metric tons of cargo excluding crude oil (NPA Calabar, 1999).

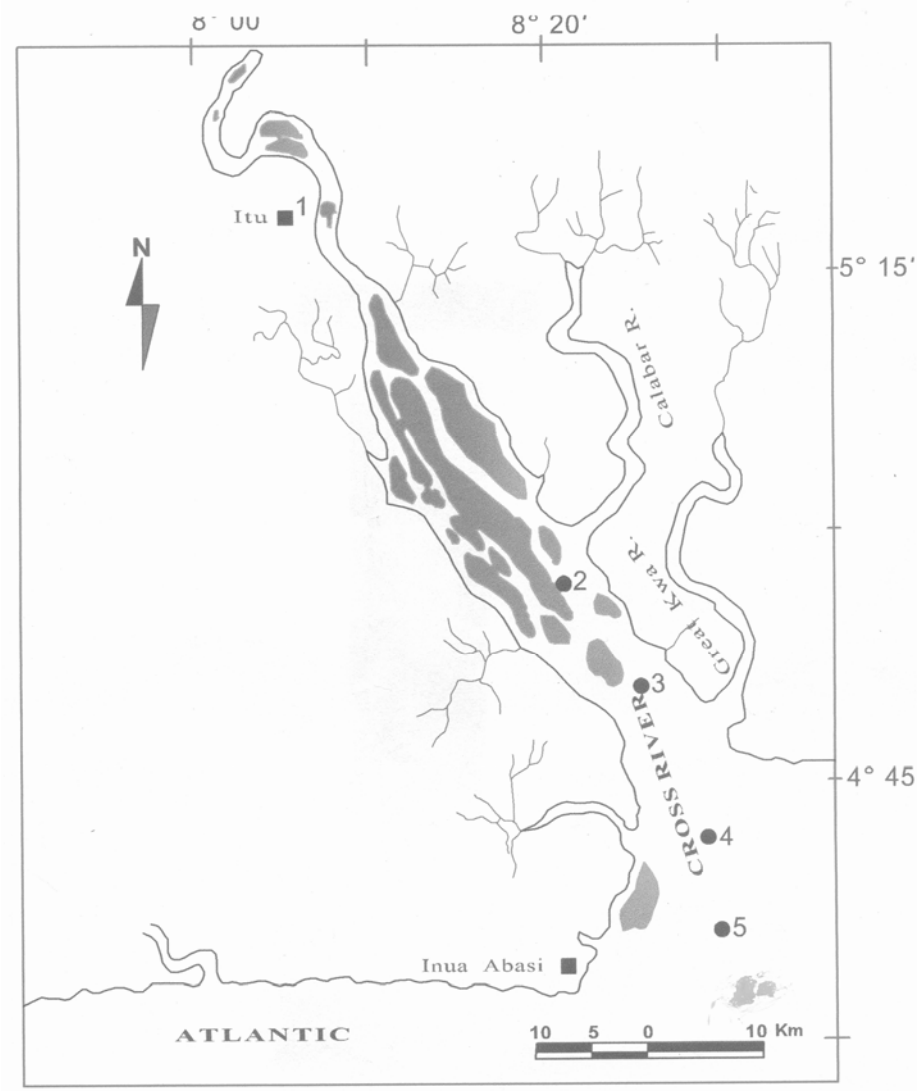

Fig 1: Map of the Cross River system showing sampling locations

2.2 Sampling Procedures

In this study only the water around the port where ships berth and water from the ships ballast tanks were collected and analysed once every month for a period of 12 months covering both the dry and wet seasons.

For the coastal water samples, five (5) sampling points were used. Samples were taken at high and low tides between 0600 hrs and 1800 hrs on each sampling day in each month. The ballast water samples were taken from tanks of ships anchored at the berths four times at three hours interval on each sampling day in each month.

2.3 Collection of Water Samples

All water samples were aseptically collected in sterile one litre capacity plastic bottles. The surface water samples were collected from the depth of 10–25 cm, while small heavy bucket tied to a rope was lowered into the water depth to fetch the water samples. Samples from the tanks were immediately emptied into the sterilized plastic bottles and closed. All containers were well rinsed at least three times with the water being sampled before collection in accordance with APHA, (1998) [2] requirements.

2.4 Treatment of Samples for Laboratory Analysis

The ballast tank water samples and the coastal water from the sampling points, each was fixed with a 1% paraformaldehyde solution for analysis.

2.5 Heterotrophic Bacteria Analysis

Nutrient agar was used in isolating heterotrophic bacteria from the water samples. 28 grams of the Agar were suspended in 1 litre of distilled water in a conical flask. This was dissolved completely by boiling over a flame and sterilized by autoclaving at a temperature of about 121 ^{0}C at 15l b pressure per square inch for 15 minutes.

2.6 Isolation of Bacteria (Pour plate method)

Serial dilutions of the water samples were made using sterile

water. Dilutions of 10^{-3} were used to prepare pour plates in triplicates thus: 1 ml of the dilution for each sample was placed on three petri dishes using a pipette, then 10 ml of the liquefied culture medium cooled to 45 ^{0}C was added after first flaming the mouth of the test tube. For this purpose, the lid of the petri dish was raised but not completely removed. The culture medium and water were mixed by circular movements of the dish, then the dish was placed on a horizontal surface and the layer of culture medium was left to set. The plates were later incubated in the incubator at a temperature of 30 ^{0}C for 48 hours.

2.7 Counting of Colonies
Colonies observed at the end of incubation were enumerated using a Gallenkamp Electronic Colony Counter – (Model no. 3340910E) at the end of which the number of colonies per millilitre of sample was determined.

2.8 Preparation of Subcultures
Cultures were examined microscopically taking into consideration the shape, edge, size and pigmentation of discrete colonies, and subcultures made from these by streaking the different colony types on nutrient agar and incubated for 24–48 hours.

2.9 Preparation of Pure Cultures
To obtain pure cultures of the isolated bacteria, nutrient agar slants were prepared. The bacteria were then cultured on the nutrient agar slants and incubated for 24 – 48 hours.

2.10 Identification of Isolates
This was done by microscopy and with the use of some biological and biochemical tests using the scheme of Shewan *et al*. (1960) and Buchaman and Gibbons. (1977) [3].

3. Result
3.1 Bacterioplankton in Calabar Coastal Waters
As indicated in table 1 below, Bacterioplankton in Calabar Coastal Waters station 1 was dominated by *Bacillus sp* (8,918 cfu/ml), *Corynebacterium spp* (6,204 cfu/ml) and *Enterobacteriaceae* (5,058 cfu/ml). Station 2 was dominated by *Bacillus sp* (6,891 cfu/ml), *Vibrio spp* (6,759 cfu/ml), *Pseudomonas spp* (5,298 cfu/ml) and *Corynebacterium spp* (4,396 cfu/ml). Station 3 was dominated by *Bacillus sp* (8,285 cfu/ml), *Enterobacteriaceae* (7,241 cfu/ml), *Pseudomonas spp* (6,986 cfu/ml) and *Corynebacterium spp* (5,163cful/ml). Station 4 was dominated by *Bacillus sp* (8,574 cfu/ml), *Vibrio spp* (5,830 cfu/ml), *Corynebacterium spp* (5,422 cfu/ml) and *Pseudomonas* (5,201 cfu/ml). Station 5 was dominated by *Bacillus sp* (21,609 cfu/ml), *Pseudomonas spp* (18,257 cfu/ml). *Enterobacteriaceae spp* (18,096 cfu/ml) and *Corynebacterium spp* (17,503 cfu/ml). Across the stations, station 5 recorded the highest of (104,124 cfu/ml), while across the bacterioplankton, *Bacillus sp* recorded the highest of (54,277 cfu/ml) followed by *Corynebacterium spp* with (38,688 cfu/ml), *Pseudomonas spp* (38,381 cfu/ml) and *Enterobacteriaceae* with (33,936 cfu/ml). The ANOVA result indicated that, there is no significant difference in the bacterioplankton composition between the different stations.

Table 1: Bacterioplankton composition, distribution and abundance in Calabar Coastal Waters July 2013 – June 2014

Species	Station 1 (cfu/ml)	Station 2 (cfu/ml)	Station 3 (cfu/ml)	Station 4 (cfu/ml)	Station 5 (cfu/ml)	Total
Bacillus sp	8,918	6,891	8,285	8,574	21,609	54,277
Vibrio spp	1,330	6,759	2,717	5,830	2,394	19,030
Enterobacteriaceae	5,058	321	7,241	3,220	18,096	33,936
Micrococcus spp	3,269	3,315	843	2,449	3,816	13,692
Corynebacterium spp	6,204	4,396	5,163	5,422	17,503	38,688
Aeromonas spp	2,937	-	1,196	2,483	8,537	15,153
Pseudomonas spp	2,639	5,298	6,986	5,201	18,257	38,381
Acinetobacter spp	1,014	1,076	146	2,803	1.324	6,363
Alcaligenes spp	313	328	1,032	-	7,426	9,099
E. coli	2,561	2,415	3,576	2,613	2,217	13,382
Salmonella spp	1,699	3,351	2,998	3,505	1,730	13,283
Proteus spp	1,428	267	1,465	527	1,215	4,902
Total	**37,370**	**34,417**	**41,648**	**42,627**	**104,124**	

3.2 Bacterioplankton in Ship Ballast Waters
Table 2 shows that, in tank 1 *Vibrio spp* recorded (28,475 cfu/ml) followed by *Bacillus sp* with (26,893 cfu/ml), *Corynebacterium spp* (23,936 cfu/ml) and *Pseudomonas spp* (21,950 cfu/ml). Tank 2 had *Vibrio spp* with (12,227 cfu/ml), *Bacillus spp* (11,398 cfu/ml), *Pseudomonas spp* (8,464 cfu/ml) and *Flavobacterium spp* with (8,067 cfu/ml). Tank 3 recorded (23,423 cfu/ml) for *Bacillus sp*, 22,375 cfu/ml *of Vibrio spp*, *Pseudomonas spp* (14,281 cfu/ml) and *Achromobacter spp* (8,867 cfu/ml). In tank 4, *Bacillus* recorded (21,922 cfu/ml), *Vibrio spp* had (16,496 cfu/ml), *Pseudomonas spp* recorded (12,847 cfu/ml) and *Alcaligenes spp* (8,717 cfu/ml). Tank 5 had (14,356 cfu/ml) of *Vibrio spp*, (12,952 cfu/ml) for *Bacillus spp*, and (12,616 cfu/ml) of *Pseudomonas spp*.

Across the tanks, tank 1 recorded the highest figure of (119,059 cfu/ml) and tank 3 with (84,879 cfu/ml). *Bacillus sp* had the highest of (96,588 cfu/ml) and *Vibrio spp* with (93,929 cfu/ml).

The ANOVA result indicated that, there is no significant difference in the bacterioplankton composition between the different tanks.

Table 2: Bacterioplankton composition, distribution and abundance in Ship Ballast Waters between July 2013 – June 2014

Species	Tank 1 (cfu/ml)	Tank 2 (cfu/ml)	Tank 3 (cfu/ml)	Tank 4 (cfu/ml)	Tank 5 (cfu/ml)	Total
Bacillus spp	26,893	11,398	23,423	21,922	12,952	96,588
Pseudomonas spp	21,950	8,464	14,281	12,847	12,616	70,158
Vibrio spp	28,475	12,227	22,375	16,496	14,356	93,929
Alcaligenes spp	10,835	3,658	4,905	8,717	4,069	32,184
Corynebacterium spp	23,936	786	5,240	5,777	4,124	39,863
Flavobacterium spp	401	8,067	673	4,999	3,935	18,075
Achromobacter spp	498	67	8,867	714	1,988	12,134
Salmonella spp	5,995	1,799	4,986	1,994	1,498	16,272
E. coli	-	994	129	-	1,800	2,923
Proteus	76	881	-	-	1,265	2,222
TOTAL	**119,059**	**48,341**	**84,879**	**73,466**	**58,603**	

IMO Regulation: Vibrio <1 cfu/100ml, *E. coli* <250 cfu/100 ml, Enterococci <100 cfu/100 ml.

Comparative analysis of Bacterioplanktons in coastal waters and ballast waters (Table 3) shows that *Bacillus sp, Vibrio spp, Corynebacterium spp, Pseudomonas spp, Alcaligenes spp, E. coli, Salmonella spp* and *Proteus spp* were observed both in Calabar coastal water and ship ballast water. While, *Enterobacteriaceae spp, Micrococcus spp, Aeromonas spp* and *Acinetobacter spp* were seen only in coastal waters. *Flavobacterium spp* and *Achromobacter spp* were seen only in ship ballast waters.

Table 3: Comparative Analysis of Bacterioplanktons in Calabar Coastal and Ship Ballast Waters.

	Calabar Coastal Waters	Ship Ballast Waters
1	*Bacillus spp*	*Bacillus spp*
2	*Vibrio spp*	*Vibrio spp*
3	*Corynebacterium spp*	*Corynebacterium spp*
4	*Pseudomonas spp*	*Pseudomonas spp*
5	*Alcaligenes spp*	*Alcaligenes spp*
6	*E. coli*	*E. coli*
7	*Salmonella spp*	*Salmonella spp*
8	*Proteus spp*	*Proteus spp*
9	*Enterobacteriaceae spp*	-
10	*Micrococcus spp*	-
11	*Aeromonas spp*	-
12	*Acinetobacter spp*	-
13	-	*Flavobacterium spp*
14	-	*Achromobacter spp*

The T-test result at 5% significant level showed that, the mean bacterioplankton composition in Calabar coastal water is less than the mean bacterioplankton composition in Ship ballast water (Figure 2).

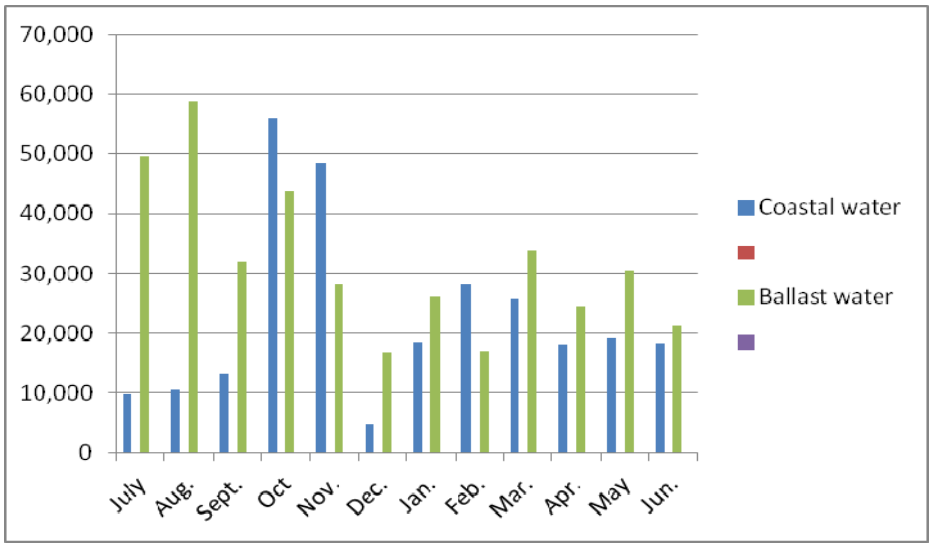

Fig 2: Relationship between Bacterioplanktons abundance in Coastal and Ballast Waters July 2013 – June 2014.

4. Discussion

Drake *et al.*, (2002) [13] identified bacteria, virus in ship ballast tanks in USA, which resulted in the dominance of flavobacterium in the coastal waters of the California Current region. The study by Burkholder *et al.*, (2007) indicated that, species richness was higher in ballast tanks with coastal water, and in tanks containing Atlantic or Pacific Ocean source waters rather than Indian Ocean water. Bacterial abundance was significantly lower in ballast tanks with Atlantic than Pacific Ocean source water. This was further supported by the studies of Karmer *et al*. 2001; that estimated that the world ocean contains 3.1 x 10^{28} bacterial cells and added that, they are very important in the cycling of carbon in the ocean and as the source of long food webs.

Bacterioplankton numerical abundance in the upper mixed layer of the Western Subarctic Pacific was about 1-2 million cells per ml, with a biomass of 15-46 mg C $m^{-3.}$ As compared to the total number of bacterioplankton in the upper mixed layer of the Bering Sea during spring to early summer ranged from 1-4 x 10^{6} per ml, with a biomass of 10-40 mg C m^{-3}. The numerical abundance of planktonic ciliates in the Western Subarctic Pacific, which feed upon bacterioplankton, was estimated to be on the order of 400-4,500 cells per litre [37].

At least one of the four pathogenic eubacteria (*E. coli, Mycobacterium spp, Pseudomonas spp*) was detected in 48% of the ballast tanks, but toxigenic strains of *Vibrio cholera* were not detected. This is in line with the result of this study where most of the samples collected were from ships that passed through the Pacific Ocean to Calabar Port. According to Nagata *et al*. 2001 [32].

According to Anil *et al*. (2002) [1], ballast waters offers conducive situation for micro-organisms such as bacteria, viruses and dinoflagellates to translocate into far away regions and cause deleterious effects to local flora and fauna through their toxigenic, proliferative and over-competitive characteristics. Certain viruses and the bacteria that cause human epidemic cholera have been detected in ballast water (Ruiz *et al*., 2000) [12]. Bukola *et al*., (2006) [4] reported the presence of *E. coli, Enterobacter, Pseudomonas, Micrococcus, Salmonella and Bacillus* in periwinkles samples from Oron and Ishiet creeks. Simberloff, (1981) reported that, even a small proportion of discharged species can proliferate, spread and persist, leading to potentially significant negative effects for the environment, economy and human health.

The study by Fernando *et al*. (2009) [14] concluded that, species found in both coastal waters and ballast waters were alien species introduced into the coastal waters by the ships ballast water discharges, species found only in the coastal waters were regarded as indigenous species, while species found only in ballast waters were alien species introduced into the coastal waters but probably could not survive due to unfavourable environmental conditions. Based on number of colony forming units per millilitre reported in this work which far above the IMO standard of less than 250 cfu/100 ml and the quantity of ballast water discharged into our waters, it is obvious that Nigerian coastal water is not safe for human consumption, bathing and recreational activities.

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