EFFECT OF TIME INTERVAL OF DEPURATION ON NUTRIENTS, HEAVY METALS AND MICROBIAL CONTENTS OF PERIWINKLES FROM ELECHI CREEK, RIVERS STATE, NIGERIA

[1]OJINNAKA, M.C., [2]A.N. OKEREKE, [1]P.C. OJIMELUKWE, [1]O.M. ANI, [1]N.N. KANU, [1]E.C. NJOKU, [1]S.O. OCHIOGU, [1]O.C. OKEREAFOR, AND [1]N.C. ONYEJIUWA

[1]Department of Food Science and Technology
Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
[2]Department of Fish Product Development, Nigerian Institute of Oceanography and Marine Research/African Regional Aquaculture Center,
Port Harcourt, Rivers State, Nigeria
Corresponding Author: mcojinnaka@yahoo.co.uk

ABSTRACT

The present study investigated the effects of time interval of depuration on nutrients, heavy metals and microbial contents of periwinkles from Elechi creek using tap water. The physico-chemical parameters of the tap water used for depuration were also determined. There were decreases in the microbial populations from 0 h to 24 h depuration. The vibrio bacteria reduced from 0 h to 48 h (6.66 x10^{7}cfu/ml^{-1} to 1.16 x10^{7}cfu/ml^{-1}). The faecal coliform bacteria were not detected (0.00 x10^{2}cfu/ml^{-1}). The total heterotrophic bacteria was highest at 0 h depuration with 33.6 x10^{7}cfu/ml^{-1} but reduced to 3.43 x10^{7}cfu/ml^{-1} at 24h depuration process. The heavy metals (chromium, cadmium,lead) investigated were of lower values howeverlead and chromium were not detected from the depurated and non-depurated periwinkle samples. Cadmium contents were in the range of 0.97 – 0.11 mg/kg. The protein content of the periwinkle samples increased as the depuration time progressed (14.42 – 14.72%). The moisture content ranged from 81.41 – 82.28%. The results from the physico-chemical parameters showed that there were significant (P>0.05) increases in the pH (6.80 – 7.77), dissolved oxygen (4.82 – 5.89 mg/l) and salinity (4.28 – 5.72 mg/l) while the temperature decreased from 28.0 – 27.8^{o}C. There were no turbidity values detected.

Keywords: Depuration, nutrients, microbial, Elechi creek

INTRODUCTION

There are many creeks in the Niger Delta areas of Nigeria and most of them are highly polluted because of the large amount of wastes that accumulate from the surrounding. Being an oil-rich region, a lot of wastes emanate from the different oil companies situated in the region as well as from discharge of human faeces and domestic origins. This causes a lot of pollution in these creeks by affecting the sediments and water which is the habitat of most shellfish (Okereke et al., 2017). The increase in environmental pollution leads to increase in contamination of the shellfish; and this equally affects the human population who consume the seafoods. The Elechi creek is one of those creeks and lie between Latitude 4^{o}25 to 4^{o}45 North and Longitude 7^{o}00 to 7^{o}15 East. The Elechi creek is close to the Eagle Island, extending to the Iloabuchi street water bank in Diobu, Port Harcourt. It receives indiscriminate effluents discharges from the heavily industrialized and highly populated Port Harcourt Metropolis. It is characterized by high sea inflow and low freshwater input from adjoining swamp forest and municipal sewers within the Diobu area of Port Harcourt (Otene and Ukwe, 2018).

Bivalve molluscan shellfish concentrate contaminants from the water column in which they grow. These contaminants may then cause illness to humans who depend on them as their source of food. This is because, they are often eaten raw or relatively lightly cooked (Lee et al, 2008). Many aquatic organisms like the periwinkles have the ability to accumulate and biomagnify contaminants like heavy metals, Polycyclic Aromatic Hydrocarbons (PAH) and Polychlorinated Biphenyls (PCB)in the environment. The ingestion of these contaminants may affect not only the productivity and reproductive capabilities of these organisms, but ultimately affect the health of man that depends on these organisms as a major source of protein (Davies et al., 2006).

Depuration is a process employed in many parts of the world for the removal of contaminants contaminated bivalve shellfish by placing them in tanks of clean seawater for them to undergo their normal activity for a period of time that may range from several hours to days(Okereke et al., 2017).Depuration of shellfish is the process by which shellfish are placed in a clean water environment for a period of time to allow purging of contaminants (FAO, 2008). In practice, commercial depuration operations are licensed by the food authority and the conditions for license approval are strict, to ascertain that the harvested shellfish from "restricted" and polluted waters are safe to eat. Depuration help in preventing water and food borne diseases such as typhoid fever and other illnesses attributed to sewage– borne bacteria and other contaminants (Okereke et al., 2017; le Guyer, 2006).

Some shellfish for example oyster and periwinkle are highly nutritional food and are good sources of proteins, minerals like calcium, sodium, phosphorus, iron and some vitamins but are low in

fats and cholesterol (Ifon and Umoh ,2007). The ingestion of these contaminants by these organisms will not only affect the productivity and reproductive compatibility of these organisms but also affect the health of humans who depend on these organisms as a source of food (Chaerun *et al*., 2004).Periwinkles are marine mollusks that are represented in mangrove swamps, lagoons and estuaries by two genera *Tympanotonus* and *Pachymelania (*Omenwa *et al.,* 2011). T*ympanotonus fuscatus* are shellfish dominantly found in brackish waters of the riverine areas of Nigeria, where they are highly prolific and this feature makes them a cheap source of protein in many homes when compared to other conventional protein sources (Bassey and Ayuk, 2007).

The main objective of this research was to study the effect of depuration using tap water on the nutrients, heavy metals and microbial load of periwinkle (*Tympanotonus fuscatus*) samples harvested from Elechi creek in Rivers State, Nigeria as well as the study of the physicochemical properties of the tap water used in the depuration process.

MATERIALS AND METHODS

Description of study area

Elechi creek is one of the several adjoining creeks of the Upper Bonny River estuary in the Niger Delta. It lies between Latitude 4º25 to 4º45 North and Longitude 7º00 to 7º15 East (Fig 1). The Elechi creek is close to the Eagle Island, extending to the Illoabuchi street water bank in Diobu, Port Harcourt. The Eagle Island is found on the south-west of Port Harcourt and delimited on the North by the Rivers State University of Science and Technology in Nkpolu-Oroworukwo area of Diobu. The Elechi creek is a brackish water system influenced by periodic events of tidal fluxes. It has mangrove vegetation (Otene and Ukwe, 2018).

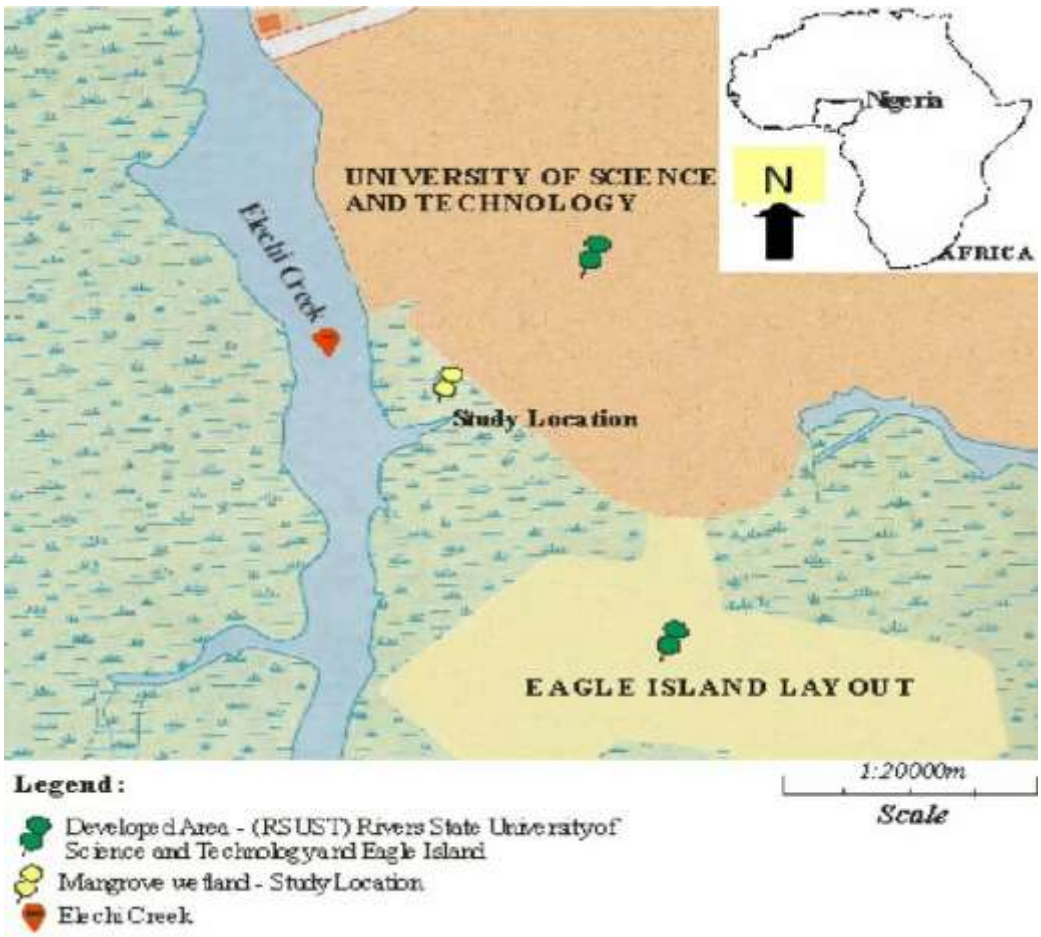

Fig1: Map showing the study area

Source of raw materials

The samples (periwinkles, *Tympanotonus fuscatus*) used during this study were harvested during the rainy season (June, 2018) from Elechi creek, Rivers state, Nigeria.

Sample preparation

The fresh *Tympanotonusfuscatus* samples were collected in a sterile bag and transported to the laboratory in Africa Regional Aquaculture Centre (ARAC), Aluu, Rivers State, where they were prepared for analysis by first scrubbing and washing

off dirt, debris and surface contaminants using tap water.

Depuration of *Tympanotonus fuscatus*

The samples after cleaning were emptied into the depuration tank for the depuration process as described by (Okereke*et al*., 2017). After every 24 h of the depuration process, few depurated samples were picked randomly and removed from their shells aseptically with the aid of a specially fabricated sterile needle and then collected in sterile sample bags labelled 24, 48, 72 and 96 h for the respective sample, then refrigerated at 4^{o}C prior to analysis.

Plate 1: Harvested periwinkle samples (*Tympanotonus fuscatus)*

Plate 2: Constructed depuration tank with tap head for water supply.

Plate 3: Depurated periwinkle (*Tympanotonus fuscatus*) samples.

Collection of water samples

Water samples were collected before the depuration process from the main water source and during the 48h and 96 h of the depuration process from the depuration tank in sterile bottles and labelled as non-depurated (0 h) and depurated (48 h and 96 h) respectively for the physicochemical analysis (pH, temperature, dissolved oxygen, turbidity, salinity).

Microbiological analysis of periwinkle samples

The periwinkle samples were mashed using sterile mortar and pestle, and homogenized with 100ml of sterile distilled water. Standard/Aerobic Plate Count method was used for enumeration of Total Heterotrophic Bacteria (THB), Total Heterotrophic Fungi, Total Coliform Bacteria (TCB), Faecal Coliform Bacteria (FCB), Hydrocarbon Utilizing Bacteria (HUB), Hydrocarbon Utilizing Fungi (HUF) and Vibrio Bacteria (VB). Serial dilution procedure as described by Harrigan and McCance (1990); Obire and Wemedo (1996); Ofunne (1999) was employed for cultivation, enumeration and isolation of the physiological groups of bacteria in the periwinkle samples. The ten-fold serial dilution was used to obtain appropriate dilutions of the sample: initial dilution was 10g of samples in 100ml of sterile distilled water, and subsequent dilutions were 1ml of initial dilution in 1ml of normal saline up to 10^{-5} dilution. Aliquots (0.1ml) of the required dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} ,10^{-5}) were spread plated in duplicates onto the surface of dried sterile nutrient agar plates (for total heterotrophic bacteria), potato dextrose agar plates (for total heterotrophic fungi), MacConkey agar plates (for faecal coliform bacteria), oil agar plates (for hydrocarbon utilizing bacteria and fungi) and TCBS agar plates (for Vibrio bacteria). All inoculated plates were incubated at 37^{o}Cfor 24 - 48 h except the plates for hydrocarbon utilizing bacteria and fungi incubated for 3 – 7 days and faecal coliform bacteria incubated at 44.5^{o}C.

Heavy metals analysis of the periwinkle samples

Heavy metal concentration in soft tissue of *Tympanotonus fuscatus* were analysed according to the method described by (Adekenya, 1998) using an Atomic Absorption spectrometer (Perkin-Elmer model 2380).

Wet digestion in soft tissue of *Tympanotonos fuscatus*

i. About 1 g of the sample was weighed into a clean 250 ml conical flask.
ii. 5 ml of concentrated nitric acid and 2 ml of 52% perchloric acid was added.
iii. The mixture was heated on an electro thermal heater hot plate until a clean solution of the digest was observed.
iv. The digest was allowed to cool to room temperature and the solution was diluted to 50 ml with distilled water.
v. The diluted solution was filter into sample bottle through Whatman filter paper N0 541.
vi. The digest solution was analysed for metal ion by AAS (Atomic Absorption spectrophotometer).

Lead determination

Lead ion was analysed by an Atomic Absorption Spectrophotometer at 283.3 nm wavelength. The wavelength was selected with a narrow slit with, air and acetylene gas flow was adjusted. Other settings as recommended for the instrument employed was attended to and regulated. Hallow Cathode lamp was given adequate time to stabilized before aspirating standards for equipment calibration. After calibrating the equipment with standard lead concentrations, the aspiration tubing and burner system were flushed with distilled water severally before aspirating the test sample solution on the sample experimental condition used for standard. The concentration of lead ion in the sample was extrapolated from the standard graph of lead ion plotted.

Chromium determination

The Atomic absorption spectroscopy was used in the determination of chromium (Defew*et al.,* 2004) was first engaged and left for about 15 minutes to stabilize wavelength of 357.9 nm recommended for chromium ion absorption was selected. Air and acetylene flow into the burner system was adjusted and regulated. Other essential settings as was recommended in the standard operational manual were adjusted. The Hallow Cathode lamp was allowed adequate time to stabilised. Standard chromium solution was aspirated into the burner system and their equivalent absorbance was obtained. The burner chamber, the aspirator tubing were flushed thoroughly with distilled water, then the test sample solution was aspirated, and the corresponding absorbance was obtained. The concentration of chromium in the sample was extrapolated from chromium standard graph plotted by the recording system.

Cadmium determination

Wavelength of 229 nm was selected using the appropriate knob, acetylene gas flow and air was adjusted as to regulate in-flow into the burner chamber. Other standard operational setting as required for the instrument used was adjusted as recommended. Hallow cathode lamp was allowed to stabilize adequately before aspiratory standard solutions of cadmium. At the end of aspirating the standards, the aspirating tubing and the system were flushed through by aspirating distilled water until no trace of cadmium was indicated on the measurement system. The machine at this point read zero

cadmium. The sample solution was at this point aspirated into the burner system and the concentration of cadmium present was displaced on the recording system or screen as against the corresponding absorbance. The actual concentration of cadmium in the sample was extrapolated from the standard graph of cadmium plotted by the Atomic Absorption recorded.

Proximate analysis of periwinkle samples

The proximate compositions (moisture, total ash, crude protein, crude fat, and crude fibre) of the periwinkle samples were determined using the method of AOAC (2010).

Determination of Physicochemical Properties of Tap Water

pH (Hydrogen-ion concentration)

The pH of water sample was determined in an electronic pH meter Horiba water checker (model u-10[a]). The electronic probes were dipped into the water sample after standardization with buffer solutions, and the reading of the value which was displayed on the digital board was taken (APHA, 2008).

Dissolved oxygen

This is the concentration of oxygen in the water sample. Dissolved oxygen was determined using the unmodified Winkler procedure as described by (APHA, 1992). The procedure entails the oxidation of manganous hydroxide in a highly alkaline medium. On acidification in the presence of an iodide, the manganic hydroxide dissolves and free iodine is liberated in an amount equal to the oxygen originally dissolved in the sample. The iodine is titrated with a standard sodium thiosulphate solution using starch as an indicator.

Procedure

i. The 300 ml BOD bottle was rinsed with the sample and filled up. The bottle was then stopped when all the air bubbles, if any have been allowed to rise out of the BOD bottle.
ii. The glass stopper is removed and 2 ml of $MnSO_4$reagent is added followed by 2 ml of KOH + KI (Alkali-Iodide-azide reagent). The stopper was replaced, mixed by inverting bottle at least 15 times and then a brown precipitate was allowed to settle down. 100 ml 0f concentrated H_2SO_4 was added and mixed to give homogenous solution (i.e. to dissolve all brownish precipitate). Then 203 ml of sample was measured and poured into a 250 ml conical flask and was titrate with 0.02 N standard sodium thiosulphate ($Na_2S_2O_3$) titrate to a faint or pale colour.
iii. Add 1-2 ml of starch indicator and continued the titration until the solution changes from blue to colourless end point and reading was taken.
iv. Calculation;

DO in mg/l= $\frac{\text{ml titrant x 0.025 N x 8 x 1000mg/l}}{\text{Vol of sample} \times \frac{\text{vol.of sample}}{\text{vol.of conical flask}}}$

Where ml titrant =Volume of $Na_2S_2O_3$ used in titration

N = Normality of $Na_2S_2O_3$

8 = Oxygen concentration equivalent to ml. of 1 N Na_2S_2O3

100 = Conversion factor to 1 litre.

Salinity

Salinity is the total concentration of all ionic constituents present in a water sample. It is an important measurement in the analysis of certain industrial wastes seawater. Associated terms are chlorinity**,** which includes chloride, bromide and iodide all reported as chloride. Salinity was determined by (APHA, 1992). Salinity is measured by argentiometric titration (silver nitrate method) to get the chloride concentration which is then converted to salinity after using the factor S% 0-1.85 × CL % + 0.03. The sample under test was titrated against silver nitrate solution using potassium chromate as indicator. The 100 ml portion was neutralized to the phenolphthalein endpoint, using either the dilute solution of sulphuric acid or caustic soda. It was only at neutral or slightly alkaline solution that potassium chromate can indicate the end point of the silver nitrate ($AgNO_3$) titration of chloride. One (1 ml) of the potassium chromate solution (5%) and then titrate with 0.05 N Silver nitrate solution until a faint pink colour is obtained.

Calculation: Sodium Chloride (NaCl) ppm = v × 29.2

Chloride (Cl) ppm = v × 17.7

Where v = amount of 0.05 N silver nitrate used in titration.

Temperature

Twenty (20 ml) of water sample was poured into 100 ml beaker and mercury-in-glass thermometer was dipped into the beaker filled with sample and allowed to stabilize. The temperature value reading was taken after 2 minutes. And recorded as ^{0}C.

Turbidity

The turbidity was determined by the method described by (APHA, 1992). A Secchi disc is a round circular metal disc, (20 cm in diameter), coloured black and white, and was used for measurement of turbidity. The disc was set down into the water until it is no longer visible. The depth at which the disc is no longer visible, disc is lifted up and at the point where it became visible again was noted. The mean of the two readings gave the transparency of the water. Turbidity can also be measured by placing 25 ml of water sample in a cuvette and read with a spectrophotometer at 425

nm. This is expressed as NTU (Nephelometric turbidity unit).

Statistical analysis

The result of the laboratory analysis was analysed using IBM SPSS Inc. software (version 21.0). All experiment was conducted in duplicate and mean values were compared using one-way ANOVA

RESULTS

Microbial load of periwinkle samples

Table 1 shows the microbial contents of depurated periwinkle samples using tap water. There was significant difference(P>.0.05) in the total heterotrophic bacteria of the samples at different time interval. The total heterotrophic bacteria (THB) was highest at 0h depuration (33.6 $x10^7$cfu/ml^{-1}); then decreased to 3.43 $x10^7$cfu/ml^{-1} after 24 h depuration process. The level of THB of depurated periwinkle samples increased between 46 h and 96 h but not compared to the 0 h depuration time.

There was significant difference ($p<0.05$) for depurated periwinkles at different time intervals. The total heterotrophic fungi (THF) decreased as the depuration process progressed. At 0 h, the THF was 2.6 $x10^3$cfu/ml^{-1}. At 24 h the THF reduced to 0.7 $x10^3$cfu/ml^{-1}) and dropped to 0.0 $x10^3$cfu/ml^{-1} at 48 h and 72 h respectively.

Table 1: Microbial populations of periwinkle samples (cfu/ml)

Time interval (h)	Total Heterotrophic Bacteria (THB) ($x10^7$cfu/ml^{-1})	Total Heterotrophic Fungi (THF) ($x10^3$cfu/ml^{-1})	Hydrocarbon Utilizing Bacteria (HUB) ($x10^3$cfu/ml^{-1})	Hydrocarbon Utilizing Fungi (HUF) ($x10^2$ cfu/ml^{-1})	Total Coliform Bacteria (TCB) ($x10^2$ cfu/ml^{-1})	Faecal Coliform Bacteria (FCB) ($x10^2$cfu/ml^{-1})	Vibrio Bacteria ($x10^7$cfu/ml^{-1})
0	$33.6^a \pm 0.33$	$2.6^a \pm 0.03$	$0.6^a \pm 0.08$	$2.20^a \pm 0.05$	$4.0^a \pm 0.03$	0.00 ± 0.00	$6.66^a \pm 0.08$
24	$3.43^b \pm 0.12$	$0.7^b \pm 0.05$	$0.6^a \pm 0.03$	$0.36^c \pm 0.06$	$6.0^c \pm 0.03$	0.00 ± 0.00	$3.06^b \pm 0.03$
48	$4.06^c \pm 0.06$	$0.0^c \pm 0.00$	$0.16^b \pm 0.00$	$0.43^c \pm 0.08$	$5.0^b \pm 0.05$	0.00 ± 0.00	$1.16^c \pm 0.08$
72	$8.30^d \pm 0.05$	$0.0^c \pm 0.00$	$0.20^b \pm 0.05$	$0.33^c \pm 0.03$	$3.9^a \pm 0.03$	0.00 ± 0.00	$1.56^d \pm 0.03$
96	$9.16^e \pm 0.08$	$0.13^c \pm 0.03$	$1.16^c \pm 0.08$	$0.00^b \pm 0.00$	$5.1^b \pm 0.06$	0.00 ± 0.00	$2.26^e \pm 0.03$

**Values are means ± standard deviation of duplicate determination. Means with different superscript within the same column are significantly different (P<0.05).*

There was no significant difference in the hydrocarbon utilizing bacteria between the 0h to 24 h depuration. The hydrocarbon utilizing bacteria were of the same value for 0 h and 24 h(0.6 $x10^3$cfu/ml^{-1}) depuration. Then decreased to 0.16 $x10^3$cfu/ml^{-1} at 48h. The HUB was highest at 96 h depuration with 1.16 $x10^3$cfu/ml^{-1}

The hydrocarbon utilizing fungi (HUF) was highest at 0 h depuration (2.20 $x10^2$cfu/ml^{-1}) and decreased to 24 h (0.36 $x10^3$cfu/ml^{-1}); increased at 48 h (0.43 $x10^2$cfu/ml^{-1}) and reduced drastically from 72 h (0.33 $x10^2$cfu/ml^{-1}) to 96 h (0.00 $x10^2$cfu/ml^{-1}). This result shows that the HUF was reduced at 24 h depuration but on further depuration it reduced after 72 h depuration.

The vibrio bacteria also had similar trend. It reduced from 6.66 $x10^7$cfu/ml^{-1} at 0 h depuration to 1.56 $x10^7$cfu/ml^{-1} at 72 h depuration time interval using tap water. The vibrio bacteria for depurated periwinkles reduced from 24 h to 72 h (6.66 $x10^7$cfu/ml^{-1} to 1.56 x 10^7cfu/ml^{-1}).

Heavy metal concentration in depurated and non-depurated soft tissues of periwinkle samples. The results for heavy metal concentration in 96 h depurated and non-depurated soft tissues of periwinkle samples are shown in Table 2. Chromium and lead were not detected in both the 96h depurated and non-depurated periwinkle samples.

Table 2: Heavy metal concentrations of depurated and non-depurated soft tissues of periwinkle sample (mg/kg).

Time interval (h)	Chromium	Cadmium	Lead
0 (non-depurated)	0.00	0.97	0.00
96 (depurated)	0.00	0.11	0.00

Proximate composition of soft tissues of depurated and non-depurated periwinkle.

Proximate composition of depurated (48 h, 96 h) and non-depurated periwinkles are shown in Table 3. The moisture content of the periwinkle samples ranged from 81.41% for non-depurated periwinkles to 82.28% for 96 h depurated periwinkles, showing significant variation in their moisture content.The protein content of the depurated (48 h and 96 h) periwinkle samples were in the range of 14.57% and 14.72% respectively; while the non-depurated periwinkle sample had protein value of 14.42%.

It was also observed that the periwinkle samples showed low fat content (0.45 – 0.64%). The carbohydrate contents of the periwinkle samples as shown in Table 3 are low; 1.43% (96 h depurated

periwinkle), 1.96% (48h depurated periwinkle) and 2.49% (non-depurated periwinkle).

Table 3: The proximate composition of depurated and undepurated periwinkle samples (%)

Time interval(h)	Moisture content	Ash content	Crude protein	Crude fat	Crude fiber	Total carbohydrate
0	81.41^{a}±0.16	1.05^{a}±0.01	14.42^{a}±0.26	0.64^{c}±0.02	0.00^{a}±0.00	2.49^{c}±0.41
48	81.84^{b}±0.00	1.09^{b}±0.00	14.57^{b}±0.00	0.54^{b}±0.00	0.00^{a}±0.00	1.96^{ab}±0.00
96	82.28^{c}±0.04	1.13^{c}±0.01	14.72^{b}±0.05	0.45^{a}±0.00	0.00^{a}±0.00	1.43^{a}±0.00

**Values and means ± standard deviation of duplicate determinations; means with different superscript within the same column are significantly (P<0.05) different.*

Physicochemical properties of tap water used for depuration process

The result of the tap water pH showed that the water is weakly acidic to weakly alkaline with values ranging from 6.80 (0 h, before depuration) to 7.77 (water used for depuration at 96 h).

The results of the tap water temperature ranged from 27.8˚C to 28˚C. The highest temperature was recorded from the tap water before depuration (natural tap water, 28˚C) while 27.8˚C and 27.9˚C were recorded from water used in depuration at 48 h and 96 h respectively

The results of the measurements of mean salinity of the tap water shows that the values ranged between 4.28mg/l – 5.72mg/l with the highest being from tap water after 96 h depuration.

Table 4 also shows that the values of the Dissolved Oxygen (DO) from the tap water source. Dissolved oxygen (DO) is a measure of the degree of pollution by organic matter, the destruction of organic substances as well as the self purification capacity of the water body (APHA, 1992). The DO values were in the range of 4.82mg/l (before depuration) and 5.36mg/l (after 48 h depuration and 5.89mg/l (after 96h depuration). The values recorded for tap water in this study are similar to those recorded for water form Elechi creek.

The turbidity values gotten in this study were insignificant.

Table 4: Physicochemical properties of tap water used for depuration

Time interval (h)	pH	Dissolved oxygen (mg/l)	Salinity (mg/l)	Temperature (^{0}C)	Turbidity (NTU)
0	6.80^{c} ±0.00	4.82^{c}±0.04	4.28^{c} ± 0.04	28.0^{a} ± 0.00	0.00^{a} ± 0.00
48	7.28^{b} ±0.00	5.36^{b} ±0.00	5.00^{b} ± 0.00	27.9^{a} ± 0.00	0.00^{a} ± 0.00
96	7.77^{a} ±0.02	5.89^{a} ±0.04	5.72^{a}± 0.12	27.8^{a} ± 0.35	0.00^{a} ± 0.00

**Values are means ± standard deviation of duplicate determination. Means with different superscripts within the same column are significantly different (P<0.05)*

DISCUSSION

There was reduction in the total heterotrophic bacteria. Okereke et al. (2017) observed total heterotrophic bacteria count of oysters at Abuloma during the wet season to be 2.6 x 10^{5}cfu/g at 24 h, 48 hand 72 h respectively. The tap water was responsible for the change in THB The THF values were low compared to other similar studies. However, the rate of reduction was similar to the results by Okereke*et al.* (2017). Okereke*et al.* (2017) stated that the high population of total fungal count recorded during rainy seasons in their study could be due to their heterotrophic nature which enable them to utilize nearly all-natural organic matters. The high fungal count could also be as a result of fungal activities such as biogeochemical cycling of nutrients in the water column, degradation of compound and contribution to the food web in the creek. Lee et al., (2003) reported the occurrence of higher fungal population during rainy seasons and also noted high population of fungi when the salinity was as low as 1.8 – 16.8%The values increased sharply from 48 h to 96 h depuration process. This is because microorganism is ubiquitous in all types of natural waters (El-Hissy et al., 2000; Okereke et al.,2017). Okereke et al. (2017) reported higher values of HUB from two of the selected sites (Okwujagu and Slaughter) for oysters compared to the lower values reported in our study on periwinkles. Earlier studies by different authors suggests that rainfall pattern in a particular zone may influence the bacteria invertebrate interactions in the aquatic environment, possibly by changing the concentration of bacterial organisms in surface waters, feeding habits of the aquatic organisms and depuration dynamics (Makinde*et al.*, 2009). El-shenawy (2004) also reported the ability of some.

The reduction in (HUF) supports the study conducted by Obodai*et al.*, (2010) which stated that depuration reduced the concentration of bacteria count after 76 h.Okereke et al. (2017) reported higher values of HUF in depurated oyster samples collected from different sites (Slaughter, Abuloma, Okujiagu and Oginigba respectively) compared to the results obtained in this study. This could be attributed to the source of water used for depuration.

The seawater used for depuration in the other studies could be high in HUF count because of incidences of exposure of the study area to industrial wastes, human faeces as well as discharge of animal blood from the slaughter and oil spills (Davies *et al.*, 2008). In this study however, depuration has been found to reduce HUF from 0 h to 24 h; then after 48 h was able to reduce further to 0.00 $x10^{2}cfu/ml^{-1}$ at 96 h time interval. This shows the success of depuration in the removal of fungi from some shellfish.

The faecal coliform bacteria could not be detected from the samples possibly due to the source of water used in depuration process. The source of water used for depuration (tap water) is not known to be polluted with industrial wastes and faeces as reported in other studies. This result is contrary to other results obtained from studies using seawater for depuration process. The high values reported in the depuration processes in other studies could be due to creeks that are known to receive nutrient laden waste materials from sewage and inland waters, which enrich the water and sediment thereby providing a sufficient source of nutrient for proliferation of these organisms (Okereke et al.,2017) The faecal coliforms for depurated oysters have been reported to have reduced from 24 h to 96 h and this might be due faecal coliforms being deactivated faster than they can reproduce (Okereke *et al.*, 2017; Richards, 1988). It has been reported that high concentration of faecal coliform counts can be efficiently eliminated through depuration process (Okereke *et al.*, 2017; Mur three and Tamplin 1995).

This reduction in vibro content could be as a result of the tap water used for depuration knowing that contaminants will be much in the other microbial compositions. Winfield and Groisman (2003) reported that increased level of human activities could bring about high microbial load in the water, which indicates the input of microorganisms from domestic and industrial sources .High vibrio count could be as a result of human activities such as bathing, washing of clothes, boats or other materials, disposal of faecal matters, sewage discharge done in those locations.

Chromium and lead were not detected in both the 96 h depurated and non-depurated periwinkle samples. This could be as a result of the time of harvesting of the periwinkles (June, 2018) from the Elechi creek. This period is known as peak rainy season in this area and after 96 h depuration using tap water, none of the heavy metals (chromium and lead) were identified. Otene and Ukwe (2018) in their study on evaluation of heavy metals in water and sediment from Elechi Creek, Port Harcourt, Nigeria reported chromium and lead content of water samples from Elechi creek to be 0.160µ/l and 0.472 µ/l respectively in the month of June (peak rainy season). This goes to show that the period of harvest (rainy season) could have affected the presence of the heavy metals in the tissues of the periwinkle samples. The rain at this period could have helped in washing away pollutants that have deposited in the creeks thereby reducing the concentration of chromium and lead. Davies et al. (2006) reported highest concentrations of chromium in normal and depurated periwinkles though the concentrations are below the recommended limits for human consumption. This could be as a result of period of harvest of the periwinkles from the creek. Dry seasons are known to give highest concentration of most of these heavy metal accumulation by the shellfish. However, the cadmium content before depuration using tap water was 0.97mg/kg while after 96 h depuration the cadmium content reduced to 0.11mg/kg. The tap water used in depuration could also have led to the decreased concentrations of the heavy metals determined. Otene and Ukwe (2018) also reported cadmium concentration of 0.350 µ/l during the rainy season (June). The low concentrations of these heavy metals in the soft tissues of periwinkles show that the period of harvest (rainy season) could have affected their reduction as reported by Otene and Ukwe (2018). Okereke et al. (2017) in their study of the effect of depuration on heavy metal contents in periwinkle (*tympanatonus fuscastus*) from a polluted creek in Rivers State, Nigeria also observed that depuration reduced heavy metals faster in periwinkles harvested during rainy season than those harvested during the dry season and this they opined led to satisfactory results when compared to the WHO standards. However, the WHO/USEPA standard limits for chromium, cadmium and lead are 0.1mg/l, 0.01mg/l and 0.05mg/l respectively.

These higher moisture content values recorded by the depurated periwinkle samples could be due to the continuous tap water used during the depuration process. Also observed is the increase in moisture content as depuration process time progressed. Kiin-Kabari et al. (2017) reported moisture content of 80.22% and 84.80% in smooth and rough periwinkles (*Tympanatonus fuscastus and Pachymelania*) respectively. Similar values were also reported by Varadharajan and Soundarapandian (2014). This could also be attributed to environmental effects as opined by Osibona et al. (2006). Shellfish tends to absorb water from the external environment into their cells which are of higher concentration in order to balance the osmotic pressure between the cell and the surrounding water.

Depuration process using tap water increased the protein content at 96 h depuration. The results of this present study were in line with the report of Adewoye and Omotosho (1997) which stated that protein forms the largest quantity of dry matter in seafoods.

According to the study of Ackman (1989), seafood generally can be grouped into four categories according to their fat content: lean fish

(<2%), low fat (2 to 4%), medium fat (4 to 8%) and high fat (>8%). This present study confirms the periwinkle samples fall into the category of lean fish.

The carbohydrate values were observed to be decreasing as the depuration process progressed. The low carbohydrate values obtained from the periwinkle samples indicates the need for its consumption to be supplemented with energy-rich foods to balance the energy-protein intake requirement (Kiin-Kabari et al., 2017); thereby helping in reducing protein-energy malnutrition especially in children.

The relatively stability of the pH could be as a result of the activities of the microorganisms which make their environment more alkaline by generating ammonia through amino acid degradation or production of acidic or basic metabolic waste products (Efiuvwevwere and Ezeama, 2004; Obodai et al., 2010; Efiuvwevwere and Amadi, 2015). These values compares favourably with the water samples from Elechi creek which were reported to be within the range of 6.30 – 7.70 (Obire et al., 2006). The pH profile from the tap water samples fall within the desirable limit for the survival of fishes. A lower pH value will not support aquatic life (Ngah et al., 2017). Efiuvwevwere and Amadi (2015) also reported alkaline values for both tap water used for depuration during the rainy and dry season. Zhang et al. (1995) reported that the pH of brackish water falls within the range 7.48 and 8.89 respectively.

Ngah et al., (2017) reported temperature of water from Elechi creek to be in the range of 27˚C to 29˚C collected from different periods with a surface water temperature profile mean value of 29˚C.Bhatnagar et al., (2004) suggested the levels of temperature as 28˚C - 32˚C to be tolerable to fish.Efiuvwevwere and Amadi (2015) also reported similar values in tap water used for depuration of mangrove oysters.

The salinity values are low compared to those reported for Elechi creek from where the periwinkles were harvested 7.00(‰) to 17.20(‰) (Ngah et al., 2017). The differences reported could be due to seasonal variations. However, Efiuvwevwere and Amadi (2015) in their study of the bacteriology and physico-chemical parameters of microcosms used in the depuration of mangrove oysters harvested from estuary of Rivers State Nigeria, reported lower salinity values (< 3 ppt) in tap water used for depuration during rainy and dry seasons respectively.

The values recorded for tap water in this study are similar to those recorded for water form Elechi creek. The DO of Elechi Creek mean values range from 3.23 mg/l to 4.65 mg/l in the dry season and 6.25 mg/l to 6.53 mg/l in the wet season at the study area (Ngah et al., 2017). The relationship between the concentration of organic materials and DO in marine water has been a concern and has been investigated. High levels of organic matter are usually associated with low DO concentrations. Ekubo and Abowei (2011) recommended that fish can die if exposed to less than 0.3 mg/l of DO for a long period of time, minimum concentration of 1.0 mg/l. Dissolved oxygen of not less than 2.0mg/l has been recommended as conducive for depuration of bivalves (Lio-Po, 1990; Efiuvwevwere and Amadi, 2015)

The turbidity values gotten in this study were insignificant and could be as a result of the design of the depuration tank used in this study as described by (Okereke et al., 2017) which involved continual passage of the depurated water under minimized pressure through a channel which in turn did not allow the retaining of accumulated turbid matter purged out overtime.Efiuvwevwere and Amadi (2015) reported lower values for turbidity. Lio-Po (1990) had previously reported 77NTU as the maximum allowable turbidity in a depuration system.

CONCLUSION

The physico-chemical parameters of the tap water used for depuration had influence on the microbial activity of the periwinkles and also provides information on the quality of tap water needed for depuration. There were decreases in the microbial populations from 0 h to 24 h depuration using tap water as evidenced in most depuration processes using sea (habitat) water.

Conflict of interest

No conflict of interest declared.

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