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Bioaccumulation of total hydrocarbon in shell and tissue of periwinkles (*Tympanotonusfuscatus*) in lowly and highly polluted mangrove forest, Niger Delta, Nigeria

Numbere A*

Department of Animal and Environmental Biology, University of Port Harcourt, Choba, Nigeria

***Corresponding author:** Numbere A, Department of Animal and Environmental Biology, University of Port Harcourt, Choba, Nigeria, Tel: +2348056002989, E-mail:aroloyen@yahoo.com

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Abstract

Bioaccumulation of pollutants up the food chain is detrimental to humans, who consume sea food harvested from polluted waters. In this study it was hypothesized that periwinkles, which is an integral part of the local meal possess the potential of causing food poisoning as result of its ground-dwelling behavior. One hundred periwinkles (n=100) were picked up from the forest floors in highly and lowly polluted sites. The samples were bagged and labeled and sent to the laboratory. The inner tissues were detached from the shell, oven-dried at 70 °C for 48 hours and crushed to fine powder. Two (2) grams of each sample was analyzed using spectrophotometric method with HACH DR 890 calorimeter (wavelength ~420 nm) for total hydrocarbon content (THC). The result indicate that there is no significant difference in THC concentration between parts and between highly and lowly polluted sites ($P>0.05$). However, the THC in shell (0.252±0.002mg/kg) is slightly higher than the THC in tissue (0.244±0.002mg/kg). For shell size, there is significant difference, and periwinkles from highly polluted site (width: 2.27±0.03cm and length: 3.83±0.07cm) is larger than periwinkle from lowly polluted site (width: 1.83±0.03cm and length: 3.14±0.04cm). There is a little correlation between THC concentration and periwinkle size ($R^2 = 0.13$). This means bigger periwinkles have lower THC concentration whereas smaller periwinkles have higher THC concentration. This implies that the bigger the periwinkle the better it is for consumption.

Keywords: Periwinkle; Total Hydrocarbon; Bioaccumulation; Niger Delta; Shell; Food Chain; Pollution

Introduction

The Niger Delta area is situated at the southern part of Nigeria bordering the Atlantic Ocean [1]. It is endowed with many aquatic organisms that serve as sea food, and supplies the local people with their daily protein intake. Mangrove is the dominant forest in this locality, and it is a biodiversity hot spot that harbors shelled and unshelled organisms. Within the mangrove forests there are a conglomeration of benthic and pelagic organisms [2,3]. They include crabs, periwinkles, hermit crabs, mud skippers, mussels etc. These organisms are mostly found on the forest floors and seen during low tide or attached to tree stems[4]. They are often picked up by local inhabitants who consume or use them for commercial purpose. Out of the organisms found in the mangrove forest the periwinkles are unique because they dwell inside the mangrove swamp and are easily captured unlike other species that beat a fast retreat when seen. Their outer shell protects the fleshy body within from impact of surging tidal current. But, the shell hardly protects their tissues from absorbing contaminants and pollutant especially when they are in a polluted environment because of their filter feeding habit [5].

The mangrove forest of Okrika where the research was done is close to the Jetty that evacuates crude oil abroad from the Port Harcourt Refinery Company (PHRC). Long term study of this forest had shown that the forest floor is constantly coated with spilled oil, which comes from industrial activities in the area. The spillages are mainly caused by mechanical failure, corrosion of aged pipelines or natural expansion and contraction of pipes due to weather condition [6]. Spillages also occur during the transfer of crude oil from pipelines into ocean going ships that are berthed at the jetty. However, spillages are also caused by sabotage and artisanal refineries operated illegally by local militia [7]. Here pipes are secretly punctured and the crude oil siphoned into private barges and sold into the black market. During this process minor oil spills occur (0-250 barrels), which may not have great impact on the environment. Despite the spillages that occur the mangroves continue to grow without dying because of their resilience [8]. However, the mangroves shed their leaves to expel the pollutants absorbed via their root [9]. This is known as defoliation, which is the expulsion of leaves that contain pollutants or high salt content. Similarly, their adventitious roots grow above the soil, which also helps to reduce contact with oil spills while the part of the root embedded within the swamp prevents the absorption of pollutants. This is called the shut-down mechanism of mangrove roots and it prevents the intake of excess salt by the mangrove [10]. Another action that reduces pollution in mangrove forest is tidal waves, which flushes out and cleans the shore of oil spills. Low and high tides in this region fluctuate every six hours. During high tides the rush of water from the sea into the mangrove forest and creeks flushes away oil spillages that had clogged the roots and floors of the mangrove forest into the sea thus, reducing their impact on the mangroves.

The action of the tide cleans up the forests because during high tide the spills that are flushed out are carried away into the sea and distributed along the tributaries. The reduction of pollution by tides does not prevent the bioaccumulation of pollutants in the body of periwinkles and other aquatic organisms that reside in the forests [11]. To worsen the situation the filter feeding behavior and the ground-dwelling habit of periwinkles expose them to contamination. Furthermore, the low mobility of periwinkles exposes them to danger when a sudden spillage occurs in their environment. Periwinkles are made up of different sizes from small to large, which influence their commercial quality. The large periwinkles are usually more attractive and more lucrative than the small periwinkles in the market. But we do not know whether their size influence their ability to bioaccumulate pollutants in the mangrove forest. It is important to study bioaccumulation of hydrocarbons in periwinkles because almost every house hold uses them for cooking. Thus, it is very important to study the concentration of total hydrocarbon content in their shell and tissue, and also to find out whether size has anything to do with bioaccumulation of THC. I thus hypothesized that the ground-dwelling habit of periwinkles will predispose them to bioaccumulating THC. The objectives of this study include: (1) to determine the THC concentration of shell and tissue of periwinkle; (2) to compare the THC concentration of periwinkles in highly and lowly polluted sites and (3) to determine the relationship between the size of periwinkles and the THC concentration.

Materials and methods

Description of study area

Buguma(4°45'N, 6°53'E) is chosen as the lowly polluted site because it has low oiling activities. It is the headquarters of the Asari Toru Local Government area and base of Kalabari kingdom in Rivers state (Figure 1).Buguma is an island that is surrounded by water and a host to a large supply of mangrove forest. The climate of the area is sub tropical with heavy rainfall and relative humidity. The major mangrove species found in this area are the red, white and black mangroves. The low inter-tidal zone is made up of mud flats which is bare of vegetation and covered with deposits and debris. A part of the mangrove forest was cut and sand filled some years ago, and today serves as nesting ground for periwinkles that is brought in by tidal currents. The area is dominated by fringe mangrove forest, which dots the intersection between the sea and the land.

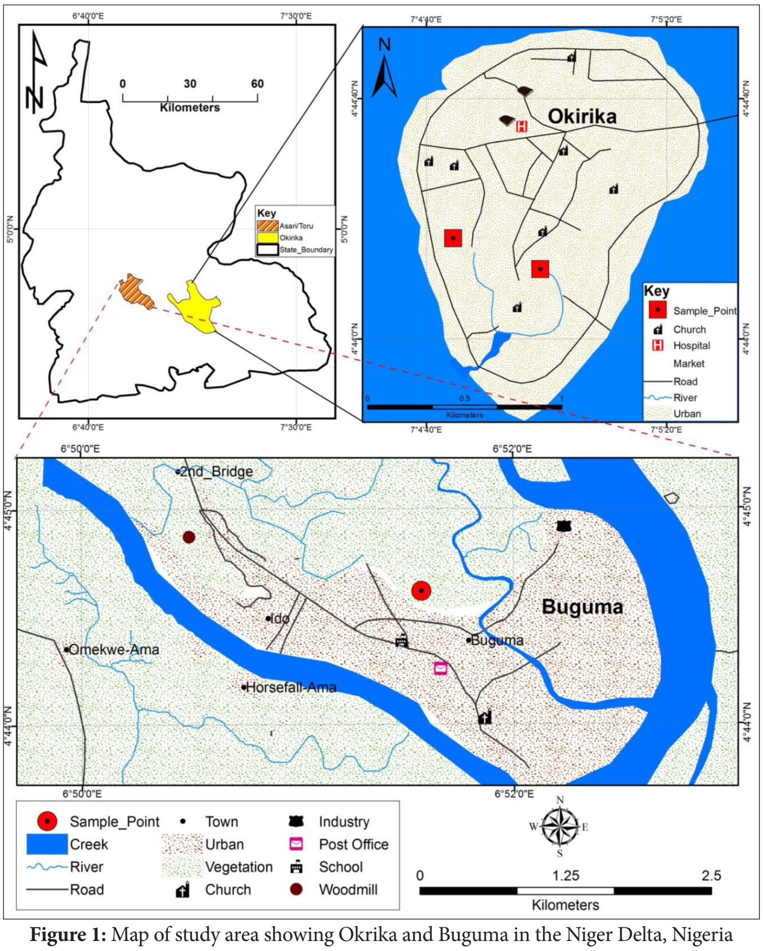

Figure 1: Map of study area showing Okrika and Buguma in the Niger Delta, Nigeria

Okrika town (4°43 N and 7°05 E) is designated the highly polluted site, and it is situated on a small island south of Port Harcourt (Figure 1). The average elevation of Okrika is 452m, and lies on the north of Bonny River. The jetty of the Nigerian National Petroleum Corporation (NNPC) is located in this community. The jetty serves as berthing point for ships that are used to evacuate crude oil to foreign countries. The area where the periwinkles were collected is on the floor of the mangrove forest few meters away from the jetty. Both sites have been extensively studies and details are found in [12].

Description of study species periwinkle (*Tympanotonusfuscatus*)

The periwinkle (*Tympanotonusfuscatus*) is a free living epibenthic animal found in intertidal locations and is widely distributed in coastal and estuarine areas of the Niger Delta, Nigeria. Periwinkles are shellfish found in the littoral region of the sea, brackish or estuarine waters which are seasonally submerged regions like the mangrove swamps. They are univalve gastropods of the phylum mollusca. The genera consist of *Tympanotonus, Pachymelania* and *Merceneria*. The two species of Periwinkle commonly found in the estuarine habitat and benthos of the Niger Delta are *Tympanotonusfuscatus and Pachymelaniaaurita*.

The periwinkles species, *Tympanotonusfuscatus* is known as the West African Mud Creeper, which is a species living in brackish water, a gastropod mollusk in the family Potamididae. *Tympanotonusfuscatus* is the only extant species in the genus *Tympanotonus*.

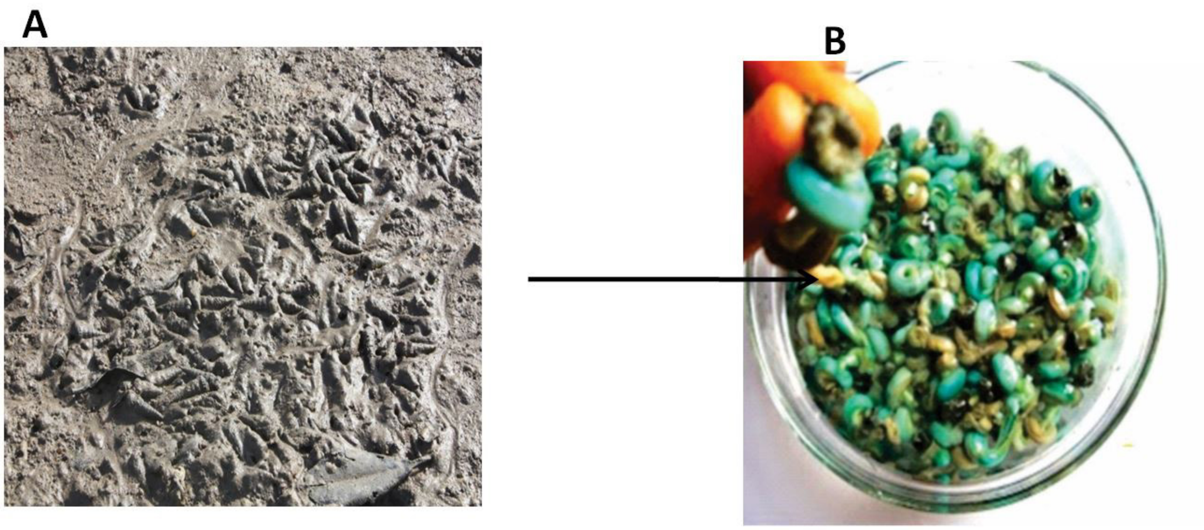

Figure 2: Periwinkles found in muddy mangrove soil in Buguma, Niger Delta, Nigeria (A) shelled and (B) without shell (tissues)

Tympanotonusfuscatus occurs in the littoral habitat of mangrove swamps and *P.aurita*inhabit sub-tidal mud beach [13,14]. The two species are morphologically different, but the *P. aurita*develops sharp spines and broader aperture. Although, the sharpness of the spines depends on the age of the organism, and the older it becomes, the blunter and thicker the spines become. The species *T. fuscatus* is characterized by turreted, granular and spiny shells with tapering ends. *T. fuscatus* tends to congregate under the roots and decaying red mangrove trees and small collection of waters during low tide. The genus Pachymelania is endemic to West Africa [15]. It is one of the most common gastropod molluscs in the Niger Delta [16].

Tympanotonusfuscatus is euryhaline and has the ability to tolerate a wide range of salinities between O.1mg/l to 25mg/l [17]. Pros branch gastropods are the most abundant and commonest molluscs in the brackish waters in West Africa, and the factors that affect their distribution in the coastal areas of West Africa include salinity, water depth, currents and nature of bottom deposits. The two genera are commonly referred to as "periwinkle" in Nigeria. They inhabit the quiet waters where the substratum is rich in decaying organic matter and mud [17]. The periwinkles feed on the mud and other decaying organic matter. They are deposit feeders. Both genera are found in most brackish water creeks and mangrove swamps in the Niger Delta area at the intertidal zone. The Periwinkles are estuarine snails and are found in the intertidal area of the mangrove edges and surfaces, therefore they could be hand-picked [14]. Periwinkles also make regular feeding excursions and trace their paths back to their former ecological niches hence remains within a short distance for many weeks [18]. They can also survive for a long period in the absence of water. . Periwinkle used reserved food when deprived of water for long time.

Benefits of *Tympanotonusfuscatus*

The Periwinkles flesh is edible and also used as bait by fisher folks. They are rich in protein (about 21%), vitamins and minerals [19]. This organism is also very medicinal for cases like endemic goiter due to its iodine content [20]. The calcium, phosphate and iron content are also recommended for pregnant women. The periwinkle shell is grounded for several purposes such as powder for pimples, cleansing, as fertilizers, and as calcium source in animal feed [21]. Other uses include building construction, ornamentals and cosmetics. These molluscs are important food delicacy among the indigenes of communities of the Niger Delta and an important source of animal protein.

Sample collection and preparation

Periwinkle samples (n= 100) were randomly collected by hand picking method from the mangrove forest floor. Within the same area soil samples were collected 5 cm below the surface. The soil samples were put in polyethylene bags and sent to the laboratory for physico-chemical analysis Table 1. The periwinkles were put in a transparent nylon bag and put in a cooler and later sent to the laboratory for analysis. The soft part of the periwinkles was obtained by cracking the shells. Approximately 10g were placed into a clean mortar, then ground with pestle to powder form. Two grams of the sample were weighed into 120ml glass bottle for extraction, 10ml extractant (organic solvent) were added and extracted for 1hour, the solid was allowed to settle and filtered into a clean bottle using a glass funnel stuffed with little cotton wool. The sample was then analyzed with spectrophotometer (see below for procedures) to determine the total hydrocarbon content (THC). The same measure was also used to get the total hydrocarbon content in the tissue, after it has been oven dried and grounded to powder.Moreover, the thickness i.e. width and length of the various samples were taken using venier caliper and meter rule.

Study site						Metals (mg/kg)				
	Salinity (%)	pH	THC	Cd	Pb	Zn	Cu	Mn	Ca	K
Okrika	2.26±0.1	6.10±0.2	607.3.45±2.3	0.90±0.01	20.07±2.01	4.30±1.1	20.42±0.4	2.2±1.3	528.5±3.5	142.7±96.2
Buguma	1.67±0.01	6.65±0.1	227.56±25.5	0.76±0.4	14.0±7.1	60.3±26.0	19.4±11.2	39.7±17.8	380.7±289.9	232.8±87.6

Table 1: Soil physico-chemical characteristics of different mangrove soil for highly (Okrika) and lowly (Buguma) polluted sites, Niger Delta, Nigeria

Laboratory analysis

Total hydrocarbon content analysis

Procedure: It was a spectrophotometric method using the HACH DR 890 calorimeter (wavelength ~420 nm). The shells of the periwinkle were carefully broken with a hammer in order to get the edible part. The crabs were carefully crushed as well to obtain the fleshy part out of its exoskeleton. The samples were oven-dried at 70°C for 48 hours to get rid of the moisture. The samples were crushed and 2gram of the crushed samples was weighed into a glass beaker and 20 mLof hexane were added and with the aid of a glass rod, the mixture was homogenized by stirring. Afterwards, the sample was filtered in a glass funnel packed with cotton wool, silica gel and anhydrous sodium sulphate. After this, 10 mLof the filtered organic extract were transferred into a 10 mLsample curvet and inserted into the calorimeter.

Statistical analysis

An analysis of variance (ANOVA) was conducted to determine whether there was a significant difference in THC concentration in different parts of the periwinkles. The data was first log transformed to ensure that they were normal and the variances were equal. Since the periwinkle samples were small in number (n =100), a non-parametric separate variances (Welch's) t-test was performed to test the null hypothesis that the THC is the same for both highly and lowly polluted plots [22]. For the relationship between THC of periwinkle and size of shell, a Pearson's correlation coefficient test was performed where the null hypothesis states that the correlation coefficient of periwinkle size and THC concentration equals zero. Bar graphs were then used to illustrate the significance and difference in concentration in shell and tissue of periwinkle in highly and lowly polluted sites. All analyses were done in R [23].

Results

Concentration of THC in shell and tissue of periwinkle (*T. fuscatus*)

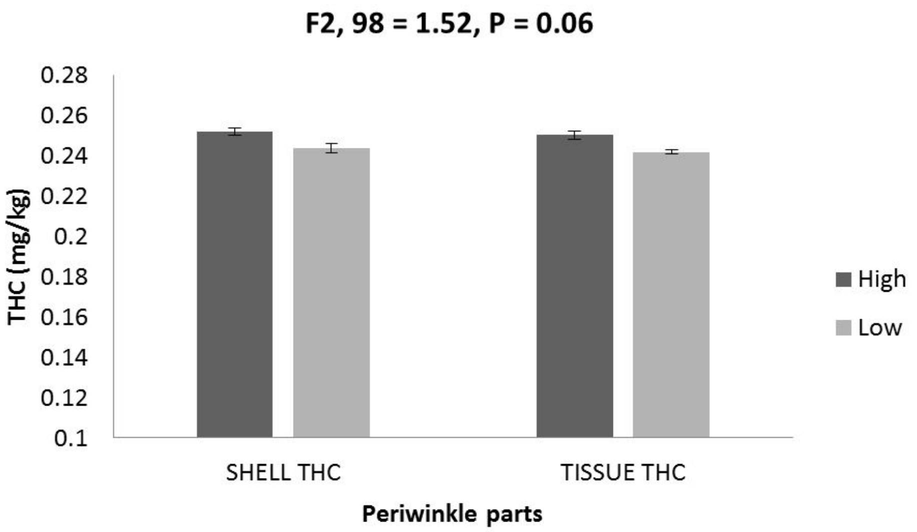

Figure 3: Total hydrocarbon concentration of shell and tissues of periwinkle (*T. fuscatus*) in highly and lowly polluted sites, Niger Delta, Nigeria

The result indicate that there is no significant difference in THC ($F_{2,}$ 98 = 1.52, P = 0.06) between shell and tissue, and also between highly and lowly polluted sites (P> 0.05) (Figure 3). The THC concentration was slightly higher in shell than in tissue for both highly and lowly polluted sites (Table 1). For the shell, highly polluted plot had a slightly higher concentration of THC (0.252±0.002mg/kg) than lowly polluted site (0.244±0.002) (Figure 3). Similarly, for the tissue, highly polluted site had a slightly higher THC (0.250±0.002mg/kg) than lowly polluted site (0.242±0.001mg/kg) (Figure 3).

Comparison of shell sizes of periwinkle (*T. fuscatus*) in highly and lowly polluted sites

The result shows that there is significant difference in length and width of periwinkles (P<0.05). Periwinkle picked up from highly polluted location was slightly larger than those picked up from lowly polluted site. Periwinkles from highly polluted site (width: 2.27±0.03cm and length: 3.83±0.07cm) is larger than periwinkle from lowly polluted site (width: 1.83±0.03cm and length: 3.14±0.04cm).

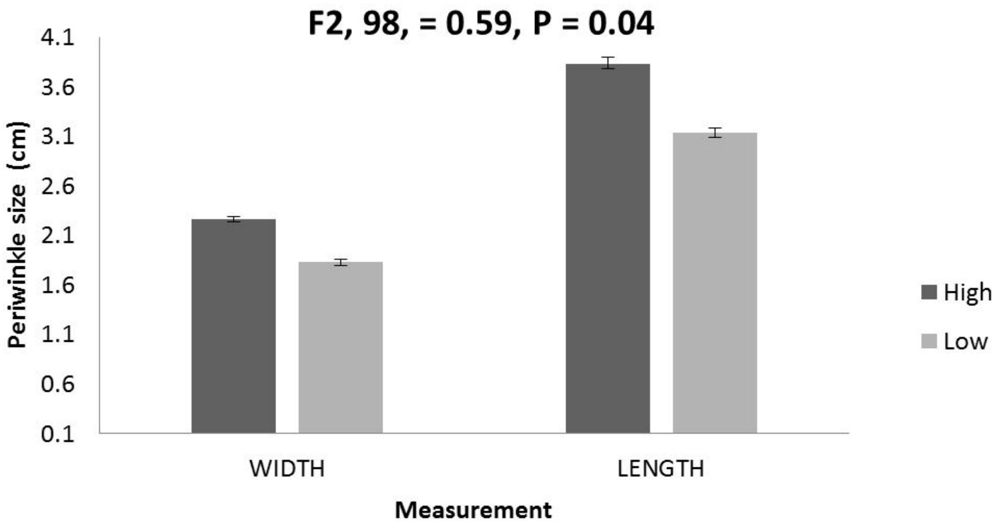

Figure 4: Mean size of periwinkle collected from highly and lowly polluted sites in the Niger Delta, Nigeria

Correlation between periwinkle length and width (i.e. area) and THC concentration

To derive the correlation data for both plots (i.e. high versus low and shell versus tissue) at each site THC concentration and area of periwinkle data were combined. The result shows that there is little correlation (R^2 = 0.13) between the area cm^2 (length × width) of periwinkle and the THC concentration (mg/kg) (Figure 5). The result shows that as the size of periwinkle increases the THC concentration reduces. This means bigger periwinkle have lower THC concentration whereas smaller periwinkle have higher THC concentration.

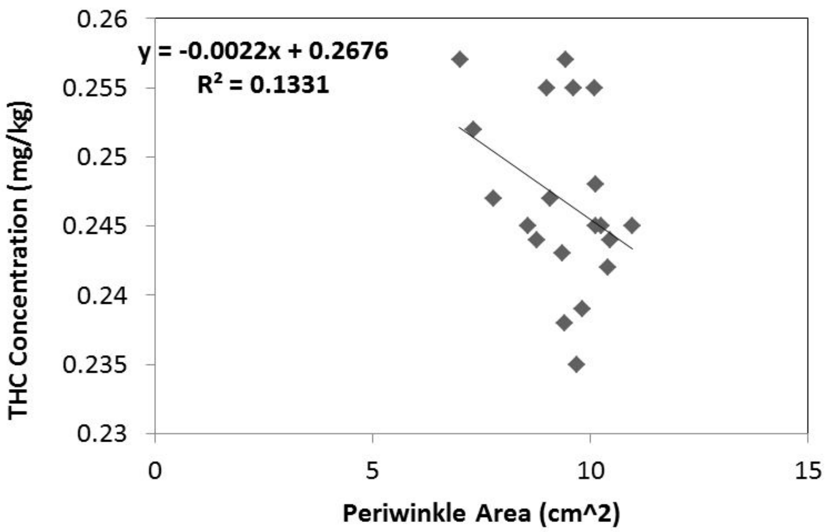

Figure 5: Correlation between THC concentration and area of periwinkle (*T. fuscatus*)collected from highly and lowly polluted sites in the Niger Delta, Nigeria

Discussion

Concentration of THC in shell and tissue of periwinkle (*T. fuscatus*)

THC concentrations in shell and tissue of periwinkle picked up from highly and lowly polluted site is not significantly differently because both locations are interconnected through a common river system, and face similar problem of hydrocarbon pollution.

This study is in line with the results of [5] who found high contamination of heavy metals in tilapia fish and periwinkle in a disturbed river in the Niger Delta[5]. Although, highly polluted site has more oiling activities due to the presence of a refinery, but it seems higher pollution in that site did not cause high bioaccumulation in the body of the periwinkle. Since the water systems are interconnected, therefore pollutants released at one end of the creek is transported to other ends of the river channel. High THC in shell has no effect on man because it is not the part consumed, but, high THC in tissue would be harmful to humans because it is the part of the periwinkle consumed. A range of 0.24-0.25 mg/kg is below the WHO standard of 0.5 mg/kg [24]. Gradual intake of these pollutants into the body may biomagnify and lead to toxic effect.

Comparison of shell sizes of periwinkle (*T. fuscatus*) in highly and lowly polluted locations

The periwinkle in highly polluted site is larger probably because of their exposure to more food materials such as litter from fallen leaves, which had decomposed to form brown manure. The hydrology of the highly polluted site also allows constant cleaning of the periwinkle shells from oil films, which may be detrimental to their growth and development. During field work it was observed that the site is constantly bathed by tidal flood, which washes the mud flat from oil spills that come from leaked pipes. The thick mud formed from the decomposed litter also neutralizes the effect of pollution, and also provide a safe hiding place for the periwinkles. According to previous studies salinity influences the growth of the periwinkles, the laboratory analysis of the salinity shows that highly polluted site was more saline than lowly polluted site (Table 1). Other physico-chemical parameters show high levels in highly polluted site. Very importantly, periwinkles in the highly polluted site are larger in size because in this location people are prevented from coming in to collect them, which allows the periwinkles to grow to full sizes as compared to the lowly polluted site that is easily accessible.

Correlation between periwinkle area and THC concentration

This study is in line with previous studies that show that size influences the THC concentration. But this study is in contrast with previous study that show that the smaller the crab *Goniopsispelii* the lower the THC concentration [24]. Here the male crabs were larger than the female crab and had higher THC bioaccumulation. The big size of the periwinkle means that there will be more re-distribution of THC within the body, which had resulted in lowering the concentration to negligible level. This implies that large periwinkle will be better for consumption in terms of THC bioaccumulation than small periwinkle.

Conclusion

This study revealed that THC bioaccumulate in the shell and tissue of periwinkle in negligible amount, which is below the WHO standard for human consumption. The study also revealed that the chemistry of the soil can influence the intake of pollutants by the periwinkles. In addition size also matters in the absorption of THC, which implies that the bigger the periwinkle the better it is for consumption because small sized periwinkle bioaccumulate higher THC. There should thus be constant monitoring of periwinkles for THC and other heavy metals because their ground-dwelling habit can predispose them to quickly absorbing pollutants. Thus, pollutants in the body of periwinkle and other sea food are transferred up the food chain and eventually get to humans when consumed. Future studies would consider investigating THC levels in other ground dwelling mangrove organisms such as oyster (*Crassostreagasar*) and anadara (*Seniliasenilis*).

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