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THE EFFECT OF CRUDE OIL ON INTESTINAL MICROBIAL POPULATIONS OF A FRESHWATER SNAIL

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Abstract

The effect of a Nigerian crude oil (Bonny light) on the aerobic microbial populations in the digestive tract of a freshwater snail (*Pila ovata*) was investigated. The test organisms were exposed to sub-lethal concentrations of crude oil for 10 days. There was an initial range finding test to determine the concentrations of crude oil to be administered on the test organisms. Four concentrations of the crude oil were prepared in the definitive test as 1.0, 2.0, 3.0, 4.0 mg/l and a control experiment (0.0 mg/l). Populations of aerobic heterotrophic bacteria and fungi, proteolytic and amylolytic bacteria, hydrocarbon degrading bacteria and total coliforms present in the digestive tract of molluscs were estimated using spread plate method. Contamination of the environment of mollusc with sub-lethal concentrations of crude oil caused a decrease in the proteolytic, amylolytic and total coliform bacterial counts but resulted in an increase of one order of magnitude in the number of aerobic heterotrophic bacteria and fungi and two orders of magnitude in the number of hydrocarbon degrading bacteria in the digestive tract. The microorganisms of the digestive tract of hydrobionts, reacting to pollutant such as crude oil, can be used as a very sensitive indicative system and hydrocarbon degrading bacteria in the digestive tract of hydrobionts can be used as microbiological markers for registration of oil pollution.

Key words: Petroleum hydrocarbon contamination, hydrobiont, gut microbiota.

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INTRODUCTION

Freshwater resources all over the world are threatened not only by over-exploitation and poor management but also by ecological degradation caused by oil industry, while exploring crude oil, on water bodies. One of the major problems of the inhabitants of the Niger Delta region of Nigeria is contamination of water and aquatic lives by crude oil spillage [1].

Benthos invertebrates, such as mollusks, live and feed directly on recently deposited sediments and, therefore, are in a direct contact with pollutants, for example oil hydrocarbons.

Molluscs are common organisms in aquatic habitats and ecologically and commercially important on a global scale, many species are sedentary, fairly resistant to chemical contamination, and often reside in regions where less hardy organisms cannot survive [2].

Consequently, they are ideal invertebrate model systems for aquatic environmental monitoring and toxicology [3]. Previous studies have shown that crude oil can have both lethal and sub-lethal effects on a wide range of aquatic organisms [4, 5, 6]. Petroleum polluted sites have greater bacterial abundance and a large proportion of bacteria capable of hydrocarbon degradation than do non polluted areas [7, 8]. The oil products in such polluted sites may be toxic to some microorganisms while others can use components of oil as a source of carbon and energy and multiply. The result is a

microbial community with altered species diversity enriched with hydrocarbon degrading microorganisms [9]. The degree and duration of the change is a function of the chemical composition of the added hydrocarbon, the concentration of the hydrocarbons and the nature of particular ecosystem [9]. The digestive tract of hydrobionts is an open system that is continuously in contact with water. Microorganisms that inhabit gastrointestinal tract are specialized to survive and multiply there. They contribute to the nutrition and physiological processes of the host animal by producing vitamins, amino acids and digestive enzymes and prevent pathogenic microorganisms from multiplying [10, 11, 12]. The stability of the microflora of the digestive tract of hydrobionts is determined by the trophic structure of water bodies, their productivity, physical and chemical factors and amount of xenobiotics [9]. Pollutants can disarrange the whole micro-ecological system of the digestive tract, thus resulting in structural and functional changes in intestinal micrflora.

There is a few information on the impact of crude oil on the intestinal microflora and hydrocarbon degrading bacteria in the intestinal tract of aquatic animals [4, 13]. But there is dearth of information on the influence of crude oil or oil products on the gut microbiota of mollusc, *Pila ovata*. Therefore, we examined the effect of a Nigerian crude oil (Bonny Light) on the abundance of microflora in the digestive tract of a freshwater snail (*Pila ovata*).

MATERIALS AND METHODS

Collection and acclimatization of test organisms

Pila ovata was collected from Okpuhur Creek in Ahoada, Rivers State, Nigeria. The snails were handpicked and placed in a plastic bucket containing habitat water. On reaching the laboratory, active snails were selected for acclimatization for 10days at room temperature [14] in a vessel containing habitat water.

Bioassay

A range finding test was carried out as described by [15] to determine the sub-lethal concentrations of Bonny Light crude oil used in the definitive test. Five sub-lethal concentrations (0.0mg/l, 1.0 mg/l, 2.0mg/l, 3.0mg/l and 4.0mg/l) were prepared using water from habitat of snail. The control (0.0mg/l) was habitat water without Bonny Light crude oil. Triplicate sets of glass tanks (29 x 29 x 30cm) for each crude oil concentration were employed. Ten snails of fairly equal sizes were handpicked and carefully transferred into each test tank. The test solution in each tank was renewed every 24hours. The test was terminated after 10days and repeated three times to confirm the data.

Enumeration of viable bacteria and fungi

On the tenth day populations of aerobic heterotrophic bacteria and fungi, proteolytic and amylolytic bacteria, hydrocarbon degrading bacteria and total coliforms present in the digestive tract of molluscs in each test tank were separately estimated using spread plate method.

The snails were cleaned externally with ethanol and the intestines dissected under sterile conditions. One gram of intestinal content was aseptically transferred to 9ml sterile physiological saline in a test tube. This gave 10^{-1} dilution from where subseqent10-fold serial dilutions were carried out up to 10^{-6}. Subsamples of 0.1ml of three dilutions expected to give between 30 and 300 colony –forming units were plated on six media in triplicates. The media chosen were: Nutrient agar (for isolation of total heterotrophic bacteria), Sabouraud dextrose agar (for isolation of total heterotrophic fungi), milk agar (for isolation of proteolytic bacteria), starch agar (for isolation of amylolytic bacteria), MacConkey agar (for isolation of total coliform bacteria) and mineral salt medium (for isolation of hydrocarbon degrading bacteria). Mineral salt medium was used to selectively isolate hydrocarbon utilizing bacteria using the vapour phase transfer method [16]. The modified crude medium comprises of 10g NaCl, 0.42g $MgSO_4.7H_2O$, 0.29g KCl, 0.83g KH_2PO_4, 1.25g Na_2HPO_4, 0.42g $NaNO_3$, 20g Agar-Agar and distilled water to 1L. After inoculation of the mineral salt agar plates, Whatman (9cm) No. 1 filter papers were soaked in crude oil and introduced on the cover of the Petri dishes. The thin layer crude oil spread on the filter paper served as a hydrocarbon source and the plate with filter paper without crude oil was used as control. All the plates were incubated at 37^{o}C for 24-48 hours except for Sabouraud dextrose agar plates and Mineral salt agar plates which were incubated at 28^{o}C for 3-5 days and 30^{o}C for 5-7days respectively. The microbial colonies appearing on each plate were counted and the colony forming units per gram (cfu/g) of the intestinal contents were calculated. Proteolytic bacteria were identified according to zone of protein (casein) hydrolysis on milk agar. Amylolytic

bacteria were determined according to zone of starch hydrolysis on starch agar under the action of iodine solution.

Statistical analysis

The differences in microbial counts were assessed using the one-way analysis of variance (ANOVA). In all cases treatments were considered significantly different if $p<0.05$.

RESULTS AND DISCUSSION

The results of the abundance of total heterotrophic bacteria and fungi, proteolytic and amylolytic bacteria, hydrocarbon degrading bacteria and total coliforms in the digestive tract of *Pila ovata* exposed to different concentrations of crude oil are shown in Figures 1-6 respectively. Before contamination with crude oil, the number of total heterotrophic bacteria and fungi, proteolytic and amylolytic bacteria, hydrocarbon degrading bacteria and total coliforms in the digestive tract of the investigated mollusc were 6.60± 1.41 log g/l, 4.17± 0.17 log g/l, 5.39± 0.71 log g/l, 5.37± 0.71 log g/l, 2.54± 2.21 log g/l and 4.54± 2.12 log g/l respectively. These results have shown that a dense microbial population occurs in the intestinal tract of mollusc, *Pila ovata*. As separate groups of heterotrophic bacteria, proteolytic and amylolytic bacteria were abundant in the digestive tract of the mollusc. The abundance of total coliform and hydrocarbon degrading bacteria reflects the presence and fluctuation of pollutants in the environments [9]. These results are in accordance with those found for other molluscs [4, 9, 17, 18, 19]. Contamination of the environment of mollusc with sub-lethal concentrations of crude oil caused a decrease in the proteolytic, amylolytic and total coliform bacterial counts but resulted in an increase of one order of magnitude in the number of aerobic heterotrophic bacteria and fungi and two orders of magnitude in the number of hydrocarbon degrading bacteria in the digestive tract of *Pila ovata*. These decreases and increases were much more evident and significant ($p<0.05$) after exposure to 4mg/l of crude oil during the initial 10days of experiments (Figures 1-6). Similarly, [4] reported that hydrocarbon degrading bacteria in the digestive tract of molluscs comprised less than 0.2% of the total number of isolated heterotrophic bacteria but after exposure to a 4ppm concentration of crude oil, the value reached 3.9% in the mollusc environment. Allochthonous microflora such as hydrocarbon degrading bacteria destroys the structure of normal bacteriocenosis of the intestinal tract and the functional action of bacteria [9]. The destruction of normal bacteriocenosis destroys the processes of nutrition and digestion, changes the physiological state of hydrobionts or even causes their death [13]. Furthermore, toxic substances may be responsible for the death of sensitive microorganisms in the digestive tract of animals when the concentration of the toxic substance is not dangerous to the animal, while the animal may die from a disorder of the activity of the digestive system [20].

CONCLUSION

Contamination of the environment of mollusc with sub-lethal concentrations of crude oil caused a reduction in the proteolytic and amylolytic bacterial count. This may slow down their further development and disturb the activity of their vital functions. We, therefore, conclude that microorganisms of the digestive tract of hydrobionts, reacting to pollutant such as crude oil, can be used as a very sensitive indicative system. Furthermore, hydrocarbon degrading bacteria in the digestive tract of hydrobionts can be used as microbiological markers for registration of oil pollution since the presence of crude oil caused a significant increase in the hydrocarbon degrading bacterial count.

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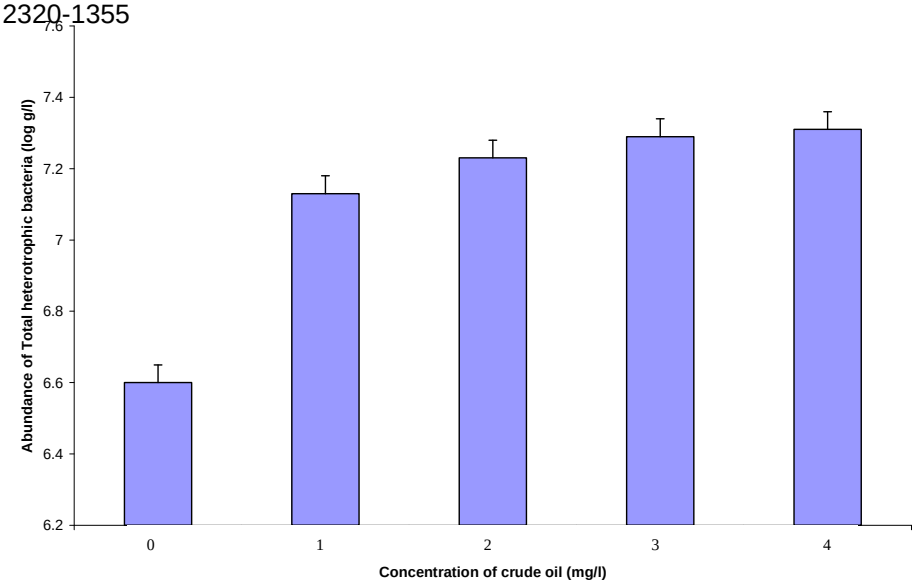

Fig.1: Abundance of total heterotrophic bacteria in the digestive tract of *Pila ovata* exposed to different concentrations of crude oil

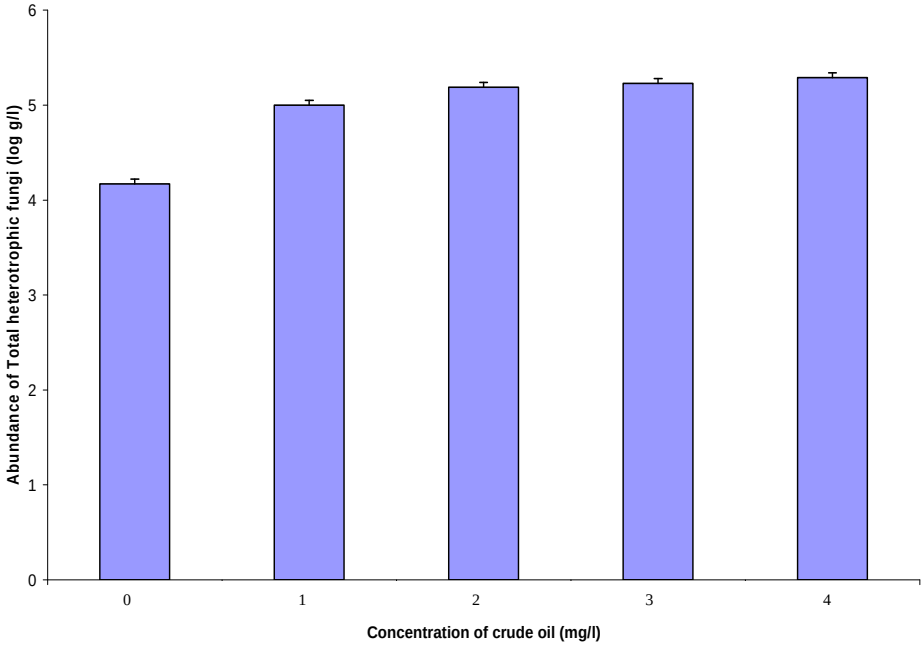

Fig. 2: Abundance of total heterotrophic fungi in the digestive tract of *Pila ovata* exposed to different concentrations of crude oil

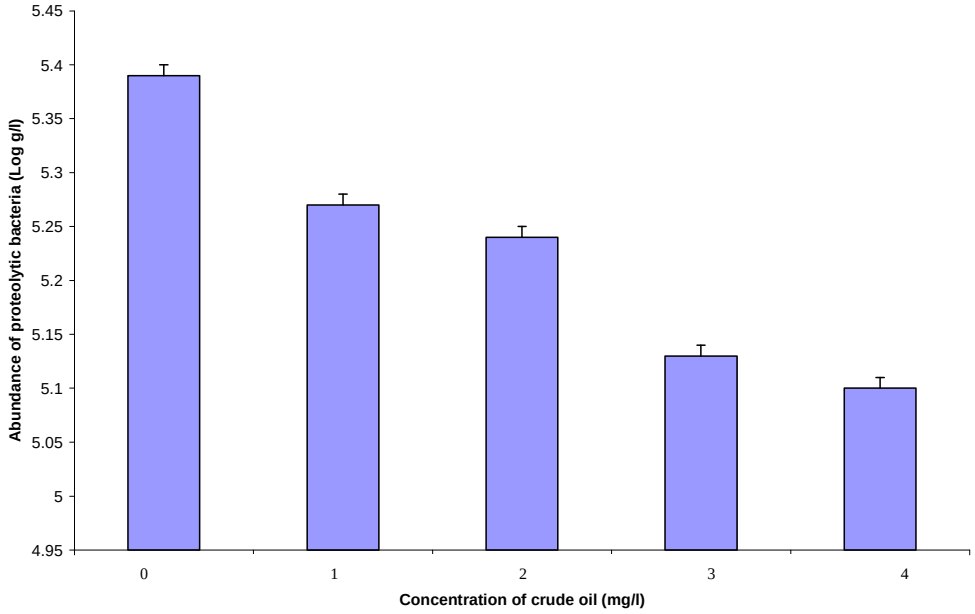

Fig. 3: Abundance of proteolytic bateria in the digestive tract of *Pila ovata* exposed to different concentrations of crude oil

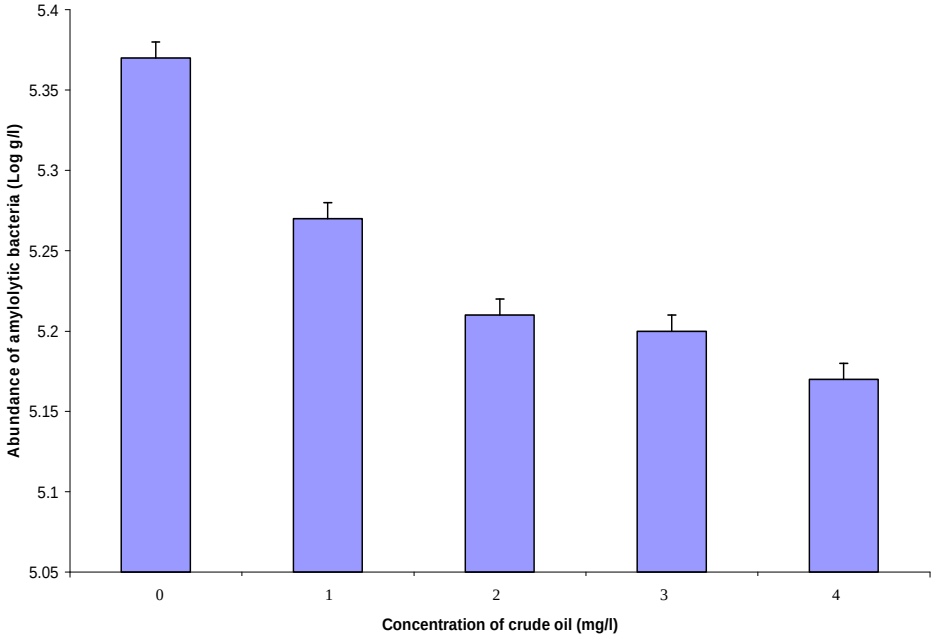

Fig. 4: Abundance of amylolytic bacteria in the digestive tract of *Pila ovata* exposed to different concentrations of crude oil

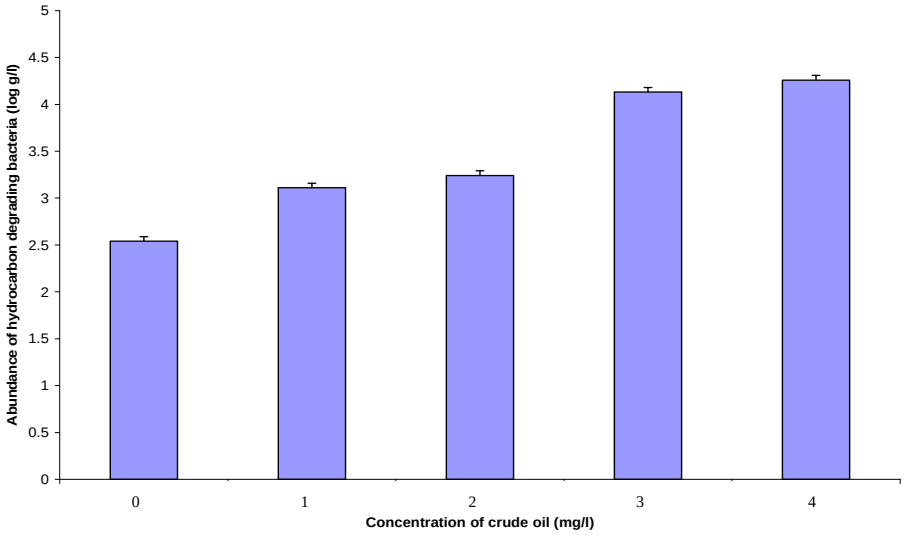

Fig. 5: Abundance of hydrocarbon degrading bacteria in the digestive tract of *Pila ovata* exposed to different concentrations of crude oil

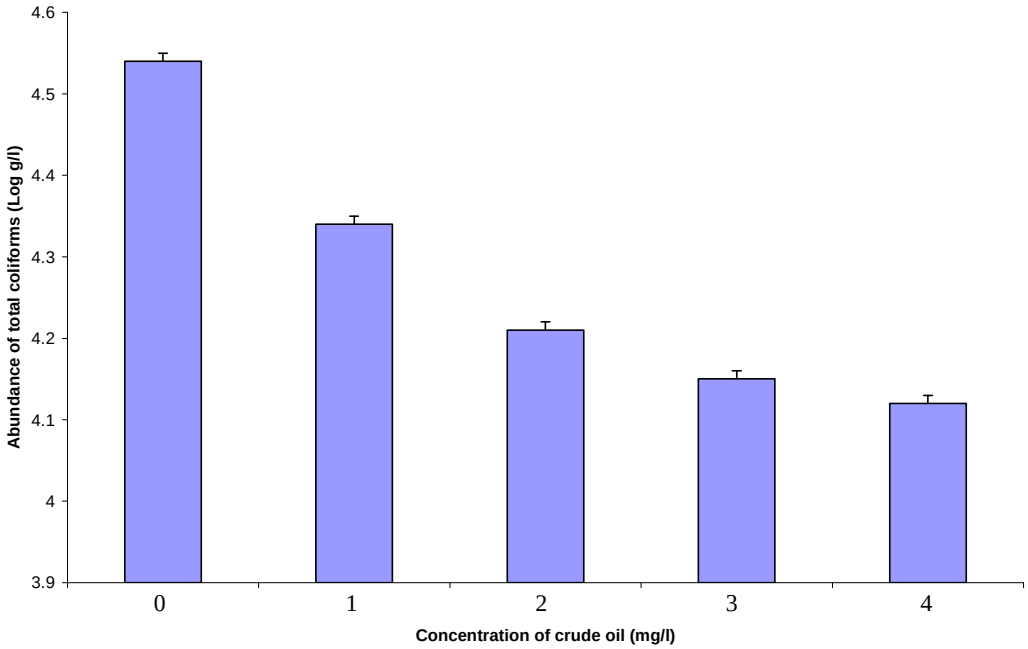

Fig. 6: Abundance of total coliforms in the digestive tract of *Pila ovata* exposed to different concentrations of crude oil