Annals of Ecology and Environmental Science
Volume 3, Issue 3, 2019, PP 25-32
ISSN 2637-5338

Bioaccumulation of Heavy Metals and Hydrocarbon by *Clibanarius africanus* (Hermit Crab) Exposed to Produced Water

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ABSTRACT

This study examined the level of heavy metals (zinc, barium, arsenic, mercury, nickel, lead, cobalt, copper, chromium, cadmium and iron) and Hydrocarbon related parameters (Total petroleum hydrocarbon, Total hydrocarbon content and Polycyclic aromatic hydrocarbon) in Clibanarius africanus (hermit crab) exposed to produced water. The Clibanarius africanus was sourced from African Regional Aquaculture Centre (ARAC), Buguma, in Rivers State, while the treated produced water was obtained from an Oil and Gas facility within Mbo LGA, Akwa Ibom State, along Calabar estuary in the Niger Delta Region of Nigeria. The Clibanarius africanus was exposed to the produced water for 28 days and heavy metals and hydrocarbon related parameters were analysed at varying time intervals: day 0, 7, 14, 21 and 28 using standard procedure. The results of the heavy metals and Hydrocarbon related parameter analyses showed a significant increase ($p<0.05$) as the exposure duration to produced water increased, suggesting bioaccumulation by the test organisms. In addition, metals such as zinc, copper, chromium and iron had their limits within the threshold recommended by Food and Agricultural Organization/Word Health Organization, Median International Standard and European Union for food fish consumed by human within the study period. Other parameters such as cadmium and lead had their values within and above the limits specified by some agencies. Since crustaceans which are part of the food chain have the capacity to accumulate these toxicants, it could end up in humans that consume these as food where they cause diseases, based on the type and concentration of the toxicants. Therefore, there is need for the agency responsible for the regulation of effluents/waste water from oil and gas facilities to review their discharge guideline towards zero harmful discharge into the water bodies like other regions so as to forestall the adverse effect of the produced water discharges on the aquatic organisms.

***Keywords:** Bioaccumulation, Clibanarius africanus, Heavy metals, Hydrocarbon, Produced water.*

INTRODUCTION

Within the past few years, the degree of environmental pollution arising from human activities has been on the increase. Authors have widely attributed this to population growth, industrialization and development (Izah *et al*., 2017). Several developmental projects are being executed all over the world, including Nigeria. But most developed countries have continued to potentially influence their environment through unending daily human activities. This however, is not the situation in many developing nations, where the law is available but poor enforcement and punishment of offenders remains a restraining factor to enjoying the usefulness of these environmental laws. Consequently, the height of environmental degradation related to human activities is quite high in developing nations than the more developed counterparts.

In a developing nation like Nigeria, environmental degradation is caused by several factors including bush burning and unsustainable agricultural practices (including use of herbicides and other chemicals). Destruction of natural ecosystem in search of forest resources especially wildlife, popularly known as bush meat is on the increasing trend. Also, environmental degradation (including soil, air and water) due to poor waste management practices is quite popular.

In Nigeria, crude oil activities are majorly carried out in marine (offshore) and to a lesser extent on land (onshore) environment (Aigberua *et al*., 2017). During crude oil exploration and production processes, several wastes streams are generated. One of the commonly generated liquid wastes from oil and gas activities is the produced water.

Petroleum drilling activities usually release saline produced water from onshore or offshore wells into the aquatic environment, leading to the acute toxicity of aquatic fauna species resulting from osmotic differences in ion distribution between organisms and the environment (Pillard *et al*., 1996). Produced water is saline or fresh underground water which has been confined for several million years with crude petroleum hydrocarbon in a geological repository. It is generally composed of solvable and suspended organic and inorganic chemical compounds. Overall, produced water is similar to sea water because of the prevalent composition of sodium and chloride ions (Neff *et al*., 2011).

Produced water is often treated and discharged into the aquatic ecosystem especially the brackish and marine waters. However, some of the parameters monitored in the treated produced water have their limits exceeded before being discharged into the ecosystem. Produced water has gained the interest of environmentalists and so have been extensively studied (Ajuzieogu *et al*., 2018; Ajuzieogu and Odokuma, 2018; Chikwe and Okwa, 2016; Isehunwa and Onovae, 2012; Onojake and Abanum, 2012; Okoro, 2010). Again, fishes and crustaceans are known to bioaccumualate toxic substances from their environment especially heavy metals (Ogamba *et al*., 2017, 2016a,b; Izah and Angaye, 2016; Aghoghowia *et al*., 2016). This may be the reason fishes have been severally used in ecotoxicological studies (Inyang *et al*., 2017a-c). Bioaccumulation of heavy metals by fish could produce useful information about the health status of individuals that consume these food from a given locality.

Fishes are typically grouped into fin fish and shell fish. Fin fishes have been widely studied for bioaccumulation in the Niger Delta (Izah and Angaye, 2016; Aghoghowia *et al*., 2016). But information about shell fish bioaccumulation in the Niger Delta appears scanty in literature. Hence, this study is focused on the bioaccumulation of heavy metals and Hydrocarbon by *Clibanarius africanus*, a shell fish exposed to treated produced water.

MATERIALS AND METHODS

Source of Test Organisms and Habitat Water/Sediment

The test organisms – *Clibanarius africanus* used for this study were procured from African Regional Aquaculture Centre (ARAC), Buguma, Rivers State. The organisms collected were uniform in size and age (with average weight and length of organism being 3.75 ± 0.5cm and 2.5 ± 1.0g respectively). Juvenile organisms were used for the test and were transported to the laboratory in a cage. All organisms were well cared for, to forestall or avoid unnecessary stress during transportation. The habitat water and sediment were also obtained from ARAC.

Source of Produced Water

The treated Produced water samples were collected into a clean labelled 25litres plastic container from an Oil and Gas facility within Mbo LGA, Akwa Ibom State, along Calabar estuary within the Niger Delta Region of Nigeria. It is slightly alkaline, with a mean pH level of 7.57. The salinity and conductivity were 8,060 mg/l and 23,440 µS/cm respectively.

Acclimatization of Test Organisms

In the laboratory, the test organisms were transferred into a plastic tank with a dimension of 72cm(l) x 48cm(w) x 48cm(h) containing their habitat sediment and allowed to acclimatize at a temperature range of 24^0C $\pm$ 2^0C in an air conditioned room for 10 days. The sediment was continuously moistened with habitat water (to retain the moisture content of the environment from where the organisms were captured) during this period. The crustacean *Clibanarius africanus* was acclimatized with ten kilograms of habitat mud/sediment from mudflats placed in the troughs. The organisms were placed in the mud, and habitat water was introduced to cover the mud and the organisms. The troughs were inclined at an angle of 30^o to enable half of the surface area of the mud to be submerged and the other half to be relatively free from water to enable organisms move from the water if need be.

Experimental Set-up and Control

Prior to the experimental set up - that is, the introduction of test organisms into the test media; baseline analysis of toxic metals and hydrocarbon content in the organisms were determined to establish their level of "cleanliness". 2,000 grams of habitat mud/sediment from mudflats of test organism were placed in previously cleaned bioaccumulation glass tanks (22 cm (l) × 15 cm(b) × 15cm (h) dimension) followed by the introduction of 1,000ml (500ml of treated produced water and 500ml of Habitat water) of the test medium for the uptake phase

– representing exposure to 50% of produced water. The test substance was placed to cover a depth of 30 mm of the troughs. Procured and acclimatized test organisms (*Clibanarius africanus)* were transferred carefully into the test medium. A total of one hundred and forty (140) test organisms were transferred into two (2) troughs, each containing seventy (70) test organisms. The test tanks were also inclined at an angle of 30^{o} to enable half of the surface area of the mud to be submerged and the other half to be relatively free from water to enable organisms move from the water if required. Crustaceans were subjected to 12h of light per day and at a temperature of 24±2^{o}C. The control set up comprised of clean test organisms in a separate test tank, containing habitat water and sediment. The volume of test media and concentrations of analytes were maintained throughout the study period. Organisms in control tanks were analyzed alongside with the test samples harvested from the bioaccumulation test tanks at varying time intervals; Day 0, 7, 14, 21 and 28. The samples were analysed for heavy metals and hydrocarbon content.

Laboratory Analysis

Heavy Metals Determination

Hermit crab tissues were oven-dried using a Memmert U27 model drying oven at 60^{o}C temperature for about 24 hours. 2 g of dried samples were further dry-ashed at 450^{o}C in clean porcelain crucibles placed in an Oceanic SX-2 model muffle furnace. Greyish samples were allowed to cool in a desiccator. Thereafter, a solution of ash was prepared by adding 5 ml of 10% hydrochloric acid (HCl) and another 5 ml of 10% nitric acid (HNO_3). A reagent blank containing the same acid mixtures used was prepared without adding samples. All samples and reagents were suctioned into the GBC Avanta PM A6600 atomic absorption spectrophotometer (FAAS), and the heavy metals (Pb, Cu, Zn, Cr, Co, As, Fe, Hg, Cd, Ni and Ba) were determined at varying wavelengths. Check standard solutions were read for quality assurance purposes. They were presented at the start, middle point and end of each analysis sequence. This was also used to assess the repeatability of acquired data (ITTA 1979, Aigberua and Tarawou, 2017).

Total Hydrocarbon Content Determination

Total hydrocarbon content of hermit crab tissue was determined according to IITA (1979) using the soxhlet extraction method. 10 g of oven-dried and ground samples were mixed with 10 g of anhydrous sodium sulphate. Samples were placed in an extraction thimble and covered with cotton wool. Extraction of sample was done for a period of 4 hours using n-hexane solvent. Thereafter, the organic extract was filtered through grease-free cotton, into a pre-weighed boiling flask. On complete distillation, recovered oil in flask was allowed to cool in a desiccator before being reweighed. The weight of oil was determined and calculated as concentration of total hydrocarbon in mg/kg units.

Total Petroleum and Polycyclic Aromatic Hydrocarbon Determination

1.0 g weight of hermit crab tissues was solvent-extracted for the isolation of organic constituents. In each case, the resultant dichloromethane/n-hexane extract was concentrated and then transferred for clean-up/separation via a chromatographic column moderately packed with glass wool, activated silica and anhydrous sodium sulfate. The eluent was collected into sample vials and injected through a hypodermic syringe into the gas chromatograph equipment to obtain the aliphatic and aromatic fractions which automatically get detected on the flame ionization detector as signals are collected based on the composition of the vapor. The resulting TPH and PAH chromatograms were reported as mg/kg units (Aigberua *et al*., 2016).

Statistical Analysis

Statistical assessment was carried out with Statistical Package for the Social Sciences (SPSS-Version 20). Data was subjected to descriptive statistics and expressed as mean ± standard deviation. One-way analysis of variance was carried out at alpha = 0.05, and Waller Duncan was used to discern the source of the observed variations.

Results and Discussion

Table 1 presents the heavy metals bioaccumulation by *Clibanarius africanus* that were exposed to treated produced water. Arsenic and mercury were not detected in the tissues of the *Clibanarius africanus* during the study period.

Zinc

Zinc level in the tissues for control and test cases were respectively recorded as 0.111 ± 0.001 mg/kg and 0.119 ± 0.011 mg/kg (day 7),

0.114 ± 0.003 mg/kg and 0.128 ± 0.002 mg/kg (day 14), 0.113 ± 0.004 mg/kg and 0.131 ± 0.002 mg/kg (day 21), 0.115 ± 0.003 mg/kg and 0.131 ± 0.001 mg/kg (day 28), while the baseline value was 0.116 ± 0.002 mg/kg. Statistically there were significant variations ($p < 0.05$). Multiple comparison showed that test cases at day 14, 21 and 28 were sources of variation showing no significant difference within them ($p > 0.05$). The significant variation is an indication of bioaccumulation. However, the values recorded were lower than the values recommended by Median International Standard (45.0 µg/g) (Philips, 1993 cited in Senarathne and Pathiratne, 2007; Senarathne *et al.*,2006), Food and Agricultural Organization/World Health Organisation (40.0 µg/g) (FAO/WHO, 1989 cited in Elnabris *et al.*, 2013), United State Environmental Protection Agency (5.0 mg/kg) (USEPA, 1986; cited in Anim-Gyampo *et al.*, 2013) World Health Organisation (5.0 mg/kg) (WHO, 2003; cited in Anim-Gyampo *et al.*, 2013) and WPCL (4.25 mg/kg) (WPCL, 2004; cited in Anim-Gyampo *et al.*, 2013) for fish food. Hence, the concentration of zinc in this study is minimal. However, it may rise if the exposure period is increased as, *Clibanarius africanus* has shown its potential to accumulate zinc from waste waters like produced water.

Table 1. *Heavy metal Bioaccumulation by Clibanarius africanus exposed to treated produced water*

Parameter	Days								
	0	7		14		21		28	
	Baseline	Control	Test	Control	Test	Control	Test	Control	Test
Zn, mg/L	0.116±0.002a	0.111±0.001a	0.119±0.011a	0.114±0.003a	0.128±0.002b	0.113±0.004a	0.131±0.002b	0.115±0.003a	0.131±0.001b
***As,mg/L**	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000
Ba, mg/L	0.121±0.000a	0.125±0.000a	0.138±0.011b	0.125±0.001a	0.145±0.004b	0.125±0.005a	0.157±0.003c	0.125±0.004a	0.156±0.004c
***Hg,mg/L**	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000	<0.001±0.000
Ni, mg/L	0.021±0.002a	0.027±0.002b	0.053±0.002d	0.038±0.002c	0.083±0.004e	0.041±0.001c	0.096±0.002f	0.039±0.001c	0.101±0.002g
Pb, mg/L	<0.001±0.000a	<0.001±0.000a	0.046±0.004b	<0.001±0.000a	0.062±0.003c	<0.001±0.000a	0.074±0.004d	<0.001±0.000a	0.073±0.004d
Co, mg/L	<0.001±0.000a	<0.001±0.000a	0.026±0.003b	<0.001±0.000a	0.034±0.005c	<0.001±0.000a	0.053±0.003d	<0.001±0.000a	0.051±0.002d
Cu, mg/L	0.113±0.003a	0.117±0.002ab	0.122±0.002bc	0.121±0.004bc	0.133±0.006d	0.124±0.005c	0.145±0.003e	0.121±0.002bc	0.145±0.004e
Cr, mg/L	<0.001±0.000a	<0.001±0.000a	0.053±0.002b	<0.001±0.000a	0.084±0.004c	<0.001±0.000a	0.127±0.003d	<0.001±0.000a	0.126±0.003d
Cd, mg/L	<0.001±0.000a	<0.001±0.000a	0.038±0.001b	<0.001±0.000a	0.077±0.003c	<0.001±0.000a	0.099±0.001d	<0.001±0.000a	0.101±0.002e
Fe, mg/L	0.122±0.002ab	0.132±0.004c	0.146±0.004d	1.281±0.001g	0.151±0.001e	0.126±0.004b	0.166±0.002f	0.119±0.001a	0.167±0.003f

The values are arranged as mean ± standard deviation (descriptive statistics) (n=3). The same letters across the row indicate no significant variations at p = 0.05 according to Waller-Duncan test statistics.

**The parameters marked as asterisks were not subjected to ANOVA because the values were uniform in the various days.*

Nickel

Nickel level in the tissues for control and test cases were recorded as 0.027 ± 0.002 mg/kg and 0.053 ± 0.002 mg/kg (day 7), 0.038 ± 0.002 mg/kg and 0.083 ± 0.004 mg/kg (day 14), 0.041 ± 0.001 mg/kg and 0.096 ± 0.002 mg/kg (day 21) 0.039 ± 0.001 mg/kg and 0.101 ± 0.002 mg/kg (day 28), while the baseline value was 0.021 ± 0.002 mg/kg. Statistically, there were significant variations ($p < 0.05$) across the test and control for most cases, an indication of nickel bioaccumulation. Elevated levels of nickel ingestible materials or substances could portend toxicity to body organs (Izah *et al.*, 2016).

Lead

Lead was not detected in the control (expressed as <0.001 mg/kg), but in the test cases, its level in the tissue were 0.046 ± 0.004 mg/kg, 0.062 ± 0.003 mg/kg, 0.074 ± 0.004 mg/kg and 0.073 ± 0.004 mg/kg at day 7, 14, 21 and 28

respectively. In addition, the baseline values also suggested that there was no lead in the tissues of the test organism. Statistically, there was significant difference ($p < 0.05$) across the various test and control cases. The result depicts that as the bioaccumulation period increased the level of lead uptake increased as well. The values of lead in this study were within the recommended limits for food fish by European Union (0.2 µg/g), (European Union, 2002 cited in Senarathne and Pathiratne, 2007; Senarathne *et al.*, 2006), Median International Standard (2.0 µg/g) (Philips, 1993 cited in Senarathne and Pathiratne, 2007; Senarathne *et al.*, 2006), FAO/WHO (0.5 µg/g) (FAO/WHO, 1989 cited in Elnabris *et al.*, 2013), USEPA (0.11mg/kg) (USEPA, 1986; cited in Anim-Gyampo *et al.*, 2013), but exceeded the limits specified by WHO (0.01mg/kg) (WHO, 2003; cited in Anim-Gyampo *et al.*, 2013) and WPCL (0.05mg/kg) (WPCL, 2004; cited in Anim-Gyampo *et al.*, 2013) for fish food. It is worthy of note that lead is highly toxic to body tissues. Hence, there is need for continuous monitoring of this toxic metal in produced water prior to its discharge into the ecosystem.

Cobalt

Like lead, cobalt was only detected in test cases across the various days. However, the values at day 7, 14, 21 and 28 were 0.026 ± 0.003 mg/kg, 0.034 ± 0.005 mg/kg, 0.053 ± 0.003 mg/kg and 0.051 ± 0.002 mg/kg respectively. There was significant difference ($p < 0.05$) across the various test and control cases, an indication of cobalt bioaccumulation. In addition, the level of cobalt was low in the test organisms. Although no limit has been specified, very high concentrations could be injurious to humans that consume fish food contaminated by cobalt.

Copper

Copper level in the tissues for control and test cases were respectively recorded as 0.117 ± 0.002 mg/kg and 0.122 ± 0.002 mg/kg (day 7), 0.121 ± 0.004 mg/kg and 0.133 ± 0.006 mg/kg (day 14), 0.124 ± 0.005 mg/kg and 0.145 ± 0.003 mg/kg (day 21), and 0.121 ± 0.002 mg/kg and 0.145 ± 0.004 mg/kg (day 28), while the baseline value recorded was 0.113 ± 0.003 mg/kg. There was significant difference ($p < 0.05$) across the various test and control cases, an indication of copper bioaccumulation. The values observed in this study were far lower than the regulatory limits for fish food as recommended by Median International Standard (20.0 µg/g) (Philips, 1993 cited in Senarathne and Pathiratne, 2007; Senarathne *et al.*,2006), FAO/WHO (30.0 µg/g) (FAO/WHO, 1989 cited in Elnabris *et al.*, 2013), USEPA (2.25 mg/kg) (USEPA, 1986; cited in Anim-Gyampo *et al.*, 2013) WHO (2.25 mg/kg) (WHO, 2003; cited in Anim-Gyampo *et al.*, 2013) and WPCL (2.0 mg/kg) (WPCL, 2004; cited in Anim-Gyampo *et al.*, 2013).

Chromium

Chromium was not detected in the control but in the test cases. Concentrations recorded were 0.053 ± 0.002 mg/kg, 0.084 ± 0.004 mg/kg, 0.127 ± 0.003 mg/kg and 0.126 ± 0.003 mg/kg for day 7, 14, 21 and 28, respectively. Statistically, there was significant increase ($p < 0.05$) across the various cases. This is an indication of bioaccumulation. The concentrations in this study were within the recommended limit for fish food as specified by Median International Standard (1.0 µg/g) (Philips, 1993 cited in Senarathne and Pathiratne, 2007; Senarathne *et al.*, 2006).

Cadmium

Like chromium, cadmium was not detected in the control. However, test cases recorded concentrations of 0.038 ± 0.001 mg/kg, 0.077 ± 0.003 mg/kg, 0.099 ± 0.001 mg/kg and 0.101 ± 0.002 mg/kg for day 7, 14, 21 and 28 respectively. Statistically, there was significant increase ($p < 0.05$) across the various cases. This suggests that there is bioaccumulation of the toxicant. The values of cadmium in this study were higher than the recommended limits for fish food by European Union (0.05 µg/g) (European Union, 2002 cited in Senarathne and Pathiratne, 2007; Senarathne *et al.*, 2006) except for day 7. All values were higher than the limits recommended by USEPA (0.01 mg/kg) (USEPA, 1986; cited in Anim-Gyampo *et al.*, 2013), WHO (0.01 mg/kg) (WHO, 2003; cited in Anim-Gyampo *et al.*, 2013) and WPCL (0.003 mg/kg) (WPCL, 2004; cited in Anim-Gyampo *et al.*, 2013), but lower than the limits specified by Median International Standard (0.3 µg/g) Philips, 1993 cited in Senarathne and Pathiratne, 2007; Senarathne *et al.*, 2006), FAO/WHO (0.5 µg/g) (FAO/WHO, 1989 cited in Elnabris *et al.*, 2013) for fish food.

Iron

Iron level in the tissues for control and test cases were respectively recorded as 0.132 ± 0.004

mg/kg and 0.146 ± 0.004 mg/kg (day 7), 1.281 ± 0.001 mg/kg and 0.151 ± 0.001 mg/kg (day 14), 0.126 ± 0.004 mg/kg and 0.116 ± 0.002 mg/kg (day 21), and 0.119 ± 0.001 mg/kg and 0.167 ± 0.003 mg/kg (day 28); while the baseline value was 0.122 ± 0.002 mg/kg. Statistically, there was significant difference ($p < 0.05$) across the various test and control cases, an indication of iron bioaccumulation. The concentrations in this study were lower than the concentration recommended for fish food by USEPA (0.5 mg/kg) (USEPA, 1986; cited in Anim-Gyampo *et al*., 2013) WHO (0.30 mg/kg) (WHO, 2003; cited in Anim-Gyampo *et al*., 2013) and WPCL (0.45 mg/kg) (WPCL, 2004; cited in Anim-Gyampo *et al*., 2013).

Hydrocarbon Related Parameters

Bioaccumulation of hydrocarbon-related compounds by *Clibanarius africanus* on exposure to produced water is presented in Table 2. The total petroleum hydrocarbon level in tissues for control and test cases were respectively recorded as 0.877 ± 0.015mg/kg and 0.623 ± 0.025mg/kg (day 7), 1.000 ± 0.000mg/kg and 0.767 ± 0.025mg/kg (day 14), 1.013 ± 0.015mg/kg and 1.033 ± 0.035mg/kg (day 21), 0.947 ± 0.035 mg/kg and 0.177 ± 0.015mg/kg (day 28), while the baseline value was 0.576 ± 0.015mg/kg. Statistically, there was significant variation ($p < 0.05$) between the control and test cases across the study period.

The total hydrocarbon content of tissues for control and test cases were respectively recorded as 2.763 ± 0.025 mg/kg and 1.943 ± 0.035 mg/kg (day 7), 3.063 ± 0.025 mg/kg and 2.570 ± 0.020 mg/kg (day 14), 4.147 ± 0.035 mg/kg and 3.523 ± 0.025 mg/kg (day 21) and 3.257 ± 0.035 mg/kg and 3.027 ± 0.015 mg/kg (day 28), while the baseline value were 0.576 ± 0.015 mg/kg and 1.827 ± 0.025 mg/kg. Statistically, there were significant variations ($p < 0.05$) between the control and test cases across the study period.

Polycyclic aromatic hydrocarbon was not detected in the control but the test cases had concentrations of 0.470 ± 0.000mg/kg, 0.677 ± 0.015mg/kg, 0.800 ± 0.000mg/kg and 0.851 ± 0.002mg/kg at day 7, 14, 21 and 28 respectively. Statistically, there was significant increase ($p < 0.05$) across the various cases. The values of polycyclic aromatic hydrocarbons, total hydrocarbon content and total petroleum hydrocarbons depict the tendency to be bioaccumulated in *Clibanarius africanus* tissues and though the concentrations are not quite large, they may still portend an adverse health impact on humans.

Table 2. *Bioaccumulation of hydrocarbon related parameters by Clibanarius africanus exposed to treated produced water*

Day	Experimental groups	TPH, mg/L	THC, mg/L	PAH, mg/L
0	Baseline	0.576±0.015b	1.827±0.025a	<0.001±0.000a
7	Test	0.877±0.015e	2.763±0.025d	0.470±0.000b
	Control	0.623±0.025c	1.943±0.035b	<0.001±0.000a
14	Test	1.000±0.000g	3.063±0.025e	0.677±0.015c
	Control	0.767±0.025d	2.570±0.020c	<0.001±0.000a
21	Test	1.013±0.015g	4.147±0.035h	0.800±0.000d
	Control	1.033±0.035g	3.523±0.025g	<0.001±0.000a
28	Test	0.947±0.035f	3.257±0.035f	0.851±0.002e
	Control	0.177±0.015a	3.027±0.015e	<0.001±0.000a

The values are arranged as mean ± standard deviation (descriptive statistics) (n=3). The same letters along the column indicate no significant variations at p = 0.05 according to Waller-Duncan test statistics.

Conclusion

This study examined the uptake of heavy metals and hydrocarbons by *Clibanarius africanus* from treated produced water. The results of hydrocarbon and heavy metals bioaccumulation by *Clibanarius africanus* from the produced water showed that zinc, barium, nickel, lead, cobalt, copper, chromium, cadmium, iron, total petroleum hydrocarbon, total hydrocarbon content, poly cyclic aromatic hydrocarbon in the tissues of the test organisms increased significantly ($p<0.05$) as the exposure duration to treated produced water increased.

This is suggestive of toxicant uptake by the test organisms (*Clibanarius africanus*). Therefore, there is need to effectively treat the produced water with regard to these toxicants before releasing the produced water into the aquatic

ecosystem, since these parameters are harmful to aquatic organisms above the threshold limits and can be harmful to higher organisms in the food chain.

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Citation: *O. S. E. Opete et.al, "Bioaccumulation of Heavy Metals and Hydrocarbon by Clibanarius africanus (Hermit Crab) Exposed to Produced Water". Annals of Ecology and Environmental Science 3(3), pp.25-32*