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HEMATO-BIOCHEMICAL PROFILING OF A BURROWING CRAB EXPOSED TO POLYSTYRENE MICROPLASTIC CONTAMINANT

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ABSTRACT
The study evaluated the effect of polystyrene microplastic contaminant on hemato-biochemical parameters of *Cardiosoma guanhumi* from the mangrove area of Lagos Lagoon using standard methods. Total Haemocyte Count (THC) ranged between 3050.00±0.00 mL and 4200.00±0.05 mL with control crabs having the highest value of 4200.00±0.05 mL. There was significant difference between the treatment groups and the control ($p<0.05$). Hemocyte sub-population variables showed that crabs fed the control and 0.01g polystyrene microplastic diets had higher granulocyte and monocyte populations but a decrease in agranulocytes. The crabs fed with the polystyrene microplastic contaminated diets were significantly higher in serum protein (32.50±0.05-32.90±0.10 gL^{-1}) than the crab fed with control diet (20.09±0.05 gL^{-1}). Crabs fed 0.01g and 0.02g polystyrene microplastic based diets recorded the same aspartate aminotransferase mean value (19.30 μI^{-1}) which was significantly higher than the mean value (11.50 μI^{-1}) recorded in crabs fed control diet. Crab fed 0.01 g polystyrene microplastic based diet recorded most of the highest values for biochemical parameters: Alanine aminotransferase (13.00±0.05 μI^{-1}), alkaline phosphatase (113.70±0.10 μI^{-1}), lactate dehydrogenase (148.60±0.05 μI^{-1}) and urea (40.41 ±0.15 μI^{-1}). The changes in the crabs' hematological and serum biochemical profiles in this study provide reliable and discriminatory data on the effect of microplastic contaminant calling for long-term monitoring to assess the eco-health of the mangrove system.

Keywords: Microplastic Contaminant, Land Crab, Pollution, Lagos Lagoon, Nigeria.

INTRODUCTION

Mangrove ecosystems fringe tropical and sub-tropical coastlines throughout the world and function as nurseries for a wide variety of vertebrate and invertebrate marine species (Olafsson *et al*., 2002). Ecosystem functions underpin ecosystem services but until now, research on the impacts of contaminants on marine ecosystem functioning has been sparse, and predominantly conducted without an ecological context (Newman and Clement, 2008). Contaminants are a pressing global concern and threaten the ecosystem services upon which billions of humans rely (Barbier *et al*., 2011). Unregulated discharge of huge amounts of domestic and industrial wastes from increasing human activities has led to unprecedented contamination and subsequent pollution of surrounding coastal waters (Usese *et al*., 2019).

Microplastics are widespread in the marine environment and small numbers of microplastics have been reported in commercial species of finfish and shellfish from field observations, and in fishery and aquaculture products (FAO, 2017). Concerns have been raised that the presence of microplastics, and their associated chemicals, represents a risk for fish productivity, fisheries resources and may result in contamination of foodstuffs (i.e. implications for seafood safety). Estuaries are sinks for pollutants where the anoxic conditions and other physical characteristics lead to the slower break down of plastics and longer residence times (Vermeiren *et al.*, 2016). The studies of the immunotoxicological effect of microplastic on an organism are of special concern since their bioaccumulation potential increases with decreasing size.

Burrowing crabs of the genus *Cardiosoma*, family Gecarcinidae, are an important element of the fauna of many tropical coastal and estuarine areas. Burrowing crabs are possibly one of the most important components of mangrove fauna not only because of their burrowing activities which can affect nutrient cycling and forest productivity but also their role as a link in the food web in mangrove ecosystems (Moruf and Ojetayo, 2017). Consequences of this burrow and bioturbation include increased vertical and horizontal movement of sediment and detritus and stimulation of microbial activity (Lawal-Are *et al*., 2018a). So any problematic variable which affects the crabs can have major effect on the habitat and ecosystem (Pandya and Vachharajani, 2011).

Studies have been done to assess the health of the Lagos Lagoon and other interconnected ecosystems affected by different kinds of pollutions using brachyuran crabs as an indicator species (Moruf and Lawal-Are, 2018; Usese *et al.*, 2018). However, there is paucity of information on the immune responses of burrowing crabs inhabiting microplastic contaminated locations of Lagos Lagoon, Nigeria. The main thrust of the present study was to examine the haematological and biochemical profiling of a burrowing crab, *Cardiosoma guanhumi* exposed to polystyrene microplastic contaminant.

MATERIALS AND METHODS

Collection of Samples

Samples of *C. guanhumi* (Plate 1) were collected from the mangrove area of University of Lagos Lagoon front (Figure 1) which is part of the Lagos Lagoon. It has a co-ordinate of latitude 6°26°N and 6°39°N and longitude 3°39°S and 3°50°S. The mangrove swamp is a typical estuarine water zone with

extensive mangrove but low transparency and alkaline (pH>7) in nature (Moruf and Lawal-Are, 2015). The samples were hand-picked between the hours of 19 and 22 from different stations within the mangrove swamp. They were immediately placed in styrofoam bags without water and transported to the laboratory for acclimatization.

Plate 1: *Cardiosoma guanhumi* (Latreille, 1825)

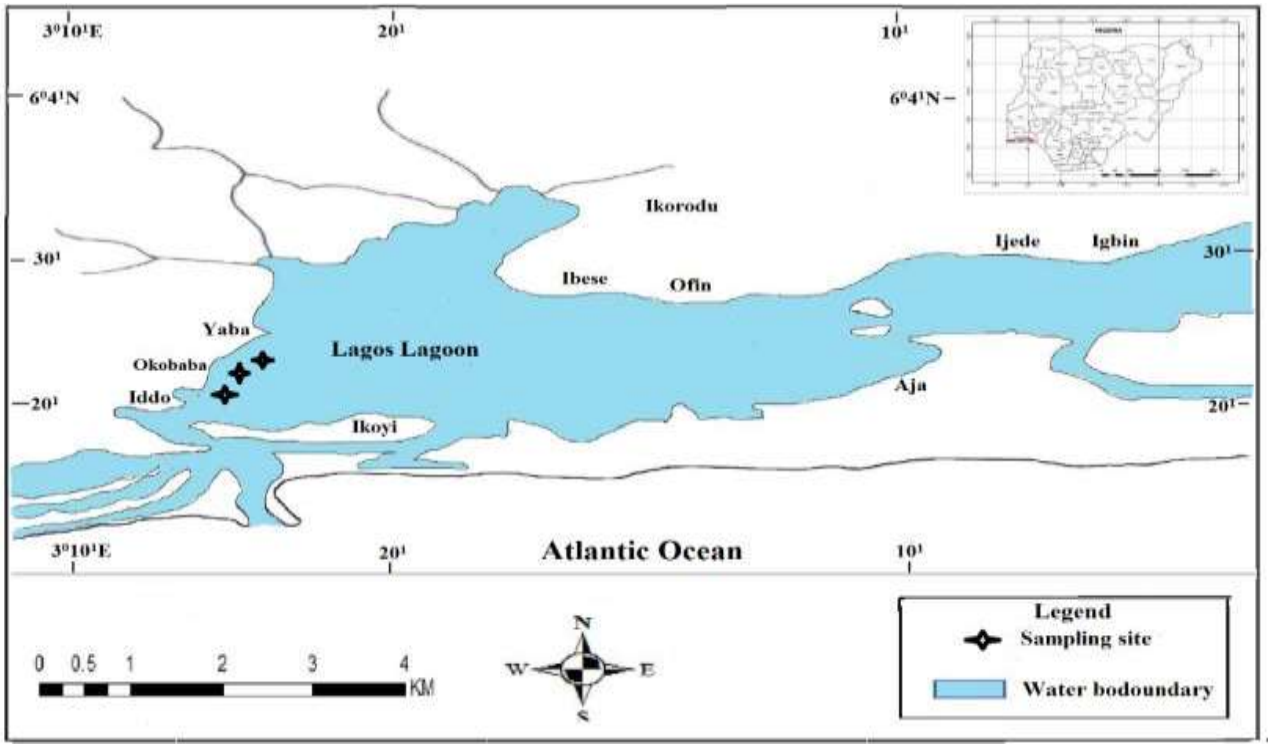

Figure 1: Lagos Lagoon showing the sampling location

Experimental Diet

Three isonitrogenous (45% crude protein) diets were formulated using person square methods. The diet has fishmeal and soybean meal as the main protein source while wheat bran and noodle (indomie) waste as its main carbohydrate source. The control feed diet does not contain the polystyrene microplastic while the test diets contained polystyrene microplastic at two different concentrations (Table 1). The first treatment contains polystyrene microplastic of 5g per 500g of feed and the second treatment contains polystyrene microplastic of 10g per 500g of feed. The feed ingredients were ground using a locally fabricated grinding machine after properly mixing them homogenously and with the polystyrene microplastic. Water (50cl) was added with the ingredients and pelleted through a 4mm die using a pelletizer. The pellets were dried at room temperature for two days. They were packed in properly labeled cellophane bags and stored in a refrigerator at 4°C before the commencement of the feeding trial.

Table 1: Feed Ingredients Showing Diet Composition

Feed Ingredient	CP	T1	T2	Control
Fishmeal	72%	3.01	3.91	3.01
Fishmeal 2	65%	1.60	1.60	1.60
Soy bean meal	44%	2.05	2.05	2.05
Wheat bran	11.2%	1.52	1.52	1.52
Indomie waste	12%	1.80	1.80	1.80
Soy oil	0	0.01	0.01	0.01
Vitamin premix	0	0.03	0.03	0.03
Polystyrene Microplastic	0	0.01	0.02	0

Experimental Procedure and Treatment

In the experiment, 24 specimens of *C. guanhumi* were distributed into eight 50litres square-shaped plastic tanks for two treatments and one control. Each treatment was in triplicate with different tanks for the crabs. Each tank contains Lagos Lagoon water, pebble stones and granite stones to imitate the natural environment of the crab. Acclimatization was achieved in two weeks before the start of experiment. During experimentation, animals were kept isolated in individual containers with room temperature. To prevent contamination, the water in the experimental containers was changed every 48 hours. Only healthy, uninjured animals were used. The relative health of the animals was determined by visual examination for injury. Plastic was added at two experimental concentrations (5g and 10g/500g of feed) with a set of crabs held in identical conditions without plastic to act as the controls. These concentrations were chosen to emulate the acute exposures according to the procedure of Watts *et al.* (2014). The crabs were fed at 3% body weight (using Sartorius pot loading balance) given to them in a way that is fully accessible to the crabs. The crabs were fed daily and the quantity of feed was adjusted and the feeding trial lasted for 8 weeks.

Collection of Haemolymph

Crabs were anaesthetized on ice for 10 mins and haemolymph was drawn with a 23G syringe from the juncture between the basis of the ischium (the joint connecting the fifth walking leg to the carapace) of the fifth walking leg. The haemolymph was collected into a syringe flushed with 1mL of anticoagulant (0.3 M NaCl, 0.1 M glucose, 30 mM Sodium citrate and 26 mM Citric acid), transferred into a 5mL lithium heparin bottle kept in an ice chest and haemolymph of *C. guanhumi* were analyzed immediately for haematological and biochemical indices. Haemocyte population parameters were determined immediately after sampling using an improved Neubaeur heamocytometer according to methods described by Blaxhall and Daisley (1973). For the biochemical analysis, haemolymph samples were centrifuged for 10 mins at 5000 g with a Hawskley micro-haematocrit centrifuge and the serum derived was stored at -20^{o}C for further analysis. The serum was assayed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and urea according to methods described by Coles (1986).

Data analysis

Statistical analysis was done using single factor ANOVA and Duncan Multiple Range Test (DMRT) for significant differences in means of haematological and serum biochemical parameters between the crab species. Differences in means were considered significant when $p<0.05$.

RESULT AND DISCUSSION

Hematological Profile

The effect of microplastic contaminant on the haematological indices of *C. guanhumi* is shown in Table 2. Total Haemocyte Count (THC) ranged between 3050.00±0.00 mL and 4200.00 ±0.05 mL with control crabs having the highest value of 4200.00 ±0.05 mL. There was significant difference between the treatment groups and the control ($p<0.05$). Haemocyte sub-population variables showed that crabs fed the control and 0.01g polystyrene microplastic diets had higher granulocyte and monocyte populations but a decrease in the agranulocytes. However, no significant difference is recorded among the haemocyte sub-population variables.

Table 2: Hematological Profile of *Cardiosoma guanhumi* Exposed to Polystyrene Microplastic Contaminant

Parameter		T1 (0.01g polystyrene microplastic)	T2 (0.02g polystyrene microplastic)	Control
Total Haemocyte Count (mL)		3050.00 ±0.05^{a}	3677.09 ±0.00^{a}	4200.00 ±0.05^{b}
Haemocyte Subpopulation	Granulocyte (%)	32.29 ±0.01^{a}	30.50 ±0.03^{a}	37.00 ±0.07^{a}
	Agranulocyte (%)	67.29±0.05^{a}	69.34 ±0.05^{a}	62.00 ±0.00^{a}
	Monocyte (%)	0.42 ±0.00^{a}	0.17 ±0.00^{a}	1.50±0.05^{a}

Keys: Mean±Standard Error; Values with different superscripts across row are significantly different at ($P < 0.05$)

Similar to the result of THC in the present study, the varying values between the crabs were also recorded by Wang and Chen (2005), where they asserted that wide difference in cell count may be attributed to various parameters such as diet, length at captivity, moult stage, pathological and environmental contaminants. Other reports have shown that the bilateral movement of haemocytes from tissue to haemolymph can result in an increase in THC and could be attributed to the presence of pathogens (Comesaña *et al.*, 2012) or exposure to contaminants (Amachree *et al.*, 2013). According to Adeogun *et al.* (2015), the high value of THC in crabs maybe as a result of lower dissolved oxygen. Sussarellu *et al.* (2012) reported that the increase in number of circulating haemocytes under hypoxic condition is a compensatory response to maintain oxygen tissue perfusion in crabs. The haemocytes sub-population monocytes recorded in the present study are generally low, similar to what Moruf and Lawal-Are (2018) observed in *S. huzardii* (2.50±2.12 %) and *U. tangeri* (1.00±0.12 %) from the polluted creek of Lagos Lagoon, Nigeria. Haematological and biochemical responses in crustaceans are usually harmoinized in reaction to environmental factors which results into homeostatic control within the organisms and as a result are used as diagnostic tools for assessing the health of wild populations (Giro´ n-Pe´ rez *et al*., 2008; Velisek *et al*., 2009). If this environmental factors or stressors are severe and long lasting, the response then becomes mal-adaptive and threatens the health of the organisms and its wellbeing.

Serum Biochemical Profile

The effect of microplastic contaminant on the serum biochemical profile of *C. guanhumi* is presented in Table 3. The crabs fed with the microplastic contaminated diets were significantly higher in serum protein (32.90 ±0.10 gL^{-1} and 32.50 ±0.05 gL^{-1} for Treatment 1 and Treatment 2 respectively) than the crab fed with control diet (20.09±0.05 gL^{-1}). The result of Aspartate aminotransferase (AST) showed that crab fed 0.01g and 0.02g polystyrene microplastic based diets recorded the same mean value of 19.30 μI^{-1}, which was significantly higher than the mean value of 11.50 μI^{-1} recorded in crab fed control diet. Alanine aminotransferase (ALT) results revealed that crabs fed 0.01g polystyrene microplastic based diet had the highest value of 13.00 ±0.05 μI^{-1} which was not significantly different from values of 10.40 ±0.07 μI^{-1} and 9.20±0.05 μI^{-1} obtained in crabs fed with 0.02g polystyrene microplastic based diet and control diet respectively. The results obtained for Alkaline phosphatase (ALP) revealed that crab fed with 0.01g polystyrene microplastic based diet recorded the highest value of 113.70±0.10 μI^{-1} which was significantly different from the values obtained in crabs fed 0.02g polystyrene microplastic based diet (91.50 ±0.90 μI^{-1}) and crabs fed with control diet (84.20±0.02 μI^{-1}). Also, crabs fed 0.01g polystyrene microplastic based diet had the highest lactate dehydrogenase (LDH) of 148.60 ±0.05 μI^{-1} and Urea of 40.41±0.15 $mg.dL^{-1}$ while the lowest LDH of 125.90±0.01 μI^{-1} and lowest urea of 34.50±1.05 $mg.dL^{-1}$ was recorded in crabs fed control diet and crabs fed 0.02g polystyrene microplastic based diet respectively.

Table 3: Serum Biochemical Profile of *Cardiosoma guanhumi* Exposed to Polystyrene Microplastic Contaminant

	T1 (0.01g polystyrene microplastic)	T2 (0.02g polystyrene microplastic)	Control
PRO (gL^{-1})	32.90 ±0.10^a	32.50 ±0.05^a	20.09±0.05^b
AST (μI^{-1})	19.30 ±1.02^a	19.30 ±0.18^a	11.50 ±0.15^b
ALT (μI^{-1})	13.00 ±0.05^a	10.40 ±0.07^a	9.20±0.05^a
ALP(μI^{-1})	113.70±0.10^a	91.50 ±0.90^b	84.20±0.02^b
LDH(μI^{-1})	148.60±0.05^a	143.90 ±0.50^a	125.90±0.01^b
UREA($mg.dL^{-1}$)	40.41 ±0.15^a	34.50 ±1.05^a	36.90 ±1.05^a

Keys: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), phosphatase alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and protein (PRO). Mean±Standard Error; Values with different superscripts across row are significantly different at ($P < 0.05$)

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The total serum protein recorded in the present study were relatively higher in the exposed crabs compared to the control and this could be as a result of the increased haemocyanin levels as which constitutes about 90% of total protein in crustacean haemolymph (Matozzo and Martin, 2010). This was similar to the findings reported by Davies *et al.* (2015) where loads of *Nicothoe astaci* copepods in the gills of *Homarus gammarus* lobster were associated with elevated haemolymph total protein and haemocyanin levels. The reduced values in the control crabs could be as a result of increased breakdown of serum peptidic materials and modulation of their involvement in various biological processes due to their exposure to environmental stress such as the microplastic contaminants. The changes in the AST could be attributed to the interference with the immune system of the crab, resulting to cell damage or a way in which the crabs are reacting to the exposure to microplastic. Once cells are damaged, these enzymes leak into the circulatory body fluid and it is generally accepted that an increase of these enzymes in the extra cellular fluid is indicative of even minor cellular damage (Van der Osst *et al*., 2003). The values recorded for Transaminases AST, ALT and the phosphatase ALP in the experimental crabs were similar to the findings of Adeogun *et al*. (2015) and Lawal-Are *et al*. (2018b).

CONCLUSION

The toxicity of Polystyrene microplastics increased with dose. Total haemocyte count decreased while haemocyte sub-populations varied in exposed crabs. The crabs fed with the polystyrene microplastic contaminated diets were significantly higher in serum protein and most biochemical parameters. Hence, the changes in the crabs' hematological and serum biochemical profiles in this study provide reliable and discriminatory data on the effect of microplastic

contaminant calling for long-term monitoring to assess the eco-health of the mangrove system.

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