

## HAEMATOLOGICAL AND HISTOPATHOLOGICAL RESPONSE OF AFRICAN CATFISH (*Clarias gariepinus*) EXPOSED TO SUBLETHAL CONCENTRATIONS OF PORTLAND CEMENT POWDER

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### ABSTRACT

This study was designed to assess the haematological and histopathological response of African catfish (*Clarias gariepinus*) to Portland cement powder (PCP). Aquatic systems are not the target of PCP but due to its ineluctable use in the environment, they may directly or indirectly find their way into the water body. A total number of 65 juvenile African catfish of the same brood stock and of both sexes, with mean weighing  $12 \pm 1.3\text{g}$  and mean length  $12 \pm 1.3\text{cm}$  were used for this study. The fish were distributed into five experimental groups using 100 litres capacity plastic aquaria with water quality; temperature of  $28.6^\circ\text{C}$ , pH of 7.16, electrical conductivity of  $16.1\mu\text{s}$ , dissolved oxygen of 4.1, salinity of 0.186ppt and total dissolved solute of 14.8. The experimental fish were fed with standard feed (Blue crown) at 08:00 and 16:00 hour each day and toxicant media were changed every 48 hours throughout the study period. The study lasted for a period of 3 months and at the end of each month, three fish were randomly selected from each group for laboratory analysis. The various parameters analyzed include; gill histology, PCV, Hb, WBC, heterophils, lymphocytes and eosinophils. Results indicate that there was severe distortion in the gill histology of the exposed groups while none was observed in control groups. Haematological analysis revealed that: the deleterious effect of PCP on PCV, Hb, RBC, heterophils, lymphocytes of African catfish were significant ( $P < 0.05$ ) while WBC, monocytes and eosinophils were not significant ( $P > 0.05$ ) when compared to the control groups after 3 months.

**Keywords:** Histopathological response, Haematological response, African catfish, Ecotoxicology, Aquatic life assessments

### INTRODUCTION

Cement is as a finely ground, non-metallic, inorganic powder, and when mixed with water forms a paste that sets and hardens. This hydraulic hardening is primarily due to the formation of calcium silicate hydrates, resulting from the reaction between mixing water and the constituents of the cement (Huntzinger and Eatmon, 2015).

Portland cement is a mixture of calcium oxide (CaO) (62% –66%), silicon oxide (SiO) (20% – 22%), aluminium tri oxide ( $\text{Al}_2\text{O}_3$ ) (4% –8%), ferric oxide ( $\text{Fe}_2\text{O}_3$ ) (2% –5%) and magnesium oxide (MgO) (Oleru, 2020). It also includes selenium (Hogue *et al.*, 2017), thallium (Brochhaus *et al.*, 2016) and other impurities (Short and Petsonk, 2018), as well as chromium, nickel, copper and zinc (Asubiojo *et al.*, 1991), with the addition of calcium sulphate (gypsum) during setting (Mindness and Young, 2008).

According to Ritu (2006), the highest impact of cement manufacturing on the environment is of the Mining activity. Mining activities, whether occurring within or near Protected Areas, cause a range of environmental consequences that can be severe and irreversible. It is worthy to note that cement production is known to contribute to pollution of streams and rivers from the degraded runoff discharges through atmospheric deposition of contaminated particulate matter, cooling water, leachates from stock piles raw material and effluents from quality control laboratories (El Fadel *et al.*, 2005; World Bank, 1998; Perfitini *et al.*, 2005). Pollutants in water can remain in solution or deposited to form part of drainage sediments (Gaiero *et al.*, 1997). Water quality is

dependent on the total dissolved substances and these substances when dissolved in water react to change its chemical composition. Gaiero *et al.* (1997) opined that heavy metals in river sediments are more sensitive indicator of pollution than dissolved contaminants. Fish, as aquatic organisms, are in a direct contact with the waterborne pollutants. They have a very thin epithelium and its total area is considerably larger than the total area of skin epithelium (Roberts, 1989). With regard to their delicate structure, various important organs are in direct contact with water, gills are the first organ affected by pollutants and therefore commonly used as the primary markers of water pollution (Bernet *et al.*, 1999). Adamu *et al.* (2008) reported destruction of gill lamella, epithelial hyperplasia and epithelial hypertrophy of Nile tilapia (*Oreochromis niloticus*) exposed to PCP. Blood Parameters are important in diagnosing the structural and functional status of fish exposed to toxicants, therefore, are considered as pathophysiological indicators of the whole body of fish (Adhikari *et al.*, 2004). On the other hand, several researchers reported an extreme toxicity of Portland Cement Powder on the population and diversity of flora and fauna inhabiting the environment (Farmer, 1993; Hegazy, 1996; Sharifi *et al.*, 1997; Iqbal and Shafiq, 1998; Adamu and Audu, 2008). Fish reflect the bioavailability of pollutants in the aquatic system and thus helps in assessing the true degree of pollution in the environment (Mason, 1993; Marcovecchio and Moreno, 1993). Several reports have suggested that fish are capable of accumulating significant concentration of toxicants in water below the limit of detection in routine water samples. Some of these toxicants may alter the gross and

histological architecture of the gill, depending on their type and concentration, fish species, length of exposure and other factors (Musa and Omoregie 1999).

In order to understand better, the haematological and histopathological response of aquatic lives to cement dusts in solution, African catfish *Clarias gariepinus* was selected due to its high level of adaptation and abundance. Catfish is a common freshwater mudfish widely cultivated in many African countries, particularly Nigeria, and Asian countries. It is highly proteinous and abundantly consumed by large number of people. According to Ufodike and Onusiriuka (1990), catfish is suitable for experimental purposes because it possesses accessory air breathing organs, which enable them to thrive in adverse aquatic conditions where other cultivated fish species cannot survive. Therefore, the present study was carried out to ascertain the impact of cement powder on selected haematological parameters and gill histology of African catfish.

## MATERIALS AND METHOD

### Test material

Portland cement powder, test toxicant was gotten from Dangote cement depot at No. 4B East/West Road, opposite University of Port Harcourt Teaching Hospital, Alakahia, Rivers State.

### Experimental Fish

A total number of 65 Juveniles of *Clarias gariepinus* of the same brood stock (with mean weighing  $12 \pm 1.3g$  and mean length  $12 \pm 1.3cm$ ) were used for this study, and were obtained from Faculty of Agriculture demonstration farm, University of Port Harcourt, Choba, Rivers state. They were transported in oxygenated plastic containers to the department of Animal and Environmental Biology laboratory, where they were placed in 100 liters capacity plastic aquarium (in five different groups) containing well aerated borehole water, to enable them acclimatize in laboratory condition for 14 days. The experimental media have the following water quality; temperature of  $28.6^{\circ}C$ , pH of 7.16, Electrical conductivity of  $16.1\mu s$ , Dissolved oxygen of 4.1, Salinity of 0.186ppt and Total dissolved solute of 14.8. The experiment lasted for a period of 90 days, during which the experimental fish, all fed with standard feed (Blue crown) at 08:00 and 16:00 hour each day and toxicant media changed every 48 hours.

### Experimental Groups

Sixty five (65) fish were separated into five groups, with each group having the total number of 9 fish (of both sexes) with additional number of 4 fish for replacement in case of mortality. The sublethal concentration of PCP used was in accordance with Adamu (2010) and ranging from 4.89 mg/l to 39.10 mg/l.

The Experimental groups are as follows:

- Group O: 13 fish of both sexes, unexposed (control).
- Group A: 13 fish of both sexes, exposed to sublethal concentration (4.89 mg/l) of PCP.

- Group B: 13 fish of both sexes, exposed to sublethal concentration (9.78 mg/l) of PCP.
- Group C: 13 fish of both sexes, exposed to sublethal concentration (19.55 mg/l) of PCP.
- Group D: 13 fish of both sexes, exposed to sublethal concentration (39.10 mg/l) of PCP.

During the ninety days experimental period, 3 (three) fish were taken at random from each group every thirty days for sacrifice.

### Bioassay Procedure

**Haematological Assays:** The blood sample collection was by caudal vein puncture at the caudal peduncle and then, samples introduced into heparinized micro-capillary (anti-coagulant) tube. The blood samples were used to determine the haematological parameters; PCV, Hb, WBC, RBC, Heterophils, Lymphocytes, Monocytes and Eosinophils in accordance with the method described by Blaxhall and Daisley, (2002).

**Gill Histology:** In the laboratory, the second right gill arch were removed, and fixed in ten percent formalin for 24 hours, after which decalcified in ten percent nitric acid, dehydrated in increasing concentrations of alcohols, cleared in xylene, impregnated and embedded in paraffin.  $5\mu m$  thick sections stained with hematoxylin and eosin for histological description (Luna, 2017). Gill histological alteration characterized based on the severity of each lesion (Poleksic and Mitrovic-Tutundzic, 1994). Thereafter, changes classified according to tissue damage.

### Statistical Analysis

Data obtained presented as Mean  $\pm$  Standard Deviation, then analysed using one-way analysis of variance (ANOVA). Randomized Block Design (Assistat version 7.6 beta, 2012), used to ascertain the significant difference among means of different groups and means of different months. The Statistical Package for Social Sciences (SPSS) software was for the analysis of data, and the level of significance was set at  $p < 0.05$ . Results depicted in plates and tabular form.

## RESULTS

### Haematological Analysis

Tables 3.1 and 3.2 depict the effects of PCP on haematological parameters of African Catfish in month 1, month 2 and month 3.

The mean value of PCV revealed that there was a significant reduction ( $P < 0.05$ ) in the exposed groups when compared to the control groups in month 1. Month 2 revealed that there was no significant difference ( $P > 0.05$ ) between the mean value of PCV of the exposed groups and control groups, whereas month 3 revealed that there was a significant reduction ( $P < 0.05$ ) in mean value of PCV of the exposed groups when compared to the control groups. The highest value of PCV for month 1 was  $17.00 \pm 7.55$  while the lowest value was  $4.33 \pm 1.15$ , the highest value of PCV in month 2 was  $23.33 \pm 10.12$  while the lowest value was  $11.67 \pm 2.89$ , and the highest value of PCV in



month 3 was  $15.00 \pm 2.65$  while the lowest value was  $4.67 \pm 0.58$ . Impact of PCP in month 1, 2 and 3 showed statistically no significant difference ( $P > 0.05$ ), that is, all affected equally within the groups.

The mean value of HB revealed that there was a significant reduction ( $P < 0.05$ ) in the exposed groups when compared to the control groups in month 1. Month 2 revealed that there was no significant difference ( $P > 0.05$ ) between the mean value of HB of the exposed groups and control groups, whereas month 3 revealed that there was a significant reduction ( $P < 0.05$ ) in mean value of HB of the exposed groups when compared to the control groups. The highest value of HB in month 1 was  $5.63 \pm 2.52$  while the lowest value was  $1.40 \pm 0.35$ , the highest value of HB in month 2 was  $7.73 \pm 3.35$  while the lowest value was  $4.07 \pm 1.33$ , and the highest value of HB in month 3 was  $5.67 \pm 1.42$  while the lowest value was  $1.56 \pm 0.23$ . Impact of PCP in month 1, 2 and 3 showed statistically significant difference ( $P < 0.05$ ), that is, the impact of PCP within the groups was relatively higher in month 3 when compared to month 1 and 2.

The mean value of RBC revealed that there was a significant reduction ( $P < 0.05$ ) in the exposed groups when compared to the control groups in month 1. Whereas month 2 and 3 revealed, there were no significant differences ( $P > 0.05$ ) between the mean values of RBC of the exposed groups and control groups. The highest value, of RBC for month 1 was  $8.87 \pm 2.20$  while the lowest value was  $3.23 \pm 0.97$ , the highest value of RBC for month 2 was  $4.87 \pm 1.08$  while the lowest value was  $3.63 \pm 1.43$ , and the highest value of RBC for month 3 was  $5.17 \pm 0.40$  while the lowest value was  $4.90 \pm 0.62$ . Impact of PCP in month 1, 2 and 3 showed statistically insignificant difference ( $P > 0.05$ ), that is, all affected equally within the groups.

The mean value of WBC revealed that there were no significant differences ( $P > 0.05$ ) between the mean values of WBC of the exposed groups and control groups in month 1, 2 and 3. The highest value of WBC for month 1 was  $4.73 \pm 0.35$  while the lowest value was  $4.30 \pm 0.56$ , the highest value of WBC for month 2 was  $5.97 \pm 0.31$  while the lowest value was  $3.93 \pm 1.79$ , and the highest value of WBC for month 3 was  $8.50 \pm 1.80$  while the lowest value was  $4.33 \pm 1.44$ . Impact of PCP in month 1, 2 and 3 showed no significant difference ( $P > 0.05$ ), that is, all affected equally within the groups.

The mean values of Heterophils revealed that there were no significant differences ( $P > 0.05$ ) between the means of the exposed groups when compared to the control groups in month 1 and 2, whereas there was a significant reduction ( $P < 0.05$ ) in the mean of the exposed groups in comparison with the control groups. The highest value of Neutrophils for month 1 was  $35.33 \pm 4.51$  while the lowest value was  $27.67 \pm 3.06$ , the highest value of Heterophils for month 2 was  $22.67 \pm 12.50$  while the lowest value was  $7.33 \pm 6.66$ , and highest value of Heterophils for month 3 was  $18.00 \pm 4.58$  while the lowest value was  $5.67 \pm 1.15$ . Impact of PCP in month 1, 2 and 3 revealed statistically

significant difference ( $P < 0.05$ ), that is, the impact of PCP within the groups was relatively higher in month 1 when compared to month 2 and 3.

The mean values of Lymphocytes revealed that there were no significant differences ( $P > 0.05$ ) between the means of the exposed groups when compared to the control groups in month 1 and 2, whereas there was a significant reduction ( $P < 0.05$ ) in the mean of the exposed groups in comparison with the control groups. The highest value of Lymphocytes for month 1 was  $69.00 \pm 2.00$  while the lowest value was  $58.67 \pm 5.50$ , the highest value of Lymphocytes for month 2 was  $86.33 \pm 2.08$  while the lowest value was  $72.67 \pm 13.50$ , and highest value of Lymphocytes for month 3 was  $91.00 \pm 2.65$  while the lowest value was  $77.33 \pm 6.66$ . Impact of PCP in month 1, 2 and 3 showed statistically significant difference ( $P < 0.05$ ), that is, the impact of PCP within the groups was relatively lower in month 1 when compared to month 2 and 3.

The mean values of Monocytes revealed that there were no significant differences ( $P > 0.05$ ) between the means of the exposed groups when compared to the control groups in month 1, 2 and 3. The highest value of Monocytes for month 1 was  $5.33 \pm 2.08$  while the lowest value was  $2.00 \pm 2.65$ , the highest value of Monocytes for month 2 was  $3.00 \pm 1.00$  while the lowest value was  $1.00 \pm 1.00$ , and highest value of Monocytes for month 3 was  $4.33 \pm 2.31$  while the lowest value was  $2.00 \pm 1.00$ . Impact of PCP in month 1, 2 and 3 showed statistically insignificant difference ( $P > 0.05$ ), that is, all equally affected within the groups.

The mean values of Eosinophils revealed that there were insignificant differences ( $P > 0.05$ ) between the means of the exposed groups when compared to the control groups in month 1, 2 and 3. The highest value of Eosinophils for month 1 was  $5.00 \pm 7.00$  while the lowest value was  $1.00 \pm 1.73$ , the highest value of Eosinophils for month 2 was  $3.33 \pm 4.16$  while the lowest value was  $2.00 \pm 1.73$ , and highest value of Eosinophils for month 3 was  $3.33 \pm 2.08$  while the lowest value was  $1.33 \pm 0.57$ . Impact of PCP in month 1, 2 and 3 showed statistically no significant difference ( $P > 0.05$ ), that is, all affected equally within the groups.

**Table 3.1:** Impact of Portland Cement Powder on Haematological Parameters of African Catfish (*Clarias gariepinus*) for month 1, 2 and 3. (Mean ± Standard Deviation)

Concentration (mg/l)		MONTHS		
		1	2	3
PCV (%)	0.00 mg/l	9.33 ± 1.15 <sup>abc</sup>	11.67 ± 2.89 <sup>a</sup>	15.00 ± 2.65 <sup>b</sup>
	4.89 mg/l	4.33 ± 1.15 <sup>a</sup>	14.67 ± 9.29 <sup>a</sup>	4.67 ± 0.58 <sup>a</sup>
	9.78 mg/l	6.67 ± 1.53 <sup>ab</sup>	23.33 ± 10.12 <sup>a</sup>	17.00 ± 4.36 <sup>b</sup>
	19.55 mg/l	15.67 ± 2.52 <sup>ab</sup>	12.33 ± 4.04 <sup>a</sup>	14.33 ± 2.52 <sup>b</sup>
	39.10 mg/l	17.00 ± 7.55 <sup>c</sup>	14.33 ± 13.65 <sup>a</sup>	9.67 ± 4.51 <sup>ab</sup>
HB (g/L)	0.00 mg/l	3.07 ± 0.40 <sup>abc</sup>	3.87 ± 0.98 <sup>a</sup>	5.00 ± 0.89 <sup>b</sup>
	4.89 mg/l	1.40 ± 0.35 <sup>a</sup>	4.87 ± 3.09 <sup>a</sup>	1.56 ± 0.23 <sup>a</sup>
	9.78 mg/l	2.17 ± 0.51 <sup>ab</sup>	7.73 ± 3.35 <sup>a</sup>	5.67 ± 1.42 <sup>b</sup>
	19.55 mg/l	5.20 ± 0.85 <sup>bc</sup>	4.07 ± 1.33 <sup>a</sup>	4.80 ± 0.85 <sup>b</sup>
	39.10 mg/l	5.63 ± 2.52 <sup>c</sup>	4.73 ± 4.59 <sup>a</sup>	3.23 ± 1.50 <sup>a</sup>
RBC (10 <sup>6</sup> /μL)	0.00 mg/l	8.27 ± 0.60 <sup>ab</sup>	3.90 ± 0.53 <sup>a</sup>	7.27 ± 2.20 <sup>a</sup>
	4.89 mg/l	3.23 ± 0.97 <sup>a</sup>	3.63 ± 1.43 <sup>a</sup>	5.17 ± 0.40 <sup>a</sup>
	9.78 mg/l	4.57 ± 1.83 <sup>ab</sup>	4.87 ± 1.08 <sup>a</sup>	5.10 ± 0.72 <sup>a</sup>
	19.55 mg/l	8.27 ± 2.18 <sup>b</sup>	4.00 ± 1.22 <sup>a</sup>	5.13 ± 1.26 <sup>a</sup>
	39.10 mg/l	8.87 ± 2.20 <sup>b</sup>	4.23 ± 2.75 <sup>a</sup>	4.90 ± 0.62 <sup>a</sup>
WBC (10 <sup>3</sup> /μL)	0.00 mg/l	3.07 ± 0.40 <sup>a</sup>	3.90 ± 0.89 <sup>a</sup>	5.43 ± 1.55 <sup>a</sup>
	4.89 mg/l	4.30 ± 0.56 <sup>a</sup>	5.97 ± 0.31 <sup>a</sup>	5.80 ± 3.41 <sup>a</sup>
	9.78 mg/l	4.73 ± 0.35 <sup>a</sup>	4.40 ± 0.60 <sup>a</sup>	8.50 ± 1.80 <sup>a</sup>
	19.55 mg/l	4.23 ± 0.61 <sup>a</sup>	3.93 ± 1.79 <sup>a</sup>	6.93 ± 1.16 <sup>a</sup>
	39.10 mg/l	4.40 ± 1.87 <sup>a</sup>	4.93 ± 0.32 <sup>a</sup>	4.33 ± 1.44 <sup>a</sup>

Means in columns with the same letters are not significantly different (P > 0.05)

**Table 3.2:** Impact of Portland Cement Powder on Haematological Parameters of African Catfish (*Clarias gariepinus*) for month 1, 2 and 3. (Mean ± Standard Deviation)

Means in columns with the same letters are not significantly difference (P > 0.05)

**Gill Histology**

Summary of histopathological examination of the gill of African Catfish (*Clarias gariepinus*) for month 1, 2 and 3 presented in the tables below with various plates showing

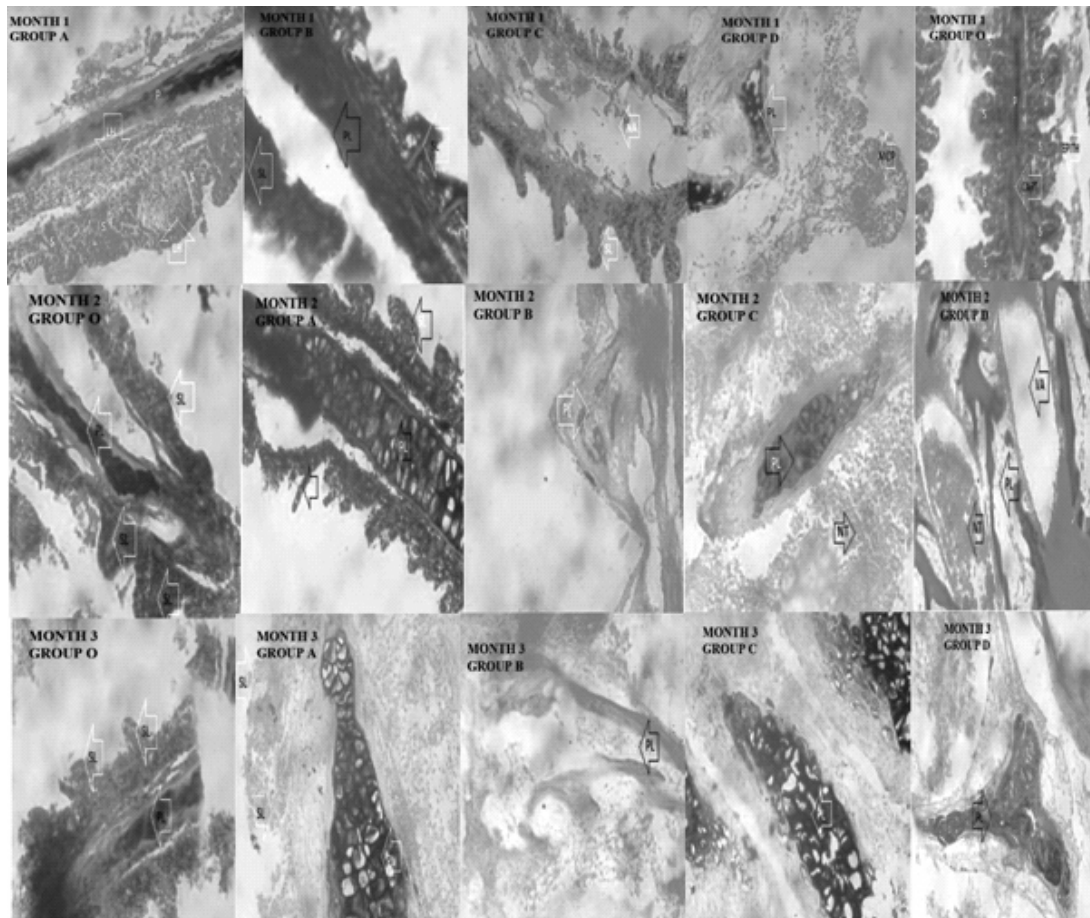
the level of histological alteration across the groups. There was no histological alteration recorded in various control groups while the exposed groups showed mild to severe histological alteration.

Concentration (mg/l)		MONTHS		
		1	2	3
HETEROPHILS (%)	0.00 mg/l	36.00 ± 9.85 <sup>a</sup>	11.67 ± 3.51 <sup>a</sup>	14.33 ± 4.16 <sup>ab</sup>
	4.89 mg/l	30.67 ± 9.45 <sup>a</sup>	7.33 ± 6.66 <sup>a</sup>	5.67 ± 1.15 <sup>a</sup>
	9.78 mg/l	30.33 ± 14.36 <sup>a</sup>	14.67 ± 11.24 <sup>a</sup>	16.00 ± 3.60 <sup>ab</sup>
	19.55 mg/l	27.67 ± 3.06 <sup>a</sup>	22.67 ± 12.50 <sup>a</sup>	15.67 ± 5.03 <sup>ab</sup>
	39.10 mg/l	35.33 ± 4.51 <sup>a</sup>	13.67 ± 11.93 <sup>a</sup>	18.00 ± 4.58 <sup>b</sup>
LYMPHOCYTES (%)	0.00 mg/l	58.33 ± 11.59 <sup>a</sup>	88.67 ± 2.08 <sup>a</sup>	80.00 ± 4.00 <sup>ab</sup>
	4.89 mg/l	60.67 ± 8.08 <sup>a</sup>	86.33 ± 2.08 <sup>a</sup>	91.00 ± 2.65 <sup>b</sup>
	9.78 mg/l	66.33 ± 17.61 <sup>a</sup>	81.66 ± 13.80 <sup>a</sup>	77.33 ± 6.66 <sup>ab</sup>
	19.55 mg/l	69.00 ± 2.00 <sup>a</sup>	72.67 ± 13.50 <sup>a</sup>	77.00 ± 7.81 <sup>ab</sup>
	39.10 mg/l	58.67 ± 5.50 <sup>a</sup>	83.33 ± 14.57 <sup>a</sup>	75.33 ± 5.69 <sup>a</sup>
MONOCYTES (%)	0.00 mg/l	2.00 ± 2.65 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	3.00 ± 0.00 <sup>a</sup>
	4.89 mg/l	5.33 ± 2.08 <sup>a</sup>	3.00 ± 1.00 <sup>a</sup>	2.00 ± 1.00 <sup>a</sup>
	9.78 mg/l	2.33 ± 2.08 <sup>a</sup>	1.67 ± 1.53 <sup>a</sup>	4.33 ± 2.31 <sup>a</sup>
	19.55 mg/l	2.00 ± 2.65 <sup>a</sup>	1.00 ± 1.73 <sup>a</sup>	4.00 ± 2.65 <sup>a</sup>
	39.10 mg/l	4.33 ± 3.51 <sup>a</sup>	1.00 ± 1.00 <sup>a</sup>	3.33 ± 1.15 <sup>a</sup>
EOSINOPHILS (%)	0.00 mg/l	3.67 ± 2.08 <sup>a</sup>	1.33 ± 0.58 <sup>a</sup>	2.67 ± 1.15 <sup>a</sup>
	4.89 mg/l	3.33 ± 2.08 <sup>a</sup>	3.33 ± 4.16 <sup>a</sup>	1.33 ± 0.57 <sup>a</sup>
	9.78 mg/l	1.00 ± 1.73 <sup>a</sup>	2.00 ± 1.73 <sup>a</sup>	2.33 ± 2.08 <sup>a</sup>
	19.55 mg/l	1.33 ± 1.53 <sup>a</sup>	2.67 ± 1.53 <sup>a</sup>	3.33 ± 2.08 <sup>a</sup>
	39.10 mg/l	5.00 ± 7.00 <sup>a</sup>	2.00 ± 1.73 <sup>a</sup>	3.33 ± 2.08 <sup>a</sup>



MONTHS	GROUP O	GROUP A	GROUP B	GROUP C	GROUP D
1	Histologically normal gill showing: primary and secondary lamella, Primary lamella containing cartilage, secondary lamella is lined by simple epithelial cell	Mildly distorted gill showing: primary and secondary lamella, lamella haemorrhage	Histologically distorted gill indicating: normal secondary lamella replaced with cavity	Histologically distorted gill showing: normal secondary lamella, primary lamella contain a necrotic area devoid	Severely distorted gill shows: destroyed primary lamella with ring-like stump of cartilage, covered with epithelial cells
2	Histologically normal gill showing: primary and secondary Lamella	Mildly distorted gill indicates: primary and secondary lamella, cavity in the primary lamella	Severely distorted gill showing: primary lamella stump	Severely distorted gill shows: primary lamella stump surrounded by necrotic tissue	Severely distorted gill showing: primary lamella stump surrounded by necrotic tissue
3	Histologically normal gill showing: primary and secondary Lamella	Histologically distorted gill indicating: primary and secondary Lamella	Severely damaged gill shows: primary lamella	Severely damaged gill showing: primary lamella	Severely damaged gill indicates: primary lamella stump

**Table 3.3:** Histological alteration in the gill of African Catfish (*Clarias gariepinus*) for month 1, 2 and 3.



**Plate 3.1:** Photomicrographs of the Gill of African Catfish (*Clarias gariepinus*). Hematoxylin and eosin X400 stain.

KEY: S/SL= Secondary Lamella, P/PL = Primary Lamella, EPITH = Epithelial Cells, CART= Cartilage, LH= Lamella Haemorrhage, NA/VA = Necrotic Area, MCP= Stump of Cartilage, NT= Necrotic Tissue



## DISCUSSION

Haematological analysis revealed that PCP had significant effect on PCV and Hb of the exposed groups when compared them to the control groups in month 1 and 3 while the effect was insignificant in month 2. The effect on RBC was significant in month 1 and insignificant in month 2 and 3. PCP has no significant effect on WBC, Monocytes and Eosinophils both in month 1, 2 and 3 whereas the effect on Heterophils and Lymphocytes was significant in month 3 and insignificant in month 1 and 2. Heterophils, Lymphocytes, Monocytes and Eosinophils are the major components of WBC characterized by the presence of cytoplasmic granules, that is, a specialized secretory vesicles containing various cytotoxic molecules, enzyme and peptidase (Lattimer, 2011). Findings have revealed that haematological components are good indicator for assessing the health status of fish (Aldrin *et al.*, 1994). In the same vein, blood serves as an indicator of physiological condition of the animal (Kefas *et al.*, 2015). Conversely, haematological studies are excellent tools for investigating physiological changes caused by environmental toxicants (Zaghloul, 2001; Zaghloul *et al.*, 2005).

The impact of PCP on Packed Cell Volume was obvious in exposed group with the least concentration. PCV is a biomarker used to ascertain the ratio of plasma to corpuscles in the blood and oxygen-carrying capacity of the blood Akinrotimi *et al.* (2011). The decrease in the PCV level of the exposed groups observed in this study, could be the result of gill damage and/or impaired osmoregulation, which bring about anaemia and haemodilution attributed to an immune response to the toxicants (George *et al.*, 2017). Similar observations were reported by; Oriakpono *et al.* (2012), Adamu and Audu (2008), Omoregie *et al.* (1998) and Tort and Torres (1996). Haemoglobin level of the exposed group with the least concentration has the major deleterious effect. Hb is oxygen-carrying component in the blood of fish; its concentration can be used as an indicator of anaemia (Blaxhall and Daisley, 2002). The decrease in haemoglobin caused by exposure to toxicant brings about haemodilution, the mechanism that reduce the concentration of the toxicant present in the circulatory system (Smith *et al.*, 1993). Therefore, the significant decrease in Hb level observed in this study could be an indicator that anaemic condition occurred during exposure. There were similar reports by Adamu and Audu (2008), Adeyemo (2005) and Kori-Siakpere *et al.* (2005).

The exposed group with the lowest concentration were the most affected in terms of Red Blood Cell count. The Red Blood Cells have a unique function of haemoglobin transport, which carries oxygen to all tissues in the body (Hibiya, 1994). The decrease in RBC, observed in the exposed groups could because of aggregation of red blood cells in damaged gills (Singh and Singh, 1992). It could also be because of swelling of the erythrocytes caused by decrease in the erythropoietic activity of the kidney (Santhakumar *et al.*, 1999). This is in line with the present study and in conformity with that of Adamu and Audu (2008), Adeyemo (2005), and Lipika and Patra (2006).

The exposed groups showed varying responses in leucogram values (WBC, Heterophils, Lymphocytes, Monocytes and Eosinophils) when compared to the control groups. In other words, PCP had obvious impact on Heterophils and Lymphocytes of the exposed groups in comparison with the control groups whereas there were no obvious impact on WBC, Monocytes and Eosinophils of the exposed groups in comparison with the control groups. The exposed group with least concentration received the greatest impact in terms of Heterophils while the exposed group with highest concentration had the greatest impact in terms of Lymphocyte. Leucogram values have the capacity of inducing some non-specific immune response in animals, which enable them to resist potential pathogens (Oriakpono *et al.*, 2012). Das (1998) channelled the decrease in leucogram values to protective response of fish to stress. They play an important role in the regulation of immunological functions of the body (Santhakumar *et al.*, 1999) and long-term exposure of toxicant induces failure of leucogram production, which brings about non-specific immunity of fish that result to low productivity, as fish exposed to toxicants cannot survive environmental stress (Svabodova *et al.*, (1994); Misra *et al.*, 1993). The decrease in some leucogram values observed in the present study may be because of bio-concentration of the test toxicant in the liver and kidney (Agrawal and Srivastava, 2008). Similar results have been reported by; Prasad *et al.* (2012), Omoregie (1998), Musa and Omoregie (1999), Mgbenka *et al.* (2003) and Oluah and Mgbenka (2004).

Photomicrographs of the gills studied revealed that the histological alterations were conspicuous in the gill of exposed fish whereas there was none observed in the control fish. The gill of the exposed fish compared with the control fish indicate varying degree of alterations across the groups. These alterations range from mildly distorted gill with lamella haemorrhage (in exposed group with the least concentration), to severely distorted gill, primary lamella with ring-like stump of cartilage (in exposed group with highest concentration) in month 1. Then mildly distorted gill with cavity in the primary lamella (in exposed group with the least concentration) to severely distorted gill with primary lamella stump surrounded by necrotic tissue (in exposed group with highest concentration) in month 2. Finally histologically distorted primary lamella (in exposed group with the least concentration) to severely damaged gill with primary lamella stump (in exposed group with highest concentration) in month 3. These histological alterations observed could be the gill's defense mechanism against toxicants (Winkaler *et al.*, 2001). Previous histological examination in fish exposed to toxicant indicate that epithelium lifting was the most common lesion observed in all analyzed gills of exposed groups, followed by lamella epithelium haemorrhage, lamella stump and necrosis of the tissues (Fracário *et al.*, 2003). Epithelium lifting is one of the earliest injuries found in fish and it is been characterized by displacement of the lining epithelium of the secondary lamella, which bring about the formation of a space called oedema, linked to the presence of toxicants (Winkaler *et al.*, 2001). In this study,



the histological alterations observed in the gill of the exposed fish are in line with that of Débora *et al.* (2014), Martinez *et al.* (2004), Meletti *et al.* (2003), and Winkaler *et al.* (2001).

## CONCLUSION

It can be concluded that exposure of African Catfish to Portland cement powder for 90 days resulted in gill and haematological defect which can affect their health status and in turn poses a significant threat in their growth and development.

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