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Haematological Indices in Fish Plasma as Indicators of Exposure to a Commonly Used Herbicides

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ABSTRACT

Background: Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. **Objective:** The exposure of chemical pollutants can induce either increase or decrease in haematological levels; this study is to access the level of haematological alterations of *Clarias gariepinus* exposed to varying concentration of glyphosate herbicides. **Results:** Haematological alterations showed significant differences ($P < 0.05$; 0.01) with elevations in RBC, HCV, MCH, Platelets and WBC counts compared to the control. The fluctuation in RBC (significant increase ($P < 0.01$) in HCV, ($P < 0.05$) in MCH), WBC (Significant increase ($P < 0.05$) in neutrophils) and a significant elevation ($P < 0.05$) in Platelets was an indication of post juvenile *C. gariepinus* response to the toxicity of glyphosate herbicide. **Conclusion:** the results from this study shows that the indices surveyed are relevant in examining the health of organisms in the ecosystem and such extent of alterations are important biomarkers of stressors.

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INTRODUCTION

The exposure of fish to several types of chemical agents may induce changes in several haematological variables (Heath, 1995), which are frequently used to assess fish health (Martnez and Souza, 2002). The study blood parameters in fishes have been widely used for the detection of physiopathological alterations in different conditions of stress (Nussey *et al.*, 1995). Haematological parameters such as haematocrit, haemoglobin, number of erythrocytes and white blood cells are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Sancho *et al.*, 2000; Barcellos *et al.*, 2003). Several workers have investigated haematological changes of fish exposed to pesticides (Santhakumar and Ramudu, 1999; Das and Mukherjee, 2000b; Aengwanich and Tanomtong, 2004; Adedeji *et al.*, 2008; Ramesh and Saravanan, 2008; Ojezele and Abatan, 2009). Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology (Rambhaskar and Srinivasa Rao 1986; and Xiaoyun *et al.*, 2009). The analysis of blood indices has proven to be a valuable approach for analysing the health status of farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani *et al.* 2001). Fish live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components (Wilson and Taylor, 1993). In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels.

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MATERIALS AND METHOD

Experimental fish specimen and chemicals:

One hundred and twenty normal post juvenile *Clarias gariepinus* of both sexes with a mean weight of 135.44 ± 1.99 g and mean length of 28.32 ± 0.844 cm were purchased from Osayi farms in Benin City, Edo state. They were kept in 60 l aquaria at 27.5 ± 0.4 °C, pH 7.3, with 12:12 h photoperiod. They were left unfed in the first 2 days to adapt to a change in environment before feeding them with the fish diet. Laboratory aquaria were well aerated and provided with external filtration and a layer of gravel on the bottom. Fish were normally fed once a day with pelleted commercial food (Durante Aquaculture fish concentration-2mm). They were allowed to acclimate to captivity conditions for two month prior to taking the blood samples. Careful netting and handling was implemented to minimize stress. The commercial formulation of glyphosate (360 g/l-41 w.wt IPA) at five nominal concentrations 72, 54, 32 and 18 mg/L were used. These concentrations were defined taking into account: the result of the range finding test.

Haematological analysis:

The peripheral blood was collected by puncture of the genital opening with a heparin-coated 21 gauge \times 0.5 in. needle, attached to a 5 ml syringe. The blood was placed immediately into separate EDTA bottles for further analysis using standard procedures.

RESULT AND DISCUSSION

Haematological response of post juvenile *C. gariepinus* exposed sublethal concentration of glyphosate herbicides showed no significant difference ($P > 0.05$) of Erythrocyte count (RBC), Haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC) but significant difference ($P < 0.01$) of Haemoglobin Corpuscular Volume (HCV), significant difference ($P < 0.05$) of Mean Corpuscular Haemoglobin (MCH

Haematological parameters in fish can significantly change in response towards chemical stressors; however, these alterations are non-specific to a wide range of substances. Some of these changes may be the result of the activation of protective mechanisms (Cazenave *et al.*, 2005) such as the results of the blood parameters observe in the present work.

Erythrocytes are produced in the haematopoietic tissue, which is situated in the spleen and head kidney (Bond, 1979; Hoffbrand and Pettit, 1980; Smith, 1982; Grey and Meyer, 1988; Kita and Itazawa, 1989; Heath, 1995). It is well known that a reduced quantity and quality of erythrocytes and a decreased haemoglobin level as seen in the present study led to a deteriorated oxygen supply. In addition to transport of oxygen, erythrocytes have other functional tasks in the body; therefore an insufficient quantity and quality of erythrocytes would consequently have several additional effects on metabolism beyond the simple oxygen supply for tissue metabolism. Prolonged reduction in haemoglobin content has been reported to be deleterious to oxygen transport and any blood dyscrasia and degeneration of the red blood cells could be ascribed as pathological conditions in fishes exposed to toxicants (Buckley *et al.*, 1976). The non significant difference ($P > 0.05$) in Erythrocyte count of Juvenile Catfish exposed to various concentration of glyphosate herbicide compared to the control in this experiment disagree with the findings of Ikpesu, (2010) of *C. gariepinus* exposed to Endosufan. Although, there was an increase in erythrocyte count with increasing concentration, but it was not enough to cause a significant difference when compared to the control. Fish exposed to the higher concentration of glyphosate for 28 days showed an increase in haematocrit and in the number of erythrocytes, indicating the release of erythrocytes from blood deposits and/or from haemopoetic tissues into the blood stream (Svodova *et al.*, 1994). This finding disagrees with the results reported by Gluszcak *et al.*, (2006), who observed a decrease in haematocrit, number of erythrocytes and haemoglobin of *Leporinus obtusidens* exposed to different concentrations of Roundup_ original (3, 6, 10 and 20 mg/L) for 96 h. This difference in response may be explained by the fact that Roundup Transorb, Roundup original, and others are herbicides with different formulations, although they use the same active principle, glyphosate. Besides the xenobiotic type, it is also important to point out that the effects of environmental toxicants on haematological characteristics of fish vary according to the target species (Elahee and Bhagwant, 2007). This is another explanation for the variation on the haematological responses observed in this work and in the works by Gluszcak *et al.*, (2006); Modesto and Martinez, (2010) using another fish species *C. gariepinus*.

Contrary to the observations of Ikpesu, (2010) and Safahieh *et al.*, (2012) no significant difference ($P > 0.05$) was observed for Haemoglobin (Hb) and Packed Cell Volume of *C. gariepinus* exposed to various concentration of glyphosate.

The haematological profile of *C. gariepinus* exposed to sublethal concentrations of glyphosate herbicide observed showed significant difference ($P < 0.01$) for Haemoglobin Corpuscular Volume (HCV), ($P < 0.05$) for Mean Corpuscular Haemoglobin (MCH) and no significant difference ($P > 0.05$) for MCHC. The calculated haematological indices, MCHC, MCH, and HVC have a particular importance in the diagnosis of anaemia in most animals (Coles, 1986). The perturbations in these haematological indices (increase HCV, MCH) in the

present study may be attributed to a defence against the toxic effect of glyphosate through the stimulation of erythropoiesis or may be related to the decrease in RBCs, Hb and Hct due to exaggerated disturbances that occurred in both metabolic and haemopoietic activities of fish exposed to sublethal concentrations of pollutants (Mousa, 1999).

The increase in MCV coupled with low haemoglobin content indicate that the red blood cells have shrunk, either due to hypoxia or microcytic anaemia; microcytosis been due to the decrease in the haematocrit values. The fluctuation in the MCH values clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control over the exposure period, thus indicating an anaemic condition.

The MCHC is a good indicator of red blood cell swelling (Wepener *et al.*, 1992). The MCHC, which is the ratio of blood haemoglobin concentration as opposed to the haematocrit, is not influenced by the blood volume nor by the number of cells in the blood but can be interpreted incorrectly when new cells, with a different haemoglobin concentration, are released into blood circulation (Soivio and Nikinmaa, 1981). The significant decreases in the MCHC values in the exposed fish are thus probably an indication of swelling of the red blood cells and/or a decrease in haemoglobin synthesis.

White Blood Cell Indices:

The white blood cells in fish respond to various stressors including infections and chemical irritants (Christensen, 1978). Thus increasing or decreasing numbers of white blood cells are a normal reaction to the exposure of toxicants (Kori-Siakpere, 2006). Differential White Blood cell profiles are sensitive to physiological changes and health status in animals (Ritchie *et al.*, 1993). White blood cell numbers can also be affected by a variety of physiological and environmental factors and the responses normally found when fish are subjected to an array of toxicants are lower percent lymphocytes and higher percent neutrophils (Witeska, 2005).

There are five main types of White blood cells (lymphocytes, monocytes, neutrophils, eosinophils and basophils), each playing a different role in responding to the presence of foreign organism or substance in the body. In the present investigation the fluctuation in WBC (leukocytosis) may have resulted from the excitation of defence mechanism of the fish to counter the effect of the toxicant (Gabriel *et al.*, 2009).

However, fish exposed to glyphosate showed a different pattern of response, with an insignificant increase in lymphocytes and monocytes followed by a significant decrease ($P < 0.05$) in neutrophils. These alterations can be the result of the activation of the immune system in the presence of contaminant, which in turn may be an adaptive response of the organism resulting in a more effective immune defense (Barreto-Medeiros *et al.*, 2005). Significant difference ($P < 0.05$) was observed in neutrophils for the treated fish compared to the control throughout the period of investigation and this agrees with the findings of Modesto and Martinez, (2010).

Conclusion:

The results from this study shows that the indices surveyed are relevant in examining the health of organisms in the ecosystem and such extent of alterations are important biomarkers of stressors.

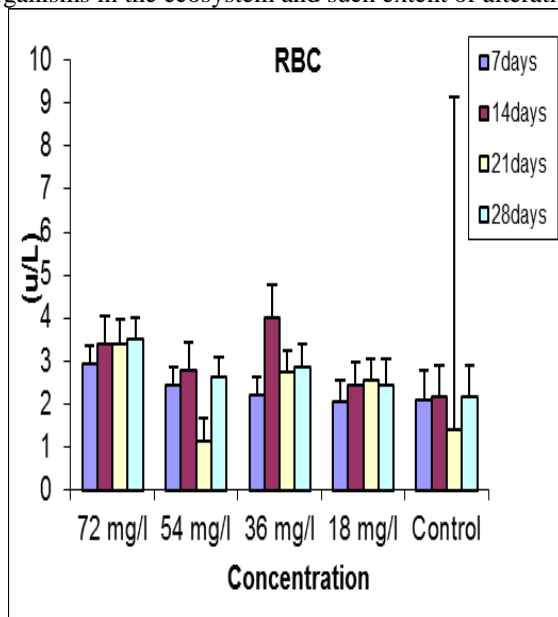


Fig 1a

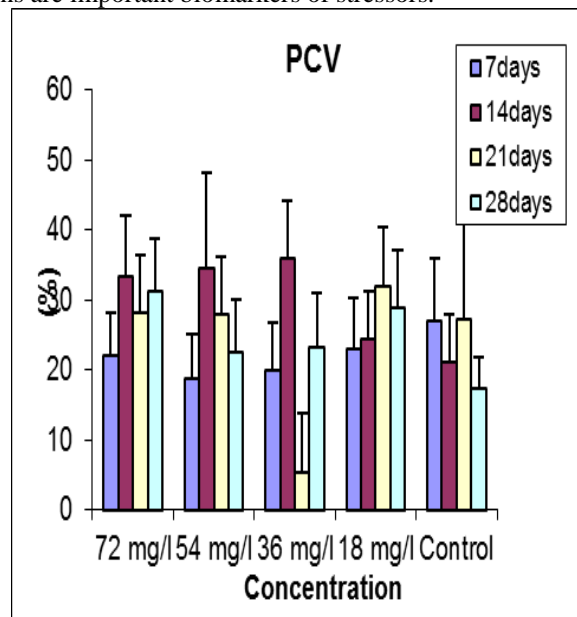


Fig 1b

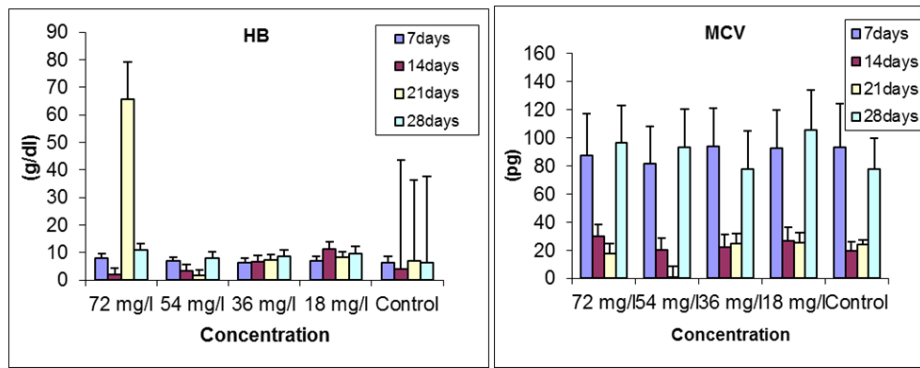


Fig 1c

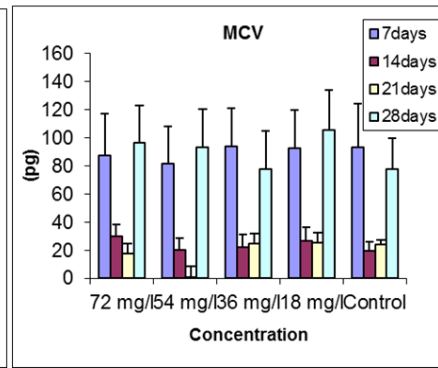


Fig 1d

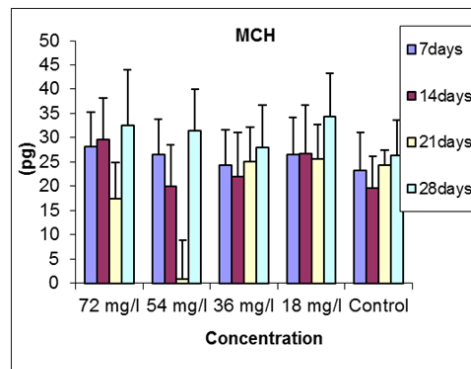


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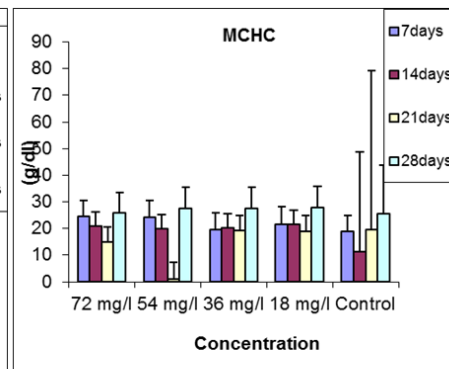


Fig 1f

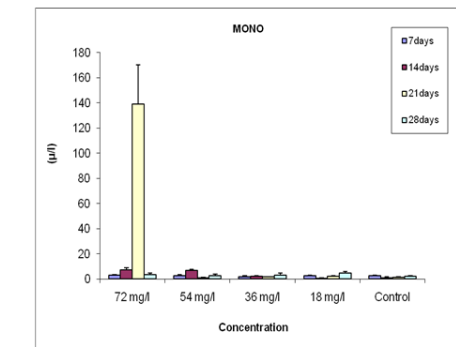


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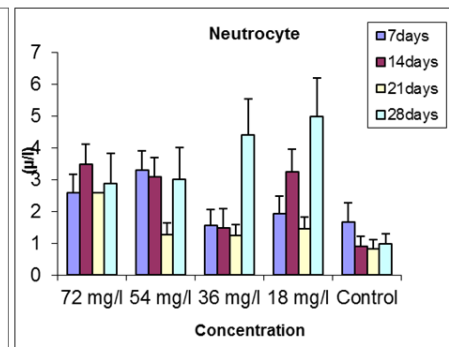


Fig 1h

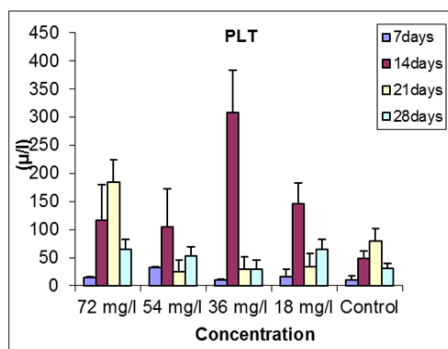


Fig 1k

Fig. 1a–k: showing the alterations in haematological indices of *C. gariepinus* exposed to varying concentration of glyphosate for 28 days.

Table 1: Summary Table of Haematology Indices Variation for Four Week.

RBC	Conc.	72 mg/L	54 mg/L	36 mg/L	18 mg/L	Control	P-value
		$\bar{X} \pm S.E$	$\bar{X} \pm S.E$	$\bar{X} \pm S.E$	$\bar{X} \pm S.E$	$\bar{X} \pm S.E$	
RBC	7days	2.92±0.4394	2.435±0.4156	2.195±0.4383	2.0575±0.4953	2.1±0.7012	P>0.05
	14days	3.4±0.6351	2.775±0.6669	4.025±0.7445	2.4325±0.5636	2.175±0.7296	P>0.05
	21days	3.415±0.5677	1.14±0.5349	2.75±0.4823	2.56±0.5007	1.425±7.7201	P>0.05
	28days	3.52±0.4823	2.625±0.4849	2.8475±0.5413	2.44±0.6031	2.175±0.7296	P>0.05
WBC	7days	35.875±10.35	28.2±10.4448	27.275±11.1369	35.675±12.2368	41.1±11.0484	P>0.05
	14days	47.725±25.254	45.9±12.2355	4.025±9.1868	36.325±10.4295	2.1±0.7047	P>0.05
	21days	24.775±12.414	2.7±1.3.270	41.15±12.1284	46.375±12.4128	41.175±10.9831	P>0.05
	28days	44.75±11.7221	30.175±11.6159	41.925±12.2459	46.1±12.4667	27.2±8.4528	P>0.05
PCV	7days	22±6.27277	18.75±6.457	20±6.8693	23±7.3813	27±9.03696	P>0.05
	14days	33.4±8.6531	34.65±13.5860	35.9±8.224556	24.4±6.7579	21.15±6.8638	P>0.05
	21days	28.15±8.2185	27.9±8.3483	5.4275±8.4425	31.9±8.4054	27.15±17.9435	P>0.05
	28days	31.15±7.5902	22.65±7.4062	23.15±7.7859	28.9±8.2898	17.4±4.4351	P>0.05
HB	7days	7.925±1.5749	6.85±1.6037	6.35±1.6857	6.925±1.7961	6.4±2.1342	P>0.05
	14days	2.2±2.1739	3.45±2.2354	6.55±2.3960	11.35±2.6824	4.2±39.5101	P>0.05
	21days	65.65±13.7161	1.65±2.0465	7.475±1.9485	8.2±2.0558	7.125±29.3394	P>0.05
	28days	11.05±2.2556	8.1±2.2332	8.675±2.3934	9.625±2.5409	6.45±31.3048	P>0.05
HCV	7days	87.625±29.4599	81.825±25.9523	94.025±26.6845	92.8±26.6771	93.075±31.0250	P<0.01
	14days	29.65±8.4410	19.95±8.6039	21.95±9.1986	26.65±10.0016	19.65±6.5505	P<0.01
	21days	17.5±7.3930	0.9±7.9212	25.025±7.0545	25.525±7.1942	24.3±3.1672	P<0.01
	28days	96.1±26.884	93.075±26.9119	77.45±27.1312	105.175±28.5599	77.925±21.7998	P<0.01
MCH	7days	28.075±7.1618	26.575±7.1839	24.325±7.2731	26.575±7.5459	23.325±7.7751	P<0.05
	14days	29.65±8.4410	19.95±8.60395	21.95±9.19855	26.65±10.0017	19.65±6.5505	P<0.05
	21days	17.5±7.3931	0.9±7.9211	25.025±7.054452	25.525±7.1942	24.3±3.1673	P<0.05
	28days	32.5±10.8543	31.425±8.5899	28.025±8.6764	34.3±9.0506	26.25±7.4609	P<0.05
MCHC	7days	24.4±6.2534	24.3±6.3064	19.475±6.31108	21.55±6.6808	18.825±6.0582	P>0.05
	14days	21.025±5.2911	19.95±5.31773	20.225±5.3814	21.7±5.2891	11.4±37.4398	P>0.05
	21days	14.8±5.9200	0.9±6.3496	19.175±5.6608	18.85±5.8922	19.65±59.5744	P>0.05
	28days	25.9±7.51332	27.625±7.7387	27.625±7.8501	27.85±7.9978	25.65±18.2564	P>0.05
PLT	7days	15±12.38055	32±13.3762	10.5±12.6201	15.5±14.4087	10±7.0711	P<0.05
	14days	116.65±63.325	104.45±67.9542	308.95±74.4680	145.9±36.4168	48.9±13.4682	P<0.05
	21days	184.9±38.6517	25.65±20.6241	29.9±21.8842	33.4±23.4139	78.9±23.2743	P<0.05
	28days	65.4±16.7215	53.4±15.9199	29.15±16.1496	64.9±17.4036	30.15±9.7829	P<0.05
LY	7days	34.525±9.7719	25.125±9.8036	26.975±10.5237	32.575±11.4903	39.225±13.0814	P>0.05
	14days	40.05±12.2941	37.275±12.5364	40.55±13.0561	3.325±13.2283	33.3±10.0946	P>0.05
	21days	22.225±11.5876	2.25±12.5113	4.55±12.5647	43.6±11.7469	39.375±39.0579	P>0.05
	28days	40.9±9.82342	26.4±9.4350	35.75±9.9224	37.45±9.9312	24.585±7.8163	P>0.05
MONO	7days	2.875±0.6089	2.725±0.6127	1.85±0.6376	2.425±0.7117	2.475±0.7294	P>0.05
	14days	7.225±1.5338	6.6±1.25346	2.15±0.3622	0.55±0.2473	0.9±0.7287	P>0.05
	21days	139.15±30.8452	0.975±0.43464	1.55±0.3525	2.125±0.4009	1.2775±0.5482	P>0.05
	28days	3.325±1.1774	2.625±1.2884	3.125±1.4097	4.525±1.6006	2.0475±0.5608	P>0.05
NEUT ROCY TE	7days	2.575±0.6007	3.3±0.5959	1.55±0.5023	1.925±0.5408	1.65±0.6104	P<0.05
	14days	3.475±0.6238	3.075±0.6086	1.475±0.6101	3.25±0.6994	0.9±0.3109	P<0.05
	21days	2.575±0.0009	1.275±0.3719	1.25±0.3276	1.45±0.3563	0.825±0.2869	P<0.05
	28days	2.875±0.9399	3±1.01851	4.4±1.1419	4.975±1.2172	0.9675±0.3327	P<0.05

Note:

P>0.05 - There is no significant difference

P<0.01 - There is highly significant difference

P<0.05 - There is significant difference

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