GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES, VOL.8, NO. 3 JUNE 2002:305 - 309
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EFFECTS OF INDUSTRIAL EFFLUENTS ON SOME HAEMATOLOGICAL PARAMETERS OF *SAROTHERODON MELANOTHERON AND TILAPIA GUINIENSIS*

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(Received 2 July 2001; Revision accepted 10 January 2002)

ABSTRACT

The effects of petroleum refinery and fertilizer plant effluents on some haematological parameters (packed cell volume - PCV, Total Leucocyte Counts - TLC and Differential Leucocyte Counts) in two species of cichlids; *Sarotherodon melanotheron* and *Tilapia guiniensis* were studied. This was with a view to assessing the usefulness of these parameters as indicators of pollution in Niger Delta Estuaries. Average Packed Cell Volume (PCV) ranged from 24.4 ± 3 to 24.4 ± 4.4 cm^3 in *T. guiniensis* while the values for *S. melanotheron* ranged form 24.3 ± 3 to 26.5 ± 4.7 cm^3. PCV is probably not a good indicator for contaminants in both species. There were high significant differences ($p<0.0001$) in Total Leucocyte Counts of the two species from the various sites. The highest values for both *T. guiniensis* (18427 ± 3896 cells/l) and *S. melanotheron* (17776 ± 3656 cells/l) were found in samples from creeks receiving fertilizer plant effluents, while values for samples from the control creek were the lowest giving 9042 ± 2859 cells/l for *T. guiniensis* and 8662 ± 2985 for *S. melanotheron*.

Key words: Haematology, *Tilapia*, Packed Cell Volume, leucocyte counts, Industrial effluents.

INTRODUCTION

Contaminants have been noted to affect many levels of biological organization, and a number of these levels can be used to monitor the effects of toxic materials on aquatic organisms (Giesy and Allred, 1985, Cairns 1986). Studies on the effects of contaminants in fishes indicate detectable changes in fish physiology (Sindermann, 1988).

The haematological parameters used in the assessment of fish health conditions include variations in leucocyte counts, differential white blood cell counts, antibody titration, macrophage phagocytic activity and the oxidative activity of neutrophils (Sunyer and Tort, 1995). In this study we examine the responses of some of these haematological parameters in *Sarotherodon melanotheron* and *Tilapaia guiniensis*, to fertilizer plant and oil refinery effluents with a view to selecting these species as potential indicators of pollution in the Niger Delta.

MATERIALS AND METHODS

Three sites (within the creeks of the Bonny Estuary) were chosen for this study (Fig 1). Two of the creeks receive industrial effluents; one (Okwetoru creek) from the National Fertilizer Company of Nigeria (NAFCON) and the other (Ekerekana creek) from the Port Harcourt Refinery Company. The third site located near George Ama, ca. 4 km upstream of the refinery creek served as a control. The study was carried out over a four-month period from June to September (1999).

SAMPLING AND ANALYSIS PROCEDURES

Water

Water samples were collected for the analyses of pH, ammonia, phosphorus, biological oxygen demand (BOD), oil and grease. BOD was analysed according to the procedures of APHA (1992) while pH, ammonia, phosphorus and oil were analysed using the methods outlined in ASTM (1989).

Fish

Live specimens of *Tilapia guiniensis* and *Sarotherodon melanotheron* used for the study were obtained live from the creeks (Fig 1), with the aid of chicken wire mesh traps. The length of

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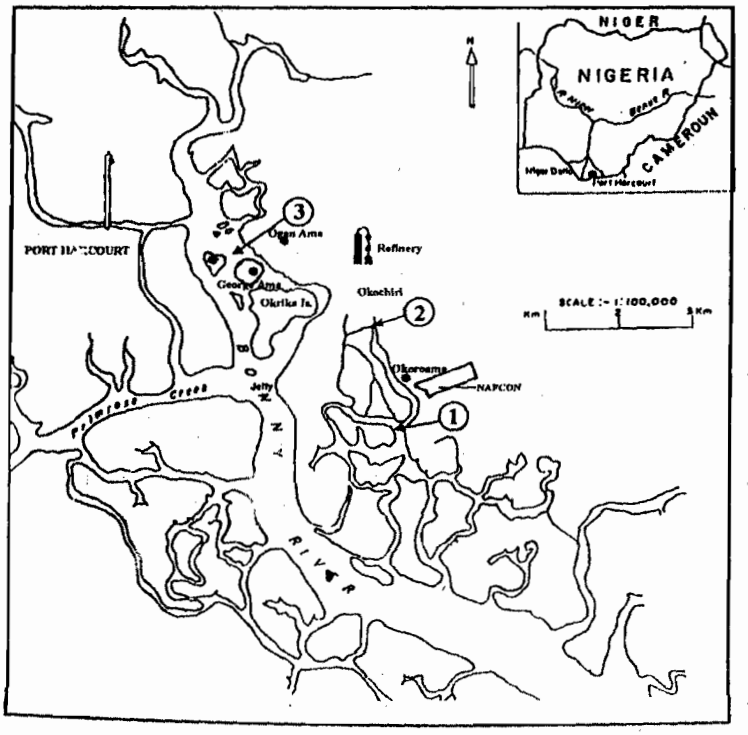

Fig. 1. Location of sample collection sites in the Upper Bonny Estuary

individual fish collected were measured, weighed and sexed. Each live fish was dissected ventrally (in order to prevent lysis of the blood), to open up the abdomen. A drop of anti-coagulant (Ethylene Diamine Tetra-acetic Acid - EDTA) was introduced into the dorsal blood vessels through the opened abdomen using plastic syringe (Blaxhall and Daisley, 1973). Blood was quickly drawn into an anti-coagulant bottle with a plastic suction syringe (Smith *et al.*, 1952). Blood samples taken were preserved in ice chest at about 4°C in the field and transported to the laboratory where they were stored in a refrigerator until analysis. The following haematological characteristics were examined:

Packed Cell Volume (PCV)

Plain capillary tubes were used to collect anti-coagulated blood for the estimation of PCV using the microhaematocrit method (Harper, 1965).

Total Leucocyte Counts (TLC)

The method described by Baker *et al.* (1966) was used in this estimation. In this method, 0.02 ml of blood was drawn with pipette and mixed with 0.38 ml of white cell diluting fluid (Turk's solution) to make a dilution of 1 in 20. White blood cells were then counted using the improved Neubauer counting chamber. The number of cells in two of the square-millimetre grids at the corners of the counting chamber was multiplied by 100 to obtain the total count (Baker *et al.*, 1966). To correct the total leucocyte counted, a correction factor (Turgeon, 1988) was used following the equation below:

$$WBC = \frac{\text{total leucocyte count}}{100 + \text{no. of nucleated RBC/100 WBC in differential count}} \times 100$$

Where WBC = white blood cells; RBC = red blood cells

DIFFERENTIAL LEUCOCYTE COUNT

The proportions of different types of white blood cells were determined. To do this, thin blood films were prepared and stained using Leishmann stain (freshly prepared) according to Baker *et al.* (1966). The films were observed by the battlement method using the oil immersion objective (x100) of a binocular microscope.

DATA ANALYSIS

The data were subjected to Analysis of Variance (ANOVA) to test for significant differences in the various haematological parameters between sites. Where significant differences were found, further analyses were carried out using Tukey tests (Zar, 1984) to compare the control site with the other sites. All data sets were transformed prior to analysis.

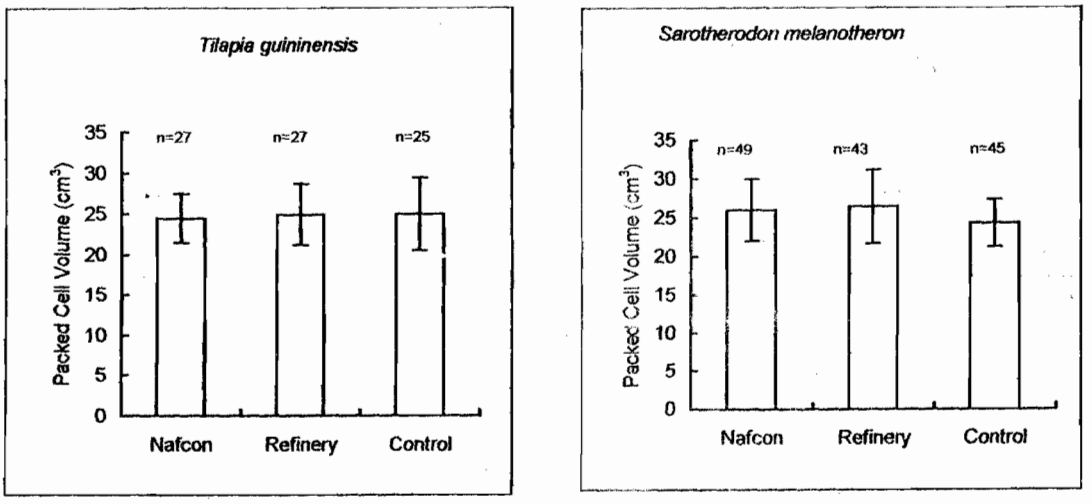

Fig 2: Packed cell volume (cm) in *T. guiniensis* and *S. melanotheron* from industrially contaminated and control sites

RESULTS

The physico-chemical characteristics of the three study sites are presented in Table 1. pH values ranged from 6.7 ± 0.3 at the Ekerekana (Refinery) creek to 8.5 ± 0.5 at the Okwetoru (NAFCON) creek. Analysis of Variance showed significant differences in pH between sites ($F_{2,6}$=25.5, p<0.001). The BOD values for the Oketoru (21.3 ± 6.4 mg/l) and Ekerekana (12.3 ± 0.6 mg/l) Creeks were much higher than that of the control with ANOVA showing significant differences between sites ($F_{2,6}$=39.5, p<0.001). The concentrations of ammonium-nitrogen varied from 0.01 ± 0.01 mg/l to 0.14 ± 0.02 mg/l, showing significant inter-site differences ($F_{2,6}$=7350, p<0.0001). The level of phosphorus was an order of magnitude higher in the Okwetoru creek with an average of 5.90 ± 0.02 mg/l than the Ekerekana (0.13 ± 0.01 mg/l) and Control (0.20 ± 0.02 mg/l) creeks, and ANOVA showed significant differences in the concentrations of phosphorus between sites ($F_{2,6}$=156, p<0.0001).

Fig 2 is a presentation of the values of PCV in *Tilapia guiniensis and Serotherodon melanotheron* from the three study sites. Average PCV ranged from 24.4 (± 3.0) to 24.9 (± 4.4) cm^3 in *T. guiniensis* while the values obtained for *S. melanotheron* were 24.3 (± 3.0) to 26.5 ± (4.7) cm^3. Analysis of Variance did not show any significant differences in PCV values between sites for *T. guiniensis* but was significantly different in *S. melanotheron* (p<005,). Tukey tests however, detected significant differences only between the samples from the Refinery creek in comparison with control individuals. No significant difference existed between the NAFCON and control sites with respect to PCV in *T. guiniensis*.

There were obvious differences in the Total Leucocyte Counts of the two study species from the various sties (Fig 3). The highest values for both *T guiniensis* (18427 ± 3896 cells/l) and *S. melanotheron* (17776 ± 3656 cells/l) were found in the samples from the NAFCON creek while the

Table 1. Water quality parameters (mean ± standard deviation) of the study sites

Parameter	Nafcon creek	Refinery creek	Control creek
pH	8.5 ± 0.5	6.7 ± 0.3	7.0 ± 0.2
Conductivity (mS/cm)	33767 ± 5198	25667 ± 9851	20833 ± 987
BOD (mg/l)	21.3 ± 6.4	12.3 ± 0.6	2.7 ± 0.6
NH_3-N (mg/l)	5.90 ± 0.02	0.13 ± 0.01	0.20 ± 0.02
PO_4 (mg/l)	0.14 ± 0.02	0.01 ± 0.01	0.02 ± 0.01
Oil content (mg/l)	<4	23.8 ± 24.1	<4

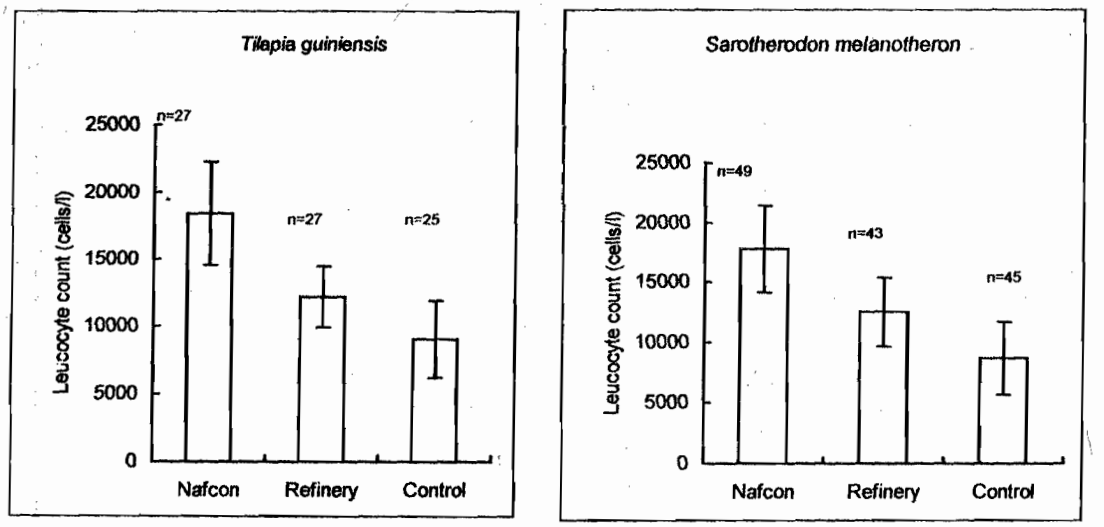

Fig 3: Total leucocytye counts (cells/l) in *T. guiniensis* and *S. melanotheron* from industrially contaminated and control sites

Table 2. Mean (± standard deviation) differential leucocyte counts (%) in *Tilapia guineensis* and *Sarotherodon melanotheron*

	Tilapia guineensis			*Sarotherodon melanotheron*		
	Nafcon	Refinery	Control	Nafcon	Refinery	Control
Lymphocytes	66.8 ± 3.5	67.6 ± 3.4	66.4 ± 3.4	67.8 ± 3.5	67.5 ± 3.1	68.1 ± 3.4
Netrophils	31.9 ± 4.1	31.6 ± 3.6	33.1 ± 3.9	31.7 ± 3.3	31.9 ± 3.2	31.3 ± 3.2
Eosinophils	1.2 ± 1.6	0.7 ± 1.0	0.5 ± 0.8	0.4 ± 0.9	0.6 ± 1.1	0.5 ± 1.0
Monocytes	0.2 ± 0.4	0.1 ± 0.3	0.1 ± 0.2	0.1 ± 0.3	0.1 ± 0.2	0.1 ± 0.3

lowest counts (9042 ± 2859 cells/l for *T. guiniensis* and 8662 ± 2985 cells/l for *S. melanotheron*) were found in the samples from the control site. These differences were statistically highly significant (ANOVA, $p<0.0001$). Tukey tests also found that there were significantly higher values of Total Leucocytes for both study species between both contaminated sites and the control site.

The differential leucocyte counts, showing the proportions of four cell types (lymphocytes, neutrophils, eosinophils and monocytes) are presented in Table 2. Lymphocytes represented by far the highest proportion of cells, accounting for 66.4 ± 3.4 to 66.8 (± 3.5) % in *T. guiniensis* and 67.5 (± 3.1) to 68.1 (± 3.4) % in *S. melanotheron*. This was followed by neutrophils; eosinophils and monocytes accounted for very low proportions of the leucocytes both accounting for less than 2%. There were no significant differences between sites in the proportions of lymphocytes and neutrophils in both study species.

DISCUSSION

The pH of the control station was about neutral, while that of the Ekerekana and Okwetoru creeks were slightly acidic and alkaline respectively. The difference in pH between the control site and the other sites is probably brought about by the nature of effluents discharged into these waters. There were also significant differences ($p<0.001$) in BOD between the two sites; showing similarity in the two sites as different from the control site. This probably also indicated that the creeks where the fertilizer and refinery plants are located are contaminated (see Table 1). However, differences in the nature of contamination between the NAFCON and Refinery creeks are further demonstrated by the values of ammonium-nitrogen, phosphorus and hydrocarbon content. The concentrations of ammonium-nitrogen and phosphorus were significantly higher in the Okwetoru creek where NAFCON is located ($p<0.0001$) than in the other creeks. We suspect that the high concentrations of NH_3-N and P are caused by the effluents from the fertilizer plant. In a similar manner, the Refinery effluents, which discharged into Ekerekana creek, contained high levels of total hydrocarbon compared to the levels of hydrocarbon measured in Oketoru creek and the control. In an earlier study, Falomo (1998)

had measured high concentrations of total hydrocarbons, nitrates and phosphates in these sites.

PCV did not appear to be a good indicator of contaminants in both species, although there appeared to be a potential for its use in *S. melanotheron* where its numbers were significantly depressed in response to refinery effluents. Soivo and Oikari (1976) also found that erythrocyte counts (as approximated by heematocrit) or haemoglobin was the most useful indicator of stress in the teleost, *Essox lucius*.

The results of this study showed that, of the haematological parameters examined, only total leucocyte counts (TLC) showed obvious effects of pollution in both *T. guiniensis* and *S melanotheron*. This was true for both the refinery effluent and the NAFCON effluent, and it may therefore be a useful indicator of general stress. The absence of significant inter-site differences in the percentages of the different leucocyte cell types implies that they were all affected in a proportionate manner. Acute exposure of salmonids to pulp mill effluents has been reported by McLeay and Gordon (1977) to result in moderate to severe leucopenia. McLeay and Gordon (1977) then suggested that leucocyte stress tests might be useful in the determination of threshold concentrations of industrial effluents and a number of chemical pollutants that are acutely stressful to salmonid fish. The pattern of response observed in the cichlids examined in this study was different from that observed for salmon in that it was akin to leucocytosis and extrapolations from laboratory dose-response relationships would be required before its usefulness for the determination of threshold contamination in the field can be assessed.

ACKNOWLEDGEMENT

We are grateful to Ollor A. Ollor of the Haematology Laboratory, Health Services Department, Rivers State University of Science and Technology, Port Harcourt for technical assistance.

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