E3 Journal of Medical Research Vol. 1(8). pp.098-0102, September, 2012
Available online @ http://www.e3journals.org
ISSN 2276-9900 © E3 Journals 2012

Full length research paper

The hematological changes of fish exposed to sublethal concentration of quarry dust

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Accepted September 6, 2012

Fishes *(Clarias batrachus*) were exposed to sublethal concentration of 10mg/L, 15mg/L and 35mg/L quarry dust (QD) on 15 and 30 days of exposure, haematology changes was determined on the blood obtained respectively with Junior Haematology Analyser. The values obtained shows of white blood cell (WBC), lymphocytes count (LYM), Lymphocytes percentage (LY), platelet percentage (PCT) were higher in fishes exposed with all concentration of the QD both in the short term (15 days) and long term (30 days) exposure, while Granulocytes percentage (GR) Red blood cell (RBC), Haemoglobin (HB), Heamatocrit percentage (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet PLT were lower than the control as compared, however they statistically show that the difference in 15 days in WBC, in 10mg/L exposure HGB in 15 – 25mg/L and PCT in 10mg/L exposure were not significant P>0.05. The result also showed that there were elevation in WBC, LYM, LY, MCH, MCHCH, PLC, PCT in fishes exposed for long term (LTE) compared to the short term exposure (STE), while all other parameter were decrease. Thus, the significant changes in some of the haematological parameters in most the concentrations administered suggest that quarry dust may have accumulative effect if exposed to quarry dust at higher concentration or lower but for long period of time.

Keywords: Exposure; Haematology; short term; long term; sublethal concentration

INTRODUCTION

Quarry dusts are fractionated rocks into small particle sizes which undergo further treatment converting these particle sizes into powdered forms. They are most reactive and mostly used by many manufacturing industries for various industrial processes (Ajayi and Aso, 1999). Quarry dust can have significant health and environmental effects. It is one of the dust that causes respiratory diseases, irritation of the eyes, skin, mucous membranes, cough, sneezing, rhenorrhea lacrimation (discharge of tears). A case of pneumoconiosis without pulmonary fibrosis attributed to limestone dust was reported in an office worker, whose unventilated office where he had worked for 24 years was situated within limestone mines quarry, that pneumoconiosis was attributed to the presence of silicates up to 10% (Crummy, 2004).

In Nigeria, a lot of quarry operations are going on at various parts of the country; products from these operations are mainly used for the road construction, cement production and refining for other essential metals. These operations lead to the production and discharge of large volume of dust into the atmosphere, on plants, water bodies or dust particles often inhaled by human and animals and could also settle on plants, buildings, water bodies and farm lands. Hence, environmental and health implications of these dusts on fauna and bio-fauna cannot be adequately ascertained except a thorough biochemical and physicochemical analysis.

The natural aquatic system may extensively be contaminated with heavy metals released from quarry dust (Velez and Montoro, 1998). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environmental and a diversity of aquatic organisms (Farembi, *et al*., 2002; Vosyliene and Jankaite 2006; Ashraj, 2005). Among the animal species, fishes detrimental effects of these pollutions (Olaita *et al*., 2004; Clarkson, 1998). Fishes are widely used to evaluate the health of aquatic ecosystem because pollutants build up in the food chain and are responsible for adverse effects and

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health in the aquatic system (Farkas *et al.,* 2002; Yusuf and El-Shahawi, 1999).Most studies on the effect of quarry dust/cement dust (Mojiminiyi *et al*., 2008; Alakija *et al*., 1990; Noor *et al*., 2000; Meo *et al*., 2002; Laraqui *et al*., 2001) are mostly on exposure in humans. Haematological examinations have been used as indicator of the physiological stress response to endogenous or exogenous changes and product systematic relationships and physiological adaptations of animals (Lermena *et al*., 2004; Kocabotmaz and Ekingen, 2000).

Thus this work aim to test the haematological changes manifestations associated with the dust hazards on the species of fish.

MATERIALS AND METHODS

Sample Collection

About 35 fishes (*Clarias batriachus*) were obtained from a private fish farm (Wahab fish farm, staff quarter, Kogi State University Anyigba, Nigeria). They were kept in 300 litre capacity plastic jar for 5 days in Biochemistry experimental animal house, Kogi State University, Anyigba Nigeria for acclimatization. They were fed with their normal feed obtained at Feedtop product, Akure.Ondo state, Nigeria.Fish of both sexes were used without discrimination.

Quarry Dust

About 200g of quarry dust was obtained at quarry site operation, Obajana cement industry, Oyi – Iwa, Lokoja Government Area, Kogi State, Nigeria following the permission by the factory management.

Experimental Animal Grouping

Initial weight of the fishes was carried out prior to the grouping; the average weight obtained was between 80 – 99.77g. The fishes were divided into five groups of six (6) fishes in each and exposed to different concentrations of quarry dust for 15 days at first instance and 30 days at second instance.The groups were labelled A, B, C, D and E where the first 4 groups were exposed to 10 mg/L, 15 mg/L, 25 mg/L and 35 mg/L of quarry dust respectively while group labelled E as control (no quarry dust added) in 30 L capacity plastic jar covered with net. The fishes were fed *ad libitum*. The water was replaced every two days intervals with the same concentrations of quarry of quarry dust as stated above.

Haematological Assay

At 15 and 30 days period, about 15 ml blood was collected from 3 fishes each by cardiac puncture into EDTA (Ethylene Diamine Tetraacetic acid) bottle from each group and was immediately analysed for haematological parameters according to the methods of Diatron group of company (1995) using Abascus Junior Haematology Analyser 2.75 (Diatro Count 3 Haematology EC Diatron, MJ PCC, Hungary).Red blood cell (RBC), Haemoglobin (Hb). Haematocrit (HCT), white blood cell (WBC), platelets count (PLT), lymphocytes count (LYM), pack cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), GRA, lymphocyte percentage (Ly).

Statistical Analysis

All data obtained were subjected to one-way analysis of variance (ANOVA) using Graphpad Instat Computer Program Software (Graphpad software; San Diego, CA, USA).

RESULTS AND DISCUSSION

The result in table 1 and 2 showed the values of the haematological parameters analysed on the blood of cat fish (*C. batrachus*) on the exposure of quarry dust to water samples.The haematological parameters, white blood cell (WBC), platelets count (PLT), platelets percentage (PCT), Lymphocytes count (LYM), lymphocytes percentage (LY%) values obtained increased from day 15 to day 30 of dust introduction in group A – D compared to control group E.On the other hand, Red blood cell (RBC), Haematocrit (HCT), haemoglobin (HGB), MCV, MCH and MCHC values were lower in treated fish blood samples.WBC increased for $8.54 \pm 26.80 \times 10^9$ (/l) in group B at 15 days to $82.20 \pm 0.80 \times 10^9$ (/l) in group C at 30 days. The values of RBC ranged between $21.00 \pm 2.09 \times 10^9$ (/l) and 6.49 ± 3.18 in control group E compared to $3.18 \pm 0.56 \times 10$ (/l) and $2.44 \pm 1.00 \times 10^2$ (/l) in treated group C and D in table 1 and 2 respectively. Lym has the lowest value $30.81 \pm 3.80 \times 10^9$ (/l) in control group E while the highest value $44.83 \pm 6.69 \times 10^9$ (/l) was measured in the blood sample in group B (Table 2). There was an increment in PLT $199.00 \pm 0.80 \times 10^9$ (/l) in control group E compared to $452.60 \pm 82.21 \times 10^9$ (/l) in group B.The HCT values ranged between $27.75 \pm 1.80\%$ to $39.00 \pm 3.80\%$. The highest value was recorded for control group E compared to the lowest value recorded in group D (Table 2). The highest values of HGB 129.67 ± 2.08 (g/l) was recorded in blood of fish in group E while the lowest value 108.00 ± 0.80 (g/l) was recorded in sample present in group C (Table 2). On the other hand, HGB value decreases from 50.00 ± 10.00 (g/l) in group A compared to 80.10 ± 9.61 in control group E (Table 2).Ly % value increased from 3.8

TABLE 1. Changes in the haematological parameter of fish exposed and quarry dust for 15 days

GROUP	DAY	WBC X 10^9 (g/l)	LYM X 10^9 (g/l)	LY (%)	GRA X 10^9 (g/l)	RBC X 10^9 (/1)	HGB (g/l)	HCT (%)	MCV (ft)	MCH (Pg)	MCHC (g/L)	PLT X 10^9 (g/l)	PCT (%)
A	15	40.16±2.20	36.18±5.20ns	35.10±2.3 ns	7.50±2.10 ns	5.47±3.10ns	121.67±4.16 ns	28.19±0.80ns	100.00±0.80	60.60±0.80	287.00±0.80	386.00±0.80	2.29±0.80
B	15	8.54±26.80ns	44.83±6.70ns	28.73±7.48ns	5.06±1.60	5.91±2.0ns	102.50±3.28 ns	30.65±10.10 ns	125.50±6.06	53.35±2.84	432.00±2.00	452.60±82.21	3.38±2.67
C	15	40.17±9.06	43.56±3.03	77.95±4.95	3.50±80ns	3.18±1.56ns	108.00±0.80	27.88±9.20ns	129.50±9.66	53.45±6.86	417.50±11	343.00±65.51	1.27±0.93
D	15	19.69±8.35ns	43.56±3.99	49.85±19.07	3.33±092 ns	15.32±15.66 ns	109.00±9.54	27.75±1.80ns	94.00±43.03	34.70±14.09	457.50±44.21	335.50±10.90	3.97±2.89
E	15	20.20±2.84	30.81±3.80	28.73±3.92	6.24±1.92	21.00±2.08	129.67±2.08	39.00±3.80	130.00±2.84	61.30±0.80	460.00±89.00	524.00±0.84	0.0±0.04

n = 3; ±SD. The mean with **ns** in the same column are not significant (P>0.05) to the control, while mean with and are moderately and extremely significant (P<0.01&P,0.001) respectively to the control.

TABLE 2. Changes in the haematological parameter of fish exposed and quarry dust for 30 days

GROUP	DAY	WBC X 10^9 (g/l)	LYM X 10^9 (g/l)	LY (%)	GRA X 10^9 (g/l)	RBC X 10^9 (/1)	HGB (g/l)	HCT (%)	MCV (ft)	MCH (Pg)	MCHC (g/L)	PLT X 10^9 (g/l)	PCT (%)
A	30	66.49±26.80ns	50.44±0.95ns	80.25 ±9.97 ns	5.49±1.62	3.00±1.34ns	50.00±10.00	20.76±1.63ns	79.52±3.41ns	54.85±7.65	445.50±57.16	571.00±66.19ns	3.40±1.01ns
B	30	72.71±2.31ns	38.98±1.39ns	3.80± 1.24	3.95±2.23	4.59±2.62ns	78.00±8.89	19.07±2.01ns	117.00±17.06ns	61.75±1.36 ns	448.50±75.00ns	383.00±73.63ns	4.38±2.67ns
C	30	82.20±0.80	46.67±2.90ns	75.50 ±0.80 ns	3.50±0.80ns	6.37±0.80ns	79.50±8.68	19.78±0.80	99.00±0.80ns	48.10±0.80 ns	482.00±0.80	386.33±1.17 ns	4.30±1.80ns
D	30	60.06±14.81ns	49.20±22.41ns	90.20 ±1.40 ns	1.35±0.97ns	2.44±1.00ns	54.00±5.29	19.37±1.25ns	123.65±37.66ns	46.65±13.88ns	554.67±15.28ns	514.00±58.10ns	3.75±3.89ns
E	30	37.95±15.10	36.60±6.56	96.20 ±7.28	5.96±3.18	6.49±0.96	80.10±9.16	30.03±12.9	407.00±518	77.87±1.944	638.00±29.00	359.67±29.02	2.03±0.87

n = 3; ±SD. The mean with **ns** in the same column are not significant (P>0.05) to the control, while mean with and are moderately and extremely significant (P<0.01&P,0.001) respectively to the control.

± 9.97% in group D at 15 days to 96.20 ± 7.28% in control group E at 30 days.GRA value 3.33 ± 90 × 10^9 (/l) was designated D relative to high value of 5.49 ± 1.63 × 10 (/l) in control group.

The control group showed elevated blood level of MCV (130.00 ± 2.84), MCH (61.30 ± 0.80) and MCHC (460.00 ± 89.00), while the administration of dust lowered the level of this indices to 94.00 ± 43.03 (group D), 34. 70 ±14.09 (group D) and 287.00 ± 0.80 (group A) table 2 respectively. Table 2 also shows that there was significant difference in the treated groups A – D when compared to the controls.The results of the haematological analyses from the blood of *C.batrochus* on exposure to quarry dust demonstrated that there were changes in the haematological parameters from day 15 to 30 of the fish exposure to quarry dust.The values of RBC, HGB, HCT, MCV, MCH, MCHC and GRA decreased significantly (P<0.05) while WBC, PLT, PCT, LYM and LY increased (P<0.01) compared to the control, in tables 1 and 2. This study is in accordance with the findings of Kori-siakpere (1998) Das and Mukherjee (2000), and Patnaik and Patra (2006).There was significant (P<0.01) reduction in the value of HGB and RBC in treated groups compared to controls in day 15, this is an indication of severe anaemia, which may be as a result of exposure of quarry dust.

There were significant (P<0.01) decrease in HGB and RBC levels after a long time exposure to sublethal doses in day 30. Therefore, this may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production (Ahmad *et al*., 2008). It may also be due to an increase in the rate at which HGB is destroyed as a result of long time exposure to quarry dust.WBC functions majorly to fight infections, defends the body against invasion by foreign substances and to produce, transport and distribute antibodies in immune response. The increase in WBC s compared to control noted in the result (Table 1) may be as a result of stimulation of the immune system of the affected fish to fight the toxicity of quarry dust. This finding was in accordance with the report that infections and intoxication stimulate WBC in fish. Shakoori *et al.* (2009), Aziz *et al.,* (2006) and Kumar *et al.,* (2001) reported increase in the WBC count from different fish species to different fish species. It is also noted from the results (Tables 1 and 2) that there was significant (P<0.001) increase in WBC level, in fish administered with quarry dust for 15 days compared to the one administered for 30 days in all the groups.The increase n lymphocytes in the administered group compared to the control on day 15 may be attributed to immunological response of the fish to the toxicant (quarry dust), which may indicate a condition known as lymphocytosis (Williams *et al.,* 1989). The lymphocytes percentage increases significantly (P<0.01) in day 15 compared to day 30.

There was a significant (P<0.05) decrease in the levels of platelets in the treated group A – D administered with various concentrations of dust compared to the control group E in day 15. Platelets are nuclear and discoid; they measured 1.5 – 3.0 micro meter in diameter. The body has a very limited reserve of platelets, so they can be rapidly depleted when there is little infection (Wagner and Burger, 2003). This shows that increase in the concentration of quarry dust administered to the fish may lead to continuous drops in the level of platelets thereby causing infections.There was increase in day 30 compared to day 15, suggesting that the accumulation of metals (Nickel, Zinc, Lead) has stimulatory effects on platelets production, possibly by enhancing thrombopoieteinsis secretions (Malomo *et al.,* 2002).

There was decrease in HCT values in treated groups compared to controls in 15 and 30 days of quarry dust exposure. This shows that there was shrinking in cell sizes and it may be due to metals intoxication (Ahmed *et al.,* 2008). However, statistical analyses show that there was no significant difference (P<0.05) between the groups A – D and control groups E in 15 and 30 days.RBC indices such as MCV, MCH and MCHC decrease significantly (P<0.05) in treated groups compared to the control group E which indicates a sign of hypochromic microcytic anaemia which may occur due to the destruction or the inhibition of erythrocytes production as a result of accumulation of metals in the blood stream (Tonnesen *et al.,* 2006).There was increase in the value of MCV, MCH and MCHC in the day 30 compared to day 15 and this may due to prolonged exposure to quarry dust. In contrast to this finding, there was increase in RBC indices values in another species of fish (*Clarias gariepinus*) exposed to cadmium chloride (Aziz *et al*., 2000) and *C. idella* exposed to sublethal doses of mercuric chloride (Shakoori *et al.,* 2002).With these foregoing it is obvious that if fish is exposed to this dust for long period of time it may have some haematological damage which may lead to stimulation of other disease. To safeguard these, it is suggested, that the cement factory management should be containment or restriction of dust emission by the use of dust filters and this should be monitored with dust particles monitoring devices.

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