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Some Organic Content and Effects of Administered Dusts on Haematological Parameters in Apparently Healthy Goats

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Abstract: Organic composition and toxicity of dusts collected in and around Maiduguri metropolis were investigated. High performance liquid chromatographic technique was used to determine the presence of aromatic hydrocarbons (anthracene, naphthalene, benzene and pyridine) in the samples. Haematological parameters, Packed Cell Volume (PCV), White Blood Cell counts (WBC), Haemoglobin estimation (HB) and Red Blood Cell (RBC) counts were used as biomarkers to identify possible toxic effects of exposure to dust samples. Low concentrations (0.0157 ± 0.002 and $0.0133 \pm 0.004 \text{ mg g}^{-1}$) of anthracene and naphthalene were measured on the dust samples while benzene and pyridine were not detected. Intravenous and intratracheal administration of dust decreased significantly ($p < 0.05$) the RBC, Hb and PCV in goats when compared to the control group. The values of WBC however increased when dust was administered intratracheally and intravenously. The result obtained do not suggest any evidence of substantial anthropogenic pollution of this environment

Key words: Organic content, haematological parameters, dust, intratracheal, intravenous

INTRODUCTION

Particles in the atmosphere are made up of a variety of in-organic and organic materials which include polycyclic aromatic hydrocarbon, nitro aromatic hydrocarbons, heterocyclic quinones, aldehydes and aliphatic hydrocarbons and trace amount of heavy metals (Stanley, 2005). This is of grave concern due to the health hazards of these substances. On a global scale, particulate matter is becoming one of the most prevalent atmospheric pollutants: Increased population and the associated industrialization, urbanization and motorization have led to an increase in the release of these pollutants into the atmosphere (Lee *et al.*, 2003).

Evidence is emerging that long term exposure to particulate matter in air is associated with mortality and other chronic effect (Ogugbuaja *et al.*, 2004) Such effects have been suggested to be associated with long-term average exposures that are low. These studies suggest that the public health implications of particulate matter exposure may be indeed serious.

Maiduguri, (Lat. $11^{\circ} 50'N$, Long $13^{\circ} 10'E$) is located in Borno State. It occupies North east position of Northern Nigeria. Located in the Chad basin, the city has an open flat topography and this allows for the incursion of dust into the area. The state is known for its high commercial activities with its few scattered industries located in the state capital (Maiduguri). The state share borders with Chad, Cameroon and Niger Republic and records a lot of transportation activities within and to the neighbouring countries by heavy trucks. Consequently, toxic waste from exhaust of cars, motorcycles and trucks are emitted into the atmosphere. Welding and construction of metal doors and windows is

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commonly seen by the major roads in Maiduguri. These welding activities release a lot of metal particles into the environment. Lack of steady power supply in Maiduguri and elsewhere in the state, compelled business activities to rely on generator sets as alternative power source which in turn emit some pollutants into the atmosphere. Studies in Maiduguri, Nigeria, revealed persistence of particulate matter in the atmosphere (Moses *et al.*, 2007). The studies revealed that there are some anthropogenic contributions to heavy metals (Fe, Al, Ti, Pb, Cd, Cr, Cu, Mn, Zn, Ni and Co) in the dusts. The objective of this study is to determine the presence of naphthalene, anthracene, pyridine and benzene from dust collected around Maiduguri Metropolis and to estimate the effect of the dust on haematological parameter on healthy goats.

MATERIALS AND METHODS

This study was conducted in Maiduguri, Nigeria. Particulate matter present in Maiduguri atmosphere was collected throughout the periods of January to December 2006, by gravity deposition according to the method described by McTainsh and Walker (1982). Samples were collected from low and high density areas of the town; collection was made twelve times a month for each location for a period of one year. Monthly samples were pooled for subsequent analyses.

Extraction of Sample

Fifty grams of dust sample was weighed into a 1000 mL beaker and 500 mL of the extracting solvent (toluene) added. The mixture was extracted for 72 h using soxhlet apparatus. It was thereafter removed, cooled and filtered. The filtrate was evaporated on a water bath until the volume reduced to about 5 mL. Then 50 mL of water/acetonitrile/tri-aceticacid (75/15/10%) mobile phase was added to the extract. From this, 2 mL was further pipetted into another 10 mL volumetric flask and made up to the mark with the solvent system.

Standard Preparation

Accurate weight of 10 mg of naphthalene and anthracene were collected into a 100 mL volumetric flask. About 50 mL of the solvent system was added. It was then sonicated for 10 min, removed and allowed to equilibrate for 5 min. This was made up to the mark with the solvent system. A 2 mL portion was further pipetted into another 25 mL volumetric flask and made up to the mark with the solvent.

Ten milliliters each of benzene and pyridine were measured into 100 mL volumetric flask and about 50 mL of the solvent was added and sonicated for 10 min. This was removed and allowed to equilibrate for 5 min. A 2 mL portion was further pipetted into another 25 mL and made up to the mark with the solvent. The prepared standard solutions were mixed together in a 50 mL volumetric flask.

Determination of Organic Content in Dusts Sample from Maiduguri by High Performance Liquid Chromatographic (HPLC)

A reverse phase HPLC apparatus was used for separation. The apparatus consisted of an Altex pump model 110, a Rheodyne 7125 injector valve with a 100 μ L loop. The HPLC instrument was warmed for 5 min, flushed and stabilized with 20 μ L of mobile phase to read 90% full-scale deflection on the UV detector under the following conditions:

Column system: C-18 (packed with SiO₂ stationary phase)
Mobile phase: water/acetonitrile/tri-aceticacid (75/15/10v/v/v)
Mobile phase flow rate: 1.5 mL min⁻¹
Pressure drop: 170 bar (250 psi)

UV detector wavelength: 254 nm
UV detector sensitivity: 0.2 au/s
Temperature: ambient room temperature (37°C) (Knox, 1986)

The injector, column and detector were mounted in a cage and the chromatograms resulting from the sample elution were recorded by an integrated chromatographic trace. The standard for anthracene, naphthalene, benzene and pyridine were run individually and their retention times were noted. The combine standards were run on the high performance chromatography column and the chromatogram (peak) obtained. All samples were run in duplicate.

Concentration of sample in extract was estimated as described by Knox (1986).

Experimental Animals

Fifteen clinical healthy adult Sahel goats of both sexes were used for this study. The goats were fed with green leaves and husk of grains and water was provided *ad libitum*. The goats were separated into three equal groups. The first two groups were treated with 50 mg kg⁻¹ dust sample dispersal in phosphate buffered saline intravenously and intratracheally respectively, the third group received phosphate buffered saline intravenously. All the animals were handled according to the International Guiding Principles for Biomedical Research Involving Animal (Council for International Organizations of Medical Sciences, 1985) and certified by the Animal Ethical Committee of the Faculty of Veterinary Medicine, University of Maiduguri.

Haematological Parameters

Collection of Blood Samples

Five millimeters of blood samples were collected, following administration through the right jugular vein using a syringe and needle. Samples were collected at 0, 6, 12, 24, 72, 168, 336 and 672 h post exposure. Blood samples were kept in EDTA bottles for estimation of blood parameters.

Estimation of Red Blood Cells (RBC)

Preparation of Diluting Fluid

Formal-citrate (red blood cells diluting fluid) was prepared by adding 10 ml L⁻¹ of 1% formalin and 40% formaldehyde in 31.3 g L⁻¹ trisodiumcitrate.

Counting by Visual Method

A 1:200 dilution was made by adding 20 µL of blood to 4 mL of diluting fluid, in a test-tube (75×12 mm). The content of the test-tube was then thoroughly mixed by tilting at an angle of 120° combined with rotation. The counting chamber (Improved Neubauer counting chamber) was charged. The chamber was then left for about two minutes for the cells to settle. The cells were counted under the microscope using a x 10 objective lens.

Haemoglobin Estimation

Method

Blood sample (20 µL) was added to 4 mL of diluting fluid (Drabkins solution) in a tube. The test-tube was closed with a rubber bung and inverted several times and allowed to stand at room temperature for 5 min. The absorbance of HiCN standards and test samples were read at a wavelength of 540 nm using a colorimeter against a blank.

Calibration Curve

The absorbance of reference HiCN solution was similarly measured against a blank (ferricyanide reagent) at 540 nm and cell path of 1 cm.

The standard solution was diluted with the reagent 1 in 2, 1 in 3, 1 in 4 and 1 in 5. The absorbance readings were plotted against Hb concentration (g L^{-1}) to obtain the calibration curve. Sample Hb concentrations were obtained from the plot and recorded.

Calculations

N = number of cells in 80 small squares, 0.1 mm in depth (0.02 mL in volume):

$$\begin{aligned}\text{Red cell count per } \mu\text{L} &= N \times \frac{1}{0.02} \times 200 \\ &= N \times 10^4 \\ &= N \times 10^6 \text{ }^{-1}\end{aligned}$$

Determination of Packed Cell Volume (PCV)

The blood sample was collected directly into capillary tube (75×1 mm) by capillarity pressure, leaving about 15 mm unfilled. The tube was then sealed by heating the dry end in a fine flame. The tube was centrifuged using haematocrit centrifuge for 15 min and the packed cell was measured using a micro haematocrit reader.

Estimation of White Blood Cells

Preparation of Diluting Fluid

A 100 mL volumetric flask was half-filled with distilled water and then 2 mL of concentrated acetic acid was pipetted into the flask with a rubber safety bulb. The solution was then coloured to a pale violet colour with gentian violet.

Counting

The blood was diluted 1:20 by adding 20 μL of blood sample to 0.38 mL of diluting fluid in a 75×10 mm tube. The tube was then corked tightly and the suspension mixed for 1 min. The Neubauer counting chamber was filled using a Pasteur pipette and the cells counted using a microscope at x10 objective lens.

Calculations

$$\begin{aligned}\text{WBC counts per litre} &= N \times 10 (\text{x20 dilution}) \times 10^6 \\ &= N \times 200 \times 10^6 \\ &= N \times 20 \text{ per } \mu\text{L}\end{aligned}$$

Statistical Analysis

Test of significance between mean parameters was performed using analysis of variance (ANOVA) and the null hypothesis rejected at 5% level of probability.

RESULTS AND DISCUSSION

The concentrations of anthracene and naphthalene in the dust extract were generally low (0.0157 and 0.0133 mg g^{-1}), respectively. Pyridine and benzene were however not detected in the dust samples (Table 1). This indicates clearly that the concentration of anthracene was slightly higher than that of naphthalene in the dust extract. Only two peaks with retention times 3.88 and 8.76 were obtained. There were no peaks to suggest the presence of benzene and pyridine. The percentage peak area for anthracene and naphthalene were 4412 and 44758, respectively.

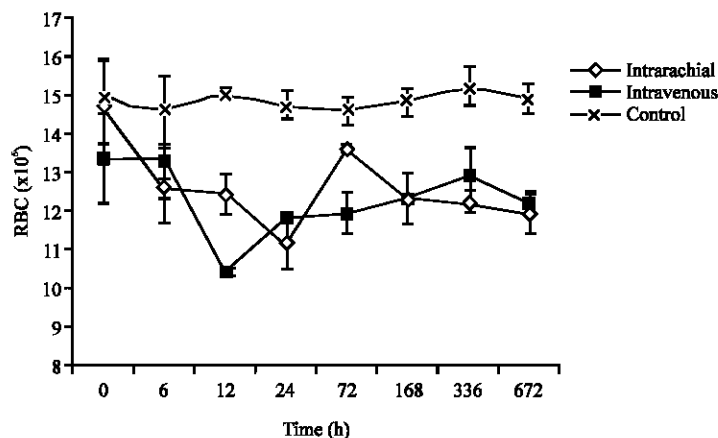


Fig. 1: Effect of dust sample (50 mg kg⁻¹) treatment on the RBC counts of goats

Table 1: HPLC determination of anthracene, naphthalene pyridine and benzene in dusts obtained from Maiduguri metropolis

Compound	RT	Area	Height	Conc. (mg mL ⁻¹)
Anthracene	3.89	4412	352	0.0157
Naphthalene	8.77	44758	2390	0.0133
Pyridine	12.29	-	-	-
Benzene	10.03	-	-	-

(Pyridine and benzene were not detected), RT= Retention Time

The RBC counts variations of the effect of dust sample (50 mg kg⁻¹) which ranged from 11.7×10⁶-14.6×10⁶ for intratracheal by administered animals and 10.5×10⁶-13.4×10⁶ for intravenously treated animals (Fig. 1). From the result of this experiment the RBC of intratracheally treated goats decreased following the administration of 50 mg kg⁻¹ of dusts. The same trend was observed for intravenously treated goats. Treatment with dust sample significantly (p<0.05) decreased the RBC counts of the treated goats when compared to the control.

The values ranged from 6.40-8.70 g dL⁻¹ for intratracheal treated animals and 6.5-8.5 g dL⁻¹ for intravenous treated animals (Fig. 2). From the result of this study the Intravenous and intratracheal treatment of goats with dust sample also decreased significantly (p<0.05), the values of haemoglobin concentration (Fig. 2), however with the termination of treatment the values of the haemoglobin concentration appear to improve, though not to the pre-treatment values.

The PCV concentration ranged from 0.23-0.26 g dL⁻¹ for intratracheally treated animals and 0.25-0.19 g dL⁻¹ for intravenously treated animals. The PCV of intravenously and intratracheally treated goats decreased significantly (p<0.05) following treatment when compared with the control. The PCV values were observed to recover following termination of treatment (Fig. 3).

Intratracheal and intravenous administration of 50 mg kg⁻¹ of dust to goats increased significantly (p<0.05) the white blood cells counts of the treated animals (Fig. 4) when compared with the control. The WBC concentration range from 13.00×10⁶-17.80×10⁶ for intratracheally treated goats and 11.9×10⁶-16.0×10⁶ for intravenously treated goats. Following termination of treatment, the WBC values were observed to decrease to about the pre-treatment values (Fig. 4).

From the results obtained in this study, there was no evidence of substantial contribution to organic pollution arising from anthropogenic or biogenic activities in the Maiduguri metropolis. The presence of anthracene and naphthalene in the dust samples (Table 1), although in small amounts, was an evidence of some form of anthropogenic contributions to these substances. Single ring aromatic compounds are important constituents of lead-free gasoline, which has largely replaced leaded gasoline

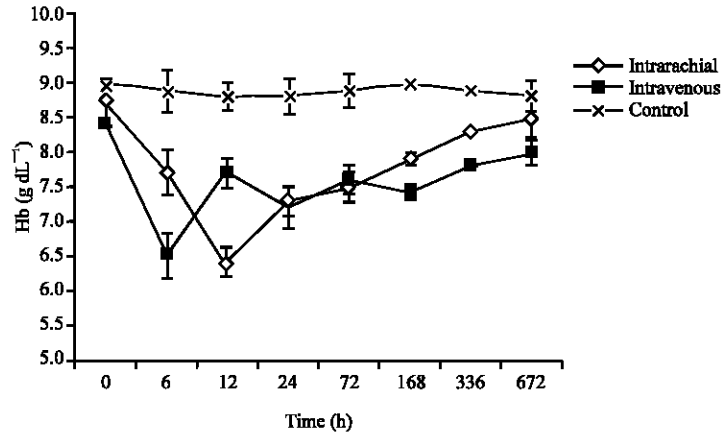


Fig. 2: Effect of dust sample (50 mg kg^{-1}) treatment on the HB of goats

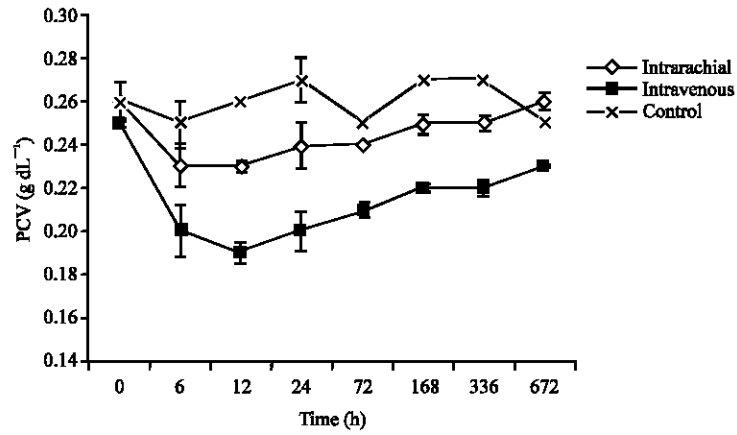


Fig. 3: Effect of dust (50 mg kg^{-1}) treatment on the PCV values of goats

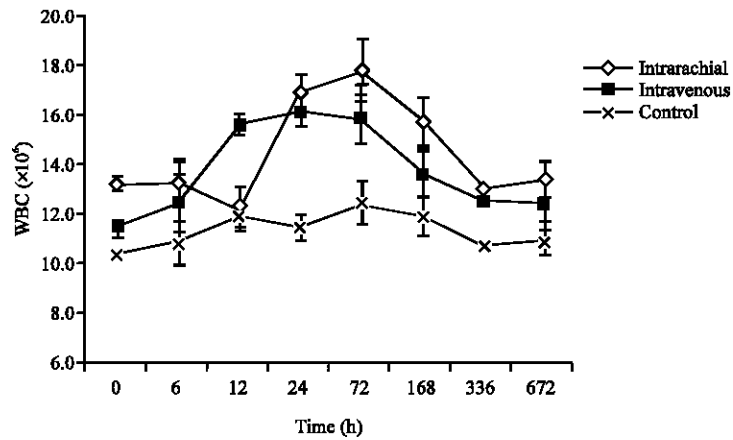


Fig. 4: Effect of dust sample (50 mg kg^{-1}) treatment on the WBC counts of treated goats

in Nigeria. They are also used as solvents in industry and are primary ingredients of tobacco smoke (Stanley, 2005). The absence of pyridine and benzene in the dust extract may suggest that these organic compounds are not prominent in the studied area. The specific sources of anthracene and naphthalene to the Maiduguri environment is however not clear.

The climatic condition around Maiduguri is likely to contribute substantially to the low concentration of organic compounds in dusts collected from around the area. In areas of high temperatures, aromatic hydrocarbons undergo partitioning and remain almost indefinitely in the gaseous phase (Stanley, 2005). It is therefore expected that most organic compounds in the area of study will preferentially remain in the surrounding gases rather than the solid particulates.

This study has shown that intravenous and intratracheal administration of dust resulted in reduction of RBC values as well as reduced Hb and PCV concentrations. Ogugbuaja *et al.* (2004) observed a similar decrease in haematological parameters of rats treated with fly ash. The decrease in PCV, Hb and RBC values in the present study indicates anaemia, which may have occurred as a result of inhibition of RBC production by the bone marrow or haemolysis. Chemicals are known to induce decreased bone marrow activity and/or cause RBC haemolysis (Turgeon, 1993). The increase in the WBC counts in intratracheally and intravenously treated goats compared to that of control may be as a result of possible stimulation of immune defence system. Furthermore, persistent antigen load in the body, results in increased WBC counts. The white blood cells are responsible for protecting the animals against foreign invasion by substances such as dust.

The effect of administration of dusts on the haematological parameters studied appears to be independent of the route of administration. The observation from the present study differs from that suggested by Ogugbuaja (1984) who administered dust orally. The observed differences in the two studies may be due to the different routes of administration used. The reactivity of the dust samples, though not clear seems to point to the tendency of inorganic and organic constituents of the dust to initiate and induce the formation of free radicals. Free radicals are reported to react and impede normal body metabolic functions. Metals such as Al, Fe, Co, Ni, As and Cr earlier reported to be present in the dust samples usually catalyse free radical formation and activate the formation of reactive oxygen species (Dreher *et al.*, 1997; Valleyathan and Shi, 1997; Smith *et al.*, 2000).

CONCLUSION

Results obtained from this analysis indicate that dust from around Maiduguri did not show evidence of substantial contribution to organic pollution arising from anthropogenic or biogenic activities; although the dust did show some negative effect on some haematological parameters following its administration.

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