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Some biochemical parameters in *Gallus domesticus* and heavy metal content of oil –polluted areas of Yenagoa, Bayelsa state, Nigeria.

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ABSTRACT
Serum glucose and liver ascorbic acid levels in Gallus domesticus , and heavy metal content of some parts of oil – polluted soil of Yenagoa city were determined in order to access the level, and the effect of pollution in those areas . Random sampling method was employed for the selection of the free-range birds used for the experiment. Spectrophotometric analysis was used to determine the hydrocarbon and glucose levels; while absorption spectrophotometry was used for the assessment of the heavy metals in the various soil samples. Our results showed that the hydrocarbon and heavy metal contents from contaminated soil were above levels recommended. The ascorbic acid levels in the control and test birds were 3.6±0.48 and 1.4 ± 0.32 mmole/L respectively. The values for serum glucose were 4.93 ± 0.39 and 10±0.89 mmole/L respectively. These differences are significant ($p<0.05$) and suggest the presence of stressors in the environment. Further studies are necessary to determine the effect of this pollution on the residents of Yenagoa.

Keywords: *Pollution, Gallus domesticus, toxic metals, ascorbic acid, soil ,glucose*

INTRODUCTION

The level of pollution of the environment due to oil spillage has steadily been on the increase in the Niger Delta region of Nigeria. It was estimated that about 2,500 cubic meters of oil was being introduced into the environment yearly, with an average of 300 individual spills occurring annually (Wales, 2009). According to this report, not less than 100 million barrels of oil was spilled between 1960 and 1997. Apart from these accidental oil spillages, the process of production, transportation and ultimate disposal or delivery to various depots could also introduce significant amount of oil into the environment.Pollutants can affect the biological system at different levels of biological organization. They include cellular, whole organism, and population/community. Biomarkers comprise biochemical, physiological and histological end-points which reflect an organism's attempt to compensate for or tolerate stressors in the environment. One set of biochemical markers is the biochemical parameters in the plasma, serum, or whole blood. Pollution of soil and water could be devastating and hence demand adequate attention. The reason is that man, and infact all living things, depend on soil /water (directly or indirectly) for their very existence and therefore when polluted, soil and water become ready media for transmission of diseases, and organic /inorganic toxic substances such as lead, mercury, cadmium and arsenic. The world health organization (WHO) in 1977 showed that drinking water, river, stream and coastal areas could be prone to pollution through dumping of refuse, sewage, industrial wastes, heavy metals and herbicides. Some of these pollutants are toxicants which eventually get into man by either exposure through the air and water, or indirectly by the ingestion/consumption of contaminated foods.

SCOPE

The present work covered Yenagoa (oil polluted) and Amassoma (non-oil polluted) areas of Bayelsa State. Soil samples from the two areas were determined for heavy metal contents and hydrocarbon levels. Also

analyzed were serum glucose and tissue ascorbic acid content of ten free-range *Gallus domesticus* obtained from both areas. These parameters serve as biochemical indices for the presence of stressors in the environment.

MATERIALS AND METHODS

Soil samples and free range *Gallus domesticus* were randomly collected from Yenagoa and Amassoma areas for analysis for hydrocarbon and heavy metal contents. The liver and blood samples of these fowls were collected for the purpose of determining the ascorbic acid and serum glucose levels respectively.

Preparation of Samples

Whole blood specimens from *Gallus domesticus* were collected into fluoride specimen containers after the birds were sacrificed by decapitation. The blood was allowed to clot, and then centrifuged at 2000rpm to obtain the serum, which was used for glucose determination, (Tilton *et al*, 1992).Two grammes of the liver was homogenized with 20mls of 10% acetic acid solution in porcelain mortar. The homogenate was centrifuged at 2000rpm for 10 minutes at ambient temperature, (Onyeike and Osuji, 2003), and the supernatant used for the determination of ascorbic acid.

Determination of Glucose in Serum

Glucose determination was carried out according to the method described by Tilton *et al* (1992). This entails the oxidation of glucose to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The hydrogen peroxide formed was reduced by peroxide-dye indicator, 4-aminophenazone. The oxidized dye was coloured and at such was measured quantitatively in a spectrophotometer. Into 1 ml serum sample were added, 3mls each of glucose colour reagent and phenol reagent. The solution was mixed for ten seconds and allowed to stand for ten minutes at room temperature. The absorbance of the solution was read at exactly 10 minutes at 600nm in a spectrophotometer against a reagent blank containing 1ml distilled water (in place of the serum samples), 3mls each of the glucose- colour reagent and phenol reagent. The standard was prepared by adding into 1ml glucose standard, 3mls each of glucose colour and phenol reagents. Glucose concentration was proportional to the absorbance at 600nm according to Beer- Lambert Law. This was calculated by dividing the absorbance of test sample by that of the standard and then multiplied by the concentration of the glucose standard.

Determination of Ascorbic Acid in Liver Tissue

The determination of liver ascorbic acid was carried out by indophenol method described by Onwuka (2005). Five millimeter supernatant obtained from liver homogenate was titrated with 2,6-dichlorophenol-indophenol to a faint pink colour end point. The ascorbic acid content was calculated by dividing the value of test by that of the standard and then multiplied by the concentration of the standard ascorbic acid solution.

Standardization of the indophenol solution was done by titrating 5ml of standard ascorbic acid solution with indophenol solution until a faint pink coloured persisted for 15 seconds.

Preparation of soil samples for analysis.

Sample Preparation:

Sample preparation involved the removal of large particles (those particles greater than 2mm in diameter) and sample homogenization. Large inorganic particles such as gravel, pebbles and rocks were removed due to their lack of contribution to THC and their chemical inertness. Samples were properly sealed and stored at 4^0C prior to analysis. This was necessary to minimize losses of organic compounds through microbial degradation.

Determination of Total Hydrocarbon (THC) Content in Soil Samples.

Determination of THC was carried out by the modified rapid oxidation method of Walkley-Black as explained by Charles and Simmons, (1986). One gramme soil sample containing dichromate and concentrated sulphuric acid was heated to 150^0C for 30 minutes and allowed to cool after which water was added to stop the reaction. The sulphuric acid was necessary for the removal of inorganic carbonate interferences, whereas the application of heat to the system was done to achieve a complete digestion of the organic carbon in the sample, a process which eliminates the use of correction factor. The temperature was strickly controlled so as to avoid the heat decomposition of acid dichromate solution.

Spectrophotometric Determination of THC Content of Soil Samples.

The spectrophotometric quantitation of THC was based on the measurement of the colour change that resulted from the presence of Cr^{3+} in solution. After sample digestion, the digest was filtered using Whatman number 1 filter paper. The filtrate was analyzed by measuring the absorbance at 601nm in UV-visible spectrophotometer which was previously calibrated using Bony light crude oil. Quantitation

was done by comparing the results against a standard curve prepared using known THC concentrations of Bony light crude oil.

Determination of Heavy Metals in Soil samples

About 2g soil sample was transferred into Kejaldah flask, followed by the addition of 20ml mixture of concentrated nitric acid and sulphuric acid (4:1). Sample was pre-digested by heating gently for ten minutes and thereafter for about 30-40 minutes. Digestion was stopped when a clear digest was obtained. The flask was cooled and the content transferred into a 50ml volumetric flask and made up to mark with distilled water. The resulting solution was filtered with Whatmann number 1 filter paper to remove the suspended particles, and then used for heavy metal analysis.

AAS analysis of soil samples.

The heavy metal analysis was done using Perkin 311 model Atomic Absorption Spectrophotometer, as described by Burtis and Ashwood (2001). From the stock solution of each element containing 10 parts per million (10ppm), three different standard solutions were prepared. A blank was prepared using water and nitric acid only. The blank was first aspirated into the flame to give a reading of zero absorbance. Thereafter each of the three standards was aspirated in-turn, starting from the solution with the lowest concentration. Each standard gave an absorbance value that corresponded to its concentration. With the standard curve the unknown concentration of the particular cation in the soil was obtained.

Air-acetylene gas was used as fuel, while the following wavelengths were used for the cationic estimations- Lead 283.3nm, cadmium 228.8nm, arsenic 193.70nm, mercury 253.65nm, zinc 213.8nm, and copper 324.8nm. All determinations were done in triplicates while student's t-test was used to test for significant differences between values.

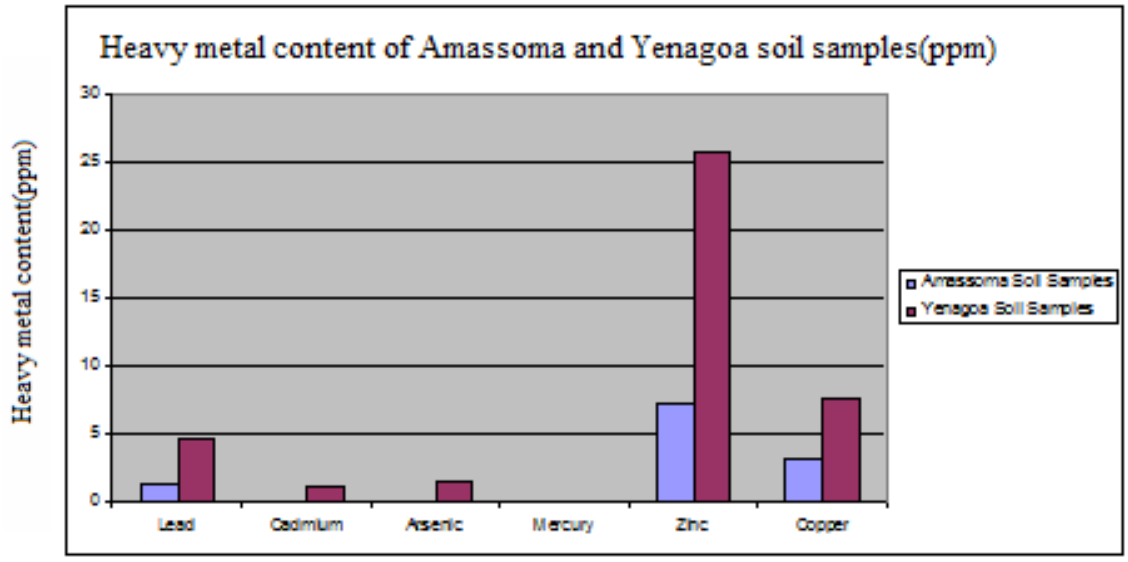

Figure 1: Heavy metals Content of Amassoma and Yenagoa Soil Samples (ppm)

RESULTS AND DISCUSSION

Soil Hydrocarbon level

Analysis of the soil samples showed that the mean (± SD) of total hydrocarbon content of Yenagoa (study area) and Amassoma (control area) were 192.87± 20.03 and 32.13 ± 2.20 mg/kg respectively.Total hydrocarbon content determined from Yenagoa-oil spilled areas was significantly higher ($p< 0.05$), than that from Amassoma non-oil spilled part and exceed the lower limit given in the literature (Wong,1996).

High levels of THC could be deleterious to health when consumed or by mere contact with the skin. Associated toxicological problems linked with the exposure to high levels of hydrocarbons include blood and kidney problems when inhaled, disorders of the central nervous system due to loss of myelin, and dermatitis when in contact with the skin, (Bhatia, 2006).

Toxic Metals in Soil

The heavy metal contents were 4.5 ± 0.02 for lead (Pb), 1.02 ± 0.01 for cadmium (Cd), 1.53 ± 0.03 for arsenic(As) and < 0.002 ± 0.01 for mercury, from Yenagoa study soils; while those from Amassoma control were 1.24± 0.21 for Pb, < 0.002± 0.0 for Cd, <0.001 ±0.0 for As, and < 0.002± 0.0(ppm) for Hg (Fig. 1).

Statistical comparison showed that the levels of the metals, Pb and Cd in Yenagoa soil were higher than the levels in Amassoma soil ($p < 0.05$). The mercury levels, less than 0.002ppm in both areas, were lower than the levels given in the literature (Wong, 1996)

Lead (Pb) is known to be widely distributed as metallic lead, inorganic and organometallic compounds. Most known toxic organo-metallic Pb compound is tetraethyl lead, used as an octane-boosting gasoline additive. Lead in this form has high affinity for lipid and for sulphydryl groups of many proteins. Evidence showed that Pb, at toxic levels could inhibit the activity of the enzyme, carbonic anhydrase - which functions in carbon dioxide transport by the blood. Also Pb can inhibit the synthesis of haemoglobin by blocking the ferrochelatase reaction. In the nervous system, Pb as well as mercury and cadmium can act as inhibitors of action potential, by antagonizing the specific calcium dependent process required for the release of the neurotransmitter from the presynaptic nerve terminus (Copper et al, 1984). This could be attributed to the fact that Pb can enter cells via hormone channels used by calcium. Inside the cell it binds with calmodulin and other calcium specific proteins (Linder, 1991).Systems involved with neurotransmission could be damaged in this way.

Arsenic is known to form a number of toxic compounds, which can coagulate proteins (hence can denature enzymes). It can form complexes with coenzymes and inhibits the production of high-energy compound, adenosine triphosphate, in essential metabolic pathways, (Bhatia, 2006).

Serum Glucose

The serum glucose level obtained from the birds in Yenagoa was 10.10 ± 0.89mmol/L which was significantly higher ($p<0.05$) than those in Amassoma, 4.93 ± 0.39mmol/L. Raised serum level of glucose obtained in *Gallus domesticus* in Yenagoa was viewed as an indication of abnormal metabolism of the compound. Abnormal glucose metabolism could arise from a defect in glucose uptake by the tissue cells particularly the hepatocytes. Therefore, diseases of the liver such as hepatitis, cirrhosis and obstructive disease can reduce the functional capability of the hepatocytes giving rise to raised glucose levels. Hyperglycemia has been linked with stress (Sibbergold**,** 1974). Sources of oxidative stress include raised levels of free radicals, toxic chemicals and metabolites which can overwhelm the normal body defenses and cause tissue damage. This damage give rise to various diseases depending on the tissue affected. Damage to hepatocytes is likely to affect glycogen storage functions and homeostatic control of blood glucose. On the other hand, damage to the pancreas could affect insulin production /release and subsequent glucose uptake by various cells.

Liver Ascorbic Acid

The liver ascorbic acid level determined from Yenagoa study area was1.40± 0.32mmol/L; while that of Amassoma control soil was 3.60 ±0.48mmol/L.

Ascorbic acid is one of the potential antioxidants in humans (Ayaori *et al*, 2000). Antioxidant reactions are part of the normal body defense mechanism against free radicals. Antioxidants neutralize the free radicals and protect cells against oxidative damage. Other known molecules with antioxidant functions include the metallo-enzymes superoxide dismutase, ceruloplasmin, glutathione peroxidase and catalase. Nutrients with antioxidant function include β-carotene, α-tocopherol (vitamin E) and glutathione.

Ascorbic acid is an excellent source of electron donor to free radicals thereby quenching their reactivity. It neutralizes the effects of many toxins including ozone, hydrocarbons and heavy metals (Ayaori *et al*, 2000). It can also regenerate the reduced antioxidant from vitamin E (Machlin and Bendich, 1982). Ascorbic acid is itself oxidized to dehydroascorbic acid (DHAA) in cells during the reduction of free radicals. When this occurs the body level of the vitamin decreases. Hence, abnormally low levels of vitamin C in the blood or tissues is an indication of either the presence of high levels of free radicals/reactive molecules in the body or low dietary supply. Since man cannot synthesize vitamin C, due to lack of the enzyme I- gluconolactone oxidase, it must be provided in the diet. Significantly lower levels ($p<0.05$) of ascorbic acid was determined in free-range birds from Yenagoa oil spillage areas than in those from Amassoma non-oil spillage terrain.The present work assessed the levels of total hydrocarbon (THC) and the heavy metal content of soil samples from Yenagoa oil - spilled area and Amassoma non-oil spilled areas of Bayelsa State, for the purpose of determining the level of pollution in the environment.

Marked differences were found between the levels of metals Pb, Cd and As in the areas studied, with Yenagoa having higher levels. Although the levels of the other metals, Hg, Zn and Cu were below critical literature range, it is our opinion that if adequate measures are not put in place in order to reduce the release of toxic chemicals into the environment, individuals who live in such areas will be exposed to the risk of toxicity. The raised level of serum glucose and low liver ascorbic acid concentration in the free range birds, support the fact that pollution control measures should be put in place without delay so as to forestall the harmful effects of environmental stressors to man. Further work should include the assessment of the levels of the heavy elements in individuals living in those polluted areas. Blood DHAA levels and urinary proline should be determined for proper assessment of vitamin C status. Due to the low level of the vitamin concentration in the birds, it appears that vitamin C supplementation may be ideal in the short run. Foods rich in ascorbic acid such as vegetables and fruits, particularly citrus fruits are recommended.

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