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Sublethal Toxic effects of spent Oil Based Drilling Mud and Cuttings to Earthworm *Aporrectodea Longa.*

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ABSTRACT: Sublethal toxic effects of spent oil based drilling mud collected from an abandoned oil drilling site in Mpanak, Akwa Ibom State, Nigeria were assessed in the earthworm *Aporrectodea lo*nga. The test annelid was exposed to sub-lethal Concentration of 0ppm SPP; 62,500ppm SPP; 125, 000ppm SPP; 250,000ppm SPP and 500,000ppm SPP of drilling mud for 28 days. The procedure for the exposure was according to the Organisation for Economic Co-operation and Development (OECD) recommended method for testing chemicals No 207. There were three replicate jars per treatment and 10 earthworms per jar of soil spiked with drilling mud. Control groups were also set up. At the end of the exposure, bioaccumulation of the chemical constituents of the drilling mud (heavy metals and TPH) increased ($p<0.01$) with increase drilling mud concentration. Mean weights of earthworms increased in the 62,500 and 125,000SPP and decreased in the 250,000 and 500,000SPP. Glucose levels increased with increase in the drilling mud concentration with the exception of 62,500ppm SPP. The elevated glucose levels could be due to hyperglycemia induced by the toxicity of the drilling mud. Protein levels increased with increase in drilling mud concentration except in the 125,000ppm SPP treatment. The increase in protein content could be due to enhancement of microsomal protein synthesis prompted by the toxicity of the drilling mud to test organisms. The results of this study have shown that drilling mud and cuttings could cause serious health risk to *Aporrectodea longa*. The discharge of drilling mud into the terrestrial ecosystems should be discouraged. © JASEM

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In Nigeria there are several oil and gas exploration and development activities going on. The practice of dumping spent drilling mud and cuttings into swamp cuts by oil and gas operators poses potential health risk to resident fauna (Ifeadi *et al*., 1985). While on one hand oil exploitation and exploration has improved the quality of life, on the other hand it has created a number of environmental and health hazards. The toxic chemicals associated with the activities are discharged into the environment which enter biological systems, disturb biochemical processes and alter normal organism functions.

Drilling mud/fluid is a term that refers to the lubricant used in oil drilling operations that stabilizes and lubricates the drill bit in the drilling process. They are suspension of solid in liquid emulsion and/or dissolved materials with chemical additives which are employed during exploration to remove cuttings. The impact of drilling mud discharges in the Niger Delta ecosystem of Nigeria is a current environmental concern, particularly with regards to the components that are used in formulating these drilling muds and the disappearance of the rich biodiversity of the Niger Delta, which is the key resource for sustainable development (Sikoki and Zabbey 2006). Studies have shown that drilling mud additives may contain toxic substances such as chromate, biocides, organic polymers, hydrocarbons, heavy metals and trace elements that have the tendency to bioaccumulate and interfere with normal biological activities of organisms including man (OGP, 2003; Wills, 2000). Earthworms have been used as biomarkers for assessing chemical environmental pollution (Olayinka *et al*., 2011).

Toxicological effects of spent oil based drilling mud and cuttings collected from an abandoned oil drilling site in Mpanak, Niger Delta Region of Nigeria were assessed in the earthworm *Aporrectodea longa.*

MATERIALS AND METHOD

Spent oil based drilling mud and cuttings were collected from swamp cuts close to an abandoned oil drilling site in Mpanak (point 1: 4°33'7.35"N, 8° 0'19.31"E; point 2: 4°32'41.75"N, 8° 0'1.71"E; point 3: 4°33'3.36"N, 7°59'43.19"E; point 4: 4°32'39.58"N, 7°59'34.99"E), Niger Delta Region of Nigeria. *A. longa* was collected from a semi-pristine environment at the Botanical garden of the University of Benin, Benin City, Nigeria. The test

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annelid was exposed to sub-lethal concentrations of drilling mud for 28 days after a range finding test. The procedure for the exposure was according to the Organisation for Economic Co-operation and Development (OECD) recommended method for testing chemicals No 207. Test concentrations were prepared according to Soegianto *et al*. (2008). Five sub lethal concentrations: 0ppm suspended particle phase (SPP); 62,500ppm SPP; 125,000ppm SPP; 250,000ppm, SPP and 500,000ppm SPP were prepared. SPP is the unfiltered supernatant extracted from a 1:9 mixture of the drilling mud and water that was allowed to settle for one hour. A sub sample of mud was mixed with water in a volumetric flask mud to water ratio of 1 to 9. This mud-water slurry was mixed with magnetic stirrers for 5 minutes. The slurry was allowed to settle for 1 hour. At the end of the settling period, the suspended particle phase (SPP) was decanted into a container. This decanted solution is defined as 100% SPP (equal to 1000 000 ppm SPP). Other percentages of SPP were prepared by volumetrically prepared by mixing 100% SPP with distilled water. Test soil (1kg) was spiked with a test concentration. There were three replicate jars per treatment and 10 earthworms per jar of soil spiked with drilling mud. The test jars were covered with net of vey small mesh size to allow aeration and prevent the earthworms from wriggling out of the containers. On completion of the experiment, the earthworms were removed, cleaned and voided. Earthworm samples were digested and analyzed as described by Bamgbose *et al* (2000) and Booth *et al* (2003). Thereafter, they were taken to the laboratory for heavy metal analysis (Bioaccumulation) using atomic absorption spectrophotometer and for total hydrocarbons analysis using UV/visible spectrophotometer (Searchtech 720).

Earthworm protein level was determined by the method of (Ryan and Chopra 1976). Five mls of Buiret reagent was added to 1ml of supernatant (0.2ml aliquot) of homogenate in 0.8ml phosphate buffer, pH 6.5. The tubes were incubated at 37^{o}c for 10 minutes and the absorbances were read at 540nm.

Glucose level of earthworm was determined according to Dubois *et al*., (1956). Two mls of aliquot homogenate was mixed in triplicate with 0.4ml of 80% phenol and 7.6ml conc. H_2SO_4. Earthworm samples were dissolved in 1:100 dilutions. The solution was incubated at 30 for 20 minutes and the absorbance was taken at 490nm.

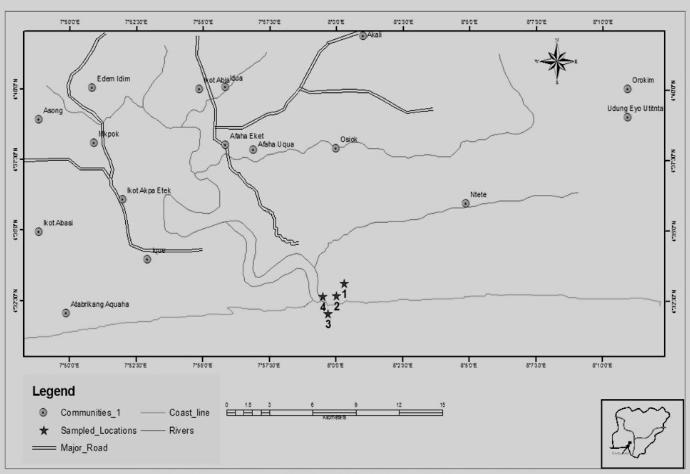

FIG. 1: MAP OF STUDY AREA SHOWING THE SAMPLED LOCATIONS

RESULTS AND DISCUSSION

At the end of the exposure, bioaccumulation of the chemical constituents of the drilling mud (heavy metals and total hydrocarbons) increased ($p<0.05$) with increase drilling mud concentration (Figures 1 and 2). All tested earthworms accumulated drilling mud but the highest bioaccumulation of heavy metals and total hydrocarbons occurred at 500,000ppm SPP and least at 62,500ppm SPP. This result shows that bioaccumulation of the constituents of drilling mud by the earthworm increased as the soil nominal drilling mud concentration increased. This is consistent with the findings of Morgan (1999) who studied the accumulation of metals (Cd, Cu, Pb and Zn) by two ecological contrasating earthworm species (*Lumbricus rubellus and Aporrectodea calignosa*). Similarly, the results of this study also agreed with that of Heikens *et al* (2001) who studied bioaccumulation of heavy metals in terrestrial invertebrates. Chemicals that persist in the environment tend to be bioaccumulated in the tissues of organisms since the transport medium and catabolic pathways for them may not have evolved.

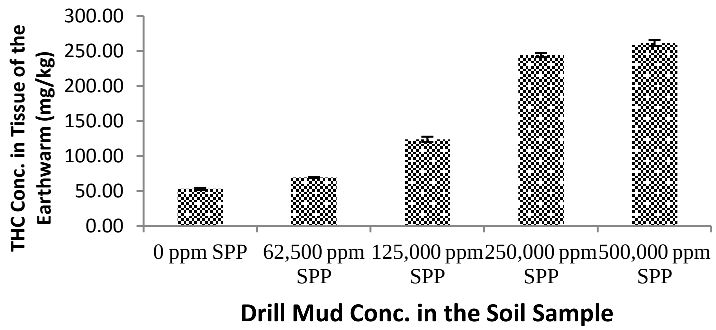

Fig 1 : Bioaccumulation of total hydrocarbons in earthworms exposed to spent drilling fluid and cuttings

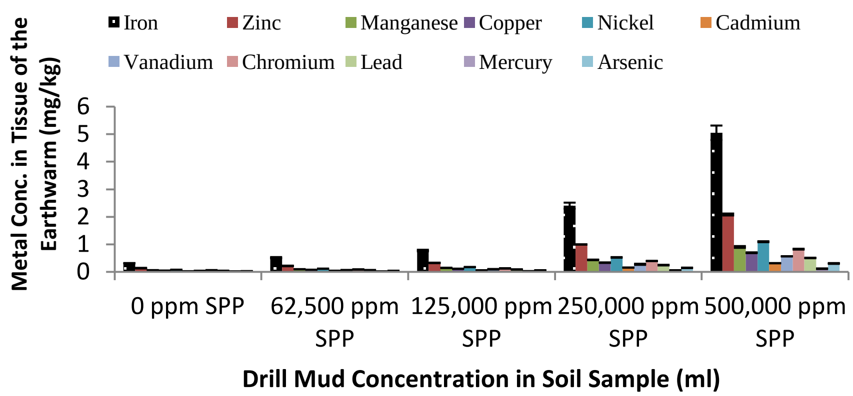

Fig 2: Bioaccumulation of heavy metals in earthworms exposed to spent drilling fluid and cuttings**.**

Glucose levels increased ($p<0.05$) with increase in the drilling mud concentration with the exception of 62,500ppm (figure 3). Previous research on glucose level in earthworm exposed to drilling mud is scarce. However, the increase in glucose levels observed in this experiment is consistent with the findings of Zikic *et al*., (1997) who studied activities of superoxide dismutase and Catalase in erythrocytes and transaminases in the plasma of Carps (*Cyprinus Carpio*. L.) exposed to cadmium and Levesque *et al*., (2002) who studied seasonal variations in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chemically exposed to metals in the field. Previous investigation proved that heavy metals, specifically cadmium modulates the metabolism of carbohydrates, causing hyperglycemia by stimulating glycogenolysis in some marine and fresh water fish species (Zikic *et al*., 1997).

Increased glucose levels observed in this study could be due to vulnerable stress induced by the heavy metals and other chemicals which resulted in hyperglycemia and synthesis of glucose from extra hepatic tissue proteins and amino acids. Increase in glucose level observed in this study also supports the findings of (Kumar and Bharthwal 1991) who observed significant increase in blood sugar level of rats fed on hexavalent chromium. The increase in the glucose level of earthworm could also be due to intensive glycogenolysis where glycogen reserves are being used in order to meet the toxic stress caused. Sobha *et al*., (2007) reported increased glucose level but decreased glycogen level in the fresh water fish, *Catla catla* (Hamilton) exposed to the heavy metal toxicant cadmium chloride

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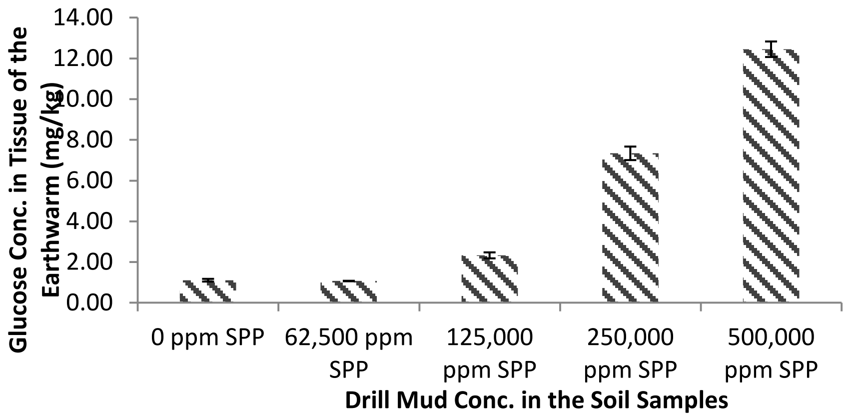

Fig 3: Glucose levels in earthworms

Protein levels increased with increase in drilling mud concentration except in the 125,000ppm SPP treatment (Figure 4). The complete function, architecture, and metabolic process of cells and tissues are built on their ability to synthesize proteins. Therefore when the level of protein in tissue or cell homogenate is measured, it provides a proximate detail of the architecture, metabolic function and pattern of gene expression. Elevation in protein contents was reported in the Air Breathing Fish, *Clarias batrachus* and in non-Air Breathing One, *Ctenopharyngodon Idellus* after treatments with cadmium (Joshi and Bose, 2002). The increase in protein content was attributed to enhancement of microsomal protein synthesis.

The decreased in protein level observed at 125,000ppm SPP concentrations which is slightly lower in comparison with control (Figure 15) could be associated with stress of toxicity of the drilling mud whereas the synthesis of stress proteins continued

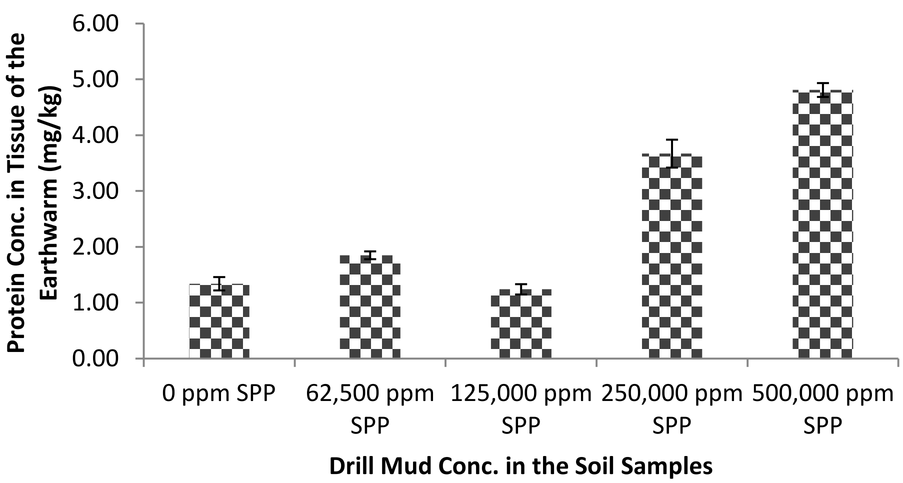

Fig 15: Protein levels in earthworm

Mean weights of earthworms increased ($p<0.05$) in the 62,500 and 125,000ppm SPP and decreased in the 250,000 and 500,000 SPP. The weight loss observed in A. longa in this study is in line with the study of Ezemonye and Enete (2004) who reported weight loss as a physiological biomarker of Cd and Pb toxicity to earthworms.

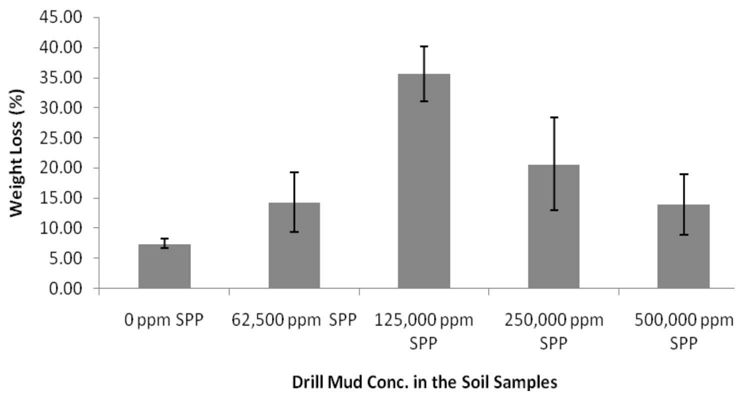

Fig 14: Weight loss (g) against concentrations (ppm SSP)

Conclusion: The earthworm *A. longa* highly bioaccumulated the constituents of the drilling mud at the end of the 28 days experimental period. Glucose levels decreased in *A. longa* exposed to the lowest concentration of drilling mud (62,500ppm SPP) and then there was a progressive elevation in higher concentrations, resulting in hyperglycemia. Protein levels increased with increase in drilling mud concentration except in the 125,000ppm SPP which showed a decline. Spent drilling mud and cuttings should not be discharged onshore where earthworms live as this will pose health risk to them. Alternatively, spent drilling mud and cuttings should be treated before disposal or dumping in terrestrial ecosystems.

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