Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
ISSN NO. 2320-5407
Article DOI: 10.21474/IJAR01/2650
DOI URL: http://dx.doi.org/10.21474/IJAR01/2650

RESEARCH ARTICLE

SERUM HEAVY METAL CONTENT OF ALBINO RATS EXPOSED TO BONNY LIGHT CRUDE OIL.

Igwe F. U[1], Orubite K. O[2] and Ogunka-Nnoka C. U[3].

1. Department of Biochemistry, Faculty of Science, Rivers State Uni. of Sci. and Tech., Port Harcourt, Nigeria.
2. Department of Pure and Applied Chemistry, Faculty of Science, University of Port Harcourt, Nigeria.
3. Department of Biochemistry, Faculty of Science, University of Port Harcourt, Nigeria.

Manuscript Info

Manuscript History

Received: 30 October 2016
Final Accepted: 29 November 2016
Published: December 2016

Key words:-
Bonny Light Crude Oil, Serum heavy metals, Dermal absorption, Bioaccumulation.

Abstract

Inhabitants of crude oil producing Niger Delta region of Nigeria are constantly exposed to constituents of crude oil due to frequent oil spills in the area. Consistent dermal contact of humans and animals with crude oil may lead to absorption of toxic heavy metals in the crude oil into systemic circulation. The level of some heavy metals in Bonny Light Crude Oil (BLCO) was evaluated using AAS. Sub-lethal doses of the BLCO were applied to shaved portion of the dorsal skin of treated male and female albino rats for 90 days while controls were similarly shaved without crude oil treatment. At 30 days intervals sets of rats were sacrificed and their blood collected for heavy metal analysis by AAS. The results showed that Cd, Pb, Ni, V and Cr were identified in the BLCO used at 0.15ppm, <0.05ppm, 3.34ppm, <0.10ppm and <0.01 ppm respectively. The analyzed serum heavy metal content showed that male albino rats had significant ($p<0.05$) increased levels of Ni, Cr, Pb and Cd at 30days post treatment and decreased levels at 90 days post treatment compared to controls, while female albino rats had significant ($p<0.05$) increased levels of Ni, Cr and Pb but significant ($p<0.05$) decreased level of Cd after 90 days treatment. These results suggest that the heavy metals in crude oil are being absorbed into the blood stream following consistent skin exposure to BLCO. This may lead to bioaccumulation of the heavy metals with possible systemic toxic effects like endocrine imbalance, organ dysfunction and hepatotoxicity.

Copy Right, IJAR, 2016,. All rights reserved.

Introduction:-

Crude oils contain a variety of complex organic and inorganic compounds including polycyclic aromatic hydrocarbons (PAHs) and heavy metals (IARC, 1989; USEPA, 2006). Over 600 potentially different hydrocarbons and metals have been reported and the chemical composition varies in different geological formation (Coppock *et al.*, 1995). The varying amounts of different hydrocarbon molecules, heavy metals and other contaminants affect the cost of processing or restrict the crude oil suitability for specific purposes (USEIA, 2006). The metal content in crude oils can also provide valuable information about the origin of crude oils and aid to identifying the source of oil spills (Jokuty *et al*, 1996).

Bonny light crude oil (BLCO) is often regarded as 'sweet' crude oil because of its low sulphur content being highly desirable for low corrosiveness to refinery infrastructure and low sulphur-linked environmental pollutants (Jokuty *et*

Corresponding Author:- Igwe F.U.
Address:- Department of Biochemistry, Faculty of Science, Rivers State Univ. of Sci. and Tech, Port Harcourt. Nigeria.

al, 1996). It has been identified to contain some of the various PAHs and heavy metals recorded as having the potential to substantially contribute to varying degrees of toxicity including carcinogenicity, mutagenicity, endocrine imbalance, organ dysfunction, etc (IARC, 1989; Eyong *et al.*, 2004; Zakrzewski, 2002; USEPA, 2006; Igwe, *et al*., 2016).

As the major foreign exchange earner of the Federal Government of Nigeria, crude oil exploration and exploitation activities and infrastructure are widespread in Nigeria especially in the Niger Delta region. Unfortunately, the health and ecology of the environment are negatively impacted as humans, animals, plants and soil organisms suffer the effect of crude oil presence in the environment. On entering the environment by spillage or other anthropogenic sources, crude oil undergoes rapid changes due to chemical reactions, photo-induced reactions, biochemical transformation and microbial degradation. Products from these reactions are sometimes more toxic than their original crude oil based sources (Bhatia 2005).

Crude oil spillages are common features in the aquatic and terrestrial landscape of the Niger Delta region. Unfortunately, ignorance, poverty and lack of basic social amenities have driven the inhabitants into unconventional and folkloric use of the readily available crude oil. Oil workers are in constant contact with it, the urban and rural populations use it for different purposes. It is sometimes orally ingested as medicine believing that it is an antidote to poisons and a cure for various gastrointestinal disturbances (Igwe and Lee, 2014). It is also liberally applied on the skin for massaging and the cure of such ailments as eczema, rashes, burns, foot rot, leg ulcers, arthritis, convulsion, witchcraft attack, etc (Orisakwe, *et al*., 2000; Otaigbe and Adesina, 2005; Igwe, *et al*., 2008).

Whenever crude oil spills occur, adequate post-spill response and remediation measures are slow or sometimes ignored. This allows the dispersion and retention of crude oil in the environment for a long time and increases the chances of human and animal contact with it. Rural farmers and fisher-folks are constantly exposed to crude oil contaminated waters, mud and soil. The ingestion and absorption of this crude oil either orally or through consumption of polluted species or by skin contact represents pathways for delivery of potential toxicants in the crude oil into the system. Other investigations on BLCO have reported that it is hepatotoxic, hematotoxic and nephrotoxic (Orisakwe *et al*., 2004; Orisakwe *et al*., 2005; Igwe *et al*., 2008).

However, there is dearth of information about systemic heavy metal levels associated with BLCO contact especially through the dermal route. Although many heavy metals are metabolically essential when present in trace quantities, they create physiological stress, generation of free radicals and production of reactive oxygen species (ROS) when present in high concentration (Adedara *et al*., 2011). Elevated levels of heavy metals can induce impairment and dysfunction in systems including the blood, liver, kidneys, colon, skin, cardiovascular, nervous and endocrine, energy production, gastrointestinal, reproductive and enzymatic pathways (Adedara *et al*., 2011).

This study investigated the dose-related, time-dependent variation in the serum level of some heavy metals following consistent topical dermal exposure of adult albino rats to BLCO. This was done to assess the potential of the heavy metals contained in the crude oil to infiltrate skin cells and become part of the systemic circulation.

Materials and Methods:-

This study was conducted using the True Experimental Design method in which the Experimental (treated) groups of animals were compared with Control (untreated) groups. The *in-vivo* biochemical experimental model was adopted with albino rats as the laboratory animals.

Sample Source:-

The Bonny Light Crude Oil sample was obtained from the Research and Development (R&D) unit of the Department of Petroleum Resources (DPR), Nigerian National Petroleum Corporation (NNPC) in Port Harcourt, Nigeria and transferred to the laboratory. The sample, contained in amber bottle, was stored in the dark at room temperature before use.

Laboratory Animals:-

Adult male and female albino rats weighing between 200 g to 250g and inbreed at the Biochemistry animal house of University of Port Harcourt, were obtained and transferred to the Biochemistry research unit of the Department of Chemistry, Rivers State University of Science and Technology, Port Harcourt. The 30 male and 30 female rats were housed in separate plastic cages, fed with rodent feed and water *ad libitum* and subjected to a well ventilated natural

12-hour light-dark cycle. They were allowed to acclimatize to this laboratory condition for 7 days before treatment commenced. The rats were randomly separated into groups (of 5 rats in each group) according to sex and duration of treatment. The treated group received sub-lethal dose of the BLCO by topical skin application. The dose (ml crude oil per cm^2 of exposed skin area) was determined using the relationship between surface area and body weight of animals (Zakrzewski, 2002). The requisite volume of BLCO was carefully smeared on a 2.0 cm^2 shaved portion of the dorsal skin of the treated groups while the control groups were also shaved but not treated with BLCO. This treatment was repeated every 48 hours for a total period of 90 days. All the rats were physically examined daily for anatomical and behavioral changes. **The investigation was carried out based on approved institutional guidelines for the care and handling of laboratory animals as** all the animals were treated in accordance with National and institutional guidelines for the protection of animal welfare during experiments (Public Health Service,1996)

Sample Collection:-

At the end of every 30 days, sets of rats (designated as Treated and Control) of each sex, were weighed, anaesthetized, sacrificed and their blood collected by cardiac puncture using sterilized syringe and needle. The blood was carefully transferred into plain poison free bottles and stored at 5^0C for serum analysis.

Determination of Heavy Metal Content in BLCO:-

Atomic absorption spectrophotometer (AAS) was used to evaluate the levels of Ni, Cr, V, Cd and Pb present in the BLCO. 10 ml of the crude oil was ashed in a furnace at 600^oC – 700^oC. After cooling to room temperature, the ash was dissolved in 10 ml of aqua regia and heated on a hot plate for digestion to take place. The digest was allowed to cool, dissolved in 20 ml of distilled water and filtered. The filtrate was subsequently injected into a GBC 902 double beam AAS which had been calibrated with the appropriate heavy metal standards.

Determination of Serum Heavy Metal Content:-

Blood in the plain poison free bottles were allowed to clot before they were centrifuged at 400 rpm for 5 minutes to get the serum. The clear serum was transferred into another plain bottle and digested in accordance with the method described by Hoenig and de Kersabiec (1996).The Ni, Cr, Pb and Cd content were then determined by aspirating the filtered digest into a GBC 902 double beam AAS recalibrated with the requisite standards.

Data Analysis:-

Results were expressed as mean of at least triplicate determinations ± standard deviation. Comparison between treated and corresponding control were made using student's t- test for equal variants. A value of $p<0.05$ was considered as statistically significant. Also, the magnitude of change of each parameter analysed over control was calculated in percentage and expressed as percent change

Results:-

Heavy Metal Content of BLCO:-

Results of the level of some heavy metals identified in the BLCO are contained in Table 1. Ni and Cd were present at 3.34 ppm and 0.15 ppm respectively while Cr, V and Pb were identified at levels below 0.05 ppm

Table 1:- Levels of Some Heavy Metals in Bonny Light Crude Oil.

Heavy Metal	Content (ppm)
Chromium (Cr)	< 0.01
Cadmium (Cd)	0.15
Lead (Pb)	< 0.05
Nickel (Ni)	3.34
Vanadium (V)	< 0.1

Serum heavy metal content:-

The serum levels of some heavy metals in the male albino rats investigated are contained in Table 2. There was significant ($p<0.05$) increase in Ni, Cr, Pb and Cd levels after 30 days exposure compared to control and significant ($p<0.05$) increase in Cr, Pb and Cd levels after 60 days exposure compared to control. However, there was significant ($p<0.05$) decrease in the serum Ni, Cr, Pb and Cd levels assayed after 90 days exposure compared to controls.

Table 2:- Serum Heavy Metal Levels of Male Albino Rats Exposed to BLCO.

Heavy Metals	Serum Levels (ppm)					
	30 days Control	**30 days Treated**	**60 days Control**	**60 days Treated**	**90 days Control**	**90 days Treated**
Ni	0.08±0.01	2.10±0.03[a] (2525)	2.43±0.02	2.29±0.02 (-5.76)	2.91±0.06	1.84±0.08[a] (-36.77)
Cr	0.02±0.00	0.95±0.01[a] (46.50)	0.53±0.01	0.84±0.01[a] (58.49)	0.81±0.02	0.45±0.01[a] (-44.44)
Pb	BDL	1.91±0.02[a] (191.0)	0.63±0.02	0.87±0.01[a] (38.10)	1.22±0.04	0.96±0.02[a] (-21.31)
Cd	BDL	0.23±0.04[a] (23.0)	BDL	0.76±0.02[a] (76.0)	0.45±0.02	BDL (-100.0)

Results are the mean ± standard deviation of 5 rats. [a] Significantly different from control ($p<0.05$).
Values in parenthesis represent percent change when compared with control. BDL= Below Detectable Limit

The serum levels of some heavy metals in the female albino rats investigated are contained in Table 3. The female albino rats showed significant ($p<0.05$) increases in serum Ni and Cr levels after 60 days exposure and significant ($p<0.05$) increases in serum Ni, Cr and Pb levels after 90 days exposure. However there was 76.92% and 27.63% decrease in Cd content after 60 days and 90 days exposure period respectively in the treated group compared to controls.

Table 3:- Serum Heavy Metal Levels of Female Albino Rats Exposed to BLCO.

Heavy Metals	Serum Levels (ppm)			
	60 days Control	**60 days Treated**	**90 days Control**	**90 days Treated**
Ni	1.58 ± 0.02	2.50±0.01[b] (58.23)	2.34 ± 0.01	2.98±0.02[b] (27.35)
Cr	0.73± 0.01	0.84±0.02[b] (15.07)	0.87 ± 0.03	1.25±0.02[b] (43.68)
Pb	0.95 ± 0.01	0.95±0.01 (0.00)	1.09 ± 0.01	1.45 ±0.04[b] (33.03)
Cd	0.65 ± 0.02	0.15±0.01[b] (-76.92)	0.76 ± 0.04	0.55±0.02[b] (- 27.63)

Results are the mean ± standard deviation of 5 rats. [b] Significantly different from control ($p<0.05$).
Values in parenthesis represent percent change when compared with control.

Discussion:-

Cellular toxicity is a function of the balance between the rate of formation of reactive metabolite and the rate of their removal. Skin absorption of environmental toxicants by mammals is being increasingly considered to be a significant exposure route. Previously the inhalation and oral routes were considered the most significant routes of exposure to environmental contaminants (Moody and Chu, 1995; Christopher *et al*., 2011). Most hydrophobic chemicals pass through the epidermal cells since it constitutes the major surface area of the skin. Passive diffusion is thought to be the main process responsible for these chemicals permeating through the skin with penetration through the *stratum corneum* being the rate determining step. Also, hair follicles and sweat glands provide further permeation routes into the dermis where the micro capillary bed of blood vessels receives the chemicals and they become bioavailable (Moody and Chu, 1995). Wester *et al.* (1990) had reported skin absorption of benzo(a)pyrene from soil contaminated with petroleum crude oil. However, mixtures of chemicals have been noted to exert synergistic or antagonistic effect on their degrees of dermal absorption as greater skin persistence of benzo(a)pyrene was observed when it was present on the skin with other PAH compounds (Dankovik *et al*., 1989).

From the results obtained in this study, BLCO contains some heavy metals (Table 1) which have the potential to exert various toxic effects. The Ni and V levels in the BLCO sample (Table 1) are below the 5.40 mg/kg and 1.90 mg/kg respectively reported by Eyong *et al*., (2004). However the Ni level of 3.34 ppm in BLCO is higher than the 2.20 ppm in South Louisiana crude oil but lower than the 10.0 ppm and 7.70 ppm levels in Prudhoe Bay crude oil and Kuwait crude oil respectively (IARC, 1989).

Heavy metals are known to elicit such toxic effects as skin irritation, ulceration, kidney damage, liver damage, endocrine imbalance as well as alteration in normal biochemical processes which can cause distortions in the synthesis of hemoglobin, effects on the gastrointestinal tract, joints, reproductive system, damage to the nervous system, decreased body weight, renal dysfunction, lung cancer, bone marrow defects, etc (Adedara *et al*., 2011; Achparaki *et al*., 2012). The significant ($p<0.05$) increases recorded for Ni, Cr, Pb and Cd especially during the initial 60 days of treatment, for the male albino rats, indicates the retention (bioaccumulation) of absorbed heavy metals circulating in the system thereby increasing the possibility of eliciting toxic effects during that period. Ni has previously been shown to induce apoptosis via pathways including generation of ROS and activation of protease enzymes involved in programmed cell death (Au *et al*., 2006). Cr is reported to be associated with allergic dermatitis (Achparaki *et al*., 2012) and Pb affects the red blood cells and the hemopoietic system, the immune system as well as causes damage to organs such as the liver, kidneys, heart and male gonads (Adedara *et al*., 2011). Exposure to Cd can cause a variety of pathological alterations in several organs and tissues as well as induce diabetic complications, hypertension and osteoporosis (Achparaki *et al*., 2012). Our result is partly in line with Adedara *et al*., (2011) who also reported the bioaccumulation of Pb in the blood of albino rats treated with BLCO. Undesirable levels of heavy metals in blood could therefore cause kidney damage which may affect the rate of elimination of the heavy metals and other xenobiotics subsequently absorbed. In this investigation, the bioaccumulation tendency in blood is more pronounced in the female albino rats compared to the male albino rats as significant ($p<0.05$) increases in the serum heavy metals levels were recorded even after 90 days exposure (Table 3) unlike the male albino rats (Table 2) where a significant ($p<0.05$) decreased levels of the serum heavy metals assayed were seen after 90 days exposure. The decreased levels may be attributed to a number of factors including increased uptake by various organs, adaptive response or increased glomerular filtration rate and reduced tubular re-absorption by renal cells as they become increasingly impacted. Adedara *et al*., (2011) reported high level of Ni accumulation in the liver and testes compared to blood in albino rats exposed to BLCO. Crude oil has also been reported to cause destruction of the renal reserve capacity and induced several pathological changes in the form of tubular necrosis on laboratory animals (Orisakwe *et al.,* 2004). Damage to renal cells could cause alteration in the steady-state concentration of blood components which may manifest in several toxic effects.

Conclusion:-

Continuous exposure of sub lethal doses of Bonny light crude on the skin of adult albino rats resulted in the absorption of heavy metals in the crude oil into the blood stream and subsequent bioaccumulation of the heavy metals in the system. The magnitude of heavy metals absorption and the extent of bioaccumulation in blood varied with the type of heavy metal, duration of exposure and sex of the animals.

After 90 days exposure, the initial rise in serum levels of Ni, Cr, Pb and Cd, decreased for the male albino rats while the levels remained higher among the female albino rats except for Cd where a 27.63% decrease was recorded. These findings suggest that chronic exposure to Bonny light crude oil via skin contact may produce systemic toxicity associated with absorption and bioaccumulation of the heavy metals in blood.

References:-

1. Achparaki, M., Thessalonikeos, E., Tsoukali, H., Mastrogianni, O., Zaggelidou, E., Chatzinikolaou, F., Vasilliades, N and Raikos, N (2012). Heavy metals toxicity. *Aristotle University Medical Journal*. 39(1): 29 -34
2. Adedara, I.A , Ebokaiwe, A.P and Farombi, E.O (2011) Tissues distribution of heavy metals and erythrocytes antioxidant status in rats exposed to Nigerian bonny light crude oil. *Toxicology and Industrial Health*.pp 1–7
3. Au, A., Ha, J., Hernandez, M., Polotsky, A., Hungerford, D.S. and Frondoza, C.G (2006). Nickel and vanadium metal ions induced apoptosis of T-lymphocyte Jurkat cells. *Journal of Biomedical Materials Research* 79: 512–521.
4. Bhatia, S. C. (2005). *Environmental Pollution and Control in Chemical Process Industries*. Khanna Publisher, Delhi (2nd ed.). pp 41 - 42
5. Christopher, Y., Tongeren, M.V., Urbanus, J and Cherrie, J.W (2011). An Assessment of Dermal Exposure to Heavy Fuel Oil (HFO) in Occupational Settings. *Ann. Occup. Hyg.,* 55(3): 319–328
6. Coppock, R. W., Mostrom, M .S., Khan, A. and Semalula, S.S. (1995). Toxicology of oil field pollutants in cattle: A review. *Vet. Hum. Toxicol.* 37: 369-576.
7. Dankovik, D. A., Wright, C. W., Zangar, R. C., Springer, D. L. (1989). Complex mixture effects on the dermal absorption of benzo(a)pyrene and other polycyclic aromatic hydrocarbons from mouse skin. *J. Appl. Toxicol*. 9: 239 - 244.

8. Eyong, E.U., Umoh,A B.I., Ebong, P.E., Eteng, M.U., Antai, B.A and Akpa, A.O (2004). Hematoxic effects following ingestion of Nigerian crude oil and crude oil polluted shell fish by rats. *Nigerian J. Physiol. Sci*. 19(1-2): 1- 6.
9. Hoenig, M, de Kersabiec, A.M (1996). Sample preparation steps for analysis by atomic spectroscopy methods: present status. *Spectrochimica Acta* B 51: 1297–1307.
10. IARC (International Agency for Research on Cancer). (1989). Crude Oil and Petroleum Products. In: *Monograph on the Evaluation of Carcinogenic Risks to Humans*. Lyon. 45. pp 126-152
11. Igwe, F. U. (2009). Systemic Toxicity Studies of the Epicutaneous Exposition of Adult Albino Rats to Bonny Light Crude Oil. *PhD Thesis*. Rivers State University of Science and Technology, Port Harcourt, Nigeria.p257
12. Igwe, F. U., Dokubo, A and Ukpaka, C.P. (2016). Overview of Crude Oil Toxicity: A Potential Health and Environmental Hazard in the Niger Delta. *Nigerian Journal of Oil and Gas Technology*. 2(2): 94 – 104.
13. Igwe, F. U. and Lee, G. D., (2014). Dermal Toxicity Studies of Adult Albino Rats Exposed to Bonny Light Crude Oil. *Journal of Nigerian Environmental Society (JNES)* 2014. 1(1): 27 – 38.
14. Igwe, F. U., Oruambo, I. F., Ekweozor, I. K. E., Oruamabo, P. P. (2008). Changes in the Blood Constituents of Adult Albino Rats following Dermal Exposure to Bonny Light Crude Oil. *Journal of Nigerian Environmental Society (JNES)* 4(4): 76 – 85.
15. Jokuty, P., Whiticar, S., Wang, Z., Fingas, M., Lambert, P., Field house, B., and Mullin, J. (1996). *A Catalogue of Crude Oil and Oil Product Properties*. Proceedings of the 14th Arctic and Marine Oil Spill Technical Seminar. Ottawa: Environment Canada, Ottawa, Ontario. pp 87-106.
16. Moody, R. P. and Chu, I. (1995). Dermal exposure to environmental contaminants in the Great Lakes. *Environ. Health Perspectives*. 103(9):103-114.
17. Orisakwe, O.E., Akumba, D. D., Afonne, O. J. and Gamanniel, K. S. (2000). Investigation into the pharmacological basis for some of the folkloric uses of Bonny light crude oil in Nigeria. *Indian J.Pharmacol*. 32: 231- 234.
18. Orisakwe, O. E., Njan, A. A, Afonne, O. J., and Akumba, D. D. (2004). Investigation into the nephrotoxicity of Nigerian Bonny light crude oil. *Int. J. Environ. Res. Pub. Health*. 1(2):106-110.
19. Orisakwe, O.E, Akumka, D.D, Njan, A.A, Afonne, O.J, and Okechi, O.O (2005). Hepatotoxic and haematological effects of Nigerian bonny light crude oil in male albino rats. *Toxicological and Environmental Chemistry*. 87(2): 215–221.
20. Otiagbe B. E. and Adesina, A. F. (2005). Crude Oil Poisoning in a 2 Year Old Nigerian –A Case Report. *Annil Aggrawal's Internet J. Forensic Medicine and Toxicology*. 6 (2).
21. Public Health Service (1996). Public Health Service Policy on Humane Care and Use of Laboratory Animals. Washington DC: US Department of Health and Human Services. (PL 99-158. Health Research Extension Act, 1985).
22. USEPA (United States Environmental Protection Agency). (2006). http://www.epa.gov/emergencies/docs/chem/title3_Oct_2006.pdf.
23. Wester, R. C., Maibach, H. I., Bucks, D. A. W., McMaster, J., Mobayen, M., Sarason, R. and Moore, A.(1990). Percutaneous absorption and skin decontamination of PCBs: *in vitro* studies with human skin and *in vivo* studies in the rhesus monkey. *J. Toxicol.Environ. Health*. 31: 235-246.
24. Yang, J. J., Roy, T. A., Krueger,A. J., Neil, W., and Mackerer, C. R., (1989). *In vitro* and *in vivo* percutaneous absorption of benzo(a)pyrene from petroleum crude – fortified soil in the rat. *Bull. Environ. Contam. Toxicol*. 43: 207-214.
25. Zakrzewski, S. F. (2002). *Environmental Toxicology*. Oxford University Press. (3rd ed).334 pp.