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Impacts of Simulated Agbabu Bitumen Leachate on Heamatological and Biochemical Parameters of Wistar Albino Rat

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ABSTRACT
The aim of this study was to investigate the short- term toxicological effects of Simulated Bitumen Leachate (SBL) on white rat. Changes brought into the haematological and biochemical parameters of the rats by the SBL were measured and used as indices of toxicity. Simulated bitumen leachate of Agbabu natural bitumen was prepared by stirring 150 g of the bitumen in 4 liters of bore-hole water for eight weeks. Some of the physicochemical properties of the SBL include Biochemical Oxygen Demand (BOD) 103.43±0.56 mg L^{-1}, total petroleum hydrocarbon (TPH) 327.89±0.23 mg L^{-1}, pH 7.42±0.04, Chemical Oxygen Demand (COD) 500. 64±0.65, SO_4^{2-} 490.27±0.47 and NO_3^- 96.37±0.32 mg L^{-1}. Various concentrations (0, 25, 50, 75, 100% v/v) of the SBL were included in the ration of the rats. Treatments were arranged in a randomized design of 5 treatments with 2 replicates each and rats were observed for 2 weeks. Haematological parameters including White Blood Cells (WBC), Red Blood Cells (RBC) and Packed Volume Cells (PVC) were higher in rats fed with SBL supplemented feed ration compared to untreated rats ($p<0.05$). Similarly, statistical analysis of biochemical parameters of treated and control rats revealed significant differences ($p<0.05$). These results have a very negative implication on the health status of the rat. It thus implies that the simulated bitumen leachate is toxic to white rat.

Key words: Short-term effect, simulated bitumen leachate, toxicological effects, heamatological and biochemical parameters

INTRODUCTION
Pollution of water occurs when a body of water receives excessive amounts of materials called contaminants, such that the quality of the water becomes adversely affected. Such water, therefore, becomes unfit for its intended use (Pollution-Issues, 2007). Pollution of water by petroleum is a universal environmental phenomenon in places where there is exploration or processing of petroleum deposits. Exploratory activities and downstream conversion in refinery generate pollutants in the form of waste water, chemicals and petroleum products which pollute the surrounding water body (Abd EL Gawad *et al.*, 2008). Petroleum polluted water causes aquatic-toxicological effects which are deleterious to aquatic life (Carls *et al.*, 1999;

Agbogidi *et al.*, 2005). According to Nwilo and Badejo (2005), pollution of water by crude oil causes modification of marine ecosystem and habitats. The consequence of this is a decrease in fishery resources and damage to wildlife especially seabirds and marine animals. Recently, the toxicological effects of water soluble fraction of crude oil on Macro benthic invertebrates (chironomus and mosquito larvae) were reported by Arimoro and Adamu (2008).

Bitumen is a mixture of organic liquids that is viscous, black and sticky. Its composition is very complex, containing a mixture of high boiling point range of compounds and molecules with a relatively low hydrogen-to-carbon ratio (Yoon *et al.*, 2009). The Canadian Encyclopaedia describes it as the heaviest and thickest form of petroleum (The Canadian Encyclpedea, 2011). A large deposit of natural bitumen occurs in the so called bitumen belt of south-western Nigeria (Adegoke *et al.*, 1991). The belt lies on the onshore areas of Eastern Dahomey (Benin) Basin, with Longitude 3°45'E and 5°45'E and Latitude 6°00'N and 7°00'N. The belt is about 120 km long and spans from Ijebu-ode in Ogun State to Siluko and Akotogbo areas near the Ondo and Edo States border (Adegoke *et al.*, 1980). Although, full exploration of the bitumen is yet to take off but seepage of the material exists, especially during the dry season when temperature is above 37°C when it occurs as a free-flowing liquid. During this period the seepage of the bitumen flows to the subsurface flowing water and the underground aquifer and contaminate them (Adegoke, 2000).

Adebiyi and Asubiojo (2008) studied the inter-elements correlation between metals and components of ecosystem in the bitumen deposit area in South Western Nigeria. Their findings showed that the ecosystem vegetations in the area accumulated the metals via soil as a result of contamination of the soil with the bitumen. They also established the relationships between various components of the ecosystem and the bitumen deposit in the area. However, to the best of our knowledge, there is no literature information on the effects of bitumen polluted water on animals in the bitumen belt of south western Nigeria.

Earlier studies carried out by Olajire *et al.* (2007, 2008), on qualitative and quantitative analyses of PAHs in the rivers and soil in Agbabu, showed an elevated Aliphatic and Polycyclic Aromatic Hydrocarbons (PAHs) levels in the water and soil. Agbabu is the major town in the bitumen belt. Two bitumen observatory wells were dug in this town in 60s during the early explorative activity on Nigerian natural bitumen (Adegoke, 2000). Thus, in continuation of the studies on the environmental impacts of Agbabu natural bitumen deposit in Agbabu, South Western Nigeria, it was decided to investigate the likelihood toxicological effects of bitumen polluted water on rat. Consequent, upon this, Agbabu bitumen leachate was simulated and the effects of this simulated bitumen leachate on the haematological and biochemical parameters of wistar albino rat were evaluated.

MATERIALS AND METHODS

Bitumen used for this investigation was collected from bitumen well-1 in Agbabu in September, 2007 and immediately transported to laboratory and kept in a fridge prior to its utilization, two weeks after collection. The experiment was conducted between September, 2007 and February, 2008.

Preparation of simulated bitumen leachate (SBL) from agbabu bitumen: About 150 g of the Agbabu bitumen was weighed into 6 L of transparent glass container and 4 L of borehole water were added unto the bitumen. The container was covered with a plastic cap which was finely perforated at the centre and this perforated area was covered with cotton so as to allow air in but prevented dust from entering. The set-up was left on a laboratory bench for eight weeks with

Table 1: Description of Treatment/Feeding of the rats on daily basis

Group	No. in group	Description of treatment/Feeding per day
Control T_0 (0% v/v)	6 (2 per cage)	75 g of feed supplemented with 100 mL of borehole water
T_1 (25% v/v)	6 (2 per cage)	75 g of feed supplemented with 100 mL of 25% (v/v) SBL
T_2 (50% v/v)	6 (2 per cage)	75 g of feed supplemented with 100 mL of 50% (v/v) SBL
T_3 (75% v/v)	6 (2 per cage)	75 g of feed supplemented with 100 mL of 75% (v/v) SBL
T_4 (100% v/v)	6 (2 per cage)	75 g of feed supplemented with 100 mL of 100% (v/v) SBL

intermittent vigorous shaking. The leachate formed was collected by decanting and stored in a fridge prior to its analysis and use. The procedure was repeated for another set-up with exception that bitumen was not included and this served as control. A representative portion of the prepared SBL was taken and analysed using recommended APHA methods (APHA, 1985). The result of the chemical analysis of the Simulated Bitumen Leachate (SBL) is as presented in Table 2.

Acclimatization of the rats: Albino rats of four weeks old were used for the toxicity studies. They were obtained from the Department of Anatomy, Ladoke Akintola University of Technology (LAUTHEC), Ogbomoso, Nigeria. The rats were fed with standard animal feed (Animal Care Feeds) supplemented with borehole water (mixing 75 g of feed with 100 mL of borehole water) for four weeks. The weights of the animals were taken before and after the acclimatization period.

Toxicological investigation of sbl on white rat (Wistar albino): The potential toxicity of the SBL on white rat (Wistar albino) was investigated by supplementing the feed given to 30 rats with varying concentrations of SBL for a period of two weeks. The various concentrations of SBL were obtained by mixing appropriate volume of bore hole water with the raw SBL using dilution formula:

$$M_1V_1 = M_2V_2$$

where, M_1 and M_2 are the initial and final concentrations of SBL respectively. V_1 and V_2 are the initial and final volumes of the SBL. At the end of the experimental period, the rats were decapitated and blood was collected for biochemical analysis.

Feeding of the rats with prepared SBL: The rats were randomly selected after the acclimatization period and grouped into five dietary/treatment groups. Each dietary group consisted of six rats in three cages. The group treatment are show in Table 1. Each pair of the rats in separate cage was daily fed with feed as described in Table 1 for a period of two weeks. The feed of the test rats had varying concentrations of SBL while that of the control rats had zero concentration of SBL.

Determination of haematological and biochemical parameters: At the end of the feeding period, the rats in each cage were killed by decapitation following chloroform anaesthesia. About 10 mL blood was collected from jugular vein during slaughter into dry clean sample vial. Three milliliter of the blood was collected into sample vial containing EDTA for haematological studies. Remaining 7 mL of the blood sample was deposited in anticoagulant free sample vial and allowed to clot at room temperature within 3 h of collection. These samples were kept in fridge (20°C) prior to analysis for biochemical parameters. The haematological parameters were determined in the Medical Laboratory Unit of LAUTECH, Health Centre, Ogbomoso. Packed Cell Volume (PCV) and Haemoglobin (Hb) were determined as described by Jain (1986). Red Blood Count (RBC) and White Blood Count (WBC) were determined with the aid of Neubaur counting chamber (Haemocytometer). The blood chemistry/biochemical parameters were determined at the Department of Chemical Pathology, University of Ilorin Teaching Hospital, Ilorin. Sera were analysed using commercial kits Randox (Laboratories Manual, 1997), for Total protein, Alkaline Phosphate (ALP), Acid Phosphate (ACP), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Albumin(Alb) determined using Doumas method (Doumas *et al.*, 1971).

Statistical analysis: Data obtained were subjected to Duncan multiple range test following One Way Variance (ANOVA). Differences at $p<0.05$ were considered significant. Values were expressed As±Standard Error of the Mean (SEM).

RESULTS AND DISCUSSION

The physico-chemical properties of the simulated bitumen leachate are as shown on Table 2 while the haematological and biochemical parameters of the rats at the end of the two weeks of feeding them with SBL supplementary feed are as contained in Table 3 and 4, respectively. The values of most of the physico-chemical properties of the SBL were well above the Nigeria Environmental Standards permissible levels for domestic water (FEPA, 1988). For instance, FEPA (1988) the permissible levels of pH, BOD, Pb, Co and SO_4^{2-} are 6.0-8.5, 4.0, 0.5, <0.5 and 250 mg L^{-1}, respectively for drinking water. Thus, the SBL may be considered to be polluted water. The concentration level (327 ppm) of Total Petroleum Hydrocarbon (TPH) in the SBL was also high and the source of which could be traced to the bitumen. Part of this petroleum hydrocarbon is made of Polycyclic Aromatic Hydrocarbons (PAHs) as earlier reported by Olajire *et al.* (2007, 2008). The carcinogenic effects of PAHs are well reported by Ngodigha *et al.* (1999), Nwilo and Badejo (2002) and Lin *et al.* (2005).

Table 2: Physico-chemical characteristics of simulated bitumen leachate (SBL)

Parameter	Mean±SE
Colour	14.15±0.05
PH	7.42±0.04
NO_3^-	96.37±0.32
SO_3^{2-}	490.27±0.47
COD	500.64±0.65
BOD	103.43±0.68
Co (ppm)	1.32±0.20
Cd (ppm)	6.85±0.11
Pd (ppm)	0.14±0.06
Ni (ppm)	2.53±0.24
Mn (ppm)	1.45±0.09
TPH (ppm)	327.89±0.23

Table 3: Haematological Parameters of Rats Fed with SBL supplemented feed

Code/conc	PCV (%)	WBC (g dL^{-1})	Hb (g dL^{-1})	RBC×10^6 mm^3
Control (0% v/v)	37.67±0.58^a	7.37±0.06^b	12.62±0.50^a	6.42±0.25^a
$T_1R_{1(25\%v/v)}$	45.67±1.53^b	6.27±0.15^a	15.27±0.38^b	7.80±0.04^c
$T_2R_{2(50\%v/v)}$	46.67±1.53^b	14.17±0. 15^e	15.94±0.09^c	8.01±0.09^c
$T_3R_{3(75\%v/v)}$	51.33±1.15^c	10.73±0.15^d	17.34±0.15^d	8.65±0.050^d
$T_4R_{4(100\%v/v)}$	44.67±0.58^b	9.53±0.21^b	15.10±0.11^c	7.51±0.050^a
Range (STD)	37-52	5-13	12.30-17.33	6.23-8.70

a, b, c, d and e: Means bearing the same alphabet along the same column are not significantly different ($p<0.05$), PCV = Packed cell volume, WBC = White blood cell, Hb = Haemoglobin, RBC = Red blood cell, STD = Standard, CACC (1980)

Table 4: Biochemical Parameters of Rats Fed with SBL supplemented feed

Code	ALP (IU L^{-1})	ACP (IU L^{-1})	SGPT (IU L^{-1})	SGOT (IU L^{-1})	Alb (g dL^{-1})	Total Protein g/100 cm^3
Control (0% v/v)	25.60±0.66^a	22.70±0.36^a	19.83±0.15^a	142.00±1.00^d	3.23±0.35^b	6.37±0.42^b
T_1 R_1 (25% v/v)	30.57±0.45^b	25.67±2.06^b	21.33±0.67^c	137.33±1.53^c	2.77±0.25^b	6.40±0.46^b
T_2R_2 (50% v/v)	33.23±0.32^b	28.27±1.11^{ab}	26.30±0.30^b	120.67±1.15^a	2.60±0.56^b	5.50±0.46^b
T_3R_3 (75% v/v)	37.07±2.97^c	32.47±0.50^{bc}	25.60±4.50^b	147.33±0.58^e	1.80±0.30^a	4.60±0.66^a
T_4R_4 (100% v/v)	37.30±1.65^c	36.20±6.17^d	24.40±4.13^a	130.00±3.61^b	1.67±0.36^a	4.60±0.36^a
Range (STD)	57-128	25.9-44.2	18-30	46-81	1.3-3.6	4-7

Result are means of 3 determinations±S.D. a,b,c,d: Means with the same superscript within the same column are not significantly different ($p<0.05$), STD: Standard alkaline phosphate ALP: Acid phosphate, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, Alb: Albumin

The haematological parameters investigated in the white rats were as shown on Table 3. The packed cell volume simply referred to as PCV is a measure of the volume occupied by red cells to the volume of whole blood in a sample of capillary or venous blood (Harper, 1975). The average PCV of the rats used in this work varied from 37.67% in control rats to 51.33% in rats treated with 75% (v/v) SBL. Analysis of variance showed that there was a significant difference in the average value of PCV of control rats and test rats ($p<0.05$). However, within the test rats, no significant difference in PCV of rats fed with 25, 50 and 100% (v/v) SBL but PCV of rats fed with 75% (v/v) SBL was significantly different from the PCV of other sets of test rats. In actual fact, the highest PCV was observed in the rats fed with 75% (v/v) SBL (Table 3).

The difference in the PCV of control and test rats might be attributable to the leachate included in the feed of test rats, since the difference in quality of feed of test and control rats was the presence of SBL in the feed of the former. The PCV of the test rats increased with concentration got to the peak (51.33) in rats treated with 75% (v/v) SBL and decreased to 44.67% in rats fed with 100% (v/v) SBL. The value of PCV is used as a measure of the degree of anaemia in animals (Table 3). High values such as obtained in the treated rats had been attributed to cell death in experimental animals as observed by Poon *et al.* (1996). The White Blood Cell (WBC) or white corpuscles is responsible for the defence action of the blood (Arthur, 1981; Mackean, 1979). The values of WBC of the rats ranged from 6.27 g dL^{-1} in rats treated with 25% SBL to 14.17 g dL^{-1} in rats treated with 50% (v/v) SBL. A significant difference exists between the values of WBC of all sets of rat fed with various concentrations of the bitumen Leachate ($p<0.05$) (Table 3).

Although the value of WBC of all sets of the rats except the one treated with 50% (v/v) SBL fell within the standard range reported for rats (CACC, 1980). However, the variation in the values of the WBC of these rats could be due to the bitumen leachate since the only difference in their feed was the inclusion of the bitumen leachate. The average value of WBC obtained for rats treated with 50% SBL was almost double that of the control but value obtained for rats treated with 25% (v/v) SBL was slightly less than the value obtained for the control rats. The value of WBC of animals is known to increase in response to invasion of animal system by foreign bodies such as bacteria or viral infection and poisonous chemicals (Baron *et al*., 1994). Thus, the difference in values of WBC of the control rats and test rats might be attributed to the presence of varying concentrations of SBL in the feed of the test rats. Probably the WBC of the test rats got elevated in attempt to respond to the SBL, as they will react to any other foreign material. The response of haemoglobin content of the test rats to SBL was not apparent. There was no significant difference in the haemoglobin content of rats treated with 50 and 100% SBL but a significant difference existed in the haemoglobin contents of control and test rats ($p<0.05$). Although, the average values of haemoglobin contents of test rats differed from the control ones, this difference may not pose any haemoglobin related physiological disorder because the values of haemoglobin of the control and test rats fell within the acceptable range (12.30-17.33) (Table 3).

This result may suggest that the physiological processes involved in the synthesis of hemoglobin of these rats are not significantly affected by the SBL. The presence of relatively large amount of cobalt in the bitumen leachate as shown on Table 1 might also be a contributory factor to the level of hemoglobin found in the test rats. Even though cobalt, is not a basic requirement for the formation of hemoglobin, its presence in blood has been suggested to enhance the formation of hemoglobin (Arthur, 1981). The effect of bitumen Leachate on the Red Blood Counts (RBC) of the test rats followed similar pattern as the hemoglobin content of the rats responded to the SBL. Rats fed with 25 and 50% SBL had similar RBC counts which were significantly different from RBC counts of rats fed with 75 and 100% (v/v) SBL. The RBC count of control rats was lower and significantly different from the RBC count of all the test rats ($p<0.05$). This result, as it was found for hemoglobin content, is not surprising, since hemoglobin is part of the RBC. The RBC count of control rats was closer to the lower limit of the standard range while that of the test rats were closer to the upper limit of the reference range; again this difference may be linked with bitumen leachate which was included in the feed of the test rats (Table 3).

The serum biochemical parameters determined in the rats used in this work were as shown on Table 4. There were significant differences in the average values of the biomarker enzymes of the control and test rats ($p<0.05$). As the concentration of SBL in the feed increased, the values of biomarker enzymes and protein increased. The ALP of rats fed with 75% (v/v) SBL did not differ from that of rats fed with 100% (v/v) SBL. The values of ALP of rats fed with 25 and 50% (v/v) SBL were also not significantly different from each other ($p>0.05$). A significant difference was observed in the values of ALP of control and all the test rats ($p<0.05$) (Table 4). Both the control and test rats, however, showed less activity than standard lower limit for rat (57-128) (Harper, 1975). Although the values of the ALP fell within the standard range, the relative increase as obtained for test rats may be of pathological concern, as Baron *et al*. (1994), had earlier linked increase in Ostreo blastic activity to a rise in ALP level.

The Acid Phosphate (ACP) of the test rats increased with increase in the concentration of the SBL in the feed of the rats. However, only rats fed with 75 and 100% (v/v) SBL have values which were significantly different from that of the control ($p<0.05$). All the test rats except ones fed with 25% (v/v) SBL had ACP which are very close to the upper limit, whereas the average value of ACP of control rats was below the lower limit of the standard range (Table 4). The value of SGPT of the test rats varied from 21.33 in rats fed with 25% (v/v) SBL to 26.30 in rats fed with 50% (v/v) SBL. No significant difference in the value of the SGPT of control and rats fed with 25 and 100% (v/v) SBL was observed. In contrast, a significant difference exists in the values of SGPT of the control and rats fed with 50 and 75% (v/v) SBL. The values of SGPT of control and all test rats fell within the standard range for rats (18-30) (CACC, 1980).

Increase in value of SGPT of animal in general is an indication of breakdown of tissues which manifests itself in form of many clinical diseases, for example, Patrick-Iwuanyanwu *et al.* (2011) reported increase in relative weight of live (hepatomegaly) and kidney in male rats fed with diets contaminated with kerosene. Thus, the health status of the test rats which have higher SGPT than the control rats might likely to have been negatively affected or deteriorated. Although, no clinical examination was performed on the rats to authenticate this claim but it was observed that the activities of the rats (such as response to feed, movement, etc.) in general decreased with the feeding periods. The average activity of Serum Glutamic oxaloacetic Transaminase (SGOT) of the control and test rats was found to be above the standard range (46-81) (Table 4). The activity of SGOT of test rats decreased with increase in concentration of SBL included in the feed except in rats fed with 75% (v/v) SBL which had SGOT activity higher than that of the control. The activities of SGOT of test rats are significantly different from each other and that of the control ($p<0.05$).

The values of albumin in the control rats and rats fed with 25 and 50% (v/v) SBL were not significantly different from each other ($p>0.05$) but these values were significantly different from the ones obtained for test rats fed with 75 and 100% (v/v) SBL. However, the albumin levels of both the test and controls rats fell within the recommended range (1.3-3.0) for the rats (Table 4). The variation in amount of protein in control and test rats followed exactly the same trend as obtained for albumin. This is not surprising since the albumin level of animal in general has a direct relationship with the nutritional status of the animal especially the amount of protein ingested. Changes in some biochemical parameters as a result of inclusion of some chemical compounds in the ration of wistar rats are well reported in literature (Ovuru *et al.*, 2004; Abdel Gadir *et al.*, 2006; Uboh *et al.*, 2008; Egbuonu *et al.*, 2010; Rhman *et al.*, 2011).

Albumin is produced in liver, so lowering of its quantity as observed in some of the test rats may be as a result of either a decrease in protein consumption or liver malfunctioning. But, since all the rats were fed with the same feed, it is reasonably suggested that consumption of SBL at higher concentration (75 and 100%) negatively interfered with ingestion of protein in rats that had lower albumin than the control rats. Another way of looking at the cause of the lower level of the albumin in some of the test rats is liver malfunctioning arising from liver damage as a result of higher concentrations of SBL in the feed of these rats.

All the test rats except the ones fed with 25% SBL supplemented feed showed a reduction in total plasma protein. This is in contrast to the findings of Akbari and Shariff (2002) who reported an increase in total plasma protein for fish fed with crude oil containing 0.107 and 0.053 ppm naphthalenes.

CONCLUSION

The simulated leachate produced from Agbabu Bitumen significantly altered the hematological and biochemical parameters of Winstar albino rat. The findings of this study will no doubt serve as reference for comprehensive toxicological study of the Agbabu bitumen on animals living in the Nigerian bitumen belt.

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