Global Journal of Earth and Environmental Science



Volume 1(1), pages 1-7, October 2016 https://doi.org/10.31248/GJEES2016.005 ISSN 2636-6002. Copyright © 2016 Article Number: BFE4FB421

http://www.integrityresjournals.org/journal/GJEES

Full Length Research

Comparative study of the fractionation and evaluation of the physical properties of bitumen obtained from Agbabu and Loda in Ilaje Area of Ondo State

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Received 29th June, 2016; Accepted 6th September, 2016

ABSTRACT: The results from the comparative evaluation of the physical properties and the chemical speciation of bitumen obtained from Agbabu and Loda in Ilaje Area of Ondo State. The results obtained are compared with some of the recent results obtained for heavy metals on Agbabu bitumen, but different methods of analysis to see the variation on the results obtained. The percentage bitumen content are; 79.80 ± 0.01 and 46.50 ± 0.01 , percentage sand content, 10.90 ± 0.01 and 53.10 ± 0.01 , penetration Test (25°C) , 44.50 ± 0.02 and 45.30 ± 0.01 , specific gravity (60°C) , 0.97517 ± 0.02 and 0.9240 ± 0.01 , Marshall stability in kg at 60°C are 849.50 ± 0.01 and 635.00 ± 0.01 , while the viscosity at 60°C (Pa/Sec) are 9.7580 ± 0.01 and 9.6415 ± 0.01 obtained from Agbabu and Loda bitumen respectively. The results obtained for the physical characteristics as indicated above where in favour of Agbabu bitumen in terms of its durability except the penetration text. The heavy metals concentrations in mg/kg are generally high in Loda bitumen for Pb, Cd, Cu, Ni, Mn, Cr, Zn and Fe. Fe is exceptionally high in the two samples with $9.201.2500 \pm 0.20$ mg/kg and $10.700.0700 \pm 0.5$ mg/kg respectively. The results obtained for Fe, Zn, Pb, Ni and Cu in this work were generally lower than the results obtained in the recent work. This may as a result of the extraction and experimental analysis method. Results of heavy metals studies on the fractionation of the heavy metals show that the concentration levels of Fe in residue fraction was higher than those in the carbonate fraction, iron and manganese fraction while the heavy metals bind to the organic fraction was low compared to the other fractions found in bitumen obtained from Loda.

Keywords: Bitumen, heavy metals, fractionation, Marshall stability, penetration test.

INTRODUCTION

According to the American Society for Testing and Materials (ASTM, 2002), bitumen is a mixture of hydrocarbons of natural or pyrogenous origin, or combination of both, frequently accompanied by their non-metallic derivatives, which may be gaseous, liquid, semi-solid, and which are completely soluble in carbon disulphide (CS₂). It is composed primarily of highly condensed polycyclic aromatic hydrocarbons, heterocyclic compounds and several heavy metals such as nickel, vanadium, lead, chromium, mercury arsenic, selenium and other toxic elements. It is the residual bottom fraction obtained by fractional distillation of crude

oil.

Naturally, bitumen exists in two main forms: either as pare bitumen accumulated down-deep below the earth surface or outcrops as oil impregnated sand, which is commonly referred to as tar sand or oil sand.

Bitumen was first discovered in Nigeria in 1910. The first bitumen well NBC-7 was drilled at Agbabu in Ondo State in the South Western part of Nigeria. Heavy metals are present everywhere in the environment (Adekola et al., 2002). Today it is generally recognized that the particular behaviour of trace metals in the environment is determined by their specific physicochemical forms rather

than by their total concentration. Speciation of metals largely determines their bioavailability and toxicity (Tack and Verloo, 1995). Speciation can be defined as identification and quantification of the different species or forms of phases in which elements occur. It is generally recognized that information about the physicochemical forms of the elements is required for understanding their mobility, pathways and bioavailability. Studies on the distribution and speciation of heavy metals in sediments can provide not only information on the degree of pollution, but specially the actual environmental impact, metal bioavailability as well as their origin (Ramirez, 2005).

Heavy metal speciation in sediment impacted with crude oil in the Niger-Delta region of Nigeria has been reported (Iwegbue et al., 2006). It was found that Cd, Pb, Zn, and Ni have high mobility indices. Cd was associated with easily exchangeable / adsorbed and carbonate fractions, Cu and Cr predominantly occur in organic and residual fractions. Zn was associated with carbonate and Fe- Mn oxide fractions (Iwegbue et al., 2006). Olajire et al., (2003) studied the levels and speciation of five heavy metals in soils of industrial Southern Nigeria and found that the highest amount of Cd was found in the exchangeable fraction and Cu and Zn were significantly associated with the organic fraction. Some studies have been carried out on the Nigerian bitumen and Agbabu bitumen deposit such as multielemental analysis (Adebiyi et al., 2006), environmental impact assessment of bitumen exploitation on animal resources of Ode-Irele (Lameed and Ogunsusi, 2002), compositional analysis of the oil component of the Nigerian bitumen (Oderinde and Olanipekun, 1991), Distribution of polycyclic aromatic hydrocarbons in soils and water from the vicinity of Agbabu bitumen field (Olajire et al., 2007), and solvent of vanadium from Nigerian (Oguntimehin and Ipinmoroti, 2007). The need for this research arose because no study has been reported on the fractionation of metals in bitumen sediment obtained from Agbabu and Loda bitumen deposit environment.

The aim of this study is to evaluate the physicochemical characteristics of bitumen obtained from Agbabu and Loda areas of Ondo State and the chemical speciation (fractionation of heavy metals) obtained from the two samples, and the objectives are to compare the characterized extracted bitumen obtained from Agbabu and Loda areas in Ilaje, Ondo State for their physical characteristics because there is a paucity of data in this aspect. It is the physical characteristics that determined their chemical composition and their durability for road construction. It is also paramount to determine their heavy metals concentration in correlation with the recent work by Fagbote and Olanipekun, 2010 and Bakare et al. (2015) for some of the heavy metals using different extraction process and digestion methods before characterization using AAS. Also, their heavy metal fractions in relation to their environment were put into

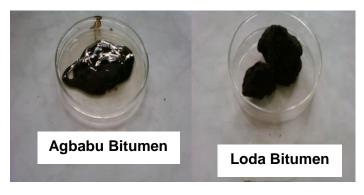


Figure 1. Raw bitumen samples from Agbabu and Loda.

consideration. The results obtained for heavy metals and their speciation will reveal the level of their environmental pollution and contaminants.

MATERIALS AND METHODS

Sample collection

Raw bitumen samples were obtained from Agbabu and Loda deposited site in Odigbo and Irele Local Government Area respectively (Figure 1). Agbabu and loda are located at Latitude 6° 35′ 16.3″N and 6° 37′ 13.9″N and longitude 4° 49′ 29.0″E and 4° 50′ 20.7″E respectively in Ondo State. They fall within the sedimentary terrain in the Dahomey basin of south western area of Nigeria.

Extraction method

The extraction method adopted was Soxhlet extraction method using acetone, 10g of the bitumen sample(w_1) was added to already weighed thimble(w_2) and placed in the Soxhlet apparatus, fixed with boiling flask which was filled to three quarter (3/4) with acetone and heated by heating mantle. Continuous extraction was done until a clear solvent was observed at the thimble containing the sample (Figure 2). The thimble and the remaining materials from the sample, which is mainly sand content was obtained (w_3). The bitumen content was calculated as weight difference (w_2 - w_3).

Parameters determined

The parameters determined were bitumen content, sand content, specific gravity and viscosity and were carried out by AOAC method (2000). The penetration test was carried out by ASTM standards (1996), and heavy metal speciation was carried out by using Tessier et al. (1985) and Abu-Kukati (2001) methods.





Agbabu

Loda

Figure 2. Extracted bitumen samples from Agbabu and Loda by Soxhlet method.

Specific gravity (AOAC, 2000)

Twenty-Five (25) ml specific gravity bottle with the lid was washed thoroughly with detergent, rinsed with clean water, oven dried at 105° C in an oven and cooled. It was weighed with the lid in analytical weighing balance and recorded (W₁). Clean water was poured into it at 60° C and the lid inserted so that the water comes out from the narrow opening in the lid. The outer side of the bottle and the lid were wiped with clean cloth and weighed (W₂). This was repeated for the bitumen sample and weighed (W₃).

Specific Gravity= $\frac{W_3-W_1}{W_2-W_1}$

Viscosity (ASTM, 1996)

Orifice type viscometers are used to indirectly find the viscosity of liquid binders like bitumen. The viscosity was expressed in seconds as the time taken by the 50 ml bitumen material to pass through the orifice of a cup. standard test conditions and temperature.10 mm Orifice Viscometer was cleaned with chromic acid solution, rinsed very well with water and drained (Figure 3). The bitumen sample was placed inside clean 10mm orifice cup and placed inside a thermosetting water bath at 60°C. It was removed and allowed to drain into 50 ml flask. The stopwatch was counting in seconds immediately the orifice opened. It was stopped when the 50 ml flask filled to the mark with bitumen and the time recorded. This was repeated for all Bitumen samples at the same temperature and the time recorded. The flow time per seconds of water at the same temperature into 50 ml flask from 10 mm orifice was equally recorded.

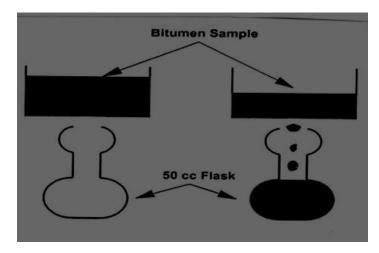


Figure 3. Orifice viscometer.

Time per seconds, T(S) = hXmXs, Where: h=hour, m= minutes, s=seconds

Viscosity (cP) $= \frac{\text{flow time of sample at 60 °C X Sg of sample X 1.002}}{\text{Flow time of H20 at 60 °C}}$

1.002 = Viscosity of H₂0 in cP at 60°C, 1 cP (centipoises), s =0.001 Pas/ sec

Marshall stability (ASTM, 1996)

Aggregate (soil) and the bitumen to be tested were weighed and heated separately up to 170°C and 163°C respectively. An increment of the bitumen by 0.5% (50.25 kg, 50.50 kg, 50.75 kg, 51.00 kg, and 51.25 kg) was mixed with 100 kg of soil each respectively. Each of the mixture was transferred to the compaction mold arranged on the compaction pedestal. 75 blows were given to the top side of the specimen with standard hammer (45cm, 4.86 kg), it was revised for the other side of the mixture, removed from the mould by gentle push after it has been cooled for few minutes and cured at room temperature overnight. Before testing, the mold was kept in water bath at 60 °C for half an hour, removed and tested for stability using Marshall stability Apparatus. A plot of %bitumen content on the x-axis against stability in kg on the y-axis was observed for maximum Marshall stability of the bitumen.

Marshall stability (kg) =
Peak of the Marshall stability from the graph(y)
Bitumen content (x)

Penetration test (ASTM, 1996)

The bitumen content was soften above the softening point (between 75 and 100°C). It was stirred thoroughly to

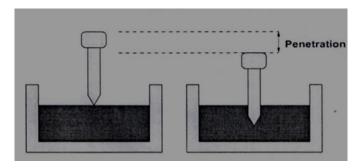


Figure 4. Penetration apparatus.

remove air bubbles and water. It was poured into a container to a depth of at least 15mm in excess of the expected penetration, cooled in an atmospheric temperature to 15-30 °C for one and half hours and then placed in a transfer dish in the water bath at 25.0 °C for one and half hours. The container was kept on the stand of the penetration apparatus (Figure 4). Its needle was adjusted to make contact with the surface of the sample and the dial reading adjusted to zero. The needle was released for exactly 5 seconds with accurate timing and the reading was taken, repeated three times and recorded the mean value in tenths of mm.

Heavy metal composition

The process for ash determination was followed by muffle furnace at 600 for 12 hours to obtain the ash. To the crucible containing the ash sample, about 5 ml of concentrated hydrochloric acid was added and the mixture boiled for 5 min on hot plate in a fume cupboard, water was added as necessary to maintain the volume. It was transferred to a beaker and the crucible washed into the beaker with distilled water. Cool and filtered through clean muslin cloth to 100 ml volumetric flask to remove insoluble materials and rinse the beaker with distilled water. It was made up to the mark with distilled water. The absorbance of the sample solution was read by standardize Atomic Absorption Spectrophotometer with appropriate lamp for the required element (AOAC, 2000).

Forms of heavy metals (Fractionation)

The procedure of Tessier et al. (1985) was selected for this study. In this method, heavy metals are separated into five operational defined fractions: exchangeable fraction, bound to carbonates, bound to iron and manganese oxide, bound to organic matter and residual fraction. The sequential extraction is as follows:

Step I, exchangeable fraction: Following Tessier et al. (1985), Samples (2 g) were extracted at room temperature for 1 hour with 16 ml of MgCl₂ solution (1 M

MgCl₂) at pH 7. Sediment and extraction solution were thoroughly agitated throughout the extraction. This is mainly an adsorption – desorption process. Metals extracted in the exchangeable fraction include weakly adsorbed metals and can be released by ion-exchange process. Changes in the ionic composition of the water would strongly influence the ionic exchange process of metal ions with the major constituents of the samples like clays, hydrated oxides of iron and manganese. The extracted metals were then decanted from the residual samples for AAS analysis while the residue was used for the next extraction.

Step II- Bound to carbonates: Following Tessier et al. (1985), the metals bound to carbonate phase are affected by ion exchange and changes of pH. The residue of fraction 1 was extracted with 16 ml of 1M sodium acetate / acetic acid buffer at pH 5 for 5 hours at room temperature. Significant amount of trace metals can be co-precipitated with carbonates at the appropriate pH. The extracted metal solution was decanted from the residual bitumen samples for AAS analysis. The residue was used for the next extraction.

Step III- Bound to iron and manganese oxides: Following Tessier et al. (1985), the residue from fraction 2 was extracted under mild reducing conditions. 13.9 g of hydroxyl amine hydrochloride (NH₂OH.HCI) dissolved in 500 ml of distilled water to prepare 0.4 M NH₂OH.HCl. The residue was extracted with 20 ml of 0.4 M NH₂OH.HCl in 25% (v/v) acetic acid with agitation at 96 in a water bath for 6hours. Iron and manganese oxides which can be present between particles or coatings on particles are excellent substrates with large surface areas for absorbing trace metals. Under reducing conditions, Fe (III) and Mn (IV) could release adsorbed trace metals. The extracted metal solution was decanted from the residual sediment for AAS analysis while the residue was used for the next extraction.

Step IV- Bound to organic matter and sulphide: Following Tessier et al. (1985), the residue from fraction 3 was oxidized as follows: 3 ml of 0.02M HNO₃ and 5ml of 30% (v/v) hydrogen peroxide, which has been adjusted to pH 2, was added to the residue from fraction 3. The mixture was heated to 85 °C in a water bath for 2hours with occasional agitation and allowed to cool down. Another 3 ml of 30% hydrogen peroxide, adjusted to pH 2 with HNO₃, was then added. The mixture was heated again at 85 °C for 3 hours with occasional agitation and allowed to cool down. Then 5 ml of 3.2 M ammonium acetate in 20% (v/v) HNO3 was added, followed by dilution to a final volume of 20 ml with de-ionized water. Trace metals may be bound by various forms of organic matter, living organisms and coating on mineral particles through complexation or bioaccumulation. substances may be degraded by oxidation leading to a release of soluble metals. The extracted metal solution

Table1.	Results	of	some	of	the	physical	properties	obtained	from	Agbabu	and
Loda bit	umen.										

Parameters	Agbabu	Loda
%Bitumen Content	79.80±0.01	46.50±0.01
%Sand Content	10.90±0.01	53.10±0.01
Penetration Test (at 25 °C)	44.50±0.02	45.30±0.01
Marshall stability(kg)(at 60°C)	849.50±0.01	635.00±0.01
Specific gravity (at 60 °C)	849.50±0.01	0.9240±0.01
Viscosity(at 60°C)(Pa/Sec)	9.7578±0.01	9.6415±0.01

±SD values of triplicate analysis.

was decanted from the residual bitumen samples for AAS analysis while the residue was used for the next extraction.

Step V - Residual or inert fraction: Following Tessier et al. (1985), residue from fraction 4 was oven dried at 105°C. Digestion was carried out with a mixture of 5 ml concentrated HNO₃ (HNO₃, 70% w/w), 10 ml of hydrofluoric acid (HF, 40% w/w) and 10 ml of perchloric acid (HClO₄), 60% w/w) in Teflon beakers. Fraction 5 largely consists of mineral compounds where metals are firmly bonded within crystal structure of the minerals comprising the sediment. Analysis was carried out with AAS using GBC Avanta PM. Ver 2.02. To validate the procedure, the instrument was programmed and it carried out metal detection by displaying two absorbance readings and what was reported was the average. Blanks were also used for correction of background and other sources of error. Apart from calibration before use, quality checks were also performed on the instrument by checking the absorbance after every ten sample runs. 2 g of the sample was extracted using 16 ml of 1 M MgCl₂ solution (pH = 7.0 with stirring at room temperature for 1 hour (continuing agitation).

RESULTS AND DISCUSSION

The comparative evaluation of the physico-chemical properties and the chemical fractionation of bitumen obtained from Adbabu and Loda revealed these physical properties from the raw bitumen after extraction; bitumen contents are $79.80 \pm 0.01\%$, $46.50 \pm 0.015\%$ and sand contents are $10.90 \pm 0.01\%$, $53.10 \pm 0.01\%$ for Agbabu and Loda respectively as shown in Table 1. Agbabu had the highest value of bitumen content and low bitumen content was recorded in Loda. This revealed the increase in yield from Agbabu bitumen for industrial utilization and high profitability, while the sand contents was lowest in Agbabu compared to Loda. Penetration test at 25°C were 44.50 ± 0.02 and 45.30 ± 0.01 respectively. Loda had the highest value compared to Agbabu bitumen. This is an indication for ability to withstand pressure that acted on it and this is higher in Agbabu bitumen. Specific gravities at

60 °C for Agbabu and Loda bitumen were 0.97517 ± 0.02 and 0.9240 ± 0.01 respectively. Agbabu has the highest specific gravity value. These revealed that Agbabu bitumen is denser than Loda bitumen. Marshall stability in kg at 60° C were 849.50 ± 0.01 and 635.00 ± 0.01 . Agbabu bitumen has the highest stability with soil than Loda with lower stability. This is an indication of high tensile stress and strain in Agbabu bitumen. Viscosities at 60°C in Pa/Sec were 9.7578 ± 0.01 and 9.6415 ± 0.01 for Agbabu and Loda bitumen respectively. These revealed that the rate of flow of Agbabu bitumen is lower than that of Loda bitumen. Since the physical characteristics have an influence on its chemical characteristics, the parameters observed for the physical composition in Agbabu bitumen is an indication for its best performance industrially. The physical parameters observed indicated that Agbabu bitumen had high durability than Loda bitumen once employed in road construction or as a roofing felt.

The heavy metals constituents in mg/kg for Pb, Cd, Cu, Mn, Cr, Zn and Fe in Loda were greater than the values obtained in Agbabu, while Ni was higher in Agbabu compared to Loda bitumen as revealed in Table 2 respectively. Some of the values obtained were compared with the values obtained by Bakare et al. (2015) from the same source (Agbabu) which were; Mn (28.40), Fe (3,633.10), Zn (17.50), pb (93.78), Ni (103.65), Cr (153.90) and Cu (17.90) in mg/kg using different extraction and digestion methods. These observed results by Bakare et al. (2015) were higher than that from Agbabu in this present work except Mn (40.28) and Fe (9,201.25) in mg/kg which were higher. The difference in the results may as a result of the extracting solvent adopted by Bakare et al. (2015) which used chloroform compared to this present work which used acetone. Furthermore, digestion procedures (HNO3, H_2SO_4 , HCI, and H_2O_2 in the ratio of 6:2:2:2 (v/v) for Bakare et al. (2015), however, HCl and HNO₃ in ratio 3:1 were used in the present work) and different equipment were used for analysis. The results of heavy metals fractionation from Agbabu bitumen in mg/kg in ascending Pb (Fr5>Fr3>Fr4>Fr1>Fr2), order (Fr5>Fr3>FR2>Fr1>Fr4), Cu (Fr5>Fr4>Fr3>Fr1>Fr2), Ni (Fr5>FR2>Fr4>Fr2>Fr51), Mn and Cr (Fr5>FR1>Fr3>

Metal Analysed (mg/kg)	Agbabu	Loda
Pb	0.7500±0.02	0.9150±0.02
Cd	0.6050±0.04	0.7710±0.05
Cu	6.8150±0.50	11.1025±0.40
Ni	6.9750±0.20	5.3526±0.10
Mn	40.2800±0.52	60.1350±0.50
Cr	40.8150±0.45	55.6143±0.20
Zn	13.6050±0.15	15.3051±0.02
Fe	9,201.2500±0.20	10,700.0700±0.50

Table2. Heavy metals constituents of bitumen obtained from Agbabu and Loda.

±SD values of triplicate analysis.

Table 3. Results of heavy metals fractionation obtained from Agbabu bitumen.

Metals Analyzed (mg/kg)	FR1	FR2	FR3	FR4	FR5
Pb	0.0025	0.0000	0.0263	0.0218	0.6975
Cd	0.0436	0.0666	0.0763	0.0242	0.3915
Cu	0.0409	0.0000	0.5998	3.0804	3.0940
Ni	0.1814	0.2372	0.6696	0.2441	5.6498
Mn	8.2474	4.2294	6.8879	3.8266	17.1465
Cr	12.7343	4.5713	5.7549	2.3265	15.3464
Zn	0.6123	1.2925	4.2312	1.3877	6.0543
Fe	64.4088	9.2013	1,748.2375	2,944.4000	4,444.2050

FR1, Fraction i – Exchangeable fraction, **FR2**, Fraction ii – Bound to carbonates, **FR3**, Fraction iii – Bound to iron and manganese oxides, **FR4**, Fraction iv – Bound to organic matter, **FR5**, Fraction v – Residual or inert fraction.

 Table 4. Results of heavy metals fraction obtained from Loda bitumen.

Metals Analyzed (mg/kg)	FR1	FR2	FR3	FR4	FR5
Pb	0.0037	0.0009	0.0274	0.0229	0.8600
Cd	0.0628	0.0713	0.1039	0.04265	0.4953
Cu	0.1333	0.2999	1.0665	5.2300	4.3754
Ni	0.1125	0.1660	9.3209	6.3142	29.0452
Mn	11.1249	4.3297	9.3209	6.3142	29.0452
Cr	15.9615	5.6172	8.2310	4.0599	21.7455
Zn	0.9492	1.7147	4.6696	0.9952	6.9814
Fe	85.6006	32.1002	1,883.2123	3,220.7211	5,478.4358

FR1, Fraction i – Exchangeable fraction, **FR2**, Fraction ii – Bound to carbonates, **FR3**, Fraction iii – Bound to iron and manganese oxides, **FR4**, Fraction iv – Bound to organic matter, **FR5**, Fraction v – Residual or inert fraction.

Fr2>Fr4), Zn (Fr5>FR3>Fr4>Fr2>Fr1) and Fe (Fr5>FR4>Fr3>Fr1>Fr2) for fraction 1,2,3,4,5 respectively as shown in Table 3. These results are generally lower than the values obtained by Fagbote and Olanipekun (2010) for Agbabu bitumen sediments except in Iron which is higher. Results of Heavy Metals fractionation from Loda bitumen in mg/kg in ascending order were; Pb (Fr5>FR3>Fr4>Fr1>Fr2), Cd (Fr5>FR3>Fr4>Fr1>Fr4), Cu (Fr4>FR5>Fr3>Fr2>Fr1), Ni (Fr5>FR3>Fr4>Fr2>Fr1), Mn (Fr5>FR1>Fr3>Fr4>Fr2)

Fr4), Zn (Fr5>FR3>Fr2>Fr4>Fr1) and Fe (Fr5>FR4>Fr3>Fr1>Fr2) for fraction 1,2,3,4,5 respectively as shown in Table 4. Agbabu Bitumen has the highest exchangeable form compared to Loda bitumen with highest innert fraction in most cases. The heavy metal fractionation indicates the rate of transformation of the metals in relation with the chemical composition and their environment. The higher the exchangeable rates, the lower the susceptibility of the heavy metal for environmental pollution and hazards. Their exchangeable

rates at different fractions left huge amount at the inert fraction (Fr5). This is the fraction that is residual in the bitumen samples and can still cause environmental pollution.

Conclusion

The results from the physico-chemical parameters revealed that the bitumen from Agbabu had high bitumen content and less sand content than Loda bitumen. The specific gravity, marshall stability and viscosity at 60°C are higher than that from Loda except the penetration test at 25°C. Since the physical characteristics have an influence on its chemical characteristics, the parameters observed for the physical composition in Agbabu bitumen is an indication for its best performance industrially and for road construction in terms of the durability. The heavy metals analysed revealed that Agbabu bitumen has lower values in Pb, Cd, Cu, and Mn except Ni in Loda bitumen. These showed that the rate of environmental pollution is higher in Loda bitumen than Agbabu bitumen due to the high concentration. The chemical fractionation indicated the fractions of the elements that are exchangeable / adsorbed from fraction 1 to 4 and the inert fraction 5. Abgabu bitumen has the highest fractions exchangeable form compared each to Loda bitumen. The fractions that are residual in both bitumen samples can still cause environmental pollution because of the high concentration in the inert fraction (Fr5). During exploitation, maximum observation and necessary precaution must be put in place to check environmental pollution.

Recommendation

Based on the results obtained from the physico-chemical evaluation of bitumen from Agbabu and Loda, it is recommended that bitumen from Agbabu could be employed for industrial and construction purposes. Therefore, care must be taken to check environmental pollution. The degree of environmental pollution was lesser in Agbabu to Loda due to lower quantities of heavy metals obtained in Agbabu.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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