COMPARATIVE ASSAY ON HEAVY METALS REMOVAL FROM SOIL USING CHEMICAL SURFACTANT, CRUDE BIOSURFACTANT AND THE PRODUCING-BACTERIA

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Abstract. Heavy metals removal from polluted soil was comparatively assayed using a chemical surfactant (CHS/SLS), two crude biosurfactants (CB) and two biosurfactants producing isolates (BPI), *Odoribacter splanchnicus* DSM 20712 isolated from waste battery dumpsite soil (WBS); an unidentified bacterium clone (BC) JX981747 isolated from cassava mill soil (CSM). The biosurfactant treatments were set up in six experimental microcosms (treatments C-H) and two controls (A-B) for 16 weeks. The initial recorded values of the test heavy metals in the soil samples were 304.40 mg/kg, 131.40 mg/kg, 724.80 mg/kg and 0.38 mg/kg for Cu, Pb, Zn and Cd respectively; but it significantly ($p<0.05$) reduced after amendments. The test soil sample treated with composite of 60ml of crude biosurfactant from BC JX981747 and 5.2g of the producing cells (G) had the lowest Pb value of 12.40 mg/kg. Zn and Cd were also reduced more in soil treated with only CB of *O. splanchnicus* cells (C). The value of 232.96 mg/kg was observed in Cu concentration of soil percolated with composite of *O. splanchnicus* and its CB (F) indicating inefficiency in Cu removal from soil, but the use of only the CB (C) depict a contrasting ability as significant reduction in the Cu value of 52.37 mg/kg was observed. Comparatively, the CHS had negligible significant difference in metal reduction ability on soil over crude biosurfactant aside those treatment with F and G. Cation exchange ability (CEC), Organic carbon, organic matter content of the test soils all increased with the peak observed in CHS, but the use of organic surfactant is encouraged due to its environmentally friendly features. This study predicts the use of the test biological surfactants in soil conservation and management.

Key words: bioremediation; biosurfactant; exchangeable cations; heavy metals; organic carbon; organic matter.

INTRODUCTION

A wide variety of metabolic and physiological factors are required for the degradation of different compounds in crude oil and its products [15]. Heavy metals are one of such known components [28] which cause biosphere pollution and presents direct risk of contamination of subsoil and groundwater. These contaminants should be treated to prevent harm to the environment since its levels in all environments with contributions from a wide variety of domestic, industrial and agricultural sources. To achieve this in an environmentally friendly manner, biological agents such as biosurfactant producing organisms and their products should be involved to help convert the hydrophobic layer into small micelles which they can engulf as a carbon source [31, 47].

Microorganisms whether autochthonous or allochthonous are expected to exhibit properties that will enable removal or degradation of these pollutants from the environment [27, 30]. All of such properties however, are not found in one organism. Monocultures can be adversely affected by negative interactions making the best approach to be the use of a consortium of biosurfactant producing microorganisms and by selecting a consortium from a contaminated environment, though the negative interactions could be minimal [26, 30]. The polluted or impacted soil and water would have to be restored to its natural or near natural state by remediation [29], a viable option that involves the use of microorganisms. It comprises the introduction of genetically engineered microorganisms or the augmentation of the activity of the native microorganisms to remediate the environment.

Synthetic detergents used to clean up these spillages have often led to more destruction of the environment from an environmental view point, it is important that all substances released into the environment be degradable. Their potential for causing environmental damage should be assessed and the possibility of future harm due to build-up in the environment should be taken into consideration. Owing to their xenobiotic nature, synthetic surfactants have the potential disadvantage of persisting in the environment, long after they are applied for a remedial measure [25]. Also, some of the synthetic surfactants are comparatively more toxic to human health [7].

The addition of biosurfactants can be expected to enhance hydrocarbon biodegradation by mobilization, solubilization or emulsification. The mobilization mechanism occurs at concentrations below the biosurfactant critical micelle concentration (CMC) measured with a tensiometer. At such concentrations, biosurfactants reduce the surface and interfacial tension between air/water and soil/water systems [32]. Due to the reduction of the interfacial force, contact of biosurfactants with soil/oil system increases the contact angle and reduces the capillary force holding oil and soil together. In turn, above the biosurfactant CMC the solubilization process takes place. At these concentrations, biosurfactant molecules associate to form micelles, which dramatically increase the solubility of oil. The hydrophobic ends of biosurfactant molecules connect together inside the micelle while the hydrophilic ends are exposed to the aqueous phase on the exterior. The hydrophilic end which is the polar moiety forms complexes with the metal contaminants and are removed by desorption. Consequently, the

interior of a micelle creates an environment compatible for hydrophobic organic molecules [24]. The process of incorporation of those hydrocarbon molecules into a micelle is known as solubilization. Emulsification is a process that forms liquid, known as an emulsion, containing very small droplets of fat or oil suspended in a fluid, usually water [5, 14, 19, 24].

Recently interest has developed in applying bioremediation to sites contaminated with both metals and organics (co-contaminated sites). Maier *et al.*, [22] noted that in co-contaminated sites, treatments effective for concurrent removal of organics and metals need to be developed. However, since heavy metals are not easily biodegradable, and since metal-induced inhibition of normal heterotrophic microbial activity has been well documented in co-contaminated sites it may be necessary to use sequential or combined treatments that address the two contaminant types separately to achieve remediation goals, on this backdrop we developed this research to study the effect of two biosurfactants, the producing bacteria and their different treatment combinations in the removal of metals in waste metal dumpsite soil co-contaminated with engine oil.

MATERIALS AND METHODS

Collection of soil sample

The test soil was collected from waste metal dumpsite in Owerri, Imo State, Nigeria, with sterile plastic sample bags usig surface sterilized soil auger at the depth of 15cm.

Biosurfactant treatments

The different treatment types shown in Table 1 were used in the bioremediation of the waste metal dumpsite soil. These treatments were added in pulsed every week till the last week before the 16th week.

Physico-chemical analysis of the soil

The soil samples were analyzed using the methods of AOAC [3].

Bacteria for biosurfactant production

The bacteria *Odoribacter splanchnicus* DSM 20712 was isolated from waste battery dumpsite soil (WBS1) and an unidentified bacterium clone JX981747 was isolated from cassava mill soil (CMS1) from Nekede, Owerri, Imo State, Nigeria . These isolates were identified by 16S rRNA phylogenic analysis using dideoxy Sanger sequencing method in Inqaba, Pittsburg, South Africa.

Screening test to ascertain biosurfactant producing potential

Biosurfactant-producing ability in the microorganisms was screened using different assays. These assays include β-hemolysis, oil spreading test and emulsification index test (E_{24}) were determined using the methods described by Anandaraj and Thivakaran [2]; Govindammal and Parthasarathi [16]; Okore *et al*. [31 - 35]; Priya and Usharani [37]. The direct correlation of E_{24} was however, found between surface activity and emulsification index according to Denger and Schnik [8].

Table 1. Treatments combinations used in biodegradation experiment

Code identification of the samples	Percolate / Treatment type
A = S-NT (positive control)	150 g of un-percolated contaminated soil (with no treatment)
B = S-CHS/SLS	150 g soil percolated with a chemical surfactant solution (sodium lauryl sulphate (SLS), 60 ml, pH 8.5 ± 0.3, 0.333 M)
C = S-CB (WBS 1)	150 g soil percolated with 60 ml aqueous solutions of crude biosurfactant obtained from the biosurfactant producing bacterial strains *Odoribacter splanchnicus* DSM20712 (WBS 1) only
D = S-CB (CMS 1)	150 g soil percolated with 60 ml of crude biosurfactant from unidentified bacterium CMS1 without the bacterial cells
E = S-MCB(WBS 1 and CMS 1)	150 g soil percolated with mixed solution of crude biosurfactant obtained from the biosurfactant producing bacteria (30 ml + 30 ml of *Odoribacter splanchnicus* DSM20712 (WBSI) and unidentified bacterium CMSI biosurfactants)
F = S-CB + BB (WBS 1)	150 g soil percolated by mixture of both aqueous solution of crude biosurfactant and a specified quantity of the biosurfactant producing bacterial cells (60 ml *Odoribacter splanchnicus* DSM20712 (WBS 1) biosurfactant and 5.2 g bacterial cell of *Odoribacter splanchnicus* DSM20712)
G = S-CB + BB (CMS 1)	150 g soil percolated with 60 ml of biosurfactant from unidentified bacterium CMS1 with 5.2 g the bacterial cells
H = S-MCB(WBS 1 and CMS 1) + CONBB	150 g soil percolated with consortium of biosurfactant producing bacteria cells (2.6 g bacterium cells of WBS 1 and 2.6 g of unidentified bacterium CMS1) and mixed solution of their crude biosurfactant (30 ml of biosurfactant from *Odoribacter splanchnicus* DSM20712 and 30 ml of biosurfactant from unidentified bacterium CMS1)

Legend: S-NT= soil with no treatment; **S-CHS/SLS** = soil percolated with chemical surfactant/Sodium lauryl sulphate; **S-CB (WBS 1)** = soil with crude biosurfactant obtained from the biosurfactant producing bacterial strains *Odoribacter splanchnicus* DSM20712 (WBS 1) only without the cell; **S-CB (CMS 1)** = crude biosurfactant from unidentified bacterium CMS1 without the bacterial cells; **S-MCB (WBS 1 and CMS 1)** = soil with mixed solution of crude biosurfactant obtained from both biosurfactant producing bacteria; **S-CB + BB (WBS 1)** = soil with both aqueous solution of crude biosurfactant and a specified quantity of the biosurfactant producing bacterial cells; **S-CB + BB (CMS 1)** = biosurfactant from unidentified bacterium CMS1 with 5.2 g the bacterial cells; **S-MCB (WBS 1 and CMS 1) + CONCB** = soil percolated with consortium of biosurfactant producing bacteria cells of WBS1 and unidentified bacterium CMS1) and mixed solution (composite) of their crude biosurfactant.

Production and extraction of crude biosurfactants for chemical characterization

The colonies of biosurfactant producers *Odoribacter splanchnicus* DSM20712 (WBSI) and unidentified bacterium clone JX981747 (CMSI) were inoculated in 500 ml nutrient broth medium in 1000 ml Erlenmeyer flask and incubated at 30^{o}C for 7 days on a mechanical shaker. After 1 week of incubation, it was centrifuged at 4,000 rpm for 45 min then filtered to obtain cell free supernatant and sediments of cell debris. The extraction of the biosurfactant was performed by acid precipitation carried out by precipitating the supernatants with 1 M H_2SO_4 of equal volume to attain a pH of 2.0 (54.3 ml conc. H_2SO_4 poured into 1 L distilled water = 1 M H_2SO_4). This was placed in the refrigerator (4^{o}C) overnight for complete precipitation of the biosurfactant after which it was centrifuged at 4,000 rpm for 45 min. The supernatant was then filtered off and the sediment is the crude biosurfactant [31-35, 39].

RESULTS

Physico-chemical analysis

The results of the physicochemical analysis of the test soil sample before metal removal study is as shown in Table 3. The total amorphous content was 75.19% (aluminium 8.92%; silicon 63.11% and iron 3.16%). Silicon oxide (63.11%) dominated the metal oxides studied followed by Aluminium oxide (8.92%) and Iron oxide (3.16%). The soil had alkaline pH status of 9.69 with moderate 25.34 mg/kg phosphorus. Among the basic cations Ca^{2+} (7.5 cmol/kg) dominated followed by Mg^{2+} (1cmol/kg), K^{+} (0.094 cmol/kg) and Na^{+} (0.076 cmol/kg). Organic carbon (1.097%) and organic matter (1.892%) of the soil before study were rated moderate (1 - 1.5%) and very low (< 2.0%) respectively. The nitrogen (N) content of the soil was high > 0.02. The particle size distribution of the soil was dominated by sand (88.96%) compared to clay (9.76%) and silt (1.28%). The clay content is low (9.76%).

It was revealed that treatment F significantly ($p < 0.05$) increased (13.71%) the Al_2O_3 content of the soil compared to other treatments especially D that lowered the Al_2O_3 concentration from 8.92 – 8.21%. The untreated soil A also lowered the Al_2O_3 8.92 – 8.66%. The treatment types F (66.22%) G (63.24%) and H (66.11%) increased the SiO_3 concentration of the soil unlike other treatments that reduced it from 63.11-58.43%. Iron oxide (Fe_2O_3) concentration was significantly ($p<0.05$) increased (from 3.16 -11.89%) by F compared to D that had lowest (from 3.16 - 3.22%) increase (Fig. 2).

Statistically, the exchangeable basic cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) of the soils increased in all the treatment types used. Highest Ca^{2+} value of 5034.67 cmol/kg and K^{+} (132.81 cmol/kg) was evident in H treatment; Mg^{2+} (120.33 cmol/kg) in C treatment and Na^{2+} (1050.0 cmol/kg) in D treatment. The increase in these basic cations perhaps may be as a result of increase in soil pH by the treatments (Fig 3). The varying exchangeable base abundance evident in all the treatments are in the order $Ca^{2+} > Na^{+} > Mg^{2+} > K^{+}$ for treatment B and C, $Ca^{2+} > Na^{+} > K^{+} > Mg^{2+}$ for treatments F,G,H; $Na^{+} > Ca^{2+} > K^{+} > Mg^{2+}$ for treatment E and $Na^{+} > K^{+} > Mg^{2+} > Ca^{2+}$ for treatment D, hence the only treatment with lowest value of Ca^{2+}.

Organic carbon and organic matter content of the soils were also significantly ($p<0.05$) increased by the treatments, highest (4.48 and 7.86%) in G and C treatments respectively and lowest (3.18 and 5.48%) organic carbon and organic matter contents were witnessed in H treatment (Fig. 4). The total exchangeable acidity (TEA) of the soils were generally increased by the treatments used especially E (from 0.2 - 1.32 cmol/kg) (Fig. 5); while the phosphorus content of the soils were drastically and significantly ($p<0.05$) reduced by all the treatment types used, with highest values (from 25.34 – 5.65 ppm) recorded in C soil compared to lowest values of 25.34–1.18 ppm in D (Fig. 6).

Biosurfactant screening test

Odoribacter splanchnicus DSM20712 (WBS1) and CMS1 unidentified bacterium clone JX981747 showed positive β Hemolysis. WBS1 recorded highest E24 on kerosene while CMS1 recorded high E24 on crude oil and power vegetable oil. WBS1 displaced kerosene, crude oil, petrol and diesel while CMS1 showed visible displacement of only crude oil (Table 2).

Heavy Metal Removal

Heavy metal concentration of the soils were drastically and significantly ($p<0.05$) reduced by the treatment types used in the study. The initial values of metals in the test soil prior to amendment is as shown in Table 2. Among the heavy metals studied, zinc (724.8 mg/kg) dominated followed by copper (304.4 mg/kg), lead (131.4 mg/kg) and cadmium (0.38 mg/kg) (Table 2).

After amendments, statistical analysis at $p<0.05$ significant difference showed that highest value of 65.54 mg/kg and lowest value of 12.40 mg/kg for Pb was observed in D and G treatment types respectively whereas significantly ($p<0.05$) highest (64.46 mg/kg) and lowest (12.40 mg/kg) value for Zn was evident in F and C treated soils respectively. Cu concentration was not efficiently reduced treatment F that recorded 232.96 mg/kg compared to 52.37 mg/kg least copper value recorded in C treated soils (Fig. 1a). Generally, figure 1a depict that more lead (20.99), zinc (27.88) and copper (52.37) was reduced by treatment C than D.

Furthermore, treatment C also reduced more of all the metals (Cu: 52.37, Pb: 20.99, Zn: 27.88) than F (Cu: 232.9, Pb: 27.12, Zn: 64.46). Treatment G reduced Pb (12.4) and Zinc (29.9) more than D (Pb: 48.86, Zn: 48.86) whereas D reduced Copper (81.31) more than G (98.19). However, the composite of the two crude biosurfactant (E) reduced all metals (Cu: 62.56, Pb: 18.18, Zn: 40.25) more than H (Cu: 79.16, Pb: 48.16, Zn: 48.16). Generally, treatment of test soil

Table 2. Screening test for biosurfactant production

S/N	Isolate	β Hemolysis	Emulsification index (E24%) on Hydrocarbon					Oil displacement test on hydrocarbon (cm)				
			Kerosene	Crude oil	Vegetable oil	Petrol	Diesel	Kerosene	Crude oil	Vegetable oil	Petrol	Diesel
1	WBS1	+ve	78.13	0	0	22.60	0	2.50	3.00	-	2.00	2.00
2	CMS1	+ve	0	57.41	68.52	28.00	0	-	2.00	-	-	-

Legend: WBS1 = *Odoribacter splanchnicus*; **DSM20712** = from waste battery dump site; **CMS1** = unidentified bacterium clone from cassava mill soil

with G (CMS1 +BB) reduced more of all the three metals (Cu: 98.19, Pb: 12.4, Zn: 29.29) than F [WBS1 +BB] (Cu: 232.9, Pb: 27.12, Zn: 64.46) and H (Cu: 79.16, Pb: 48.16, Zn: 48.16) (Fig. 1a). Significantly ($p<0.05$) highest Cd (0.20 mg/kg) was reduced in C and lowest (0.11 mg/kg) recorded in D treated soils (Fig. 1b).

Table 3. The physicochemical analysis of some soil parameters and heavy metals of the test soil sample before heavy metal removal studies (Week 0)

Parameters	Metal waste dumpsite soil
$\%Al_2O_3$	8.92
$\%SiO_2$	63.11
$\%F_eO_3$	3.16
pH in H_2O (1: 2.5)	9.69
AvP pmm	25.34
Ca^{2+} cmol / kg	7.50
Mg^{2+} cmol / kg	1.0
K^+	0.094
Na^+	0.076
Exchangable acididty (Al + H)	0.20
Exchange.Al	Trace
% organic carbon	1.097
% Organic matter	1.892
% Nitrogen	0.139
Silt	1.28
% sand	88.96
% clay	9.76
Lead mg/kg	131.40
Zinc mg/kg	724.80
Cadmium mg/kg	0.38
Copper mg/kg	304.40

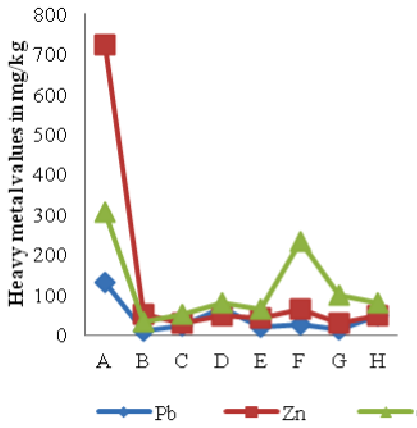

Figure 1a. The heavy metals (Pb, Zn, Cu) values of the test soil samples before (A) and after percolation (B-H)

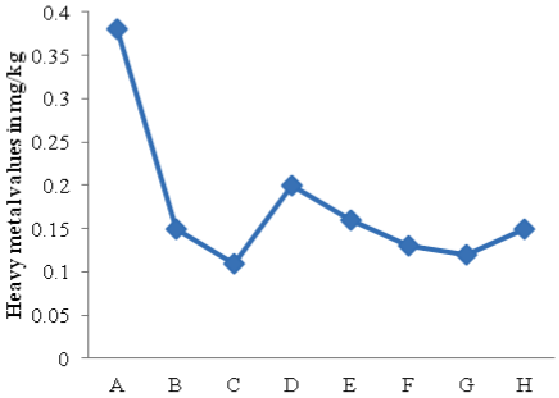

Figure 1b. The heavy metal (Cd) values of the test soil sample before (A) and after percolation (B-H)

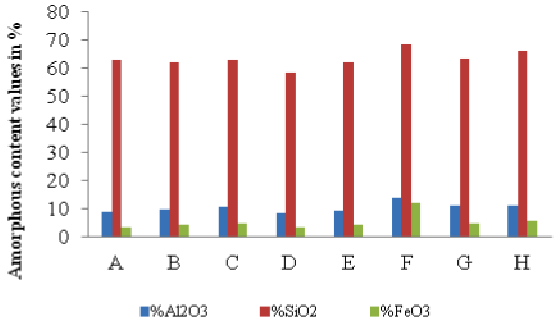

Figure 2. The amorphous content of the test soil samples before (A) and after percolation (B-H)

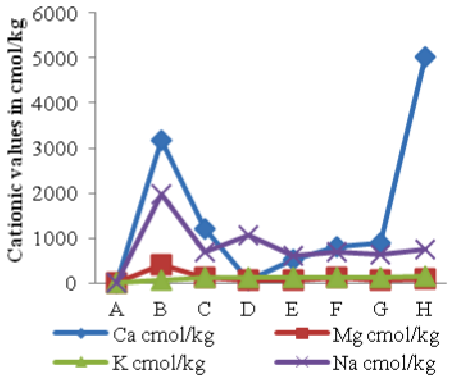

Figure 3. The exchangeable basic cations of the test soil before (A) and after percolation (B-H)

Legend: A = S-TPHU, **B** = S-CHS, **C** = S-CB (WBS1), **D** = S-CB (CMS1), **E** = S-MCB, **F** = S-CB+BB (WBS1), **G** = S-CB + BB (CMS1), **H** = S-MCB + CONBB (WBS1 and CMS1)

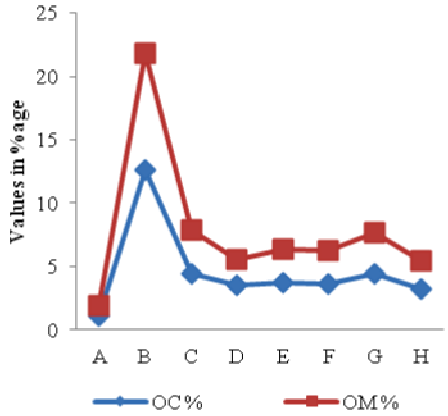

Figure 4. Percentage organic carbon and organic matter of the test soil sample samples before (A) and after percolation (B-H)

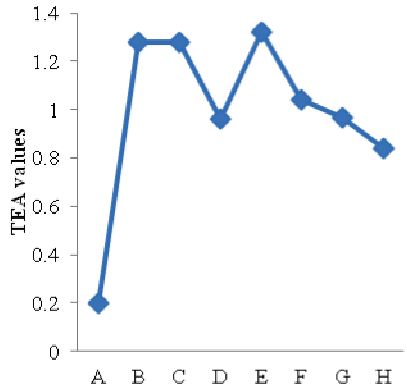

Figure 5. The Total Exchangeable acidity of the test soil before (A) and after percolation (B-H)

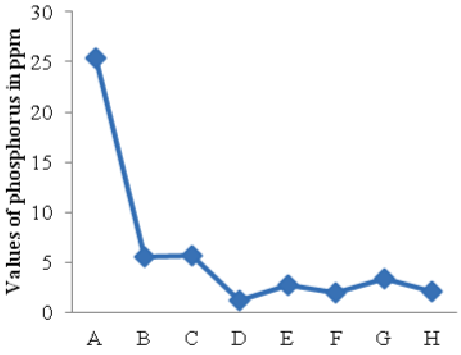

Figure 6. The phosphorus content of the test soil samples before (A) and after percolation (B-H)

Legend: A = S-TPHU, **B** = S-CHS, **C** = S-CB (WBS1), **D** = S-CB (CMS1), **E** = S-MCB, **F** = S-CB+BB (WBS1), **G** = S-CB + BB (CMS1), **H** = S-MCB + CONBB (WBS1 and CMS1)

DISCUSSION

The values of cations obtained from the analysis prior to soil treatment followed the decreasing cation magnitude $Ca^{2+} > Mg^{2+} > K^{+} > Na^{+}$ of Tope *et al.* [43] although during treatment the values increased and varied depending on treatment method involved as evident in this study. Low proportions of exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) have been reported for most Nigerian soils [1, 44] attributable to leaching losses by the high tropical rainfall as well as low content in the parent rock; and high value of Ca^{2+} was reported to be due in part to the dominant features of sub-humid regions of Nigeria such as low activity clay minerals and low organic matter content [43]. The increased value observed in this study therefore showed that probably, the parent rock /sand textured class recorded is a reflection of the parent material from which the soil was formed which is coastal plain sand. The higher the clay contents of a soil the higher the cation exchange capacity (CEC) since clay particles have the greatest surface area per unit volume of soil and therefore can hold the most cations. However, critical values of exchangeable cations have been reported by various researchers [40, 44, 46]. According to Ellis [10] organic matter is an important factor in the sorption of metals. Organic carbon and organic matter of the soil before study was rated moderate (1% - 1.5%) FAO [12, 13] and corroborates with the findings of Tope *et al.* [43]. The nitrogen content of the soil was in line with Esu [11] findings.

Furthermore, this study depict the clay content to be high (9.76%) with a low organic matter content (1.892%). Hence, the higher the clay contents of a soil the higher the cation exchange capacity (CEC) since clay particles have the greatest surface area per unit volume of soil and therefore can hold most cations. This is evident in the findings of this study and also supports the report made by Igwe and Nkemakosi [17] who studied on the nutrient element contents and cation exchange capacity in fine fractions of southeastern Nigerian soil in relation to their stability. Metal removal process is however; most efficient when applied to soils and sediments containing large proportions of sand and gravel and is relatively ineffective when applied to soils having a high silt, organic matter and clay content. Soils with a relatively high cation exchange capacity (the capacity to exchange cations for those in the polluting substances) tend to bind pollutants more tightly, which can limit the ability of the soil washing process to effectively separate the pollutants from the soil. This soil sample collected from waste metal dumpsite is a heavy sink for these metals. These metals will leach into the water table and nearby farmlands and will equally accumulate in the crops planted around these soils. This in turn will pose a health risk and overtime will trigger acute toxicity in persons who consume heavy metals bio-accumulated foods, hence the need for removal in an environmentally friendly manner using biological surfactants.

Heavy metals were evidently removed using the treatment methods employed on comparison to control (untreated soil). Similar result was recorded by Lai *et al.*, [20] who noted that biosurfactants have the potential to remove heavy metals from the soil through desorption and complexation. In addition, some microbes can mobilize the toxic metals and also accumulate them intra- cellularly. They can also change the mobility of the metals by producing various substances and by pH [4, 21] as observed in this

present study. They form complex by ionic bonds which is much stronger than the bonds formed by the soil and metals. By lowering the interfacial tensions, the metals are desorbed from the soil. Also by biosurfactants micelles, the metal ions can be removed as the polar head present in the peripheral regions of the micelles and it has the potential to mobilize the metal ions [23, 42]. It is worthy of note that heavy metal concentration of the soils after treatments were far below the EU threshold levels for Pb (300 mg/kg), Zn (300 mg/kg), Cd (3.0 mg/kg) and Cu (140 mg/kg) [6].

King *et al.* [18] noted that it is the macronutrient (N and P) that are of major concern in field application of bioremediation, because the minor and trace nutrients are usually present in sufficient amounts in the natural environment and are almost never the limiting factors in the field. This study also corroborates the report of Parr *et al.*, [36] who stated that the pH of the soil influences the solubility or bioavailability of macro and micro nutrients. Surprisingly, total exchangeable acidity (TEA) of the soils were generally increased by the treatments used especially E (from 0.2 to 1.32 cmol/kg). Although Sims & Bass [41] argued that organic matter is very important to the microbial ecology and activity of the soil, but the high cation exchange capacity and high density of reactive functional groups help bind both organic and inorganic compounds that are added to the soil. These properties also help retain the soil bacteria which can then attack the contaminants [38] as observed in this study.

This current study maintained the constant level of biosurfactants in the system by pulsed addition on weekly bases, in line with the findings of Maier *et al*. [22] whose research demonstrated that pulsed application of rhamnolipid allows bioremediation of the organic contaminant component in sites that are co-contaminated with organics and metals with no toxic residuals after treatment. With the exception of cadmium, all heavy metals before amendment exceeded the WHO range for heavy metals in agricultural soil [45] whereas there was drastic reduction during treatment justifying the effectiveness of the crude biosurfactant extracted from the test isolates. This study therefore predicts that application of this method will be of great importance in soil conservation and management, hence production of these biological biosurfactants requires scaling up.

Biosurfactant producing bacteria are ubiquitous and can be isolated from diverse environmental samples. The two bacteria used in this study were isolated from waste battery dumpsite soil and cassava mill soil respectively. These bacteria and the biosurfactant they produced removed metals from test soil samples. The treatment using only the biosurfactant produced from *Odoribacter splanchnicus* DSM 20712 removed multiple metals (copper, zinc and lead) more than the other treatments combinations.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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