RESEARCH ARTICLE

REMOVAL OF LEAD AND CHROMIUM FROM SOIL USING BIOSURFACTANTS PRODUCED BY BACTERIAL ISOLATES FROM SPENT LUBRICATING OIL CONTAMINATED SOIL.

*Munachimso Odenakachi Elemba1 and Udeme Joshua Josiah Ijah2.

1. Department of Biology/Microbiology/Biotechnology, Federal University Ndufu Alike, Ikwo, P.M.B 1010, Abakaliki, Ebonyi State, Nigeria.
2. Department of Microbiology, Federal University of Technology P.M.B.65, Minna, Niger State, Nigeria.

Abstract

Heavy metal removal using direct addition of biosurfactant (Rhamnolipid Bios-40, firmnolipid Bios-30, sulfoglycolipid Bios-25, glycolipopeptide Bios-17 and disulfoglycolipid Bios-12) solution on heavy metal spiked soils was studied. The heavy metals used were lead and chromium. 500ml of 100ppm of each metal salt solution was applied to 1kg of soil. The remediation potential of the biosurfactants was assessed using atomic absorption spectroscopy (AAS) and changes in pH of the soil after application of the biosurfactants (10mg/ml) to the metal contaminated soil. The results revealed the percentage removal of the metals in the range of 68.05%–95.5% for Lead (Pb) and 75.7%–91.0% for Chromium (Cr); Among the biosurfactants used rhamnolipid Bios-40 was most effective with percentage removal of 95.5% and 91.03%, followed by disulfoglycolipid Bios-12 (82.50% and 75.7%) for Pb and Cr respectively. This indicated that the biosurfactants favored mobilization of metals in the order of Pb>Cr. It was further observed that pH of the heavy metal contaminated soil remediated with biosurfactants was low (5.24–6.71 for Pb and 4.57–6.21 for Cr.) as compared to that of unpolluted soil (6.78) indicating high removal of metals from the contaminated soil and effective remediation of metals with the biosurfactants. The results suggest that the biosurfactants are suitable for the remediation of lead and chromium contaminated soil.

Introduction:

The presence of inorganic heavy metals such as lead, chromium, cadmium, mercury, zinc, iron, copper, and nickel in natural environments presents a potential health hazard for humans. The pollution caused by heavy metals has been a subject of concern worldwide. This is because heavy metals are non-biodegradable and because of this quality, they tend to accumulate in living organisms causing serious health effects, including reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death (D’Amore et al. 2005; Otitoloju et al. 2009). These have prompted the search for ways to mitigate this problem and one of these ways is the use of biosurfactants produced from bacteria (Herman et al. 1995; Mulligan 2005; Mulligan et al. 2007).

Biosurfactants are surface-active, degradable organic compounds, produced by microorganisms when grown on water-immiscible substrates (Bachmann et al. 2014). They have several beneficial properties in contrast with chemical surfactants, these include, lower toxicity, higher biodegradability, better environmental compatibility, higher foaming, high selectivity and specific activity at extreme temperatures, pH, and the ability to be synthesized from renewable feed-stock (Cooper and Goldenberg 1987; Mukherjee et al.2006;Thenmozhi et al., 2011., Adamu et al. 2015a). These properties give biosurfactants an edge for use in many areas such as agriculture, public health, food, health care, waste utilization, and environmental pollution control such as in degradation of hydrocarbons.
present in soil, enhanced oil recovery, remediation of organic compounds from contaminated soil as well as heavy metal removal from contaminated soil (Mulligan et al. 2007; Sanjee et al. 2012; Adamu et al. 2015b).

In bioremediation, biosurfactants effectively enable the transport of the more hydrophobic compounds, while the less hydrophobic ones are retarded due to the sorption of surfactant micelles to the soil surface. Due to their anionic nature, biosurfactants, such as rhamnolipid, are able to remove metals from soil by their ability to form complexes (Tan et al., 1994; Herman et al., 1995; Ochoa-Loza 2001).

Heavy metal contaminated soil can be remediated by batch or column washings using biosurfactants; for instance, Wang and Mulligan (2004) conducted column experiments to evaluate the feasibility of using rhamnolipid foam to remove Cd and Ni from a sandy soil and showed that the leaching behavior of the biosurfactant was different for different metals and suggested the need for more information to be established in the nature of the biosurfactant–metal complexes, this formed the bases for this study. Due to the recent challenge facing Nigeria in the area of heavy metal contamination especially in Zamfara State and Niger State, this study has become necessary. The study was conducted to evaluate the potential of biosurfactants in the removal of heavy metals (lead and chromium) from soil intentionally polluted with heavy metals in the laboratory.

**Material and Methods:**

**Collection of Samples:**

Uncontaminated soil was collected from agricultural land in Minna, Nigeria, using a soil auger into sampling bottles and transported to the laboratory. The metal compounds, lead [Pb(NO\(_3\)]\(_2\)] and chromium [Cr(NO\(_3\)]\(_3\)] used were of analytical grade with 99% purity obtained from Sigma-Aldrich, UK.

The biosurfactants used include rhamnolipid Bios-40, firmnolipids Bios-30, glycolipopeptide Bios-17, sulfoglycolipid Bios-25 and disulfoglycolipid Bios-12 produced by *Pseudomonas aeruginosa* mpe40, *Bacillus firmus* mpe30, *Pseudomonas paucimobilis* mpe17, *Acinetobacteriwoffii* mpe25 and *Micrococcus kristina* mpe12 respectively isolated from spent lubricating oil contaminated soil (Elemba and Ijah, 2016).

**Physicochemical Analysis of the Soil:**

The physico-chemical properties of the soil samples were determined using the methods described by Black (1965). The parameters determined were: pH, organic matter, permeability, porosity, cation exchange capacity (CEC), electrical conductivity (EC), moisture content, particle size distribution and metal (Pb and Cr) concentration.

**Preparation of Metal Stock Solution:**

Each metal salt (Lead trionoxitrate [Pb(NO\(_3\)]\(_2\)] and chromium nitrate [Cr(NO\(_3\)]\(_3\)] was weighed according to their molecular weight (that is, 1.599g and 4.55g respectively). This was dissolved in 1000ml of distilled water to make a standard stock solution of 1000ppm. Then 10ml of the 1000ppm was withdrawn into a 100ml volumetric flask and distilled water was added to make up to 100ml mark to make the concentration 100ppm.

**Pollution of Soil with Heavy Metal:**

The uncontaminated soil sample was crushed in a mortar with pestle and was passed through 2.0 mm sieve to remove debris. It was air dried for 48 hours at room temperature (30±2°C); then it was contaminated artificially in the laboratory using the prepared metal salts solution. One kilogram of the soil sample was weighed into 1000 capacity flasks and 500ml of 100ppm metal salt solution was added into the flask containing the soil; these were shaken vigorously using an orbital shaker (model HY-B11, USA) at 9000rpm for six hours to ensure maximum homogenous mixing, after which the mixture was allowed to stand for 20days with intermittent shaking. After 20 days, the supernatant was discarded and the contaminated soil was oven dried at 120°C for 3 hours to achieve sterilization and metal-soil binding. Some of the physicochemical properties (such as pH, Organic matter, moisture content, cation exchange capacity (CEC), electrical conductivity (EC), and permeability) of the soil were determined.

**Determination of Moisture:**

The moisture content of both the polluted and unpolluted soil was determined using the dry weight method. Four crucibles was dried in an oven at 90°C for 30minutes, cooled in a desiccator and weighed (W\(_1\)). Five grams (5g) of
the soil was introduced into the dried crucibles and the weight was taken \( (W_2) \). The crucible with the soil samples were dried to a constant weight at 90°C, allowed to cool in a desiccator and weighed again \( (W_3) \), the loss in weight of the sample during drying is the moisture content. It was calculated using the formula:

\[
\text{Moisture content} \% = \left( \frac{W_3 - W_1}{W_2 - W_1} \right) \times 100
\]

**Determination of Metal Concentration and Organic Matter:**

The metal content of the polluted and unpolluted soil was determined using the AAS (atomic absorption spectroscopy), while the organic matter content was determined using the method described by Black (1965).

**Bioremediation of Polluted Soil with Biosurfactants:**

One kilogram each of the polluted soil was placed into eight different sterile flasks (labeled mpe12, mpe17, mpe25, mpe30, and mpe40) in triplicates. To each of the flask containing the polluted soil, 10mg/ml of the biosurfactants was inoculated into the flask according to their specified labels. Control flask had soil inoculated with distilled water. The pH of the heavy metal polluted soil treated with biosurfactants was determined by suspending 10g of the treated soil sample in 100ml of distilled water in a beaker, swirled and allowed to stand for 10minutes. The pH meter (pH meter 3015, Jenway, U. K) was standardized with buffer of pH 4 and 7. The pH of the soil samples was determined by inserting the pH electrode into the solution and the reading was recorded when the reading on the pH meter was at a stable state.

Determination of heavy metal removal from soil by biosurfactant was done by removing 1 g of the treated soil into a 100ml beaker with 20ml of acid mixture containing 100ml of concentrated Perchloric \( (HClO_4) \) and 300ml of Nitric \( (HNO_3) \) acid in the ratio of 1:3. This mixture was heated at high temperature for 1h using a heating mantle (thermostat hotplate, Gallenhamp) until the soil was left only with whitish silicates. The digest was allowed to cool for 10mins, after which it was filtered into a 50ml volumetric flask using whatman No. 1 filter paper. The filtrate was diluted (i.e. water level was made up to the mark), after which the diluted filtrate was used for the measurement, using atomic absorption spectrophotometer (Shimadzu, wAArd80, Japan) with a slit no.0.2nm loaded with air/acyetylene in the ratio 60:85 as the fuel gas. The wavelengths set for the metals were: 217.0nm for Pb and 357.9nm for Cr.

**Statistical Analysis:**

Data generated were analyzed using one-way analysis of variance (ANOVA) of the Duncan descriptive test via the statistical package (SPSS) version 20. Statistical differences were set at \( p<0.05 \).

**Result and Discussion:**

**Physicochemical Properties of Heavy Metal Polluted Soil:**

The physical and chemical properties of both the metal polluted and the unpolluted soils are shown in Table 1. The pH, CEC, porosity, permeability value, and organic matter content of the heavy metal polluted soil were lower than the unpolluted soil while EC and moisture content of the polluted soil were higher than the unpolluted soil (Table 1). These could be attributed to the presence of metallic elements in the soil, showing that the metals (lead and chromium) actually bounded to the soil.

The CEC and porosity of the heavy metal polluted soil decreased as compared to the unpolluted soil. The changes in soil porosity and CEC may be due to the competitive metal sorption in soil and concentration of the heavy metals used for contamination (Juwarkar et al. 2007; Wuana et al. 2010) because the CEC is an indicator of ion availability in soil (Larson and Pierce, 1994). Schoenholtz et al. (2000) observed reduction in soil organic matter resulting from adsorption of metallic pollutant to soil surface.

The textural fraction remained unchanged even after the pollution, meaning that, the presence of the heavy metals in soil does not affect the texture of soil. Parthasarathi and Sivakumara (2011) recorded no change in particle size distribution, changes in pH value and CEC when a soil was spiked with nickel, cadmium and copper. Similar results were obtained by Liang et al. (2011). The investigators reported that the presence of charged ions on soil surface resulted in the decrease in cation exchange capacity and reduced pH value. Other researchers (Rahatgaonkar and Mahore 2008; Zhan et al., 2009; Damodaran et al., 2011) reported changes in soil physicochemical properties due to metal contamination, as obtained in this study.

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### Table 1: Physicochemical properties of unpolluted and heavy metal polluted soils.

| Properties                | Unpolluted soil | Lead polluted soil | Chromium polluted soil |
|---------------------------|-----------------|--------------------|------------------------|
| pH                        | 6.78            | 5.24               | 4.57                   |
| Organic matter (%)        | 8.09            | 2.18               | 2.67                   |
| Permeability              | $1.08 \times 10^{-3}$ | $1.02 \times 10^{-3}$ | $1.04 \times 10^{-3}$ |
| Porosity                  | 22.62           | 17.07              | 16.62                  |
| CEC (meq/100g$^{-1}$)     | 25.4            | 19.3               | 20.5                   |
| EC ($\mu$Scm$^{-1}$)      | 120             | 288                | 348                    |
| Moisture (%)              | 6.18            | 7.20               | 7.21                   |
| Particle size (%): sand   | 54.9            | 54.9               | 54.9                   |
| slit                      | 17.14           | 17.14              | 17.14                  |
| clay                      | 21.8            | 21.8               | 21.8                   |
| Metal concentration (ppm): Pb | 5.0              | 105.0              | NA                     |
| Cr                        | 0.6             | NA                 | 100.6                  |

ppm: parts per million; Pb: Lead; Cr: Chromium; CEC: cation exchange capacity; EC: electrical conductivity; meq: milliequivalents; $\mu$Scm$^{-1}$: microSiemens percentimeter, NA: Not applicable.

The results of heavy metal removal from contaminated soil by the biosurfactants are presented in Figs. 1-3.

**Biosurfactants**

Bios-12=(disulfoglycolipid Bios-12), Bios-30=(firmnolipid Bios-30), Bios-25=(sulfoglycolipid Bios-25), and Bios-17(glycolipopeptide Bios-17); Bios-40=(rhamnolipid Bio-40). ppm=parts per million.

**Fig. 1:** Effect of biosurfactants on lead polluted soil.

Results (Fig 1) show a progressive decrease in the concentration of lead (Pb) in the soil, with time (days), meaning that, as time (days) increased, the concentration of the heavy metal in the soil decreased with effectiveness of the biosurfactant in the order of Bios-40>12>30>25>17.

The concentration of lead in the soil (105.60ppm) was reduced to 18.37ppm, 46.45ppm, 33.55ppm, 25.70ppm and 4.71ppm by the biosurfactants, Bios-12, Bios-17, Bios-25, Bios-30, and Bios-40 respectively after 28 days (Fig. 1). It was generally observed that the biosurfactants, Bios-30 was faster in action than the other biosurfactants, though it did not achieve the best result (highest rate of metal removal) after 28 days while the lowest reduction rate was
observed in Bios-17. These variations in the rates of removal of heavy metals by the biosurfactants could be attributed to the rates of biosurfactant adsorption to metal which may be influenced by the molecular compositions, size and length of carbon chain of the biosurfactants.

The results (Fig 1) also revealed that the biosurfactants Bios-40 (rhamnolipid Bios-40), Bios-12 (disulfoglycolipid Bios-12), Bios-30 (glycolipid Bios-30), Bios-25 (sulfoglycolipid Bios-25), and Bios-17 (glycolipopeptide Bios-17) achieved lead removal in the order of 95.5%> 82% >75.52%> 68.05%>55.76% from soil as compared to 0.24% and 3.07% removal with distilled water and solvent (controls) respectively; the results revealed a significant decrease in lead concentration in the soil at p<0.05.

The percentage removal by the biosurfactant, rhamnolipid Bios-40 was as high as 95.5% compared to 15.3%, 88%, and 62% achieved by a rhamnolipid biosurfactant used by Neilson et al. (2002) Daharazma and Mulligan (2007), Juwarker et al. (2007), and Abdurrahim et al. (2012) respectively in the treatment of lead contaminated soil. These differences in results could be attributed to variations in concentration (10mg/ml) of biosurfactant used as compared to 80ppm used by Abdurrahim et al. (2012) and 5ppm used by Neilson et al. (2002). Also, 98.3% removal of Pb from aqueous solution was achieved by a polysaccharide produced by B. firmus as reported by Salehizadeh et al. (2003) which is higher than 75.5% removal obtained by biosurfactant (Bios-30) produced by B. firmus mpe30 in the present study; the reason being that the mobility of heavy metal in aqueous solution is different from that in soil.

In addition, the biosurfactant Bios-17 produced from P. paucimobilis mpe17 had the lowest lead removal of 55.76% as compared to the maximum removal of 95.5% by Bios-40 from P. aeruginosa indicating that biosurfactant activity varies from species to species irrespective of the fact that they are of same genus. This was proved when biosurfactant from Pseudomonas sp.CH_8 showed percentage efficiency removal of lead up to 90%, as compared to 50%, 45% and 25% removal of lead by biosurfactants of Pseudomonas CH_2,CH_6 and CH_12 respectively (Lin andHarichund, 2011).

So far, we are not aware of any report on the use of biosurfactant produced from Micrococcus species in remediation of heavy metal. However, Micrococcus sp. BRM7 with ability to produce biosurfactant had been successfully used to adsorb 17% and 34% of strontium within an incubation time of 2 and 4days respectively as compared to its non biosurfactant producing counterpart (Micrococcus sp. ARMT8) that died off after 24 hours (Issam et al., 2012). The researchers added that the use of the surfactant alone may be effective in remediation of heavy metal other than strontium (Young et al., 2010; Issam et al., 2012) and this has been proved in this present study that obtained 82% removal of lead from lead contaminated soil using the biosurfactant disulfoglycolipid Bios-12. Hence, it was deduced that biosurfactants used in the present study demonstrated metal sorption capacity with varying levels of efficiency.
A significant reduction in the concentration of metal (Cr) was observed after the addition of biosurfactant as compared to control. The concentration of chromium in the soil (100.60ppm) was reduced to 24.48ppm, 15.70ppm, 21.35ppm, 23.03ppm and 9.02ppm by the biosurfactants, Bios-12, Bios-17, Bios-25, Bios-30, and Bios-40 respectively after 28days (Fig 2). It was generally observed that as time (days) progressed the concentration of chromium in the soil decreased, meaning that, the percentage removal of the heavy metal by the biosurfactants increased. However the rates of reduction of chromium concentration differed from one biosurfactant to another. The rate of reduction by Bios-40 was faster than all the other biosurfactants and it achieved the best result (highest rate of metal removal) after 28 days. This variation in the rate of metal removal by the biosurfactants could be attributed to the size of the biosurfactant, the degree of saturation, the substituent, length of carbon chain, as well as their adsorption capacity and different sorptive interactions exhibited by the biosurfactants to the soil system (Asci et al. 2008).

The results (Fig 2) revealed that the biosurfactants, Bios-40, Bios-17, Bios-25, Bios-30, and Bios-12 achieved an effective chromium removal in the order of 91.03% >84.4% >78.8% >77.1% >75.7% as compared to 0.4% and 2.3% removal with distilled water and solvent (controls) respectively after 28days. Thus Bios-40 had the maximum removal efficiency of 91.03% which was closely followed by Bios-17 (84.4%) > Bios-25 (78.8%) >Bios-30(77.1%) while Bios-12 had the minimum removal efficiency of 75.7%. The results were significantly different at p<0.05.

The biosurfactant produced by *P. aeruginosa* mpe40 (rhamnolipid Bios-40) and *P. paucimobilis* MPE17 (glycolipopeptide Bios-17) in the present study were more effective with percentage removal of 91.03%, and 84.4% respectively after 28days. These results agree with the report of Massara et al. (2007). Similarly Singh (2012) reported 92% removal of chromium from a soil spiked with 100mg/kg heavy metal within an interval of 24 days by rhamnolipid. A batch and column experimental setup by Lidi et al. (2012) was used to evaluate the efficiency of biosurfactants (saponin and sorphorolipid) in the removal and recovery of heavy metals from sludge and the investigators observed 73.2% removal of lead, 64.2% of nickel, and 56.1% of chromium whereas 89.7%, 91.1%, and 99.1% of Pb, Ni and Cr. respectively were recovered after two weeks.

The adsorption efficiency for chromium achieved by the biosurfactant, firmnolipid Bios-30 from *Bacillus firmus* mpe30 in this study was 77.11% which is lower than 90% and 85% achieved by biosurfactant from *Bacillus subtilis* WD90 in nickel and cadmium respectively (Kaewchai and Prasertsan, 2002). Percentage removal obtained by sulfoglycolipid Bios-25 and disulfoglycolipid Bios-12 in this study was 75.67% and 78.78% respectively. Although there has been no report to the best of our knowledge on the use of biosurfactant from these species in metal remediation; it has been cited by researchers that *Acinetobacter* RAG-1 can bind up to 240µg uranium per emulsion or emulsan ([UO$_2^{2+}$]/mg emulsion or emulsan) (Zosim et al.1983; Niu et al. 1993; Miller, 1995; Abdurrahim et al., 2012) which has been confirmed in the present study.
The biosurfactants exhibited different removal efficiencies for both lead and chromium (Fig 3). Only Bios-40 and Bios-12 had higher percentage removal for lead at 95.5% and 82.50% respectively while the other three biosurfactants (in the order Bios-17 >25 >30) were more effective on chromium at 84.39%, 78.78% and 77.11% respectively. Comparing the effectiveness and trend of reduction in all the five biosurfactants on the metals, it was deduced that the biosurfactants were more effective on chromium than lead (Cr >Pb) and this is possibly due to its (Pb) strong association and adsorption with soil (Wuana et al., 2010) which contributes to its high toxicity to the environment.

Differences in Cr and Pb removal indicate that biosurfactants facilitate mobilization of metals selectively, and their adsorption is dependent on the characteristics of the metal due to the specificity of biosurfactant for each metal in the soil (Lidi et al., 2012). Ochoa-Loza et al. (2009) had also reported preferential complexion of metals in the range of Cd, Pb and Hg by biosurfactant. Similarly Lin and Harichund (2011) added that differences in affinity of metals for biosurfactants are due to charge density, attractive interaction, and types of conformation formed by these polymers with the adsorbed ions.

All the five biosurfactants produced in this study demonstrated metal sorption potential with varying levels of efficiency (Figures 1, 2 and 3). Several other researchers (Wang and Mulligan, 2004; Noghabi et al. 2007; Lin and Harichund, 2011) have reported varying efficiencies by biosurfactants in metal removal.

The pH of metal polluted soil remediated with biosurfactants was monitored and the results obtained are presented in Figures 4 and 5 for lead and chromium respectively.
It was observed that the pH of the unpolluted soil was higher (6.78) than the lead polluted soil (5.24). However, the pH of the polluted soil increased gradually from day zero to 28 days (Fig. 4). As the concentration of the metal decreased in the soil, the pH of the soil increased progressively (Fig. 4), with a range of 5.24−6.43 for Bios-12, 5.24–6.52 (Bios-17), 5.24–6.44 (Bios-25), 5.24–6.44 (Bios-30), and 5.24–6.71 (Bio-40). When the pH of the remediated soil was compared with the pH (6.78) of the original soil (before pollution with Pb) and the soil polluted with Pb (5.24), there were significant differences (p<0.05). However, there was no significant difference (p>0.05) between the treated soil and the original soil sample after 28 days, meaning that the soil was almost returning to its original state after 28 days of inoculation with the biosurfactants, signifying that the biosurfactants were effective in the metal removal. Sanjee et al. (2012) reported an increase in pH value (6.04–6.78) of a heavy metal contaminated soil when treated with di-rhamnolipid as an evidence of surfactant effectiveness in remediation of metal.

**Fig 4:** pH of lead polluted soil treated with biosurfactants.
The results (Fig. 5) showed that the pH of the unpolluted soil (6.75) was higher than the chromium polluted soil (4.57). However the pH of the polluted soil increased gradually from day zero to 28 day. It was also observed that the pH decreased from 6.78 to 4.57 as Cr was added to the soil and thereafter increased with the addition of biosurfactants. The metal concentration decreased as the day progressed the pH of the soil increased. This is in agreement with Lidi et al. (2012) who recorded that the removal efficiency of heavy metals by biosurfactants generally ascended with decreasing concentration of metal and increasing pH value. Singh et al. (2012) also recorded increased in pH value from 6.60 to 6.78 after remediation period. Significant correlation coefficient with pH and metal concentration was observed to be (r=0.922, P< 0.05) and (r=0.903, P<0.05) for lead and chromium respectively. These strong correlation values demonstrate the strength of association between pH and metal concentration in soil. This correlation can be explained by considering the fact that pH is the main soil characteristic that influences the CEC of the soil which in turn shows the association and adsorption status of metal to soil (de Matos et al., 1996; de Matos et al., 2001). Biosurfactants used in the remediation of metal contaminated soil hindered the mobility of metal, which prevented them from accumulating in soil, thus enhancing metal removal. The treatment is eco-friendly, biodegradable and eventually leads to restoration of the contaminated soil. The present study showed that the biosurfactants were efficient in preventing detrimental environmental impacts by remediating metals from contaminated soil thereby helping to restore the integrity of the ecosystem.
References:-
1. Abdurrahim, A. E., Abdulrhaman, A. A., Ali, M.E., Mokhtar, E. and Bassam, A. (2012). Removal of heavy metal contamination by biosurfactants (rhamnolipids). Journal of Chemical and Pharmaceutical Research, 4(9), 4337-4341.
2. Adamu, A., Ijah, U.J.J., Riskuwa, M.L., Ismail, H.Y. and Ibrahim, U.B. (2015a). Study on biosurfactant production by two Bacillus species. Internat. J. Scient. Research in Knowl., 3(1), 030-020.
3. Adamu, A., Ijah, U.J.J., Riskuwa, M.L., Ismail, H.Y. and Ibrahim, U.B. (2015b). Isolation of biosurfactant producing bacteria from tannery effluent in Sokoto metropolis, Nigeria. Internat. J. Innovat. Sci., Engine. Tech., 2(1), 366-373.
4. Asci, Y., Nurbas, M. and Acikel, Y. S. (2010). Investigation of sorption/desorption equilibria of heavy metal ions on/from quartz using rhamnolipid biosurfactant. J. Environ. Mgt., 91, 724-731.
5. Bachmann, R.T., Johnson, A.C. and Edyvean, R.G. (2014). Biotechnology in the petroleum industry; an overview. Internat. Biodeter. and Biodegrad., 86, 225-273.
6. Black, C.A. (1965). Method of soil analysis II, American society of agronomy, Madison, pp.573-590.
7. Charoon, S. and Siripun, S. (2010). Removal of cadmium and zinc from soil using immobilized cell of biosurfactant producing bacteria. Internat. J. Environ. Asia, 3(2), 49-53.
8. D'amore, J. J., Al-abed, S. R., Scheckel, K. G. and Ryan, J. A. (2005). Methods of speciation of metals in soils. J. Environ. Qual., 34(5), 1707-1745.
9. Dahrzama, B. and Mulligan, C.N. (2007). Investigation of removal of heavy metals from sediments using rhamnolipid in a continuous flow configuration. Chemosphere, 69(5): 705-711.
10. Damodaran, D., Suresh, G., and Mohan, R. B. (2011). Bioremediation of soil by removing heavy metals using Saccharomyces cerevisiae 2011 2nd International Conference on Environmental Science and Technology IPCBEE vol.6 (2011) © (2011) IACSIT Press, Singapore
11. De Matos, A.T., Fontes, M. P. F., Da Costa, L. M. and Martinez, M. A. (2001). Mobility of heavy metals as related to soil chemical and mineralogical characteristics of Brazilian soils. Environmental Pollution, 111, 429-435.
12. De Matos, A.T., Fontes, M. P. F., Jordao, C.P. and da Costa, L. M. (1996). Mobility and retention forms of heavy metals in a Red- Yellow Latosol profile. Revista Brasileira de Ciencia do Solo, 20, 379-386.
13. Elemba, O. M., Ijah, U.J.J. and Chibunna Miracle (2015). Isolation, characterization and meor ability of the biosurfactant produced from serratiamarcescens ueo15. Global J. Adv. Res. 2(6), 962-974.
14. Herman, D. C., Artiola, J. F. and Miller, R. M. (1995). Removal of cadmium, lead, and zinc from soil by a rhamnolipid biosurfactant. Environ. Sci. and Tech., 29, 2280-2292.
15. Juwarkar, A. A., Nair, A., Dubey, K.V., Singh, S.K and Devotta, S. (2007). Biosurfactant technology for remediation of cadmium and lead contaminated soils. Chemosphere 68, 1996-2002.
16. Kaewchai, S. and Prasertsan, P. (2002). Biosorption of heavy metal by thermotolerant polymer - producing bacterial cells and the bioflocculent. SongKlanakarin J. Sci. and Techn., 24(3), 421 - 430.
17. Larson, W. E. and Pierce, F. J. (1994). The dynamics of soil quality as measure of sustainable management. In: J.W. Doran, D.C. Coleman, D. F. Bezlick, B. A. Stewart (Eds.). Defining Soil Quality for a Sustainable Environment, pp. 37-51. Soil Science Society of America, Special Publication No. 35.
18. Lidi, G., Naoki, K., Yuichi, S., Chong, L., Shuang, Z. and Hiroshi, I. (2012). Behaviour and distribution of heavy metals including rare earth elements, thorium, and uranium in sludge from industry water treatment plant and recovery method of metals by biosurfactants application. Bioinorganic Chemistry and Applications 2012(2012) Article Id 173819, 11 Pages http://dx.Doi.org/10.1155/2012/183819.
19. Lin, J. and Harichund, C. (2011). Isolation and characterization of heavy metal removing bacterial bioflocculants. African J. Microbiol. Res., 5(6), 599 – 607.
20. Liu, J., Varonsson, H., Bergstrom, L. and Sharply, A. (2012). Phosphorus leaching from loamy sand and clay loam top soils after application of pig slurry. Retrieved from www.springerplus.com/content/1/1/53.
21. Mukherjee, S., Das, P. and Sen, R. (2006). Towards commercial production of microbial surfactants. Trends in Biotechn., 24, 509 – 515.
22. Mulligan, C. N. (2005). Environmental applications for biosurfactants. Environmental Pollution, 133,183–198.
23. Mulligan, C. N., Oghenekewye, C., Fukue, M. and Shimizu, Y. (2007). Biosurfactant enhanced remediation of a mixed contaminated soil and metal contaminated sediment. 7th Geoenvironmental Engineering Seminar, Japan–Korea–France, May 19–24, Grenoble, France.
24. Neilson, J.W., Artiola, J.F. and Maier, R. M. (2002). Characterization of Lead Removal From Contaminated Soils by Non-Toxic-Soil Washing Agents. J. Environm. Qual. 32, 899-908.
25. Ochoa-Loza, F.J., Artiola, J.F. and Maier, R.M. (2009). Stability constants for the complexation of various metals with a rhamnolipid biosurfactant. J. Environmt. Qual. 30, 479–485.
26. Parthasarathi, R. and Sivakumaar, P. K. (2011). Biosurfactant mediated remediation process evaluation on a mixture of heavy metal spiked topsoil using soil column and batch washing methods, soil and sediment contamination: An Internat. J. 20,8, 892-907.
27. Rahatgaonkar M. and Mahore, N. R. (2008). A Selective Bioreduction of Toxic Heavy Metals. Journal of Experimental Biotechnology, 47,766-769.
28. Sanjee, K., Asha, J.A Ackmez, M. and Kirti.D. (2012). Remediation of chromium-contaminated soils using di-rhamnolipid. J. Environ. Toxicol. 6(2), 100-105.
29. Schoenholtz, S.H., Miegroeth, H.V. and Burger, J.A. (2000). A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. Forest Ecol. and Mgt. 138, 335 – 356.
30. Singh, S. K., Juwarkar, A. A., Mudhoo, A. and Dubey, K.V. (2012). Remediation of chromium-contaminated soils using Pseudomonas aeruginosa strain BS2. Terrestial and Aquatic Environmental Toxicology, 6(2), 100-105.
31. Tan, H., Champion, J. T., Artiola, J. F., Brusseau, M. L., and Miller, R. M. (1994). Complexation of cadmium by a rhamnolipid biosurfactant. Environmental Science and Technology, 28, 2402–2406.
32. Thernmozhi, R., Sornalaksmi, A., Praveenleumar, D. and Nagasathya, A. (2011). Characterization of biosurfactant produced by bacterial isolates from engine oil contaminated soil. Advances in Environmental Biology, 5(8), 2402 – 2408.
33. Wang, S. and Mulligan, C. N. (2004). Rhamnolipid foam enhanced remediation of cadmium and nickel contaminated soil. Water Air and Soil Pollution, 157:315–330.
34. Wuana, R. A., Okieimen, F. E. and Imborvungu, J. A. (2010). Removal of heavy metals from a contaminated soil using organic chelating acids. International Journal of Environmental Science and Technology, 7 (3), 485-496.
35. Young, M., Artsathanov, Y., Beller, H.R., Chandra, G., Chater, K.F. and Dover, L.G. (2010). Genome sequence of the Fleming strain of Micrococcus Luteus, a simple free – living Actinobacterium. J. of Bacteriol. 192, 841 – 860.
36. Zhan, Y., Fan, C., Meng, Q., Diao, Z., Dong, L., Peng, Y, Shuyan, M. and Zhou, Q. (2009). Biosorption of Pb²⁺ by Saccharomyces cerevisiae in static and dynamic absorption tests. J. of Environt. Contamination and Toxicol. 83, 708 – 712.