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Evaluating The Performance of Indigenous Microorganism for Bioremediation of Lead Contaminated Soil

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Abstract: This work is on remediation of lead concentration (130.36 mg/kg) in soils from farm settlement at Agbabu community in Ondo State of Nigeria to below maximum allowable 100 mg/kg specified for safe agriculture by standards to ensure that farm products from this farm settlement close to area of mining are safe for human beings. Three indigenous organisms: Bacillus subtilis (B. subtilis), Escherichia coli (E. coli) and Proteus mirabilis (P. mirabilis) were engaged for the remediation study. The organisms were isolated and cultured. Optimum weights of the distinct organisms were inoculated in 5g soils each conditioned with optimum values of pH, temperature, stirring frequency and nutrient in thirty-six 50 ml beakers; and experimented for residual lead ion at times 5, 10, 15, 20, 25, 30 and 35 days in triplicate with Atomic Absorption Spectrophotometer. Lead concentration in the soil was tackled by the three organisms, they were able to bring the initial concentration of lead to below the maximum allowable concentration, but this happened at different rates and efficiencies. For the three organisms, there was a continuous decline in the soil lead concentration with time. B. subtilis was able to bring the concentration to below the maximum allowable concentration at time 10 days with efficiency of 27.64% at a residual concentration of 94.32 mg/kg. In contrast, P. mirabilis and E. coli were able to bring the initial concentration to below the maximum allowable concentration at time 15 days with different efficiencies of 32.64 % at 87.81 mg/kg residual concentration for P. mirabilis and 34.30 % at 85.85 mg/kg residual concentration for E. coli. The initial strength rating showed B. subtilis to be ahead in strength, seconded by E. coli while P. mirabilis lagged behind them. This was noticed from time 5 days to 15 days. However, after this time, P. mirabilis was observed to take the lead, seconded by B. subtilis while E. coli lagged behind them. This is obviously remarkable in their 35 days removal efficiencies of 75.12% for P. mirabilis at a residual concentration of 32.44 mg/kg; 74.26 % for B. subtilis at a residual concentration of 33.55 mg/kg; and 52.26 % for E. coli at a residual concentration of 62.24 mg/kg. This shows that removal by B. subtilis is preferred on a short-run bioremediation, while removal by P. mirabilis is preferred on a long-run pollution control.
Keywords: Remediation, Indigenous Organisms, Lead Concentration, Pollution Control

INTRODUCTION

Heavy metals are parts of the natural earth's crust. However, man's activities of diverse nature have boost their concentrations to pollution levels in the environment (Ukponge et al. 2013). Through human activities, dangerous metals have become global problem plaguing many sites (Gray et al. 2006), these metals persist due to geoaccumulation and bioaccumulation (Akpor and Muchie, 2010) and are very difficult to remove (Nanda and Abraham, 2013).

Some of these metals are relevant to the existence and performances of living organisms and other lives at the required concentrations. Above these concentrations benchmarks, they are injurious to lives (humans, plants, and other animals) on planet earth (Ukponge et al.2013).

Microorganisms have reportedly developed various methods for their survival in heavy metals polluted environments as they are known to develop and adopt different detoxifying mechanisms such as biosorption, bioaccumulation, biotransformation and biomineralization. Microorganisms are capable of breaking down many complex molecules by adaptation of their degradative enzyme system (Boonchan et al. 2000). The anthropogenic enrichment or contamination of the environment by heavy metals is caused by their dispersion. These includes: gas-dust releases into the atmosphere under high temperature technological processes

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(e.g. power plants, metals smelting, the burning of raw materials for cement etc.), waste incineration and fuel combustion like the one from motor transport, which is widely connected with the use of lead as an additive to gasoline (Galiulin et al. 2002).

Because of the serious ecological dangers of having these metals in soils, treatment of affected sites is pursuit vigorously with serious drive to understand their hot spots by studying their spatial concentration (Hua et al. 2012; Hani et al. 2014) and the best treatment alternative.

Treatments housed in physical-chemical methods are effective but with many post-treatment headaches of more toxic products in soil and high cost (Bahi et al. 2012). Besides, they are incapable of handling certain, low concentrations of metals (Dixit et al. 2015). This paved way for bioremediation that is still undergoing intensive research to have a more effective cleaning method that is friendly to the environment. Bioremediation has been reported as cost effective, environmentally healthy, and the way forward in treating heavy metals affected lands. Bioremediation, a method of soil cleansing functions on the utilization of mechanisms in-built in microorganisms and plants to remove injurious substances from the ecosystem. Bioremediation with genetically engineered; and indigenous microorganisms have yielded significant and reliable results (Gupta et al. 2016).

In this work, bioremediation of soils from farm settlement in Agbabu community in Ondo State of Nigeria was studied using three indigenous organisms (Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), and Proteus mirabilis (P. mirabilis). This was aimed at attenuating the soil Lead concentration to below 130.36 mg/kg specified as the maximum allowable for safe agriculture by standards in (Chiroma et al. 2014) to ensure that farm products from these farm settlements close to area of mining are safe for human beings.

SOURCING OF MATERIALS

These include soil sample from Agbabu community, MacConkry agar, magnetic stirrer, hydrogen peroxide, measuring cylinder, safranin, refrigerator, simon citrate ager, inoculating needles, Kovac's reagent, incubator, triple sugar iron agar, microscope, sodium hydroxide, conical flasks, nitric acid, beakers, hydrochloric acid, wire loops, Lugo's iodine, pipettes, oxidase reagent, cotton wool, methylene blue, autoclave, peptone water, petri dishes, ethanol, filter paper, perchloric acid, MacCartney bottles, sulphuric acids, hot plate, peptone water, atomic absorption spectrophotometer and crystal violent.

ORGANISMS ACQUISITION

At a microbiology laboratory belonging to Delta State University, Nigeria; microbiology analysis was conducted on the soils to acquire indigenous microorganisms.

Aliquot from serial dilution was introduced into petri dishes, covered with Mac Conkey agar (Baron et al. 1994), and incubated for 24 hours at 37^{o}C (Chessebrough,2000). Developed Colonies were recognized after they were sub cultured (Holt,1994, Chessebrough,2000)

OPTIMUM FACTORS ACQUISITION

Vital factors have been discovered to have significant influence on bioremediation process and rate (Atikpo, 2016, Murthy et al. 2012). The immense scientific significance of these factors at their optimal levels requires that they be carefully studied, screened and selected for a particular bioremediation study

Adopting the batch method in (Atikpo and Michael, 2018) pH values of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; temperature values of 10, 20, 30, 40, 50, and 60^{o}C; nutrient dosage of 2, 4, 6, 8, 10, and 12 ml; organisms' weights of 1, 2, 3, 4, 5, and 6g; and stirring frequencies of 0, 1, 2, 3, 4, 5 and 6 per week (pw) were respectively and distinctly introduced into 4g in thirty-four 50 ml beakers and inoculated with the different organisms. The soils samples separated from the organisms were tested for depletion in metal content on the 14th day with Atomic Absorption Spectrophotometer (AAS).

ION REMOVAL

Applying the method in (Atikpo and Michael, 2018), the optimum weights of the distinct organisms were inoculated into 4g soils each conditioned with optimum values of pH, temperature, stirring frequency and nutrient in thirty-six 50 ml beakers and experimented for residual zinc ion at times 5, 10, 15, 20, 25, 30 and 35 days in triplicate with AAS.

The concentration removed with time, removal efficiency, and concentration removed at equilibrium were calculated from Equations (1), (2) and (3) (Augustine et al. 2007, Igwe and Abia, 2006)

$$qt=\frac{(Co-Ct)}{m.V} \quad \text{(Equation 1)}$$

$$\text{Efficiency } (\varepsilon) = \frac{(Co-Cf)}{Co}.100 \quad \text{(Equation 2)}$$

$$qe=(Co-Ce)m.V \quad \text{(Equation 3)}$$

Where V is volume of soil used, Ce is equilibrium concentration, Co is initial concentration, m is the mass of organism, Ct is the residual concentration per time, qe removal at equilibrium, Cf is the final residual concentration, and qt is removal with time.

Two-ways (ANOVA) at ($P < 0.05$) conducted with Microsoft Excel, 2016 version was engaged to determine significant variation in removal with organisms and significant variation in removal with time.

ORGANISMS AND OPTIMUM FACTORS

The microbiology experiments revealed B. subtilis, E.coli and P. mirabilis from developed colony of 2.8 x 105 with respective biochemical properties of (positive, negative, positive, negative, positive, positive, positive and negative); (negative, negative, positive, negative, negative, positive, negative and negative); and (positive, negative, negative, negative, positive, positive, positive and positive) catalase, citrate, oxidase, indole, glucose, sucrose, motility and lactose analysis.

pH, stirring frequency, temperature, organisms' masses, and nutrient dosage were determined.

pH affects the negative charges on cells and the chemistry cell wall; and the metals physiochemistry (Lopez et al. 2000, Samarth et al. 2012), thus influencing bioremediation. From Figure 1, optimum values were 8 for B. subtilis and 6 for P. mirabilis and E. coli at respective minimum concentrations of 80.9 mg/kg, 99.8 mg/kg and 100.35 mg/kg remaining in soils.

Temperature variation influences the process significantly (Ajaykumar et al. 2009).

The influences of the tested temperature degrees are shown in Figure 2 displaying an optimum degree of 30^{o}C for the organisms. The respective minimum concentration at this optimum degree where 77.9 mg/kg, 100.71 mg/kg and 96.35 mg/kg for B. subtilis, P. mirabilis and E. coli respectively.

The supply of requisite nutrient is very essential for the stimulation of the indigenous microorganisms for effective performance (Sang-Hwan et al. 2007). Bio stimulation by nutrient supply increases the number of organisms through rapid growth and replication, and ultimately increases bioremediation rate (Thieman and Palladino 2009)

From Figure 3, an optimum nutrient dosage of 8 ml was displayed. The influence was in the decreasing order of 8 ml, 6 ml, 10 ml, 4 ml, 12 ml and 2 ml for the use of B. subtilis; 8 ml, 10 ml, 6 ml, 12 ml, 4 ml and 2 ml. for the use of P. mirabilis; 8 ml, 6 ml, 10 ml, 12 ml, 4 ml and 2 ml for the use of E. coli. The minimum concentrations at the optimum nutrient dosage was 85.9 mg/kg, 101.41 mg/kg and 101.1 mg/kg for removal by B. subtilis, P. mirabilis and E. coli respectively.

Figure 4 shows the resultant influence of 2, 3, 4, 5 and 6 grams of the respective organisms on the process with the optimum weight of 5g for the respective organisms at the respective minimum concentrations of 93.7 mg/kg, 110.14 mg/kg and 112.36 mg/kg for B. subtilis, P. mirabilis and E. coli respectively.

Influences of the weights of the organisms were in the decreasing order of 5g, 4g, 3g, 6g, 2g and 1g; 5g, 4g, 3g, 6g, 2g ; and 5g, 4g, 3g, 6g, 2g and 1g for removal by for B. subtilis, P. mirabilis and E. coli respectively. Figure 5 shows the influences of stirring frequencies on the organisms' performances, 5pw at 120 rpm for P. mirabilis; and 5pw at 150 rpm for B. subtilis and E. coli as the optimum stirring frequencies at the respective residual concentrations of 84.04 mg/kg, 101.99 mg/kg and 110.31 mg/kg for B. subtilis, P. mirabilis and E. coli respectively.

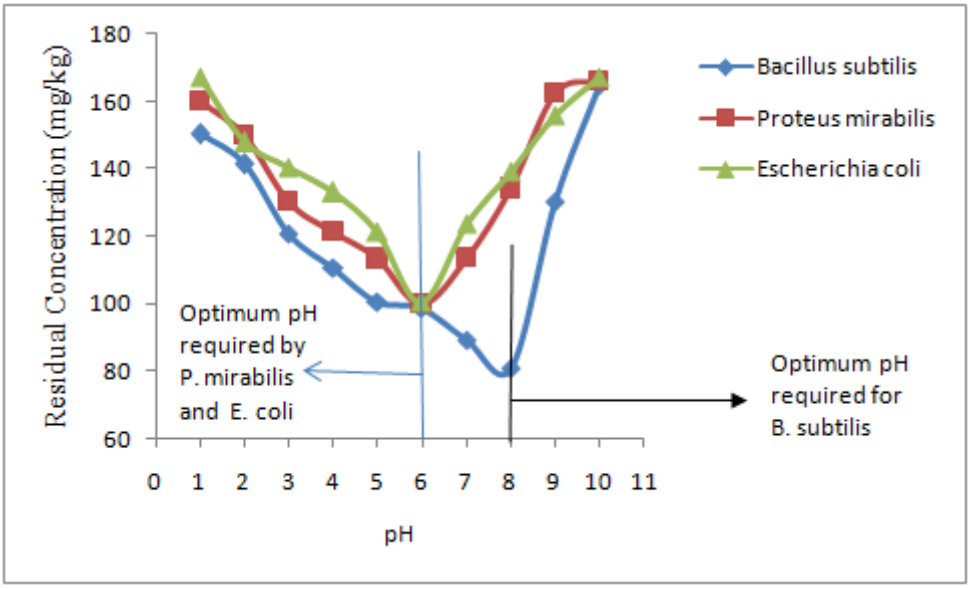

Figure 1. Impact of pH on Lead Removal

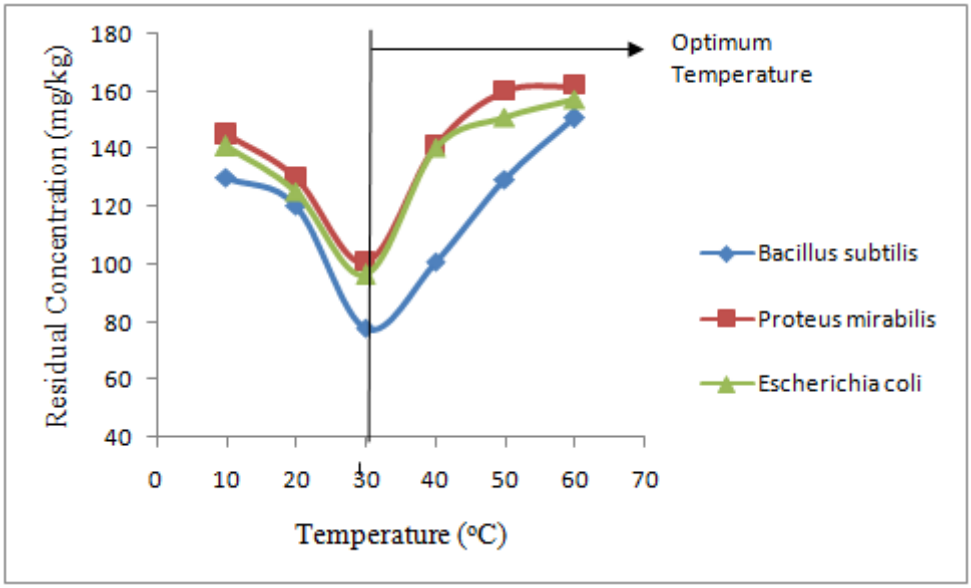

Figure 2. Impact of Temperature on Lead Removal

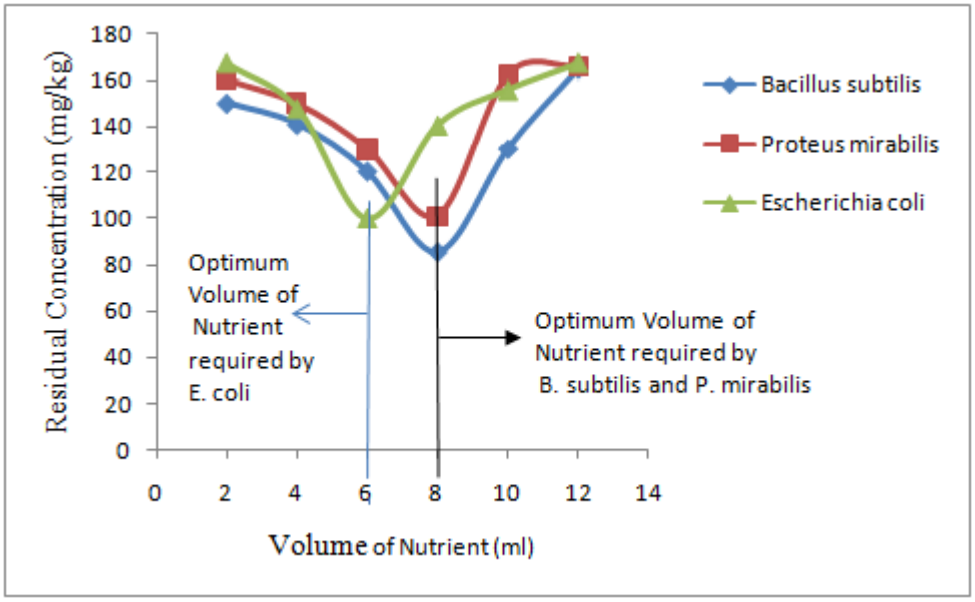

Figure 3. Impact of Nutrient Volume on Lead Removal

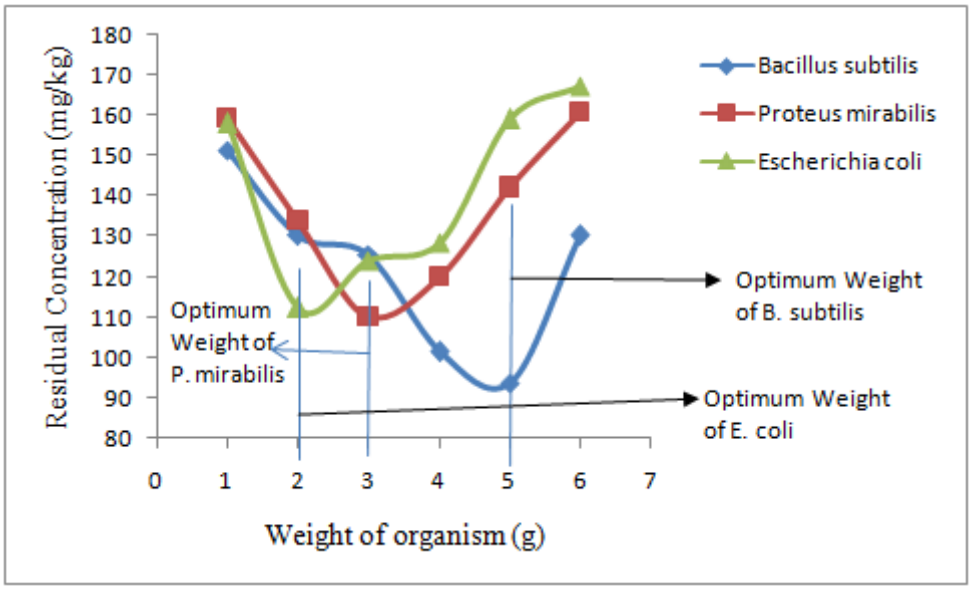

Figure 4. Impact of Organisms' Weights on Lead Removal

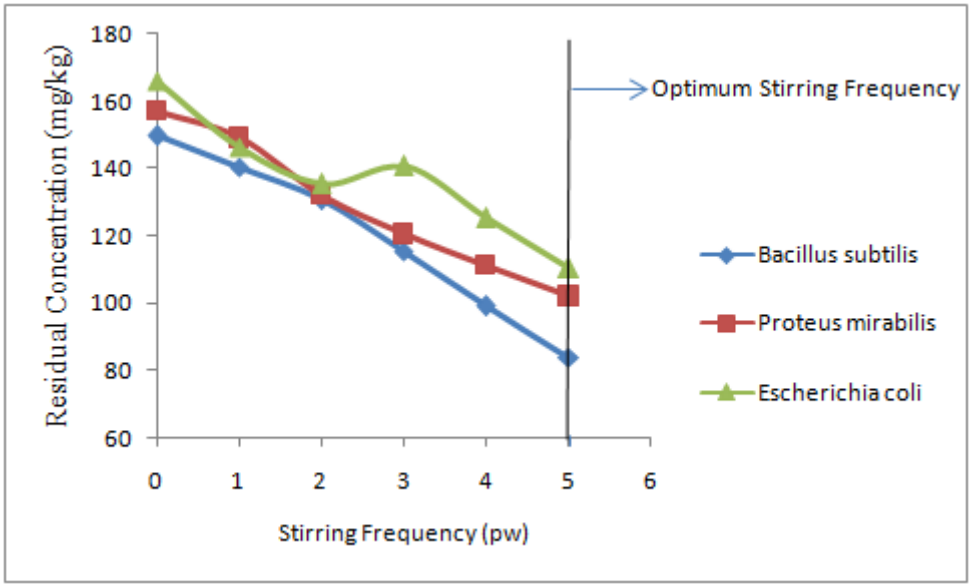

Figure 5. Impact of Stirring Frequency on Lead Removal

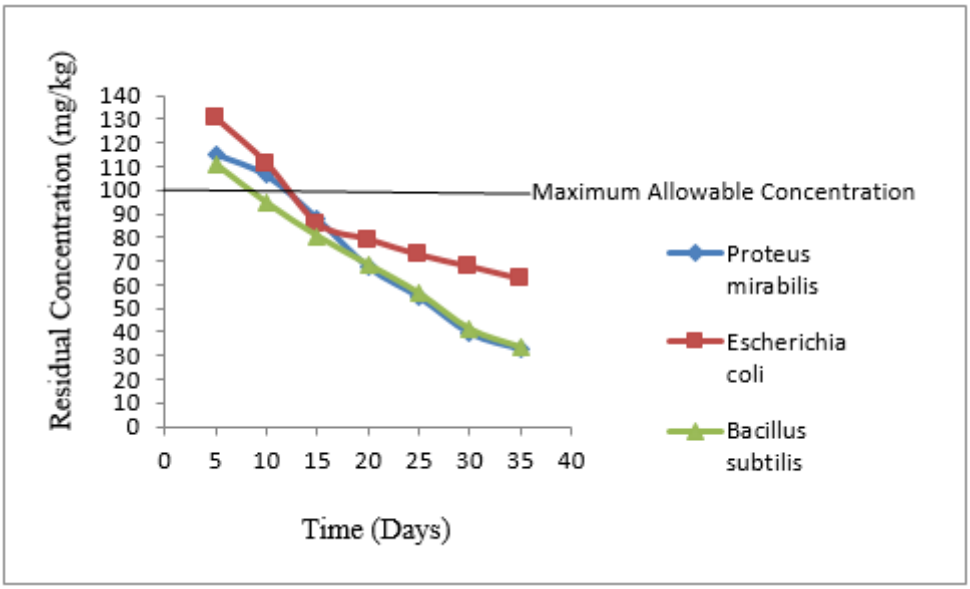

Figure 6. Comparative Removal of Lead (mg/kg)

COMPARATIVE IMPACTS OF THE ORGANISMS

In Figure 6, the organisms were able to bring the initial concentration of lead to below the maximum allowable concentration, this happened at different rates and efficiencies. B. subtilis was able to bring the concentration to below the maximum allowable concentration at time 10 days with efficiency of 27.64% at a residual concentration of 94.32 mg/kg, P. mirabilis and E. coli were able to bring the initial concentration to below the maximum allowable concentration at time 15 days with different efficiencies of 32.64 % at 87.81 mg/kg residual concentration and 34.30 % at 85.85 mg/kg residual concentration respectively.

The initial strength rating showed B. subtilis to be ahead in strength, seconded by E. coli while P. mirabilis lagged behind. This was noticed from time 5 days to 15 days. However, in their 35 Days removal efficiencies of 75.12% for P. mirabilis at a residual concentration of 32.44 mg/kg; 74.26 % for B. subtilis at a residual concentration of 33.55 mg/kg; and 52.26 % for E. coli at a residual concentration of 62.24 mg/kg was observed. This shows that removal

by B. subtilis is preferred on a short-run bioremediation, while removal by P. mirabilis is preferred on a long-run pollution control.

Significant difference at ($P < 0.05$) in the residual concentrations effected by the different organisms showed that a combination of 2 or 3 of the organisms would result in lower residual concentration. Relevant performance was shown possible at shorter times by the ANOVA at ($P < 0.05$). This was reflected by the significant difference in the residual concentrations with respect to time

CONCLUSION

The concentration of Lead in the soil before treatment where discovered to be 130.36 mg/kg. At this concentration, the soil was found polluted when compared with the maximum allowable concentration values of 100 mg/kg as stipulated.

The organisms where able to bring the initial concentration of lead to below the maximum allowable concentration at different rates and efficiencies. B. subtilis effected this at time 10 days with efficiency of 27.64% and a residual concentration of 94.32 mg/kg. P. mirabilis and E. coli were able to effect control at time 15 days with different efficiencies of 32.64 % at 87.81 mg/kg residual concentration for P. mirabilis and 34.30 % at 85.85 mg/kg residual concentration for E. coli. The initial strength rating showed B. subtilis to be ahead in strength, seconded by E. coli while P. mirabilis lagged behind them. This was noticed from time 5 days to 15 days. However, after this time, P. mirabilis was observed to take the lead, seconded by B. subtilis while E. coli lagged behind them. This is obviously remarkable in their 35 days removal efficiencies of 75.12% for P. mirabilis at a residual concentration of 32.44 mg/kg; 74.26 % for B. subtilis at a residual concentration of 33.55 mg/kg; and 52.26 % for E. coli at a residual concentration of 62.24 mg/kg. This shows that removal by B. subtilis is preferred on a short-run bioremediation, while removal by P. mirabilis is preferred on a long-run pollution control.

DECLARATION OF COMPETING INTEREST

There is no conflict of interest in this work

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