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Exploring the use of Indigenous Microorganism for the Removal of Cadmium Contaminated Soil

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Abstract

This work deals on cadmium concentration remediation of (70.21mg/kg) in soils in Nigeria to below maximum allowable 3 mg/kg specified for safe agriculture by standards to ensure that farm products 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 4g soils each conditioned with optimum values of pH, temperature, stirring frequency and nutrient in thirty-six 50 ml beakers; and experimented for residual cadmium ion at times 5, 10, 15, 20, 25, 30 and 35 days in triplicate with Atomic Absorption Spectrophotometer. Cadmium removal was a big task for the organisms within the experiment time frame of 5 to 35 days. The organisms either do not have great expertise for cadmium removal or the initial concentration of 70.21 mg/kg was too high for the organisms to bring the concentration to below the maximum allowable of 3 mg/kg early enough. In addition, the rapid drop in concentration with experiment time suggested a longer experiment time to achieve cadmium pollution control provided equilibrium has not been exceeded, the influence of the selected organisms on cadmium removal from the soil sample. Along the experiment time, no organism could remove cadmium to control level apart from B. subtilis at time 35 days with an efficiency of 96.10 % and residual concentration of 2.74 mg/kg. In the case of P. mirabilis and E. coli, high efficiency seemed not to necessarily effect control. This is obvious from efficiency (85.05%) of removal by P. mirabilis and 79.35 % of removal by E. coli. At these respective high efficiencies, the residual concentrations of cadmium were 10.50 mg/kg and 14.42 mg/kg for removal by P. mirabilis and E. coli respectively. These efficiencies were not sufficient for cadmium pollution control but for it abatement.

1. Introduction

Man relies on uncontaminated soils to cultivate his food crops [1] Therefore, the sustainable use of soils is necessary to ensure its continuous availability to support a wide range of use [1] With the growth of industrialization and extraction of natural resources, there has been a considerable

increase in the discharge of industrial waste to the environment, mainly soil and water, which has led to the accumulation of heavy metals [2]**.** Consequently, contamination of soils, groundwater, sediments, surface water, and air with hazardous heavy metals and toxic chemicals is one of the major threats facing the world, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem [2]**.**

According to recent study by [3]**.** the need to remediate these natural resources has led to the development of new technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal because of their potential to enter the food chain. Scientific report revealed that metals when present in our body are capable of causing serious health problems, by interfering with our normal functions [4]**.** Although, some of these metals are useful to the body at low concentration, they are toxic at high concentration and also cytotoxic, carcinogenic and mutagenic in nature [5]**.**

Heavy metals can be defined as metallic elements with high atomic weight which can damage living cells at low concentration and which tends to bio-accumulate in the food chain. [6]**.**
Heavy metals having relatively high density are toxic at low concentration [7]**.**
The non-biodegradability of heavy metals makes it hard to remove them from contaminated biological tissues and this is a major concern for global health because of their lethal nature [8]**.**
Due to the adverse ecological implications of soils contaminated with heavy metals, there is a need to take preventive and treatment measures [9]**.**
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 [10].

In this work, bioremediation of soils from 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 cadmium concentration to below 3mg/kg specified as the maximum allowable for safe agriculture by standards in [11] to ensure that farm products from this area are safe for human consumption

2. Methodology

2.1 Materials

These include soil sample from Contaminated area, MacConkry agar, magnetic stirrer, hydrogen peroxide, measuring cylinder, safranin, refrigerator, simon citrate ager, inoculating nidles, 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.

2.2 Method

2.2.1. Organisms Acquisition

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

Aliquot from serial dilution was introduced into petri dishes, covered with MacConkey agar [12], and incubated for 24 hours at 37ºC [13]. Developed Colonies were recognized after they were sub cultured [14, 15].

2.2.2. Optimum factors Acquisition

Vital factors have been discovered to have significant influence on bioremediation process and rate [16, 17]. 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 [18], pH values of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; temperature values of 10, 20, 30, 40, 50, and 60 ºC; 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).

2.2.3. Ion Removal

Applying the method in [18], 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) [19, 20].

$qt=\frac{(Co-Ct)}{m.V}$ ……..(1)
Efficiency $(\varepsilon) =\frac{(Co-Cf)}{Co}.100$ ……..(2)
$qe=\frac{(Co-Ce)}{m.V}$…….. (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.

3. Results And Discussion

3.1 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.
Significant determinants of effective bioremediation were carefully studied to acquire their optimum values for optimum bioremediation. These include pH, stirring frequency, temperature, organisms' masses, and nutrient dosage.

pH affects the negative charges on cells and the chemistry cell wall; and the metals physio chemistry [18, 19] thus influencing bioremediation. This makes pH a pivotal, critical influence of bioremediation [22]. pH (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) influence on the ion removal is shown in Figure 1. Optimum values were 8 for the use of B. subtilis; 6 for P. mirabilis and E. coli at respective minimum concentrations of 14.19 mg/kg, 18.67 mg/kg and 23.41 mg/kg remaining in soils.

Temperature, an indicator of heat magnitude supplied to the process is a major determinant of organisms' performances [17]. Its variation influences the process significantly [20].
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 11.79 mg/kg for the use of B. subtilis; 16.17 mg/kg for the use of P. mirabilis; and 21.41 mg/kg for the use of E. coli.

The supply of requisite nutrient is very essential for the stimulation of the indigenous microorganisms for effective performance [21]. Bio-stimulation by nutrient supply increases the number of organisms through rapid growth and replication, and ultimately increases bioremediation rate [22]. Influence of nutrient dosage of 2, 4, 6, 8, 10 and 12 ml on the organism performances is shown in Figure 3 displaying an optimum nutrient dosage of 8 ml. 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 16.17 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 is 15.79 mg/kg for removal by B. subtilis, 20.67 mg/kg for removal by P. mirabilis, and 25.01 mg/kg for removal by E. coli.

The organisms' population used in bioremediation bears direct relationship with the collective weight of the organisms brought in contact with contaminated medium. This makes it very vital to engage the optimum weight of organism in bioremediation study.
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 14.71 mg/kg for B. subtilis; 18.01 mg/kg for P. mirabilis; and 23.11 mg/kg for E. coli.

The influences of the weights of the distinct organisms were in the decreasing order of 5g, 4g, 3g, 6g, 2g and 1g for removal by B. subtilis; 5g, 4g, 3g, 6g, 2g and 1g for removal by P. mirabilis; and 5g, 4g, 3g, 6g, 2g and 1g for removal by E. coli. Oxygen diffusivity promoted by soil stirring is another essential influencer of bioremediation [17]. Stirring makes available oxygen for microorganism's aerobic activities. Figure 5 shows the influences of stirring frequencies on the organisms' performances. The study showed 5pw at 65 rpm for P. mirabilis; and 5pw at 60 and 68 rpm for B. subtilis and E. coli as the optimum stirring frequencies. These values were recognized at the respective residual concentrations of 14.19 mg/kg for B. subtilis; 19.69 mg/kg for P. mirabilis; and 24.71 mg/kg for E. coli.

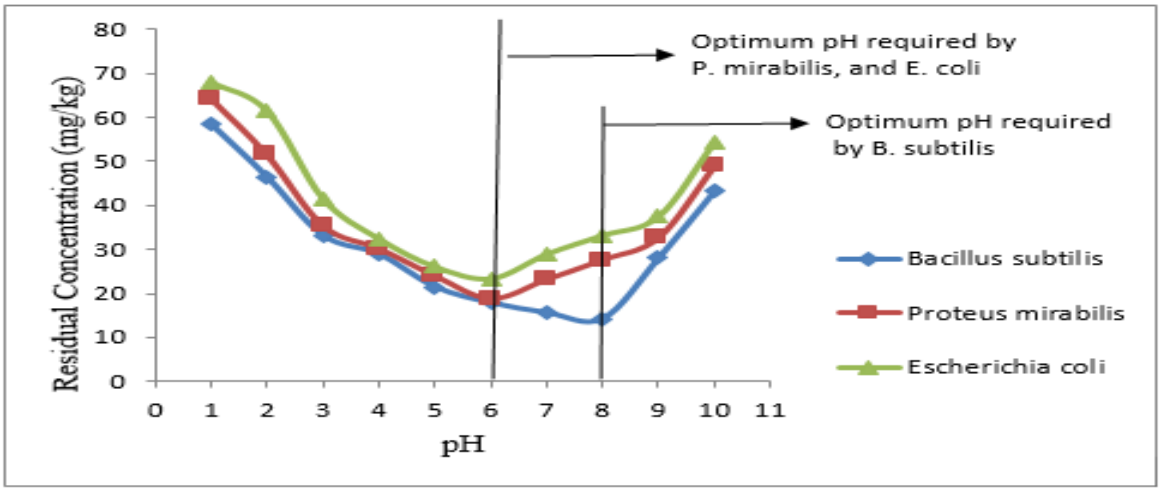

Figure 1: Impact of pH on Cadmium Removal

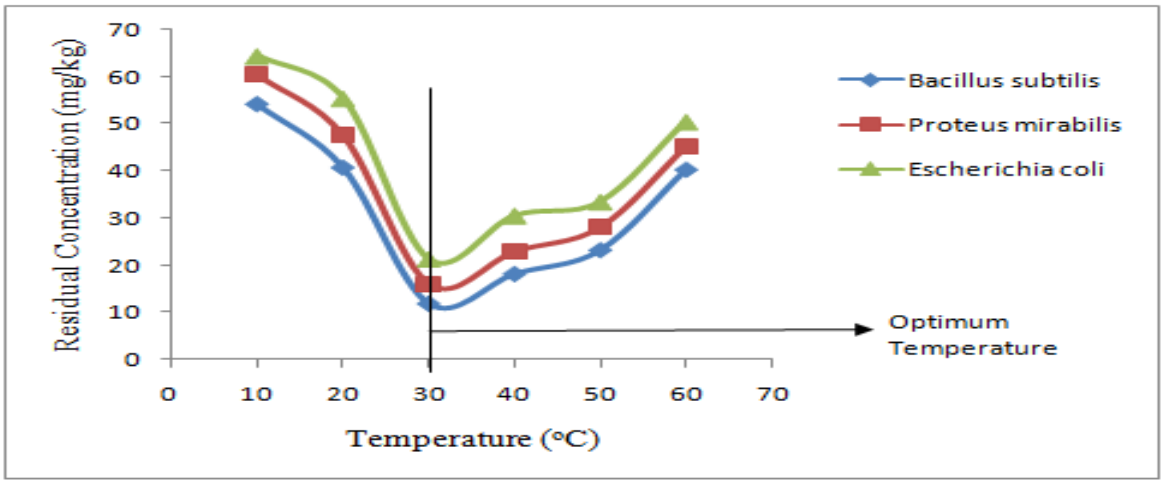

Figure 2: Impact of Temperature on Cadmium Removal

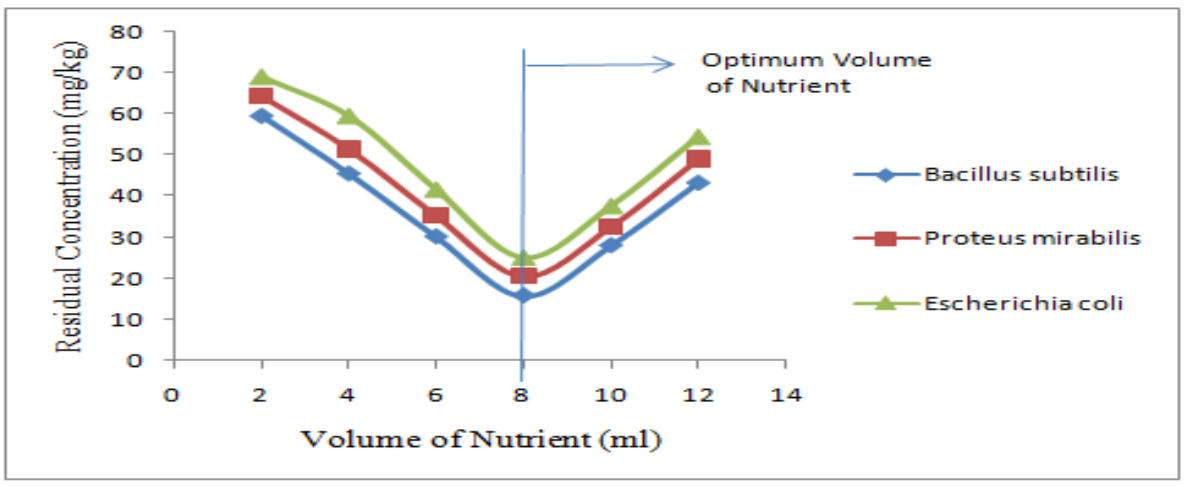

Figure 3: Impact of Nutrient Volume on Cadmium Removal

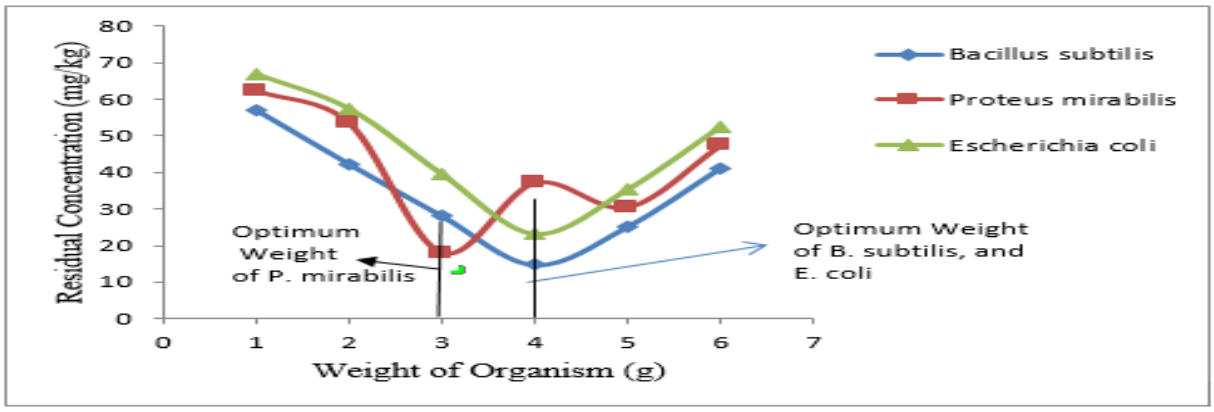

Figure 4: Impact of Organisms' Weights on Cadmium Removal

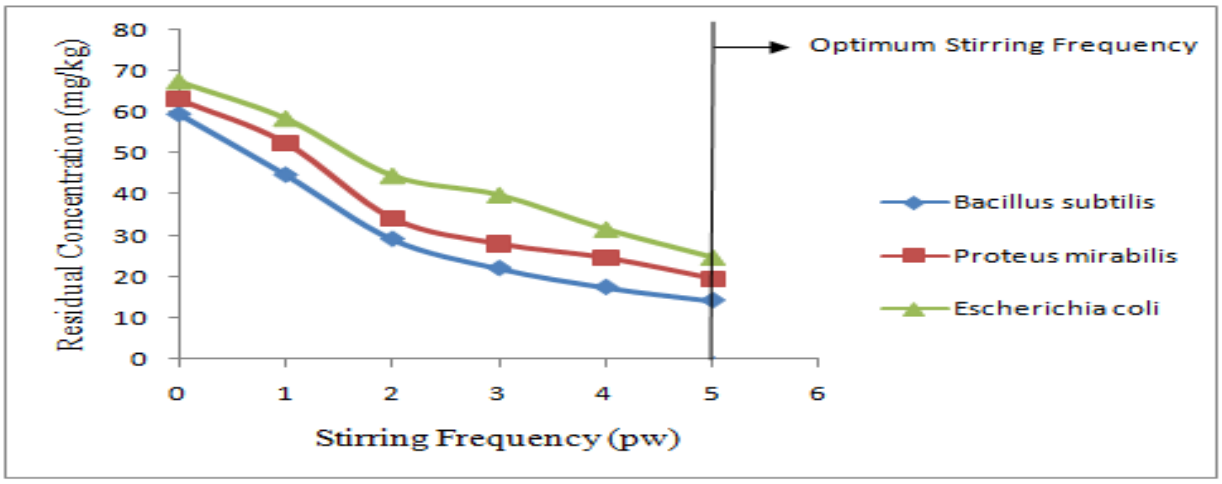

Figure 5: Impact of Stirring Frequency on Cadmium Removal

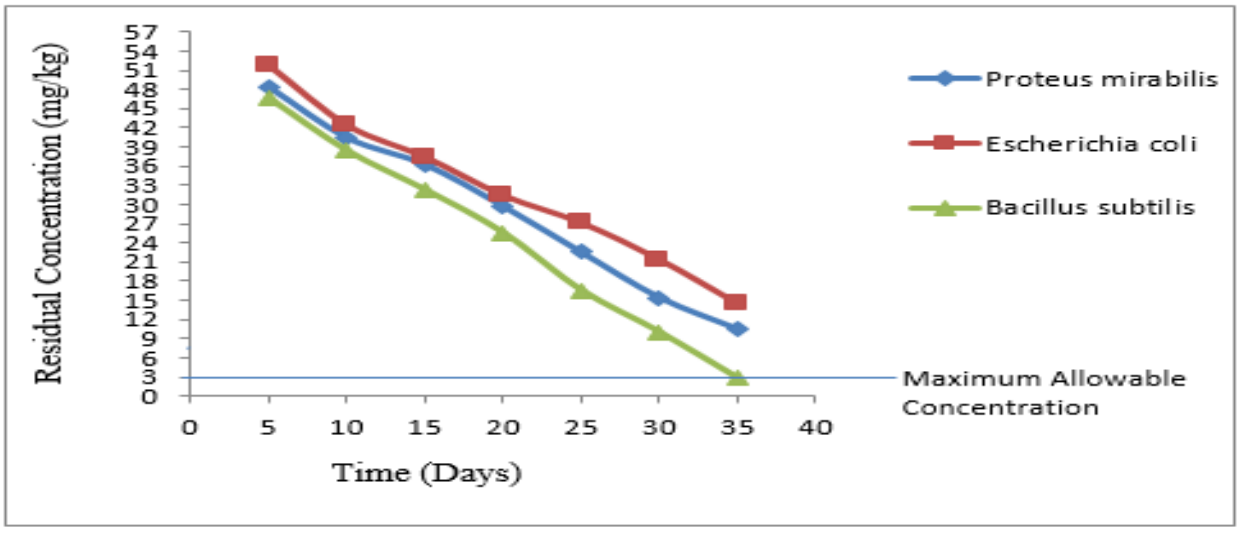

Figure 6: Comparative Removal of Cadmium (mg/kg)

3.2 Comparative Impacts of the Organisms

Cadmium removal was a big task for the organisms within the experiment time frame of 5 to 35 days. The organisms either do not have great expertise for cadmium removal or the initial concentration of 70.21 mg/kg was too high for the organisms to bring the concentration to below the maximum allowable of 3 mg/kg early enough. In addition, the rapid drop in concentration with experiment time suggested a longer experiment time to achieve cadmium pollution control provided equilibrium has not been exceeded.

Figure 6 shows the influence of the selected organisms on cadmium removal from the soil sample. Along the experiment time, no organism could remove cadmium to control level apart from B. subtilis at time 35 days with an efficiency of 96.10 % and residual concentration of 2.74 mg/kg. In the case of P. mirabilis and E. coli, high efficiency seemed not to necessarily effect control. This is obvious from efficiency (85.05%) of removal by P. mirabilis and 79.35 % of removal by E. coli. At these respective high efficiencies, the residual concentrations of cadmium were 10.50 mg/kg and 14.42 mg/kg for removal by P. mirabilis and E. coli respectively. These efficiencies were not sufficient for cadmium pollution control but for it abatement. 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.

4. Conclusion

The concentration of the metals in the soil before treatment was discovered to be 70.21 mg.kg. At this concentration, the soil was found polluted when compared with the respective maximum allowable concentration values of 3 mg/kg for Cadmium (Cd) ,stipulated in [23,23, 25]. Cadmium removal was a big task for the organisms within the experiment time frame of 5 to 35 days. Only B. subtilis could remove Cadmium to a control level at time 35 days with an efficiency of 96.10 % and residual concentration of 2.74 mg/kg.

The organisms either do not have great expertise for cadmium removal or the initial concentration of 70.21 mg/kg was too high for the organisms to bring the concentration to below the maximum allowable of 3 mg/kg early enough, but the ($C_o - C_A$) inhibited the organisms for controlling Cadmium (Cd) pollution of the soil. In addition, the rapid drop in concentration with experiment time suggested a longer experiment time to achieve cadmium pollution control provided equilibrium has not been exceeded. Figure 6, shows the influence of the selected organisms on cadmium removal from the soil sample. Along the experiment time, no organism could remove cadmium to a control level apart from B. subtilis at time 35 days with an efficiency of 96.10 % and residual concentration of 2.74 mg/kg.

In the case of P. mirabilis and E. coli, high efficiency seemed not to necessarily effect control. This is obvious from efficiency (85.05%) of removal by P. mirabilis and 79.35 % of removal by E. coli. At these respective high efficiencies, the residual concentrations of cadmium were 10.50 mg/kg and 14.42 mg/kg for removal by P. mirabilis and E. coli respectively. These efficiencies were not sufficient for cadmium pollution control but for it abatement.

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