

## Enhanced Heavy Metal Decontamination Potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* Co-Culture Isolated from Spent Engine Oil Polluted Soil

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### Abstract

Heavy metals emanating from anthropogenic activities which have the penchant for polluting the environment have been documented as imminent residues synonymous with spent engine oil (SEO). This study was staged to evaluate heavy metal decontamination potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* co-culture isolated from spent engine oil polluted soil in Dutse Mechanic village. About 1.5 kg of autoclaved soil was artificially contaminated with SEO at three levels. Subsequently, the sterilized soil was supplemented with the same quantity (150 g) of biostimulants, compost, powdered cocoa pod husk (CPH), and powdered cow dung (CD). Successively, each mixture in the experimental bag was bioaugmented with 150 mL bacterial inoculants. Concentrations of heavy metals; Cadmium (Cd), Arsenic (As), Nickel (Ni), Lead (Pb) and Chromium (Cr) were determined at the beginning, 5<sup>th</sup> and 10<sup>th</sup> week of the study. The factorial experiment was laid out adopting a completely randomized design (CRD). Results obtained indicate that at the 5<sup>th</sup> week, compost and powdered CD only enhanced more reductions (0.008 mg kg<sup>-1</sup>) of Cd compared with powdered CPH only on 5% SEO contamination level while compost enhanced the most reductions (0.004 mg kg<sup>-1</sup> and 0.005 mg kg<sup>-1</sup>) of Cd on 10% and 15% SEO contamination levels respectively. At the 10<sup>th</sup> week, both compost and powdered CPH only enhanced complete removal of Cr compared with other biostimulants. At the 10<sup>th</sup> week, powdered CD only recorded complete removal of Pb, powdered CPH only enhanced further reduction (0.005 mg kg<sup>-1</sup>) and compost recorded the most Pb reduction (0.012 mg kg<sup>-1</sup>) compared with other biostimulants on 5%, 10% and 15% SEO contamination levels respectively ( $P < 0.05$ ). Due to the significant heavy metal decontamination prowess of the bacterial inoculants employed in this study, biostimulation of these hydrocarbonoclastic bacteria is recommended with a view to expunging heavy metals inherently detected in polluted environments.

**Keywords:** Spent engine oil, heavy metals, soil, bacterial co-cultures, biostimulation, decontamination.

### Introduction

Heavy metals occur naturally in the earth and only get concentrated due to anthropogenic activities (Rajeswari & Sailaja, 2014). These authors further reported that heavy metals can be found polluting the environment due to activities involving indiscriminate disposal of mining and industrial wastes, chemical fertilizers, paints, treated woods and lead-acid batteries coupled with emissions from vehicles. Over the years, environmental pollution that is triggered by heavy metals originating from industrial and anthropogenic activities has exacted its deleterious effects on the health of the terrestrial and aquatic ecosystems (Adeleye *et al.*, 2019; Das *et al.*, 2008). In Nigeria, the citing of mechanic workshops without strict guidelines on ground has worsened the level of pollution that spent engine oil (SEO) which is as waste product derived therein exposes the environment to.

According to Zali *et al.* (2015), Chromium (Cr), Aluminium (Al), Iron (Fe), Cadmium (Cd) and Lead (Pd) that are well-known as wear metals may portray a substantial wear in the engines of automobiles. These authors submitted further that SEO should be correctly managed following the standard international regulations, and it can be equally retrieved from automobile engines that have got possible economic values.

If found in excessive concentrations in the food chain, toxic heavy metals that are of environmental concern include Chromium (Cr), Lead (Pb), Zinc (Zn), Arsenic (As), Copper (Cu), Nickel (Ni), Cobalt (Co), Cadmium (Cd) and Mercury (Hg) (Kanamarlapudi *et al.*, 2018). They reported further that these metals do not biodegrade thereby accumulating in the tissues of living organisms which can ultimately result to many diseases and disorders that can substantially threaten the existence of humans.

Over the decades, numerous orthodox treatment methods have been adopted with a view to expunging heavy metals from contaminated media. However, the methods that are normally employed include ultra-filtration, ion exchange, chemical precipitation, electro winning, phytoremediation and reverse osmosis (Azimi *et al.*, 2016; Baraket, 2011; Gunatilake, 2015; Joshi, 2017; Lakherwal, 2014). According to Mulligan *et al.* (2001) and Kadirvelu *et al.* (2002), these orthodox treatment methods employed for such removal are very expensive and not environment friendly. It is against these backdrops that this study was conducted with a view to assessing the influence of selected readily available organic waste products on enhancing possible bacterial decontamination of heavy metals of environmental concern in SEO contaminated soil.

## **Materials and Methods**

### **Study Area**

This study was conducted at the back of the Department of Soil Science, Faculty of Agriculture situated in Federal University Dutse campus, Jigawa state, Nigeria.

### **Collection and Processing of Organic Amendments**

Organic amendments, Cocoa pod husk (CPH) and Cow dung (CD) employed in this study were collected and processed following the methods described by Adeleye *et al.* (2019).

### **Collection, Preparation of Non-Polluted Soil and Inoculation with SEO**

Collection of soil was done according to the method described by Agbor *et al.* (2015). About 250 kg top soil (0-25 cm depth) that no history of pollution had been reported was collected from four (4) different areas at the back of the Department of Soil Science, Federal University Dutse main campus. The soil was subsequently air-dried and sieved with 2 mm mesh. It was later autoclaved at 121°C for 15 minutes as recommended by Soretire *et al.* (2017). About 1.5 kg of this sterile soil was then dispensed into 108 polyethylene bags followed by the addition of 75, 150, and 225 mL (w/w) of SEO obtained from a service pit in Dutse mechanic village. This implies that each soil sample represented; 5%, 10% and 15% contamination levels respectively. As recommended by Abioye *et al.* (2012), having mixed the soil and SEO comprehensively, it was then left to attain volatilization for fourteen (14) days.

### **Isolation and Identification of the Bacterial Inoculants**

About 10 g of soil that had been naturally polluted with SEO was collected at a depth of 5 cm from Dutse mechanic village. During soil collection, the ambient temperature recorded was 37.3°C. Subsequently, the bacterial isolates that were employed as co-culture inoculants in this study were isolated from the SEO polluted soil through the adoption of enrichment method involving the utilization of Trypticase Soy Broth and Agar with mineral salt medium overlaid with SEO described by Adeleye and Yerima (2019). The bacterial isolates were then identified biochemically following the recommendations of Barrow and Feltham (1993).

### **Determination of Physicochemical Properties of the Samples**

Unpolluted soil sample, SEO polluted soil sample and organic amendments were analysed for pH values, and electrical conductivity (EC) in deionized water (1: 2.5 w/v for soil, and 1: 5 w/v for organic amendments). Organic carbon was determined using the modified Walkley-Black procedure (Nelson and Sommers, 1996). Cation exchange capacity (CEC) of soil samples was determined using the summation method of Chapman (1965). Total Nitrogen and Phosphorous contents of the samples were determined

using Kjeldhal and Bray-1 method in reference to Reeuwijk (1993); Bremmer (1996). The soil mechanical analysis was similarly determined employing the hydrometer method of Bouyoucos (1962).

### **Heavy Metal Decontamination Experiment**

In this study, thirty-six (36) polyethylene bags were employed for assaying the decontaminating potentials of the bacterial inoculant. Except the nine (9) experimental bags that served as controls, about 150 g of the sterilized biostimulants (compost, powdered CPH, and powdered CD) were added individually and mixed comprehensively with each set of the soils contaminated with the SEO at 5%, 10% and 15% levels. With the exemption of the control bags, all the other twenty-seven (27) experimental bags in each set were thoroughly augmented with 150 mL of the bacterial inoculant. All the bags were later subjected to incubation at room temperature for seventy (70) days. Three (3) replicates were employed for each SEO contamination level. The contents of the experimental bags were tilled for aeration while addition of sterile distilled water (6 mL) moisture was equally done twice a week (Ayotamuno *et al.*, 2006; Chorom *et al.*, 2010). The decontamination assay was monitored for ten (10) weeks as suggested by Chorom *et al.* (2010).

### **Determination of Heavy Metals in Sampled Soils**

Following the procedures outlined by Maurya *et al.* (2018), heavy metals; As, Cd, Ni, Pb and Cr were estimated from sampled soils using Perkin Elmer Atomic Absorption Spectrophotometer (Analyst 400) at the beginning, 5<sup>th</sup> week and 10<sup>th</sup> week of this study.

### **Statistical Analysis**

All data collected from the heavy metal decontamination assay were subjected to analysis through Proc. GLM of GenStat version 17, and the means were separated using Duncan Multiple Range Test (DMRT).

## **Results**

### **Isolation and Identification of Inoculant Bacteria used in the Decontaminated Assay**

The isolation of the bacterial inoculants from the SEO contaminated soil on the enrichment medium employed in this study led to the retrieval of two principal bacterial isolates with colonial features that are depicted in Table 1. The results of the various biochemical tests conducted on these two bacterial isolates revealed *Pseudomonas aeruginosa* and *Alcaligenes faecalis* as their respective identities (Table 1).

Table 1

Identification of the Bacterial Inoculants in Accordance with their Colonial and Biochemical Features

	Status	Status
<b>Colonial characteristics</b>	Creamy white colonies	Creamy colonies with scanty growth
<b>Gram staining</b>	Gram Negative Rods	Gram Negative Rods in Chains
<b>Biochemical Tests</b>		
Motility	+	Oxidase +
Growth on MacConkey agar	+	Nitrate -
Oxidase	+	Simmon's Citrate +
Nitrate	+	Christensen's Citrate +
Simmon's Citrate	+	Urease -
Christensen's Citrate	+	Glucose -
Urease	-	Fructose -
Gluconate	+	Maltose -
Glucose	+	Mannitol -
Fructose	+	Sucrose -
Lactose	-	Xylose -
Maltose	-	Casein hydrolysis -
Sucrose	-	Tyrosine hydrolysis +
Xylose	+	Growth on Centrimide agar +
10% Glucose	+	
10% Lactose	-	
<b>Identity of bacteria</b>	<i>Pseudomonas aeruginosa</i>	<i>Alcaligenes faecalis</i>

**Note:** + = Positive; - = Negative

The physicochemical properties of the powdered cow dung, compost, powdered CPH, unpolluted soil and SEO polluted soil are presented in Table 2.

Table 2

*Physicochemical Properties of the Soils and Organic Amendments Used for the Experiment*

Parameters	PCD	CM	PCPH	Ss	Us
MC (%)	7.3	2.0	11.11	0.8	2.04
AC (%)	68.8	65	23	-	-
pH <sub>(water)</sub>	8.15	9.45	7.6	6.8	6.5
OC (%)	41.55	48.25	33.40	0.52	0.49
TN (%)	2.85	5.85	2.65	0.08	0.06
AP (mg kg <sup>-1</sup> )	1.2	1.48	0.08	9.40	11.02
EC (dS cm <sup>-1</sup> )	8.10	8.86	6.42	1.20	0.92
<b>EB (cmol kg<sup>-1</sup>)</b>					
K	80	213.16	162	0.07	0.19
Ca	0.2	4.8	1.6	0.63	1.82
Mg	1.5	3.24	2.45	0.18	0.92
Na	0.4	0.5	0.1	0.17	0.58
CEC	82.1	221.7	166.15	1.05	3.51
<b>Ps (g kg<sup>-1</sup>)</b>					
Sa	-	-	-	800	580
S	-	-	-	80	320
C	-	-	-	120	100
C+S	-	-	-	200	420
TC	-	-	-	Sl	Ls

**Note:** PCD= Powdered cow dung; CM= Compost; PCPH= Powdered Cocoa pod husk; Ss= Spent engine oil polluted soil; Us= Unpolluted soil; MC= Moisture content; AC= Ash content; OC= Organic Carbon; TN= Total Nitrogen; AP= Available Phosphorous; EC= Electrical conductivity; EB= Exchangeable Bases; K= Potassium; Ca= Calcium; Mg= Magnesium; Na= Sodium; CEC; Cation exchange capacity; Ps= Particle size; TC= Textural class; C+S= Clay + Slit; C= Clay; S= Slit; Sa= Sand; Sl= Sandy loam; Ls= Loamy sand

### Arsenic Decontamination Potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* Coculture

The potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* co-culture was examined for possible decontamination of As content of SEO contaminated soil in this study. At the fifth week, all the biostimulants did not significantly enhance the decontamination of As ( $p > 0.05$ ). However, both compost and powdered CD only enhanced more reductions ( $0.008 \text{ mg kg}^{-1}$ ) compared with powdered CPH only on 5% SEO contamination level while compost enhanced the most reductions ( $0.004 \text{ mg kg}^{-1}$  and  $0.005 \text{ mg kg}^{-1}$ ) on 10 % and 15% SEO contamination levels respectively (Table 3).

Table 3

*Effect of Biostimulants on Arsenic Decontamination ( $\text{mg kg}^{-1}$ ) At the Fifth Week*

Biostimulants	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	0.008	0.004	0.005
CPH only	0.011	0.017	0.011
CD only	0.008	0.008	0.010
Control	0.273	0.273	0.273
Significant status	Ns	Ns	Ns

Ns= Not significant ( $p > 0.05$ )

At the tenth week, both compost and powdered CD only enhanced complete removal ( $0.000 \text{ mg kg}^{-1}$ ) of As compared with powdered CPH only on 5% SEO contamination level while all the biostimulants enhanced total reductions ( $0.000 \text{ mg kg}^{-1}$ ) of As on 10 % and 15% SEO contamination levels respectively (Table 4).

Table 4

*Effect of Biostimulants on Arsenic Decontamination ( $\text{mg kg}^{-1}$ ) at the Tenth Week*

Biostimulants	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	0.013 <sup>c</sup>	0.000 <sup>d</sup>	0.000 <sup>d</sup>
CPH only	0.000 <sup>d</sup>	0.000 <sup>d</sup>	0.000 <sup>d</sup>
CD only	0.000 <sup>d</sup>	0.000 <sup>d</sup>	0.000 <sup>d</sup>
Control	0.273 <sup>a</sup>	0.274 <sup>b</sup>	0.273 <sup>a</sup>

**Note:** Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT). ( $p < 0.05$ ); 0.000= Below Detectable Limit

### Cadmium Decontamination Potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* Coculture

All the biostimulants significantly ( $p < 0.05$ ) enhanced *Pseudomonas aeruginosa* and *Alcaligenes faecalis* co-culture decontamination of Cd on SEO contamination level at the fifth week (Table 3). Powdered CD only produced the most reductions ( $0.001 \text{ mg kg}^{-1}$  and  $0.005 \text{ mg kg}^{-1}$ ) compared with other biostimulants on 5% and 10% SEO contamination levels respectively. However, powdered CPH only recorded the most decontamination ( $0.012 \text{ mg kg}^{-1}$ ) compared with other biostimulants on 15% SEO contamination level (Table 5).

Table 5

*Effect of Biostimulants on Cadmium Decontamination ( $\text{mg kg}^{-1}$ ) at the Fifth Week*

Biostimulants	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	0.028 <sup>d</sup>	0.025 <sup>e</sup>	0.024 <sup>f</sup>
CPH only	0.035 <sup>c</sup>	0.035 <sup>c</sup>	0.012 <sup>h</sup>
CD only	0.001 <sup>j</sup>	0.005 <sup>i</sup>	0.013 <sup>g</sup>
Control	0.090 <sup>b</sup>	0.090 <sup>a</sup>	0.090 <sup>a</sup>

**Note:** Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT). ( $p < 0.05$ )

At the tenth week, both compost and powdered CD only enhanced complete removal of Cd at all SEO contamination levels while complete removal of Cd was only attained on 15% SEO contamination level (Table 6).

Table 6

*Effect of Biostimulants on Cadmium Decontamination ( $\text{mg kg}^{-1}$ ) at the Tenth Week*

Biostimulants	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	0.000 <sup>e</sup>	0.000 <sup>e</sup>	0.000 <sup>e</sup>
CPH only	0.012 <sup>d</sup>	0.017 <sup>c</sup>	0.000 <sup>e</sup>
CD only	0.000 <sup>e</sup>	0.000 <sup>e</sup>	0.000 <sup>e</sup>
Control	0.090 <sup>b</sup>	0.090 <sup>a</sup>	0.090 <sup>a</sup>

**Note:** Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT). ( $p < 0.05$ ); 0.000= Below Detectable Limit

### **Chromium Decontamination Potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* Coculture**

The possibility of decontaminating Cr present in the SEO contaminated soil adopting the co-culture of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* was investigated. Results generated at the fifth week indicate that all the biostimulants significantly enhanced bacterial decontamination of Cr ( $P < 0.05$ ). Powdered CPH only influenced the most decontamination ( $0.000 \text{ mg kg}^{-1}$ ) compared with other biostimulants on 5% SEO contamination level while powdered CD only influenced the most reductions ( $0.000 \text{ mg kg}^{-1}$  and  $0.058 \text{ mg kg}^{-1}$ ) on 10% and 15% SEO contamination levels respectively (Table 7).

Table 7

*Effect of Biostimulants on Chromium Decontamination ( $\text{mg kg}^{-1}$ ) at the Fifth Week*

Biostimulants	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	0.052 <sup>i</sup>	1.226 <sup>a</sup>	0.075 <sup>g</sup>
CPH only	0.000 <sup>j</sup>	0.089 <sup>f</sup>	0.088 <sup>f</sup>
CD only	0.222 <sup>e</sup>	0.000 <sup>j</sup>	0.058 <sup>h</sup>
Control	0.344 <sup>d</sup>	0.346 <sup>c</sup>	0.348 <sup>b</sup>

**Note:** Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT). ( $p < 0.05$ ); 0.000= Below Detectable Limit

At the tenth week, both compost and powdered CPH only enhanced complete removal of Cr compared with other biostimulants. Conversely, both powdered CPH and CD recorded complete decontamination of Cr on 10% SEO contamination level compared with compost while all the biostimulants enhanced complete removal of Cr on 15% SEO contamination level (Table 8).

Table 8

*Effect of Biostimulants on Chromium Decontamination ( $\text{mg kg}^{-1}$ ) at the Tenth Week*

Biostimulants	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	0.000 <sup>e</sup>	0.112 <sup>d</sup>	0.000 <sup>e</sup>
CPH only	0.000 <sup>e</sup>	0.000 <sup>e</sup>	0.000 <sup>e</sup>
CD only	0.129 <sup>c</sup>	0.000 <sup>e</sup>	0.000 <sup>e</sup>
Control	0.343 <sup>b</sup>	0.346 <sup>a</sup>	0.347 <sup>a</sup>

**Note:** Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT). ( $p < 0.05$ ); 0.000= Below Detectable Limit

#### **Lead Decontamination Potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* Co-culture**

The addition of biostimulants employed in this study significantly ( $P < 0.05$ ) enhanced Pb decontamination potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* co-culture on SEO contaminated soil. At the fifth week, powdered CD only had the most removal ( $0.015 \text{ mg kg}^{-1}$ ) of Pb, powdered CPH only enhanced the most reduction ( $0.014 \text{ mg kg}^{-1}$ ) and compost recorded the most Pb reduction ( $0.083 \text{ mg kg}^{-1}$ ) compared with other biostimulants, on 5%, 10% and 15% SEO contamination levels respectively (Table 9).



Table 9

*Effect of Biostimulants on Lead Decontamination ( $\text{mg kg}^{-1}$ ) at the Fifth Week*

Biostimulants	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	0.101 <sup>f</sup>	0.091 <sup>g</sup>	0.083 <sup>h</sup>
CPH only	0.092 <sup>g</sup>	0.014 <sup>i</sup>	0.299 <sup>e</sup>
CD only	0.015 <sup>i</sup>	0.316 <sup>d</sup>	0.362 <sup>c</sup>
Control	0.748 <sup>b</sup>	0.750 <sup>a</sup>	0.747 <sup>b</sup>

**Note:** Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT). ( $p < 0.05$ )

At the tenth week, powdered CD only recorded complete removal of Pb, powdered CPH only enhanced further reduction ( $0.005 \text{ mg kg}^{-1}$ ) and compost recorded the most Pb reduction ( $0.012 \text{ mg kg}^{-1}$ ) compared with other biostimulants, on 5%, 10% and 15% SEO contamination levels respectively (Table 10).

Table 10

*Effect of Biostimulants on Lead Decontamination ( $\text{mg kg}^{-1}$ ) at the Tenth Week*

Biostimulants	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	0.089 <sup>d</sup>	0.081 <sup>e</sup>	0.063 <sup>f</sup>
CPH only	0.022 <sup>g</sup>	0.005 <sup>k</sup>	0.019 <sup>h</sup>
CD only	0.000 <sup>l</sup>	0.013 <sup>i</sup>	0.012 <sup>j</sup>
Control	0.748 <sup>b</sup>	0.750 <sup>a</sup>	0.747 <sup>c</sup>

**Note:** Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT). ( $p < 0.05$ ); 0.000= Below Detectable Limit

#### **Nickel Decontamination Potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* Co-culture**

The supplementation of biostimulants employed in this study significantly ( $P < 0.05$ ) improved Ni decontamination potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* co-culture on SEO contamination level. At the fifth week, powdered CD only improved the decontamination of Ni the most ( $0.025 \text{ mg kg}^{-1}$ ,  $0.046 \text{ mg kg}^{-1}$  and  $0.036 \text{ mg kg}^{-1}$ ) when compared with other biostimulants employed on 5%, 10% and 15% SEO contamination levels respectively (Table 11).

Table 11

*Effect of Biostimulants on Nickel Decontamination ( $\text{mg kg}^{-1}$ ) at the Fifth Week*

Biostimulants	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	0.077 <sup>a</sup>	0.072 <sup>b</sup>	0.071 <sup>b</sup>
CPH only	0.065 <sup>c</sup>	0.070 <sup>b</sup>	0.047 <sup>d</sup>
CD only	0.025 <sup>f</sup>	0.046 <sup>d</sup>	0.036 <sup>e</sup>
Control	0.071 <sup>b</sup>	0.071 <sup>b</sup>	0.078 <sup>a</sup>

**Note:** Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT). ( $P < 0.05$ )

This remarkable trend was further recorded at the tenth week as it did enhance the most decontaminations ( $0.021 \text{ mg kg}^{-1}$ ,  $0.031 \text{ mg kg}^{-1}$  and  $0.026 \text{ mg kg}^{-1}$ ) compared with other biostimulants adopted on 5%, 10% and 15% SEO contamination 8levels respectively (Table 12).

Table 12

*Effect of Biostimulants on Nickel Decontamination ( $\text{mg kg}^{-1}$ ) at the Tenth Week*

Biostimulants	Spent Engine Oil Contamination Levels		
	5%	10%	15%
Compost	0.070 <sup>c</sup>	0.062 <sup>d</sup>	0.050 <sup>g</sup>
CPH only	0.054 <sup>f</sup>	0.060 <sup>e</sup>	0.045 <sup>h</sup>
CD only	0.021 <sup>k</sup>	0.031 <sup>i</sup>	0.026 <sup>j</sup>
Control	0.071 <sup>b</sup>	0.071 <sup>b</sup>	0.078 <sup>a</sup>

**Note:** Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT). ( $P < 0.05$ )

## Discussion

The complete removal of As in this study might have been aided by the supply of suitable nutrients that ultimately enhanced the performance of the bacterial co-culture employed. This can be corroborated with the reports of Lukic *et al.* (2016); Kastner and Miltner (2016), which stated that organic amendments have got the ability to aid effective release of important nutrients required for optimum microbial metabolism. The results obtained in this study are in concord with the submission of Han and Gu (2010), on the capability of *Pseudomonas aeruginosa* to actively take part in the transformation of heavy metals. Again, the complete removal of As attained by *Pseudomonas aeruginosa* and *Alcaligenes faecalis* co-culture in this study could be attributed to the report of Huckle *et al.* (1993); Huston *et al.* (2002) on the powerful metal bio-sorbents obtainable in the genus of *Pseudomonas*.

In this study, the results generated from Cd decontamination prowess of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* co-culture have further supported the submissions of Wiszniewska *et al.* (2016); Lukic *et al.* (2016); Kastner and Miltner (2016) and additionally shown the influence of organic amendments on the decontamination of heavy metals when indigenous microorganisms are employed for such bioremediation project. Similar to this study, Zolgharnein *et al.* (2010) have reported surface phenomena and diffusion as the mechanisms employed by *Pseudomonas aeruginosa* strain MCCB 102 isolated from the Persian Gulf to achieve a significant adsorption of Cd. In a similar research conducted by De *et al.*

(2008), significant reductions of Cd by *Pseudomonas aeruginosa* and *Alcaligenes faecalis* were equally reported.

The results recorded in this study have shown that addition of biostimulants is important in achieving desired bio-sorption of Cr. These results are in tune with the report of Joseph and Ramya (2016), on the level of Cr decontamination achievable in a typical polluted soil that is properly enhanced with organic fertilizer. The decontamination prowess that the co-culture of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* exhibited in this study is in concord with the report of Gupta *et al.* (2009) on the versatility of *Pseudomonas* sp in achieving active removal of Cr from the industrial effluent analysed in their study. Again, the feat achieved by the bacterial co-culture is in line with the report of Wang *et al.* (2006) on the major role that some strains of *Pseudomonas* sp played in achieving bioremediation of pesticides and heavy metals in their study. Remarkably, Coelho *et al.* (2015) have implicated the chrR gene positioned on the chromosome of *Pseudomonas aeruginosa* as the gene that confers resistance to chromate on the bacterium.

Despite the established toxicity of Pb in the literature, its decontamination in this study by bacterial coculture is in line with the report of Rajapasksha *et al.* (2004). The Pb decontamination prowess that *Pseudomonas aeruginosa* and *Alcaligenes faecalis* co-culture demonstrated in this study could be attributed to their ability to resist and tolerate the deleterious effects of Pb. These results are in agreement with the submission of Rodriguez *et al.* (2006) on the ability of bacteria to exert resistance and tolerance of heavy metals by depollution mechanisms and environmental alteration of toxicity. Comparably, Zolgharnein *et al.* (2010) have implicated surface phenomena and diffusion as the mechanisms employed by a strain of *Pseudomonas aeruginosa* to accomplish a substantial adsorption of Pb in their study. A similar research conducted and reported by De *et al.* (2008), equally documented significant reductions of Pb by *Pseudomonas aeruginosa* and *Alcaligenes faecalis*.

Results generated on Ni decontamination potential of *Pseudomonas aeruginosa* and *Alcaligenes faecalis* co-culture have clearly shown that these bacteria can adequately cope with the toxicity and tolerance of this metal as significant reductions were recorded. As reported by Joseph and Ramya (2016), the reductions recorded in this study can be as a result of the bacterial co-culture's ability to bring about effective bio-sorption of Ni.

## Conclusion

Bacterial decontamination of all the heavy metals assayed in this study was enhanced significantly through the addition of the biostimulants employed in all the SEO contaminated soil levels. Moreover, the controls employed did not record any heavy metal decontamination owing to the absence of biostimulants *in situ*.

## Recommendation

Due to the significant heavy metal decontamination prowess of the bacterial inoculants employed in this study, biostimulation of these hydrocarbonoclastic bacteria is recommended with a view to expunging heavy metals inherently detected in polluted environments.

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