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Effect of Bio-enhanced *Streptococcus pyogenes* and *Enterococcus faecalis* Co-culture on Decontamination of Heavy Metals Content in Used Lubricating Oil Contaminated Soil

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ABSTRACT

This study assessed the heavy metal decontamination potential of bio-enhanced *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture in used lubricating oil-contaminated soil. The bacterial co-culture was isolated from the soil obtained from Dutse mechanic village, Nigeria. One thousand five hundred(1500) g of sterilized soil was intentionally contaminated with used lubricating oil at three levels. The sterilized soil was biostimulated with processed compost, powdered cocoa pod husk (PCPH), and powdered cattle dung (PCD). Afterward, the mixtures were bio-augmented with the bacterial co-culture (150 mL). The concentrations of Arsenic (As), Cadmium (Cd), Chromium (Cr), Nickel (Ni) and Lead (Pb) in the used lubricating oil contaminated soil were determined at the commencement, fifth and tenth week of the study. A factorial experiment which was laid out in a completely randomized design (CRD) was adopted. Results generated from the As decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture indicated that all the organic amendments significantly ($p<0.05$) enhanced its decontamination. At the fifth week, PCPH only enhanced the most Cd decontaminations (0.01020 mg kg^{-1}, 0.00220 mg kg^{-1} and 0.00150 mg kg^{-1}) compared with other organic amendments on 5%, 10% and 15% used lubricating oil contamination levels, respectively. At the tenth week, PCD only enhanced complete removal of Cd on all used lubricating oil contamination levels compared with compost and PCPH only, which attained complete removal of Cd on 5% and 15% of used lubricating oil contamination levels, respectively. The heavy metal decontamination potential of bio-enhanced *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture witnessed in this study indicates its suitability in effecting bioremediation of heavy metal impacted environments.

KEYWORDS: Bacterial co-culture, bioaugmentation, biostimulation, heavy metals, lubricating oil contaminated soil

1. INTRODUCTION

Heavy metal ions derivable from industrial effluents have been implicated as being remarkably toxic even at concentrations that are low. This is largely due to fact that its presence in the environment, even in minute quantities, intolerably disrupts the survival equilibrium of major events in the eco-system, which in turn can be weirdly harmful to human health

(Saravanan et al., 2022). Pollution synonymous with soil is categorized as having the capability of truncating life as all edible plant materials grown therein are invariably eaten by animals and humans (Iyobosa et al., 2020). Numerous authors (Sebiomo et al., 2010; Onuoha et al., 2011; Idemudia et al., 2014; Buraimoh et al., 2017; Ekanem, Ogunjobi, 2017 and Obi et al., 2022) have reported the employment of microorganisms in the reclamation of hydrocarbon impacted media culminating into tremendous success stories over the years.

Effective decontamination of toxic heavy metals by microorganisms can be accomplished through volatilization, extracellular chemical precipitation and valence conversion (Igiri et al., 2018). Bacteria, algae and fungi have the potential to decontaminate toxic heavy metals from impacted environmental media (Wisniewska et al., 2016; Lukic et al., 2016; Kastner and Miltner, 2016; Kanamarlapudi et al., 2018; Adeleye et al., 2019). These authors further reported the use of enzymes, extrusion, manufacture of exopolysaccharide and biotransformation as the mechanisms employed by microorganisms to interact and survive the toxicity of heavy metals.

In Nigeria, mechanic workshops are cited with flagrant contempt for town planning guidelines, thereby giving rise to indiscriminate pollution of the built and natural environments with used lubricating oil emanating from such workshops (Adeleye et al., 2020). According to Kanamarlapudi et al. (2018), toxic heavy metals; Mercury (Hg), Cobalt (Co), Chromium (Cr), Zinc (Zn), Arsenic (As), Copper (Cu), Nickel (Ni), Cadmium (Cd) and Lead (Pb) are inherently found in used lubricating oil. Toxic heavy metals are known not to be biodegradable and can thus accumulate in living organisms' tissues which can, in turn cause numerous human diseases and maladies (Onokebhagbe et al., 2019).

Over the past few years, countless management methods have been employed with a view to removing toxic heavy metals from impacted environments. According to Barakat (2011); Lakherwal (2014); Gunatilake (2015); Azimi et al. (2016); Joshi (2017) and Kanamarlapudi et al. (2018), orthodox methods; microfiltration, ultrafiltration, electro-dialysis, ion-exchange, photocatalysis, chemical precipitation, reverse osmosis, electro-winning, phytoremediation to ultra-filtration were reported to mitigate heavy metal concentration in soil. However, Kanamarlapudi et al. (2018) reported that these remediation methods used for the removal of heavy metals in environmental media are very costly and not eco-friendly. Against these backdrops, this study was conducted to assess the combined effect of bio-enhanced *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture on decontamination of heavy metal content in used lubricating oil contaminated soil.

2. MATERIAL AND METHODS

2.1 Study Area

This study was carried out at the Department of Soil Science, Faculty of Agriculture, Federal University Dutse, Jigawa State, Nigeria. The area lies within Latitude 11.7333N and Longitude 9.2875E (Adeleye et al., 2022a).

2.2 Processing of organic amendments

Processed organic amendments; powdered cattle dung (PCD), powdered cocoa pod husk (PCPH) and compost {fresh cocoa pod husk (CPH) and cattle dung (CD) subjected to composting} employed in this study were produced using procedures earlier outlined by Adeleye et al. (2020). Effective composting of the organic amendments was enhanced through the improved surface area that could enhance faster microbial mineralization by chopping fresh CPH into tiny pieces of less than 5 cm, as outlined by Komolafe et al. (2021).

2.3 Soil collection and processing

Two soils were used in this study. Soil that had no record of pollution (Nkereuwem et al., 2020), was collected from the Teaching and Research farm while used lubricating oil polluted soil was collected from the mechanic village in Dutse town of Jigawa state. Two hundred and fifty-kilogram soils were collected at a depth of 25 cm using a sterilized soil auger through the grid method from four spots in the center of the above-mentioned locations. The uncontaminated soil was simply used to compare the physical and chemical properties of the contaminated soil. The soil was sieved through 2.0 mm mesh size. The soil was there after autoclaved at 121°C for 15 minutes with a view to sterilizing it. One and a half kg of the sterilized soil was subsequently transferred into 36 polyethylene bags each. Three determined levels of used lubricating oil collected from a service pit in Dutse Mechanic Village was added and vigorously mixed with the sterilized soil. Explicitly, these varying levels of the used lubricating oil (75, 150 and 225-mL weight/weight), indicated 5, 10 and 15% contamination levels, correspondingly. The soil-used lubricating oil combinations were left undisturbed for 14 days to ensure the volatilization of the toxic components of the used lubricating oil (Agbor et al.,2015).

2.4 Isolation and identification of *Streptococcus pyogenes* and *Enterococcus faecalis* Co-culture used for heavy metal decontamination

The two bacteria, *Streptococcus pyogenes* and *Enterococcus faecalis* used as a co-culture for the decontamination of heavy metals present in the used lubricating oil contaminated soil in this study were isolated and identified as earlier reported by Adeleye et al. (2022b).

2.5 Experimental design and heavy metal decontamination assay

The design adopted for heavy metal decontamination assay was a 4 × 3 factorial experiment laid out in a completely randomized design (CRD) with three replications. The two factors considered were: (a) organic amendments at four levels, including the control (OA1, having no organic amendment), OA2 representing compost, OA3 representing PCPH, and OA4 representing PCD; and (b) used lubricating oil at three levels of 75 mL representing 5%, 150 mL representing 10% and 225 mL representing 15% contamination levels, labeled ULO1, ULO2 and ULO3, respectively. This experimental layout denotes that *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture (150 mL) were bioaugmented with used lubricating oil contaminated soil in each polyethylene bag except for experimental bags adopted as

control. All the polyethylene bags were then incubated at room temperature for 70 days (Chorom et al., 2010). The contents of each polyethylene bag were subjected to tilling and 6 mL of distilled water was added twice a week for effective aeration and moisture content maintenance, respectively (Abioye et al., 2012).

2.6 Determination of the physicochemical properties of soils and organic amendments

The procedures reported by American Water Works Association (AWWA) (2017); Okareh et al. (2018), were employed to determine available phosphorous and pH in water. Electrical conductivity (EC), moisture content, total nitrogen, organic carbon, texture and other related properties of the soils and organic amendments used in this study were determined using the procedures reported by Adeleye et al.(2020).

2.7 Estimation of heavy metals in the spent engine oil contaminated soil

The procedure reported by APHA (2012) was used to estimate heavy metals; Arsenic (As), Cadmium (Cd), Lead (Pb), Nickel (Ni) and Chromium (Cr) contents in the used lubricating oil contaminated soil at the commencement, 5th week and 10th week of bio-degradation assay using Perkin Elmer Atomic Absorption Spectrophotometer Analyst 400.

2.8 Analysis of Data

All data collected were subjected to analysis of variance using the procedure of the General Linear Model of GenStat Version 17.0. Significant means were subsequently separated using Duncan's new multiple range test at $p< 0.05$.

3. Results and Discussion

3.1 Physicochemical properties of the soil and organic amendments

The results of the physicochemical properties of the soils and organic amendments employed in this study are shown in Table 1.

The pH of the soils employed in this study was acidic (Table 1). It has been reported by Ibrahim et al. (2021) that natural and artificial activities can substantially influence the disparity in the pH of soils. The submission of these authors on African soils being slightly acidic can be substantiated with the current results of the pH recorded in the experimental soils employed in this current study. As witnessed in this study, Osaigbovo et al. (2013) reported that used lubricating oil did contribute significantly to the acidic nature of the soil assayed in their research. The results obtained from the unpolluted soil's mechanical analysis and lubricating oil-contaminated soil indicated them as sandy loam and loamy sand, respectively. The presence of used lubricating oil changed the natural soil texture (sandy loam) of the study area to loamy sand (Table 1). The total sum of exchangeable bases estimated in the used lubricating oil contaminated soil, compost, PCD and PCPH are 1.05, 221.7, 82.1 and 166.15 cmol $kg^{-1,}$ respectively (Table 1).

Table 1. Physicochemical properties of soil and organic amendments

Parameter	US	ULOCS	Compost	PCD	PCPH
Moisture content (%)	2.04	0.8	2.0	7.3	11.11
Ash content (%)	-	-	65	68.8	23
$pH_{(water)}$	6.5	6.8	9.45	8.15	7.6
Organic Carbon (%)	0.49	0.52	48.25	41.55	33.40
Total Nitrogen (%)	0.06	0.08	5.85	2.85	2.65
Available Phosphorous (mg kg^{-1})	11.02	9.40	1.48	1.2	0.08
EC (dS cm^{-1})	0.92	1.20	8.86	8.10	6.42
Exchangeable Bases (cmol kg^{-1})	--	--	--	--	--
Potassium	--	--	--	0.19	0.07
Calcium	--	--	--	1.82	0.63
Magnesium	--	--	--	0.92	0.18
Sodium	--	--	--	0.58	0.17
SEC	--	--	--	3.51	1.05
Particle Size (g kg^{-1})	--	--	--	--	--
Clay + Silt	420	200	--	--	--
Clay	100	120	--	--	--
Silt	320	80	--	--	--
Sand	580	800	--	--	--
Textural class	Sandy Loam	Loamy Sand	--	--	--

Note: US= Unpolluted soil; ULOCS= Used lubricating oil contaminated soil; PCPH= Powdered cocoa pod husk; PCD= Powdered cattle dung; EC= Electrical conductivity; SEC= Sum of Exchangeable Bases; -= Test not conducted

Table 2. Baseline concentrations of heavy metals in used lubricating oil contaminated soil before bioremediation

	Concentrations of heavy metals in used lubricating oil contaminated soil (mg kg^{-1})		
	5%	10%	15%
Arsenic	0.20400	0.20400	0.20400
Cadmium	0.06400	0.06500	0.06400
Chromium	0.3430	0.3460	0.3480
Nickel	0.07100	0.07100	0.08200
Lead	0.6410	0.6390	0.6360

3.2 Baseline concentrations of heavy metals at the commencement of biodegradation assay

Table 2 depicts the baseline concentrations of specific heavy metals estimated in the used lubricating oil contaminated soil based on varying concentrations of the used lubricating oil. It can be seen that As had the same concentration (0.20400 mg kg^{-1}) on 5%, 10%, and 15%, respectively. However, Pb recorded 0.6410 mg kg^{-1}(5%), 0.6390 mg kg^{-1}(10%) and 0.6360 mg kg^{-1}(15%). The presence of the various heavy metals recorded in the used lubricating oil contaminated soil assayed in this current

study had been previously reported by Stephen et al. (2012); Stephen et al. (2013); Zali et al. (2015) and Echiegu et al. (2021) in their respective studies.

3.3 Arsenic decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture

Results generated from the As decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture indicate that all the organic amendments significantly enhanced its decontamination ($p<0.05$) as shown in Table 3. At the fifth week, compared with other organic amendments, compost recorded the most As removal (0.04130 mg kg^{-1} and 0.04720 mg kg^{-1}) on 5% and 15% used lubricating oil contamination levels, respectively. This finding might be due to the considerable quantities of organic carbon and total nitrogen inherently present in the compost, which aided optimum bacterial growth and metabolic activities (Fadina et al., 2019), thereby leading to significant As decontamination witnessed in this study. However, PCD only recorded the most As decontamination (0.04450 mg kg^{-1}) on 10% used lubricating oil contamination level (Table 3). Interestingly, at the tenth week, all the organic amendments enhanced the complete removal of As on all the used lubricating oil contamination levels (Table 3). Results obtained on As decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture are in line with the report of Bhakta et al. (2014) on *Enterococcus* spp. prowess in the removal of As from water bodies. Similar to this study, Bhattacharyya1 et al. (2013), reported significant detoxification of As by *Streptococcus* spp. isolated from a waste dumping site in India. Bacterial depollution of As in this study corroborates the report of Igiri et al. (2018), on the ability of bacteria to facilitate excellent metal interactions that inevitably lead to its desirable depollution from polluted environments.

3.4 Cadmium decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture

Results generated from the Cd decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture indicated that all the organic amendments significantly ($p<0.05$) enhanced its decontamination, as shown in Table 4. At the fifth week, PCPH only enhanced the most cadmium removal decontaminations (0.01020 mg kg^{-1}, 0.00220 mg kg^{-1} and 0.00150 mg kg^{-1}) compared with other organic amendments on 5%, 10% and 15% used lubricating oil contamination levels respectively (Table 4). Results generated from the Cd decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture indicated that all the organic amendments significantly ($p<0.05$) enhanced its decontamination, as shown in Table 4. At the fifth week, PCPH only enhanced the most Cd removal decontaminations (0.01020 mg kg^{-1}, 0.00220 mg kg^{-1} and 0.00150 mg kg^{-1}) compared with other organic amendments on 5%, 10% and 15% used lubricating oil contamination levels respectively (Table 4). However, at the tenth week, PCD only enhanced complete removal of Cd on all used lubricating oil contamination levels compared with compost and PCPH only that attained complete removal of Cd on 5% and 15% used lubricating oil contamination levels,

respectively (Table 4). In line with the results obtained in this study, Eghomwanre et al. (2016) reported tolerance of *Streptococcus* spp. isolated from contaminated soils and sediments around the Warri area of Delta State, Nigeria to Cd exposure. Conversely, at the tenth week, PCD only enhanced complete removal of Cd on all used lubricating oil contamination levels compared with compost and PCPH only that attained complete removal of Cd on 5% and 15% used lubricating oil contamination levels, respectively (Table 4). The results are equally in agreement with the report of Bhakta et al. (2014) that implicated *Enterococcus* spp. in the removal of Cd from water bodies. The biostimulatory influence of the organic amendments most especially powdered CD not only enhanced complete bacterial removal of Cd in this study, it also proved influential in attaining such feat owing to the nutrients available therein. The results obtained in this study further corroborate the submission of Huet and Puchooa (2017), on the capability of *Enterococcus* spp. to remove Cd substantially from a medium contaminated with Cd, Pb and Cr.

3.5 Chromium decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture

Table 5 shows that results generated on the potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture to decontaminate Cr contents of used lubricating oil contaminated soil revealed that the organic amendments employed significantly ($p<0.05$) enhanced such.

At the fifth week, compared with other organic amendments, PCD only enhanced the completed removal of Cr on 5% and 10% used lubricating oil contamination levels, while compost facilitated its complete removal on 15% used lubricating oil contaminated soil (Table 5). At the tenth week, both compost and PCD only enhanced the complete removal of Cr on all the used lubricating oil contamination levels compared with the performance of PCPH (Table 5). The essential nutrients inherently present in the organic amendments employed for biostimulation of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture aided the optimum bacterial metabolism of Cr witnessed in this study. According to Sarkar et al. (2016), the combination of nutrients required for microbial metabolism and growth aids the biodegradation aptitude of the microbial consortium involved in the biodegradation of soils with high concentrations of pollutants. The Cr decontamination attribute that *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture recorded in this study corroborates the submission of Bhattacharyya1 et al. (2013), regarding the substantial Cr detoxification potential of *Streptococcus* spp. isolated from a waste dumping site in their study.

3.6 Nickel decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture

Results generated on the potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture to decontaminate Ni contents of used lubricating oil contaminated soil reveal that the organic amendments employed did significantly ($p<0.05$) enhance such as shown in Table 6.

Table 3. Interaction of organic amendments and used lubricating oil on bacterial decontamination of Arsenic during biodegradation assay

Treatments		Concentrations of Arsenic (mg kg^{-1})	
OA	ULO level	5th Week	10th Week
OA1	ULO1	0.20400^{a}	0.20400^{a}
OA1	ULO2	0.20400^{a}	0.20400^{a}
OA1	ULO3	0.20400^{a}	0.20400^{a}
OA2	ULO1	0.04130^{i}	0.00000^{b}
OA2	ULO2	0.04453^{g}	0.00000^{b}
OA2	ULO3	0.04720^{f}	0.00000^{b}
OA3	ULO1	0.07370^{d}	0.00000^{b}
OA3	ULO2	0.07770^{c}	0.00000^{b}
OA3	ULO3	0.08120^{b}	0.00000^{b}
OA4	ULO1	0.04240^{h}	0.00000^{b}
OA4	ULO2	0.04450^{g}	0.00000^{b}
OA4	ULO3	0.04920^{e}	0.00000^{b}

Note: OA= Organic amendment; ULO= Used lubricating oil; OA1= without organic amendment; OA2= Compost; OA3= Powdered cocoa pod husk only; OA4= Powdered cattle dung only; ULO1= ULO at 5%; ULO2= ULO at 10%; ULO3= Used lubricating oil at 15%. Means with the same letter (s) in each column are not significantly different using Duncan multiple range test (DMRT). ($p>0.05$) 0.00000= Not detected

Table 4. Interaction of organic amendments and used lubricating oil on bacterial decontamination of cadmium during biodegradation assay

Treatments		Concentrations of Cadmium (mg kg^{-1})	
OA	ULO level	5th Week	10th Week
OA1	ULO1	0.06400^{b}	0.06400^{b}
OA1	ULO2	0.06500^{a}	0.06500^{a}
OA1	ULO3	0.06400^{b}	0.06400^{b}
OA2	ULO1	0.01540^{g}	0.00000^{g}
OA2	ULO2	0.02090^{e}	0.00090^{e}
OA2	ULO3	0.03750^{c}	0.01150^{c}
OA3	ULO1	0.01020^{i}	0.00020^{f}
OA3	ULO2	0.00220^{j}	0.00120^{d}
OA3	ULO3	0.00150^{k}	0.00000^{g}
OA4	ULO1	0.01340^{h}	0.00000^{g}
OA4	ULO2	0.01760^{f}	0.00000^{g}
OA4	ULO3	0.02400^{d}	0.00000^{g}

Note: OA= Organic amendment; ULO= Used lubricating oil; OA1= without organic amendment; OA2= Compost; OA3= Powdered cocoa pod husk only; OA4= Powdered cattle dung only; ULO1= ULO at 5%; ULO2= ULO at 10%; ULO3= Used lubricating oil at 15%. Means with the same letter (s) in each column are not significantly different using Duncan multiple range test (DMRT). ($p>0.05$) 0.00000= Not detected

Table 5. Interaction of organic amendments and used lubricating oil on bacterial decontamination of Chromium during biodegradation assay

Treatments		Concentrations of Chromium (mg kg^{-1})	
OA	ULO level	5th Week	10th Week
OA1	ULO1	0.3430^{c}	0.3430^{c}
OA1	ULO2	0.3460^{b}	0.3460^{b}
OA1	ULO3	0.3480^{a}	0.3480^{a}
OA2	ULO1	0.0540^{h}	0.0000^{g}
OA2	ULO2	0.0450^{i}	0.0000^{g}
OA2	ULO3	0.0000^{j}	0.0000^{g}
OA3	ULO1	0.3120^{d}	0.2130^{d}
OA3	ULO2	0.3010^{e}	0.2020^{e}
OA3	ULO3	0.1950^{f}	0.1130^{f}
OA4	ULO1	0.0000^{j}	0.0000^{g}
OA4	ULO2	0.0000^{j}	0.0000^{g}
OA4	ULO3	0.0850^{g}	0.0000^{g}

Note: OA= Organic amendment; ULO= Used lubricating oil; OA1= without organic amendment; OA2= Compost; OA3= Powdered cocoa pod husk only; OA4= Powdered cattle dung only; ULO1= ULO at 5%; ULO2= ULO at 10%; ULO3= Used lubricating oil at 15%. Means with the same letter (s) in each column are not significantly different using Duncan multiple range test (DMRT). ($p>0.05$) 0.0000= Not detected.

Table 6. Interaction of organic amendments and used lubricating oil on bacterial decontamination of Nickel during biodegradation assay

Treatments		Concentrations of Nickel (mg kg^{-1})	
OA	ULO level	5th Week	10th Week
OA1	ULO1	0.07100^{b}	0.07100^{b}
OA1	ULO2	0.07100^{b}	0.07090^{c}
OA1	ULO3	0.08200^{a}	0.08150^{a}
OA2	ULO1	0.04230^{h}	0.02223^{l}
OA2	ULO2	0.04407^{gh}	0.02920^{k}
OA2	ULO3	0.05647^{cd}	0.03690^{h}
OA3	ULO1	0.05160^{def}	0.05020^{f}
OA3	ULO2	0.05320^{de}	0.05150^{e}
OA3	ULO3	0.06130^{c}	0.05620^{d}
OA4	ULO1	0.04663^{fgh}	0.03160^{j}
OA4	ULO2	0.04880^{efg}	0.03670^{i}
OA4	ULO3	0.05450^{de}	0.04930^{g}

Note: OA= Organic amendment; ULO= Used lubricating oil; OA1= without organic amendment; OA2= Compost; OA3= Powdered cocoa pod husk only; OA4= Powdered cattle dung only; ULO1= ULO at 5%; ULO2= ULO at 10%; ULO3= Used lubricating oil at 15%. Means with the same letter (s) in each column are not significantly different using Duncan multiple range test (DMRT). ($p>0.05$)

Table 7. Interaction of organic amendments and used lubricating oilon bacterial decontamination of Lead during biodegradation assay

Treatments		Concentrations of Lead (mg kg^{-1})	
OA	ULO level	5th Week	10th Week
OA1	ULO1	0.6410^{a}	0.6410^{a}
OA1	ULO2	0.6390^{b}	0.6390^{b}
OA1	ULO3	0.6360^{c}	0.6360^{c}
OA2	ULO1	0.2260^{i}	0.0027^{k}
OA2	ULO2	0.1470^{j}	0.0080^{j}
OA2	ULO3	0.0680^{k}	0.0380^{g}
OA3	ULO1	0.3180^{g}	0.1160^{e}
OA3	ULO2	0.0110^{l}	0.0000^{l}
OA3	ULO3	0.2730^{h}	0.1230^{d}
OA4	ULO1	0.3880^{e}	0.0130^{i}
OA4	ULO2	0.3570^{f}	0.0260^{h}
OA4	ULO3	0.3990^{d}	0.0430^{f}

Note: OA= Organic amendment; ULO= Used lubricating oil; OA1= without organic amendment; OA2= Compost; OA3= Powdered cocoa pod husk only; OA4= Powdered cattle dung only; ULO1= ULO at 5%; ULO2= ULO at 10%; ULO3= Used lubricating oil at 15%. Means with the same letter (s) in each column are not significantly different using Duncan multiple range tests (DMRT). ($p>0.05$) 0.0000= Not detected

However, at the fifth week, compared with other organic amendments, compost influenced the most reductions (0.04230 mg kg^{-1}and 0.04407 mg kg^{-1}) on 5% and 10% used lubricating oil contamination levels, respectively, while PCD only enhanced the most reduction (0.05450 mg kg^{-1}) on 15%used lubricating oil contamination level (Table 6). At the tenth week, compost significantly ($p<0.05$) enhanced the most Ni decontamination (0.02223 mg kg^{-1}, 0.02920 mg kg^{-1} and 0.03690 mg kg^{-1}) compared with other organic amendments on 5%, 10% and 15% used lubricating oil contamination levels respectively (Table 6). These results have shown that organic nutrient supplementation can enhance bacterial decontamination of Ni when present in an undesirable concentration in the environment. The bacterial decontamination of Ni in this study is in agreement with the report of Alfano and Cavazza (2020) on its importance in redox processes and microbial metabolism.

3.7 Lead decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture

Results generated on the potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture to decontaminate Pb contents of used lubricating oil contaminated soil revealed that all the organic amendments engaged significantly enhanced such ($p<0.05$) as shown in Table 7. Specifically, at the fifth week, compared with other organic amendments, compost influenced the most reductions (0.2260 mg kg^{-1} and 0.0680 mg kg^{-1}) on 5% and 15% used lubricating oil contamination levels, respectively while PCPH only enhanced the most decontamination (0.0110 mg kg^{-1}) of Pb on 10% used lubricating oil contamination level (Table 7).

At the tenth week, compared with other organic amendments employed, compost further significantly ($p<0.05$) enhanced the most reductions (0.0027 mg kg^{-1} and 0.0380 mg kg^{-1}) on 5% and 15% used lubricating oil contamination levels, respectively while PCPH only facilitated complete removal of Pb on 10% used lubricating oil contamination level (Table 7). The feat that the bacterial co-culture attained in terms of tolerance of Pb and its eventual decontamination from the used lubricating oil contaminated soil studied can be attributed to its effective supplementation with suitable nutrients that ultimately enhanced its performance. Similar finding has been reported by Adeleye et al. (2020) on the ability of effective supplementation of bacteria with organic amendments leading to significant decontamination of heavy metals from used lubricating oil contaminated soil. These results further support the report of Huet and Puchooa (2017), on the proficiency of *Enterococcus* spp. in recording the highest level of Pb removal in their study.

4. CONCLUSION

Bacterial decontamination of the heavy metal contents of varying levels of used lubricating oil contaminated soil was significantly enhanced through biostimulation with processed organic amendments in this study. However, compared with the controls employed, which recorded poor heavy metal decontamination, the concentrations of heavy metals decontaminated were significantly higher in the bio-enhanced used lubricating oil contaminated soil samples. Resultantly, the blend of bio-augmentation and bio-stimulation technologies is recommended for the clean-up of heavy metal impacted environments due to the significant removal of such recorded in this study.

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A.O.A. and M.B.Y. conceptualized the research. M.E.N and V.O.O.were actively involved in the physicochemical analysis of the soils and biostimulants. M.E.N, analyzed the data generated from the research. A.O.A. wrote the manuscript. M.B.Y and M.G.D. revised the manuscript.

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