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Effect of organic amendments on the decontamination potential of heavy metals by *Staphylococcus aureus* and *Bacillus cereus* in soil contaminated with spent engine oil

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Abstract

This study was conducted to assess the decontamination of heavy metals of an environmental concern in spent engine oil (SEO) contaminated soil, through the adoption of *Staphylococcus aureus* and *Bacillus cereus* co-cultures, isolated from Dutse mechanic village, Nigeria. About 1.5 kg of autoclaved soil was contaminated with SEO at three levels. The sterilized soil was then amended with compost, powdered cocoa pod husk (CPH), and powdered cow dung (CD). Subsequently, bio-augmentation with bacterial co-cultures (150 ml) was done. Heavy metal concentrations; Arsenic (As), Cadmium (Cd), Chromium (Cr), Nickel (Ni) and Lead (Pb) were estimated at the commencement, at the fifth and tenth week of the study. The factorial experiment was laid out in a completely randomized design (CRD). Results indicate that bio-stimulation adopted through the amendments did not have significant effects on the bacterial decontamination of soil especially at the 5^{th} week ($P > 0.05$). CD only influenced the decontamination of Cd at 5% contamination level (0.0008 mg\ kg), compared with compost that recorded the least effect (0.00360 mg\ kg) at the 5^{th} week. Meanwhile, CPH had the highest decontamination effect on Cr (0.004 and 0.000 mg\ kg) at 10% and 15% contamination levels; respectively, at the 5^{th} week. However at the 10^{th} week, complete removal of As was influenced by compost, CPH, and CD using 5%, 10% and 15% SEO contamination levels, respectively ($P < 0.05$). The decontamination efficacies of *S. aureus* and *B. cereus* recorded in this study is an indication of their potentialities for application in bioremediation of heavy metals.

Keywords: Soil, Spent engine oil, Heavy metals, Bacterial co-cultures, Bioremediation, Decontamination

1. Introduction

Environmental pollution caused by heavy metals emanating from industrial and anthropogenic activities has inflicted the substantial irrevocable impairment of the ecosystem health. According to Adeleye *et al*., (2018), the technological developments that are presently witnessed throughout the world regarding the utilization of hydrocarbon related products have increasingly brought about all forms of hydrocarbon related environmental pollution. In Nigeria, the management of mechanic workshops has been reported in the literatures as being done poorly (Adams *et al*., 2014). Moreover, these authors reported that this poor management is the main source of constant release of spent oil discharged from the crank cases of cars and motorcycles, which may thus be aesthetically unpleasant and cause severe environmental pollution.

Zali *et al*., (2015) revealed that the introduction of heavy metals analysis in engine oil aims to identify the failure of the oil components during the early stages before the emergence of major damages. They added that Iron (Fe), Chromium (Cr), Aluminium (Al), Lead (Pd), and Cadmium (Cd) known as wear metals may depict a significant wear in engine or any oil wetted compartment in an automobile. However, Aucelio *et al*., (2007) previously reported that the detection of Silicon (Si), Sodium (Na) and Boron (B) as wear metals may have arisen from dirt and antifreeze, thus resulting into failure of the system. Zali *et al*., (2015) added that SEO should be managed appropriately according to the standard international regulations, and it can be recovered also from those engines that have potential economic values. These authors further submitted that SEO that does not meet the set of standards and regulations can be categorized as wastes and must be disposed of. Vähäoja *et al*., (2005) summarized the standards and specifications of recovered SEO as; Arsenic (As) (5 ppm maximum allowed limit), Cadmium (Cd) (2 ppm), Chromium (Cr) (10 ppm), and Lead (Pb) (100 ppm).

Numerous physicochemical and biological processes are usually engaged with the removal of heavy metals from the environment. According to Mulligan *et al*., (2001); Kadirvelu *et al*., (2002), orthodox physicochemical methods employed for such removal including; ion exchange, precipitation, sorption, electrochemical treatments, osmosis and evaporation are very expensive, coupled with the fact that some of these methods are not ecofriendly.

Accordingly, this study was conducted to assess the influence of the selected organic amendments on the possible bacterial decontamination of heavy metals of an environmental concern in SEO contaminated soil.

2. Materials and methods

2.1. The area of study

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

2.2. Collection and processing of the organic amendments

About 30 kg of each of the fresh cocoa pod husks (CPH) and Cow Dung (CD) were collected from Ado Ekiti, Ekiti State, and Dutse Abbatoir, Jigawa State, respectively. After that, 15 kg of the collected CPH was air dried for 30 days and then crushed into powder using electric blender as described by Agbor *et al*., (2015). This powdered material was sieved into fine form, and then sterilized by autoclaving at 121°C for 15 min. according to Ezekoye *et al*., (2017). On the other hand, 10 kg of the collected CD was sun-dried and crushed into powder using pestle and mortar. It was equally sieved into fine form, autoclaved at 121°C for 15 min. as described by Ezekoye *et al*., (2017). They were subsequently stored in a container and labeled as powdered CPH and CD, respectively.

Compost was generated by mixing of 10 kg of each of the fresh CPH and CD. The fresh CPH was chopped into small pieces of 5 cm^2 as described by Adegunloye and Olorunnusi, (2016), and then mixed thoroughly with the CD by pounding until a homogenous mixture was perceived. This mixture was subsequently placed in a self-designed compost container and labeled "composted CPH + CD 1:1 w/w". The mixture was continuously mixed every 5 days for the duration of composting according to Vishan *et al*., (2017). Mixing of the compost was done to achieve the release of nutrients gradually through mineralization as reported by Adeleye and Sridhar, (2015). The composting process lasted for 20 days as described by Vishan *et al*., (2107). The generated compost was subsequently autoclaved at 121°C for 15 min., to exclude the extraneous influence of the microbes as recommended by Ezekoye *et al*., (2017).

2.3. Collection, preparation of non-polluted soil and inoculation with the SEO

In reference to Agbor *et al*., (2015), about 250 kg top soil (0-25 cm depth) that had no any history of pollution was collected from four different regions of the Department of Soil Science, Federal University Dutse main campus. The soil was air-dried, sieved with 2 mm mesh, and then autoclaved at 121°C for 15 min. (Soretire *et al*., 2017). About 1.5 kg of this sterile soil was placed in 108 polyethylene bags, and then 75, 150, and 225 ml (w/w) of SEO obtained from one of the service pits in Dutse mechanic village were added individually to each soil sample representing; 5%, 10% and 15% contamination levels, respectively. The soils and the SEO were mixed thoroughly, and then left for 14 days to ensure volatilization of the toxic components from the oil (Abioye *et al*., 2012; Agbor *et al*., 2015).

2.4. Determination of the samples' physicochemical properties

Samples of unpolluted soil, SEO polluted soil and organic amendments' were analyzed 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). The organic carbon was determined by the modified Walkley-Black procedure (Nelson and Sommers, 1996), whereas, the cation exchange capacity (CEC) was estimated by the summation method of Chapman, (1965). The total nitrogen and phosphorous contents of the samples were determined by the Kjeldhal and Bray-1 method in reference to Reeuwijk, (1993); Bremmer, (1996). The soil mechanical analysis was equally estimated by the hydrometer method of Bouyoucos, (1962).

2.5. Collection of naturally polluted SEO soil, isolation and identification of the inoculant bacteria

Approximately 10 g of SEO naturally polluted soil was collected at a depth of 5 cm from the mechanic village, Dutse. The ambient temperature of this polluted soil recorded during the sampling was 37.3°C. The bacterial isolates that were used as co-culture inoculants in this study were isolated from a SEO polluted soil on Trypticase soy agar, adopting the procedure of Adeleye and Yerima, (2019), and then they were identified biochemically according to Hemraj *et al*., (2013).

2.6. Heavy metals' decontamination assay

In this study, 36 polyethylene bags were used for the heavy metals' decontamination assay conducted according to Nkereuwem *et al*., (2010); Ezekoye *et al*., (2017). Apart from the 9 experimental bags that were used as controls, about 150 g of each sterilized biostimulant (compost, powdered CPH, and powdered CD) was added individually and mixed thoroughly with each set of the soils contaminated with the SEO (at 5%, 10% and 15% levels). With the exception for the 9 control bags, all the remaining 27 experimental bags in each set were augmented and mixed thoroughly with 150 ml of the bacterial co-cultures. All the bags were subsequently incubated at room temperature for 70 days. Three replicates were used for each SEO contamination level. According to Ayotamuno *et al*., (2006); Chorom *et al*., (2010), the contents of each bag were tilled twice a week for aeration. Maintenance of moisture was equally done twice a week by the addition of 6 ml of sterile distilled water (Abioye *et al*., 2012). The assay lasted for 10 weeks as recommended by Chorom *et al*., (2010).

2.7. Determination of heavy metals

The heavy metals were estimated through the employment of Perkin Elmer Atomic Absorption Spectrophotometer (Analyst 400), as described by Maurya *et al*., (2018).

2.8. Statistical analysis

All results obtained were subjected to analysis by Proc. GLM of GenStat version 17, and the means were separated using Duncan Multiple Range Test (DMRT).

3. Results

3.1. Isolation and identification of the inoculant bacteria

Isolation of the bacterial inoculants from the SEO contaminated soil on Trypticase soy agar led to the recovery of two predominant bacterial isolates with colony characteristics presented in Table (1). According to the results of biochemical identification of these two isolates (Table 1), they were identified as *S. aureus* and *B. cereus*, respectively.

3.2. The physicochemical properties of the soil samples and the biostimulants

The physicochemical properties of the unpolluted soil, SEO polluted soil and biostimulants are listed in Table (2).

3.3. The Arsenic decontamination potential of co-cultures of *S. aureus* and *B. cereus*

At the 5th week, all the biostimulants did not significantly (P> 0.05) influence the bacterial decontamination of Arsenic (As) throughout all the studied SEO contamination levels. However, CPH only recorded the highest reductions of (As) (0.009 mg\ kg and 0.024 mg\ kg) on using 5% and 15% SEO, respectively, compared with the other biostimulants. On the other hand, compost recorded significant reduction (0.044 mg\ kg) on applying 10% SEO (Table 3). Conversely, all the biostimulants significantly influenced the bacterial decontamination of (As) at the 10th week (P< 0.05). Thus, the compost, CPH and CD facilitated complete removal of (As) on using 5% and 15% SEO, while compost and CPH enhanced complete removal of (As) on using 10% SEO contamination level, compared with the controls (Table 4).

3.4. The Cadmium decontamination efficacy of co-cultures of *S. aureus* and *B. cereus*

Results obtained on the Cadmium (Cd) decontamination potential of the bacterial co-cultures indicate that all the biostimulants significantly enhanced (Cd) decontamination throughout all the SEO contamination levels (P< 0.05). However at the 5th week, CD presents the most significant decontaminations (0.001 mg\ kg, 0.001 mg\ kg and 0.005 mg\ kg) on 5%, 10% and 15% SEO; respectively, compared with the controls (Table 5). At the 10th week, compost and CD enhanced complete removal of (Cd) on 5% and 10% SEO compared with the CPH. Whereas, compost recorded complete removal of (Cd) on 15% SEO contamination level (Table 6).

Table 1: Identification of the bacterial inoculants according to the colony and biochemical characteristics

	Status		Status
Colonial characteristics	Creamy yellow colonies		Glistering white colonies
Gram staining	Gram positive cocci in clusters		Gram positive rods
Biochemical Tests			
Oxidase	_	Voges Proskauer	+
Voges Proskauer	+	Methyl Red	_
Coagulase	+	Oxidase	+
Lactose	+	Indole	_
Maltose	+	Citrate	_
Mannitol	+	Nitrate	+
Fructose	+	Urease	_
Sucrose	+	Simmon's Citrate	+
Xylose	_	Glucose	+
Cellobiose	_	Fructose	+
Mannose	+	Lactose	_
Phosphatase	+	Sorbitol	_
Nitrate	+	Xylose	_
Arginine	+	Galactose	_
Protease	+	Mannose	_
		Tyrosine hydrolysis	+
		Growth in 10% NaCl	+
Identification of the two bacterial isolates	*Staphylococcus aureus*		*Bacillus cereus*

Table 2: Physicochemical properties of unpolluted soil, SEO polluted soil and biostimulants

Parameters	Unpolluted soil	SEO polluted soil	Compost	CD	CPH
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)	11.02	9.40	1.48	1.2	0.08
EC (dS\ cm)	0.92	1.20	8.86	8.10	6.42

Exchangeable Bases [cmol\ kg]					
Potassium	0.19	0.07	213.16	80	162
Calcium	1.82	0.63	4.8	0.2	1.6
Magnesium	0.92	0.18	3.24	1.5	2.45
Sodium	0.58	0.17	0.5	0.4	0.1
CEC	3.51	1.05	221.7	82.1	166.15
Particle Size (g\ kg)					
Clay + Silt	420	200	--	-	-
Clay	100	120	-	-	-
Silt	320	80	-	-	-
Sand	580	800	-	-	-
Textural class	Sandy Loam	Loamy Sand	-	-	-

Table 3: Effect of the biostimulants on the Arsenic decontamination levels (mg\ kg) at the 5^{th} week

	SEO Contamination levels		
Biostimulants	5%	10%	15%
Compost	0.031^{b}	0.044^{a}	0.036^{ab}
CPH	0.009^{c}	0.046^{a}	0.024^{b}
CD	0.077^{ab}	0.080^{b}	0.106^{a}
Control	0.204^{a}	0.204^{a}	0.144^{b}
Significance status	Ns	Ns	Ns

where standard error (SE)= (+) 0.01735, degree of freedom (d.f.)= 22, Ns = non-significant
Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT) ($P < 0.05$)

Table 4: Effect of the biostimulants on the Arsenic decontamination levels (mg\ kg) at the10th week

	SEO Contamination Levels		
Biostimulants	5%	10%	15%
Compost	0.000	0.000	0.000
CPH	0.000	0.000	0.000
CD	0.000	0.032^{a}	0.000
Control	0.204^{a}	0.204^{a}	0.205^{a}

where standard error (SE)= (+) 0.00002843, degree of freedom (d.f.)= 22.
Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT) ($P < 0.05$)

Table 5: Effect of the biostimulants on the Cadmium decontamination levels (mg\ kg) at the 5th week

	SEO Contamination Levels		
Biostimulants	5%	10%	15%
Compost	0.036^{a}	0.028^{b}	0.029^{b}
CPH	0.043^{a}	0.039^{b}	0.036^{c}
CD	0.001^{b}	0.001^{b}	0.005^{a}
Control	0.064$^{\mathbf{b}}$	0.065$^{\mathbf{a}}$	0.064^{b}

where standard error (SE)= (+) 0.00001667, degree of freedom (d.f.)= 22.
Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT) ($P < 0.05$)

Table 6: Effect of the biostimulants on the Cadmium decontamination levels (mg\ kg) at 10th week

	SEO Contamination Levels		
Biostimulants	5%	10%	15%
Compost	0.000	0.000	0.000
CPH	0.022^{a}	0.022^{a}	0.023^{a}
CD	0.000	0.000	0.002^{a}
Control	0.064^{a}	0.065^{a}	0.064^{a}

where standard error (SE)= (+) 0.00002973, degree of freedom (d.f.)= 22.
Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT) ($P < 0.05$)

3.5. The Chromium decontamination potency of *S. aureus* and *B. cereus* co-cultures

All the biostimulants had significant influence on Chromium (Cr) decontamination powers of the bacterial co-cultures (P< 0.05) compared with the control. At the 5th week, compost enhanced the reduction level (0.080 mg\ kg) on applying 5% of SEO, whereas CPH recorded significant decontamination (0.004 mg\ kg) and complete removal on using 10% and 15% SEO; respectively (Table 7). At the 10th week and compared with the other biostimulants, compost significantly (P< 0.05) enhanced complete removal of (Cr) on 5%, 10% and 15% SEO, while CPH only facilitated complete removal of (Cr) on 15% SEO contamination level (Table 8).

3.6. The Lead decontamination activity of *S. aureus* and *B. cereus* co-cultures

Results of Lead (Pb) decontamination potential of the bacterial co-cultures demonstrated that all the used biostimulants influenced it significantly (P< 0.05) compared with the control. At the 5th week, CD enhanced the decontamination level (0.007 mg\ kg) on 5% SEO, whereas CPH caused considerable reduction levels (0.046 mg\ kg and 0.031 mg\ kg) on applying 10% and 15% SEO, respectively (Table 9). At the 10th week, compost and CD facilitated complete removal of (Pb) on the three SEO contamination levels, compared with the results of the CPH (Table 10).

3.7. The Nickel decontamination potential of *S. aureus* and *B. cereus* co-cultures

Results recorded on Nickel (Ni) decontamination potential of the bacterial co-cultures indicate that all the tested biostimulants significantly (P<0.05) enhanced (Ni) decontamination levels at the 5th week (Table 11). However at the 10th week, the biostimulants did not significantly (P> 0.05) enhance the decontamination levels (Table 12). Specifically at the 5th week, CD presented significant decontaminations levels (0.032 mg\ kg, 0.033 mg\ kg and 0.038 mg\ kg), while compost enhanced the decontamination levels to (0.003 mg\ kg, 0.001 mg\ kg and 0.005 mg\ kg) on using 5%, 10% and 15% SEO contamination levels; respectively, compared with the controls.

4. Discussion

The results of isolation of the bacterial cultures responsible for SEO utilization in the current study led to the recovery of *S. aureus* and *B. cereus* isolates, as part of those bacteria associated with hydrocarbon degradation in different soils of Kano, Nigeria. These results are in agreement with the findings of Yahaya and Bappa, (2019).

The current detection of all the heavy metals such as; As, Cd, Cr, Pb and Ni that are of serious environmental concern in the SEO contaminated soils, is in line with the previous reports of Zali *et al*., (2015). The complete removal of As by the bacterial co-cultures adopted in this study could be ascribed to the same reason reported earlier by Hamer, (1986); Hameed, (2006) concerning the presence of powerful metal bio-sorbents in the bacteria such as the genus *Bacillus*. These results are in agreement with those of Wu *et al*., (2017) regarding the ability of *B. cereus* to initiate effective bio-sorption, bioaccumulation and biosurfactant production especially when enhanced with suitable organic fertilizers, thereby leading to the removal of the heavy metals from the polluted environments. In addition, Bhakta *et al*., (2014) have reported the employment of *Bacillus* sp. and *S. aureus* for the removal of As from water bodies. The current results are line with the reports of Wu *et al*., (2017), concerning the potentiality of *B. cereus* to affect the decontamination of Cd, when enhanced with suitable organic fertilizer. Moreover, Ahemad and Malik, (2012) reported the presence of tolerance and co-resistance of *Staphylococcus* spp. and *Bacillus* spp. against Cd in their study.

Table 7: Effect of the biostimulants on the Chromium decontamination levels (mg\ kg) at the 5th week

	SEO Contamination Levels		
Biostimulants	5%	10%	15%
Compost	0.080^{c}	1.273^{a}	0.098^{b}
CPH	0.120^{a}	0.004^{b}	0.000
CD	0.221^{a}	0.211^{b}	0.198^{c}
Control	0.382^{b}	0.380^{b}	0.390^{a}

where standard error (SE)= (+) 0.0001667, degree of freedom (d.f.)= 22.
Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT) ($P < 0.05$)

Table 8: Effect of the biostimulants on the Chromium decontamination levels (mg\ kg) at the 10th week

	SEO Contamination Levels		
Biostimulants	5%	10%	15%
Compost	0.000	0.000	0.000
CPH	0.080^{a}	0.001^{b}	0.000
CD	0.134^{a}	0.134^{a}	0.115^{b}
Control	0.381^{b}	0.380^{b}	0.390^{a}

where standard error (SE)= (+) 0.0001667, degree of freedom (d.f.)= 22.
Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT) ($P < 0.05$)

Table 9: Effect of the biostimulants on the Lead decontamination levels (mg\ kg) at the 5th week

	SEO Contamination Levels		
Biostimulants	5%	10%	15%
Compost	0.099^{c}	0.134^{a}	0.110^{b}
CPH	0.026^{c}	0.046^{a}	0.031^{b}
CD	0.007^{i}	0.110^{e}	0.100^{f}
Control	0.641^{a}	0.639^{a}	0.636^{a}

where standard error (SE)= (+) 0.000458, degree of freedom (d.f.)= 22.
Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT) ($P < 0.05$)

Table 10: Effect of the biostimulants on the Lead decontamination levels (mg\ kg) at the 10th week

	SEO Contamination Levels		
Biostimulants	5%	10%	15%
Compost	0.000	0.000	0.000
CPH	0.055[a]	0.031[b]	0.021[c]
CD	0.000	0.000	0.000
Control	0.642[a]	0.639[b]	0.636[c]

where standard error (SE)= (+) 0.00549, degree of freedom (d.f.)= 22.
Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT) ($P < 0.05$)

Table 11: Effect of the biostimulants on the Nickel decontamination levels (mg\ kg) at the 5th week

	SEO Contamination Levels		
Biostimulants	5%	10%	15%
Compost	0.037[c]	0.046[b]	0.052[a]
CPH	0.063[a]	0.063[a]	0.063[a]
CD	0.032[b]	0.033[b]	0.038[a]
Control	0.075[b]	0.075[b]	0.078[a]

where standard error (SE)= (+) 0.001757, degree of freedom (d.f.)= 22.
Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT) ($P < 0.05$)

Table 12: Effect of the biostimulants on the Nickel decontamination levels (mg\ kg) at the 10th week

	SEO Contamination Levels		
Biostimulants	5%	10%	15%
Compost	0.003[b]	0.001[c]	0.005[a]
CPH	0.020[c]	0.030[b]	0.034[a]
CD	0.004[b]	0.009[a]	0.009[a]
Control	0.075[b]	0.075[b]	0.078[a]
Significance status	Ns	Ns	Ns

where standard error (SE)= (+) 0.00549, degree of freedom (d.f.)= 22, Ns = non-significant
Means with the same letters in each column are not significantly different using Duncan multiple range test (DMRT) ($P < 0.05$)

The Cr decontamination capabilities of the bacterial co-cultures exhibited in this study could be attributed to the enhancement caused by the organic fertilizers. This is in accordance with Joseph and Ramya, (2016) who reported that bio-sorption of Cr expectedly takes place upon exposure to such enhancement. As reported previously by Lovera *et al*., (1993), the ability of the bacteria to readily decontaminate Cr via its reduction from Cr (VI) to Cr (III), through Cr (V) and Cr (IV) intermediaries may be responsible for the recorded bacterial decontamination of Cr witnessed in this study. A previous study of Kanmani *et al*., (2012) employed *B. cereus* for the removal of Cr in agreement with the results of this study. Current results have clearly indicated that the bacterial co-cultures can sufficiently cope with the toxicity and tolerance of Pb, as significant removal levels were documented. The ability of the bacterial co-cultures to resist, tolerate and bring about effective bio-sorption of Pb that was reported by Rodriguez, (2006); Joseph and Ramya, (2016), may have been responsible for its decontamination level attained in this study. In agreement with the results obtained in this study, Das *et al*., (2008) reported that *Bacillus* spp. have been commercialized during the preparation of bio-sorbent, due to its great potential for sequestering heavy metals. The present detection of Ni in the SEO contaminated soil is in line with the reports of Chicarelli *et al*., (1990) on its presence in the petroleum hydrocarbons. However, the insignificant decontaminations recorded by all the biostimulants at the 10^{th} week do not make the reductions attained inconsequential. As reported by Samuelson *et al*., (2000), the increased Ni binding capabilities referred to the expression of poly-His peptides in Staphylococcus spp., may interpret the decontamination of Ni witnessed in this study.

Conclusion

In this study, bacterial decontamination of As, Cd, Ni, Cr and Pb is significantly enhanced by the addition of biostimulants in all the SEO contaminated soil levels. Moreover, the concentrations of heavy metals decontaminated are higher in the bio-augmented and bio-stimulated soil samples, compared with the controls employed. Moreover, owing to the significant removal of heavy metals that are known to be toxic, non-biodegradable and hazardous to human health, the combination of bio-augmentation and bio-stimulation is recommended as an efficient biotechnology tool to clean up such pollutions form the environment. However, further research focusing on the molecular-based characterization of the inherent heavy metal-decontaminating enzymes in the genome of the bacteria is recommended. Additionally, there is a need to subject the biostimulants employed in this study to Fourier Transmission Infra-red (FT-IR) analysis, to determine the active functional groups which exerted their influence on heavy metal chelation, and caused significant decontamination levels witnessed in this study.

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Conflict of interests

The authors declare that they have no financial or non-financial conflict of interests with regard to the current manuscript.

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