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Research article

Assessing the Potential of Mechanical Aeration Combined with Bioremediation Process in Soils and Coastal Sediments Impacted by Heavy Metals

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Abstract: Microorganisms make use of heavy metals through enzymatic, non-enzymatic processes or bioaccumulation in bacterial cells in insoluble or particulate forms and by-products. Increasing effectiveness of bioremediation is still being explored and other stimulation techniques cited by various authors used mostly EDTA, nitrogen fertiliser and other amendments. The use of mechanical aeration combined with bioremediation using *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa* and *Pseudomonas fluoresecens* offer a greener approach with more efficient remediation capabilities. Zinc exceeded the permissible limit recommended by FAO/WHO by more than two folds while other metals were close to the threshold limit posing a dangerous threat to human health. Implementation of the current package treatment showed statistically significant decreases in heavy metal concentrations in both soils and coastal sediments in a 90 days experiment under atmospheric conditions. For sediments, 21.4% to 100% bioremediation was achieved under mechanical aeration conditions representing an increase of up to 60% efficiency compared to non-aeration while for soil highest efficacy achieved was 63.1%. However, the mechanisms and pathways of bioremediation were noticed to depend according to biotic and abiotic factors. This article provides an insight on the comparison between proposed stimulation technique and other methods reported.

Keywords: effectiveness; stimulation; permissible limits; *Bacillus; Pseudomonas*; comparison

1. Introduction

Microorganisms make use of heavy metals (HM) as terminal electron acceptors and acquire energy to detoxify metals [1]. These may occur either through enzymatic, non-enzymatic processes or bioaccumulation of heavy metals in bacterial cells in insoluble or particulate forms and by-products [1]. In attempt to achieve an effective method of bioremediation, several techniques are still being explored. Bhatt et al., [2] reviewed the different biological remediation processes in which the use of oxygen was vital. In the experiment of Lin et al. [3], the authors used new bacterial consortia in order to increase efficiency of biodegradation and also bioaugmentation implemented showed a reduction in half-life of the contaminants. Similarly, Kang et al. [4] used a mixture of four bacterial strains to remediate heavy metals from contaminated soils and achieved a success rate of up to 98.3%Pb, 85.4% Cd and 5.6% Cu. In the study of Singh et al., [5] indigenous *Bacillus cereus* showed to remediate up to 72% Cr at 37 ºC and initial pH of 8.0. However it was also pointed out that the temperature range for remediation could vary from 25 ºC to 40 ºC and pH 6 to 10. Fulekar et al., [6] further conducted a laboratory experiment using bioreactors whereby the bacteria were isolated, cultured and stimulated under aerobic conditions. Bioremediation under aerobic conditions for metals Fe, Cu and Cd was conducted for 21 days and could reach 100 %, 99.6 % and 98.5 % respectively.According to Adiloğlu [7] bacterial remediation within the rhizosphere of plants was reported to be enhanced using EDTA applications. Metals such as Cr, Co, Ni and Pb could be removed more efficiently using stimulation of EDTA doses increasingly. Another similar study conducted by Shrestha et al., [8] showed that remediation can be boosted using compost which reduced significantly bioavailable fractions of metals. Another mode of bioremediation widely studied was the use of biofilm-based technology biodegradation of environmental pollutants. Biofilm-mediated remediation has also been delineated as being organized, competent option for the degradation of contaminants [9]. Lal et al., [10] studied nanotechnology and nanoparticles and reported success rates for removing toxic metals ions from water however, these were expensive methods with limited recycled-use of nano-inspired adsorbents. Since most investigations focused on laboratory analysis for stimulating remediation and incubation of bacteria under different concentrations of heavy metals, gap analysis showed limited studies to actually investigate the remediation capabilities of given bacteria on a range of heavy metals under atmospheric and natural conditions. The experiment aims to investigate whether mechanical aeration which is an easy and practical method could stimulate bioremediation process efficiently. Therefore, the objectives of the study are to contrast between bioremediation under implemented mechanical aeration conditions, natural attenuation and non-aerated treatments and to provide an insight on findings compared to other stimulation techniques reported by various authors.

2. Materials and methods

2.1. Site Selection

Fourteen sites which were suspected to be contaminated with heavy metals were inspected across the Island of Mauritius. These comprised of 7 land fields and 7 coasts (Figure 1). A site analysis was conducted based on their historical background, that is, their land uses and activities.

Soil and sediment samples (30 independent samples) were taken using the 'W' method covering maximum of the sites [11]. It was then mixed thoroughly before analysis to ensure uniformity and homogeneity of the area under investigation. Parameters assessment conducted in laboratory were done in three replicates.

Figure 1. Location of contaminated land and coastal sites.

Table 1. Coordinates of site location.

Site codes	Coordinates
S-SJD	20°13'49.4"S, 57°38'16.4"E
S-BMF	20°11'60.0"S, 57°46'50.4"E
S-M1	20°11'06.8"S, 57°28'51.7"E
S-UOM	20°14'08.2"S, 57°29'26.3"E
S-LCC	20°13'57.6"S, 57°25'50.7"E
S-MCL	20°23'21.1"S, 57°37'50.5"E
S-AIR	20°25'32.7"S, 57°40'17.5"E
C-GPS	20°19'39.4"S, 57°46'17.4"E
C-PAS	20°10'02.8"S, 57°28'20.1"E
C-TDD	20°14'21.9"S, 57°47'29.2"E
C-MER	20°08'19.9"S, 57°29'50.3"E
C-BDT	20°08'12.0"S, 57°29'51.7"E
C-FFF	20°16'43.5"S, 57°21'59.7"E
C-RIA	20°31'07.7"S, 57°28'57.6"E

2.2. Physical parameters analysis of soil/sediment

Homogenised soil samples were measured in pre-weighed envelope and placed in oven at 110 ± 5 ºC overnight and the masses were recorded every 24 hours until these were constant after being cooled in a desiccator. Iron core-ring method was also used for determining the bulk densities of the soils. Three replicates were done and measurements were recorded up to 3 decimal places. Soil textures were also determined based on Stokes' Law and Textural Triangle.

2.3. Biological parameters analysis of soil/sediment

Using Bergey's Manual of Determinative Bacteriology main species of bacteria were identified plating on specific agars and 16S rRNA gene sequencing [12] was used to identify bacterial isolates. Bacterial counts were determined using plate count method. In addition, the microbial respiration rate analyses were conducted using back-titration of unreacted sodium hydroxide as per Rowell [13].

2.4. Chemical parameters analysis of soil/sediment

2.4.1. pH (Probe method [13])

20 g of soil/sediment was measured in a container to which 50 ml of deionized water was added and shaken for 30 minutes. pH meter electrode was inserted into the sample and values were recorded to 2 decimal places.

2.4.2. Electrical conductivity (Probe method [13]

20 g of soil/sediment was measured in a container to which 50 ml of deionized water was added and shaken for 30 minutes. EC probe was inserted into the sample and values were recorded at an accuracy of ± 0.01 unit.

2.4.3. Soil Organic Matter (Colorimetric method [13])

0.1 g of sieved soil was measured in Erlenmeyer flask into which potassium dichromate and sulphuric acid were added and stirred and left overnight. The supernatant was collected and the absorption of the solution at 660 nm was measured using a photospectrometer. Accuracy of measurement was of order ± 1%.

2.4.4. Total Nitrogen (Kjeldahl method [13])

2 g of air-dried soil was weighed into Kjeldahl flask, followed by 1 tablet of catalyst and 15 ml sulphuric acid. It was digested and later allowed to cool. The solution was then back titrated with 0.01M HCl and pH indicator. Detection limit was 0.002% N with an accuracy of ± 1%. Three replicates were done.

2.4.5. Total Phosphorus (Rowell [13])

Ashed soil/sediment samples were digested in 5 ml concentrated HCl. 5 ml HNO_3 was added and transferred on hotplate. It was then diluted with deionized water, filtered and serial dilutions were made. Vanado-molybdate was pipetted in each sample and allowed to stand for 30 minutes after which absorbance were read at 430 nm. Detection limit using this method was 0.1 %.

2.4.6. Total Potassium (Rowell [13])

Filtrates obtained after acid digestion for total phosphorus were used to determine level of potassium using a flame photometer. Accuracy of measurement was of order ± 0.1 unit.

2.4.7. Heavy Metals using AAS (Rowell [13])

10g of <2mm air-dry soil was transferred to a polystyrene bottle. 50 ml of ammonium EDTA was then added and shaken for 1hr at 125 rpm on a shaking machine. The solution was then filtered and retained for analysis. Standards solutions of the prepared heavy metals were passed in the AAS spectrometer (Solar Unicam 929 AA spectrometer), followed by the soil samples, where their absorbance were read. Detection limit was of order ± 0.1 %.

2.5. Treatment allocations for bioremediation (combination of bioaugmentation and biostimulation)

Table 2. Treatment allocation.

Treatment code	Bioremediation treatment description
Before	Soil/sediment condition prior to experiment
Ctrl	No treatment
Trt PA	Bioaugmentation using *Pseudomonas aeruginosa* + aeration
Trt PA(N)	Bioaugmentation using *Pseudomonas aeruginosa* + no aeration
Trt PF	Bioaugmentation using *Pseudomonas fluorescens* + aeration
Trt PF(N)	Bioaugmentation using *Pseudomonas fluorescens* + no aeration
Trt BS	Bioaugmentation using *Bacillus subtilis* + aeration
Trt BS(N)	Bioaugmentation using *Bacillus subtilis* + no aeration
Trt BC	Bioaugmentation using *Bacillus cereus* + aeration
Trt BC(N)	Bioaugmentation using *Bacillus cereus* + no aeration

Bioaugmentation involved adding up native microorganisms (bacteria) to the contaminated soils to supply appropriate conditions for their growth. The specific bacteria (*Pseudomonas aeruginosa, Pseudomonas fluorescens, Bacillus cereus, Bacillus substilis*) were inoculated in Muller-Hinton broth and allowed to grow for 24 hours at 37 ºC. Cultures were then adjusted to a turbidity of 0.5 McFarland prior to bioaugmentation. Being among the aerobes, these bacteria were stimulated with mechanical aeration (biostimulation) to increase performance efficiency. Biostimulating the soil/ sediment involved making “tiny holes” of 1 cm diameter mechanically and depth of 20 cm in the media every week to keep them aerated and reduced compaction. Each treatment and parameters assessed had three replicates.

2.6. Statistical analysis

All parameters in the study were distributed normally. Data were expressed as mean ± standard deviation. Differences were tested by one-way ANOVA test. Pearson's correlation was used to analyse the association between all studied parameters. The values $P < 0.05$ were considered statistically significant. Statistical analysis was done using Minitab 16.2.1 statistical software.

3. Results

From the experiment, it was noted that S-SJD (petroleum station site) was the most contaminated having the highest concentrations of most heavy metals. Zinc exceeded the permissible limit recommended by FAO/WHO (1976) by more than two folds while others were close to the threshold limit posing a dangerous threat to human health. Bioremediation showed consequent decreases in heavy metal concentrations in both soils and coastal sediments. For sediments, upto 100% Cd, 21.4% Cr, 88.2% Cu, 47.7% Mn, 100% Ni, 50.3% Pb and 59.6% Zn bioremediation were achieved under mechanical aeration conditions representing an increase of 60% for Cd, 14% Cr, 25.8% Cu, 1.8% Mn, 38.1% Zn, 47% Ni and 24% Pb respectively. Similar trends were observed for soils when the same treatment was applied. Highest efficacy achieved were 63.1% Cd, 26.7% Cr, 7.3% Cu, 9.2% Mn, 11.6% Ni, 24.5% Pb and 34.1% Zn. Despite the changes in concentrations being digitally substantial, statistics using Tukey's method of comparison at 95% confidence interval revealed no significant changes. The current findings might be due to variation in distribution of the metals in the soils and sediments resulting in large standard deviation in replicates.

Table 3. Soil parameters.

Parameters	S-BMF Site	Std Dev.	Unit	S-SJD Site	Std Dev.	Unit
Moisture content	9.73	± 0.01	%	23.37	± 0.89	%
Bulk density	1.09	± 0.35	g/cm^3	1.11	± 0.39	g/cm^3
Texture	Sandy			Loamy		
pH	8.21	± 0.02		7.75	± 0.02	
EC	360	± 10	µS/cm	327	± 1	µS/cm
Org.matter	15.56	± 0.07	ppm	38.61	± 0.01	ppm
Total Nitrogen	0.17	± 0.01	%	0.32	± 0.01	%
Total Phosphorus	0.548	± 0.003	ppm	0.129	± 0.002	ppm
Total Potassium	5.3	± 0.0	ppm	44.9	± 0.3	ppm
Bacterial count	37.8×10^7	$\pm 1.4 \times 10^7$	count	23.4×10^7	$\pm 1.4 \times 10^7$	count
Microbial resp. rate	0.649	± 0.010	mg/hour	0.391	± 0.003	mg/hour
Microbial biosmass Carbon	1.778	± 0.000	g/g	0.556	± 0.112	g/g

The soil and coastal sediment physical, chemical and biological parameters were summarised in Tables 3 and 4 respectively. The remediation achieved under each treatment was tabulated in Tables 5 to 11. Hence the best treatment was selected.

Table 4. Coastal sediment parameters.

Parameters	C-PAS Site	Std Dev.	Unit	C-GPS Site	Std Dev.	Unit
Moisture content	36.74	± 0.52	%	45.67	± 0.93	%
Bulk density	1.13	± 0.26	g/cm^3	1.45	± 0.2	g/cm^3
Texture	Sandy			Sandy Clay		
pH	7.5	± 0.08		8.19	± 0.00	
EC	2810	± 2.83	µS/cm	85	± 7.07	µS/cm
Org.matter	2.64	± 0.01	ppm	10.29	± 0.07	ppm
Total Nitrogen	0	± 0.00	%	0	± 0.00	%
Total Phosphorus	0.032	± 0.002	ppm	0.129	± 0.003	ppm
Total Potassium	12.1	± 1.4	ppm	35.1	± 0.6	ppm
Bacterial count	19×10^7	$\pm 1.9 \times 10^7$	count	55.6×10^7	$\pm 1.1 \times 10^7$	count
Microbial resp. rate	0.658	± 0.010	mg/hour	0.568	± 0.002	mg/hour
Microbial biosmass Carbon	1.111	± 0.667	g/g	1.778	± 0.000	g/g

4. Discussion

According to the statement of Gupta and Diwan [14], these microorganisms defend themselves against toxicities and other forms of stress caused by heavy metals. Even though the current experiment was conducted under atmospheric conditions, results of bioremediation were comparatively in line with those stated by Pang et al., [15] where experiments reported were performed under controlled conditions. In addition, current mechanical aeration technique showed a statistically significant increase ($P<0.05$) in bacterial count in soils and coastal sediments except for S-BMF. As a result of continued exposure to high concentrations of these metals, the microorganisms have established tolerance resulting in rise in bacterial count [16]. Conversely, S-BMF with a sandy soil texture had coarser particle sizes and typically contained the freest particulate organic matter [17] to feed on as a source of energy, could probably explain the high but insignificant change in bacterial count after aeration. According to Hemkemyer et al., [18], different soil particle size fractions had dissimilar adaptive capacities of microbes governing the sorption and mineralisation of organic pollutants. Pearson coefficient showed a moderate positive relationship between bacterial counts and microbial respiration rates (MRR). Current findings also showed that there were no significant changes in MRR for all sandy soil/sediment textures which might also confirm the above statement of Hemkemyer et al., [18].

Soils contaminated with various heavy metals were reported to be more complex and more difficult to restore compared to soils contaminated with a single metal [19]. A few bacteria have uncommon properties allowing the solubilisation of phosphorus, sequestration of iron, nitrogen fixation and generation of phytohormones that improve plant development and biomass helping in phytoremediation processes [20]. The changing metal speciation is as a rule utilised to assess the remediation productivity of heavy metal in soil and sediments and to depict remediation mechanisms [21].

Table 5. Bioremediation of zinc.

C-GPS		C-PAS		S-SJD		S-BMF		Zn (ppm)
Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	
NA	0.076A ± 0.005	NA	7.620A ± 0.020	NA	129.73A ± 7.96	NA	0.721A ± 0.088	Before
5.3	0.072A ± 0.003	0.3	7.597A ± 0.132	2.6	126.34A ± 1.71	28.3	0.517B ± 0.023	Ctrl
21.1	0.060A ± 0.015	59.6	3.082C ± 0.168	49.6	65.42G ± 1.93	35.1	0.468B ± 0.128	PA
13.2	0.066A ± 0.003	21.5	5.980B ± 0.112	36.8	82.00F ± 0.80	19.6	0.580AB ± 0.047	PA(N)
15.8	0.064A ± 0.003	29.1	5.403B ± 1.250	30.1	90.74E ± 0.73	34.8	0.470B ± 0.019	PF
7.9	0.070A ± 0.000	21.1	6.012B ± 0.148	24.3	98.22DE ± 1.20	24.5	0.544B ± 0.040	PF(N)
15.8	0.064A ± 0.005	46.8	4.053C ± 0.349	20.6	102.98CD ± 1.67	64.9	0.253C ± 0.110	BS
7.9	0.070A ± 0.000	22.3	6.230B ± 0.152	14.6	110.82BC ± 0.12	30.8	0.499B ± 0.094	BS(N)
17.1	0.063A ± 0.007	46.0	4.116C ± 0.036	18.5	105.72BCD ± 1.93	39.9	0.433B ± 0.031	BC
21.1	0.060A ± 0.006	16.5	6.366B ± 0.108	14.3	111.21B ± 1.10	18.3	0.589AB ± 0.054	BC(N)

Note: ^{A,B,C,D} : Tukey's test at 95% confidence interval; mean ± standard deviation values.

Table 6. Bioremediation of copper.

S-BMF		S-SJD		C-PAS		C-GPS		Cu (ppm)
Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	
6.281^{A} ± 0.380	NA	7.642^{A} ± 0.345	NA	6.802^{A} ± 0.074	NA	8.074^{A} ± 0.43	NA	Before
5.399^{B} ± 0.051	14.0	7.150^{B} ± 0.207	6.4	6.861^{A} ± 0.112	-0.9	7.150^{B} ± 0.207	11.4	Ctrl
1.898^{CD} ± 0.023	69.8	3.675^{D} ± 0.058	51.9	0.841^{C} ± 0.035	87.6	3.675^{C} ± 0.058	54.5	PA
2.340^{C} ± 0.010	62.7	4.373^{C} ± 0.023	42.8	2.687^{B} ± 0.037	60.5	3.000^{D} ± 0.100	62.8	PA(N)

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S-BMF		S-SJD		C-PAS		C-GPS		Cu
Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	(ppm)
1.911^{CD} ± 0.070	69.6	3.844^{D} ± 0.081	49.7	0.814^{C} ± 0.062	88.0	3.843^{C} ± 0.081	52.4	PF
2.387^{C} ± 0.027	62.0	4.440^{C} ± 0.020	41.9	2.627^{B} ± 0.041	61.5	2.700^{E} ± 0.050	66.6	PF(N)
1.723^{CD} ± 0.236	72.6	3.103^{E} ± 0.040	59.4	0.840^{C} ± 0.073	88.2	3.102^{D} ± 0.040	61.6	BS
2.180^{CD} ± 0.020	65.3	4.692^{C} ± 0.021	38.6	2.558^{B} ± 0.012	62.4	3.005^{D} ± 0.250	62.8	BS(N)
1.517^{D} ± 0.621	75.8	3.810^{D} ± 0.081	50.1	0.820^{C} ± 0.020	87.9	3.810^{C} ± 0.081	52.8	BC
2.220^{CD} ± 0.044	64.7	4.401^{C} ± 0.079	42.4	2.660^{B} ± 0.500	60.9	3.100^{D} ± 0.050	61.6	BC(N)

Note: ^{A,B,C,D}: Tukey's test at 95% confidence interval; mean ± standard deviation values.

Table 7. Bioremediation of chromium.

S-BMF		S-SJD		C-PAS		C-GPS		Cr (ppm)
Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	
0.150^{A} ± 0.000	NA	24.99^{A} ± 0.91	NA	1.686^{A} ± 0.065	NA	7.379^{A} ± 0.261	NA	Before
0.121^{A} ± 0.245	19.3	25.32^{A} ± 2.13	0.0	1.752^{A} ± 0.123	0.0	7.589^{A} ± 3.250	0.0	Ctrl
-0.021^{A} ± 0.563	100.0	17.52^{B} ± 3.25	29.9	1.326^{A} ± 1.247	21.4	6.171^{A} ± 3.685	16.4	PA
0.040^{A} ± 0.020	73.3	23.36^{A} ± 0.01	6.5	1.562^{A} ± 0.002	7.4	7.508^{A} ± 0.082	0.0	PA(N)
0.120^{A} ± 0.122	20.0	21.77^{AB} ± 3.25	12.9	1.397^{A} ± 0.684	17.1	6.880^{A} ± 0.055	6.8	PF
0.050^{A} ± 0.005	66.7	23.20^{A} ± 0.00	7.2	1.505^{A} ± 0.500	10.7	7.265^{A} ± 0.005	1.5	PF(N)
-0.092^{A} ± 2.011	100.0	23.19^{A} ± 2.13	7.2	1.610^{A} ± 0.246	4.5	6.880^{A} ± 1.230	6.8	BS
0.045^{A} ± 0.015	70.0	24.05^{A} ± 0.05	3.8	1.590^{A} ± 0.045	5.7	7.257^{A} ± 0.843	1.7	BS(N)
-1.101^{A} ± 2.132	100.0	21.77^{AB} ± 1.23	12.9	1.468^{A} ± 0.000	12.9	6.880^{A} ± 3.254	6.8	BC
0.050^{A} ± 0.010	66.7	24.35^{A} ± 0.30	2.6	1.598^{A} ± 0.028	5.2	7.302^{A} ± 0.068	1.0	BC(N)

Note: ^{A,B}: Tukey's test at 95% confidence interval; mean ± standard deviation values.

Table 8. Bioremediation of cadmium.

S-BMF Mean Concentration (ppm)	S-BMF Percentage remediation achieved (%)	S-SJD Mean Concentration (ppm)	S-SJD Percentage remediation achieved (%)	C-PAS Mean Concentration (ppm)	C-PAS Percentage remediation achieved (%)	C-GPS Mean Concentration (ppm)	C-GPS Percentage remediation achieved (%)	Cd (ppm)
0.046^{A} ± 0.001	NA	0.026^{A} ± 0.001	NA	0.035^{A} ± 0.000	NA	0.074^{A} ± 0.097	NA	Before
0.041^{A} ± 0.006	10.9	0.025^{A} ± 0.000	3.8	0.034^{A} ± 0.000	2.9	0.018^{A} ± 0.006	75.7	Ctrl
0.025^{A} ± 0.019	45.7	0.002^{A} ± 0.011	92.3	0.018^{A} ± 0.006	48.6	0.006^{A} ± 0.009	91.9	PA
0.030^{A} ± 0.010	34.8	0.017^{A} ± 0.019	34.6	0.022^{A} ± 0.001	37.1	0.010^{A} ± 0.001	86.5	PA(N)
0.025^{A} ± 0.025	45.7	0.005^{A} ± 0.025	80.8	0.018^{A} ± 0.014	48.6	0.019^{A} ± 0.011	74.3	PF
0.031^{A} ± 0.004	32.6	0.012^{A} ± 0.001	53.8	0.082^{A} ± 0.107	0.0	0.012^{A} ± 0.008	83.8	PF(N)
0.025^{A} ± 0.010	45.7	0.009^{A} ± 0.027	65.4	0.022^{A} ± 0.014	37.1	0.012^{A} ± 0.005	83.8	BS
0.031^{A} ± 0.004	32.6	0.013^{A} ± 0.001	50.0	0.025^{A} ± 0.001	28.6	0.014^{A} ± 0.006	81.1	BS(N)
0.002^{A} ± 0.096	95.7	0.009^{A} ± 0.027	65.4	0.000^{A} ± 0.011	100.0	0.012^{A} ± 0.005	83.8	BC
0.031^{A} ± 0.008	32.6	0.014^{A} ± 0.001	46.2	0.021^{A} ± 0.000	40.0	0.013^{A} ± 0.002	82.4	BC(N)

Note: A: Tukey's test at 95% confidence interval; mean ± standard deviation values.

Table 9. Bioremediation of manganese.

S-BMF		S-SJD		C-PAS		C-GPS		Mn (ppm)
Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	
1.861^{A} ± 0.155	NA	46.88^{A} ± 0.10	NA	1.833^{A} ± 0.000	NA	1.504^{A} ± 0.180	NA	Before
1.672^{AB} ± 0.079	10.2	40.92^{A} ± 3.56	12.7	1.700^{A} ± 0.045	7.3	1.329^{AB} ± 0.094	11.6	Ctrl
1.352^{ABC} ± 0.509	27.4	18.09^{D} ± 2.62	61.4	1.226^{B} ± 0.065	33.1	0.827^{C} ± 0.149	45.0	PA
1.640^{AB} ± 0.010	11.9	22.40^{BCD} ± 0.05	52.2	1.380^{B} ± 0.120	24.7	0.920^{BC} ± 0.080	38.8	PA(N)
1.101^{C} ± 0.129	40.8	19.23^{D} ± 1.71	59.0	1.135^{B} ± 0.017	38.1	0.787^{C} ± 0.112	47.7	PF

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S-BMF		S-SJD		C-PAS		C-GPS		Mn (ppm)
Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	
$1.520^{ABC} \pm 0.020$	18.3	$23.15^{BCD} \pm 0.05$	50.6	$1.245^{B} \pm 0.005$	32.1	$0.813^{C} \pm 0.012$	45.9	PF(N)
$1.107^{C} \pm 0.064$	40.5	$23.23^{BCD} \pm 4.94$	50.4	$1.329^{B} \pm 0.295$	27.5	$0.873^{C} \pm 0.084$	42.0	BS
$1.550^{ABC} \pm 0.050$	16.7	$26.44^{BC} \pm 0.02$	43.6	$1.324^{B} \pm 0.034$	27.8	$0.810^{C} \pm 0.290$	46.1	BS(N)
$1.170^{BC} \pm 0.129$	37.1	$19.80^{CD} \pm 2.62$	57.8	$1.101^{B} \pm 0.107$	39.9	$0.873^{C} \pm 0.183$	42.0	BC
$1.720^{A} \pm 0.010$	7.6	$27.20^{B} \pm 0.10$	42.0	$1.305^{B} \pm 0.015$	28.8	$0.996^{BC} \pm 0.004$	33.8	BC(N)

Note: ^{A,B,C,D}: Tukey's test at 95% confidence interval; mean ± standard deviation values.

Table 10. Bioremediation of nickel.

S-BMF		S-SJD		C-PAS		C-GPS		Ni (ppm)
Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	
$0.757^{A} \pm 0.000$	NA	$0.749^{A} \pm 0.013$	NA	$0.720^{A} \pm 0.032$	NA	$0.494^{A} \pm 0.027$	NA	Before
$0.661^{A} \pm 0.018$	12.7	$0.651^{B} \pm 0.031$	13.1	$0.610^{A} \pm 0.064$	15.3	$0.374^{A} \pm 0.031$	24.3	Ctrl
$0.333^{B} \pm 0.018$	56.0	$0.362^{D} \pm 0.047$	51.7	$0.240^{C} \pm 0.065$	66.7	$-0.038^{D} \pm 0.117$	100.0	PA
$0.412^{B} \pm 0.028$	44.4	$0.452^{CD} \pm 0.010$	39.7	$0.427^{B} \pm 0.010$	40.7	$0.210^{BC} \pm 0.000$	57.5	PA(N)
$0.333^{B} \pm 0.018$	56.0	$0.363^{D} \pm 0.064$	51.5	$0.240^{C} \pm 0.047$	66.7	$-0.069^{D} \pm 0.175$	100.0	PF
$0.420^{B} \pm 0.120$	44.5	$0.435^{CD} \pm 0.005$	41.9	$0.445^{B} \pm 0.072$	38.2	$0.182^{BC} \pm 0.002$	63.2	PF(N)
$0.374^{B} \pm 0.031$	50.6	$0.374^{CD} \pm 0.031$	50.1	$0.209^{C} \pm 0.018$	71.0	$0.034^{CD} \pm 0.031$	93.1	BS
$0.428^{B} \pm 0.028$	43.5	$0.442^{CD} \pm 0.024$	41.0	$0.412^{B} \pm 0.031$	42.8	$0.171^{C} \pm 0.000$	65.4	BS(N)
$0.363^{B} \pm 0.035$	52.0	$0.363^{D} \pm 0.035$	51.5	$0.209^{C} \pm 0.065$	71.0	$-0.151^{D} \pm 0.000$	100.0	BC
$0.430^{B} \pm 0.097$	43.2	$0.465^{C} \pm 0.025$	37.9	$0.429^{B} \pm 0.000$	40.4	$0.232^{BC} \pm 0.001$	53.0	BC(N)

Note: ^{A,B,C,D}: Tukey's test at 95% confidence interval; mean ± standard deviation values.

Table 11. Bioremediation of lead.

S-BMF		S-SJD		C-PAS		C-GPS		Pb (ppm)
Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	Mean Concentration (ppm)	Percentage remediation achieved (%)	
$1.210^{A} \pm 0.000$	NA	$2.94^{A} \pm 0.06$	NA	$1.22^{A} \pm 0.02$	NA	$1.71^{A} \pm 0.01$	NA	Before
$1.175^{AB} \pm 0.043$	2.9	$2.85^{A} \pm 0.11$	3.1	$1.03^{B} \pm 0.09$	15.6	$1.22^{B} \pm 0.01$	28.7	Ctrl
$0.706^{E} \pm 0.043$	41.7	$1.99^{BC} \pm 0.04$	32.3	$0.78^{D} \pm 0.04$	36.1	$0.88^{D} \pm 0.04$	48.5	PA
$0.940^{BCDE} \pm 0.060$	22.3	$2.20^{B} \pm 0.05$	25.2	$0.96^{BC} \pm 0.02$	21.3	$1.13^{C} \pm 0.03$	33.9	PA(N)
$0.731^{DE} \pm 0.043$	39.6	$2.11^{B} \pm 0.04$	28.2	$0.81^{D} \pm 0.04$	33.6	$0.93^{D} \pm 0.04$	45.6	PF
$0.930^{BCDE} \pm 0.070$	23.1	$2.11^{B} \pm 0.04$	28.2	$0.95^{D} \pm 0.03$	22.1	$1.23^{B} \pm 0.01$	28.1	PF(N)
$0.805^{CDE} \pm 0.043$	33.5	$1.99^{BC} \pm 0.19$	32.3	$0.83^{CD} \pm 0.07$	32.0	$0.90^{D} \pm 0.00$	47.4	BS
$1.001^{ABC} \pm 0.174$	17.3	$2.18^{B} \pm 0.12$	25.9	$0.99^{B} \pm 0.01$	18.9	$1.22^{B} \pm 0.01$	28.7	BS(N)
$0.805^{CDE} \pm 0.114$	33.5	$1.62^{C} \pm 0.37$	44.9	$0.78^{D} \pm 0.04$	36.1	$0.85^{D} \pm 0.04$	50.3	BC
$0.970^{ABCD} \pm 0.130$	19.8	$2.34^{B} \pm 0.02$	20.4	$0.95^{BC} \pm 0.01$	22.1	$1.26^{B} \pm 0.01$	26.3	BC(N)

Note: $^{A.B,C,D}$: Tukey's test at 95% confidence interval; mean ± standard deviation values.

Bioavailability played a vital role in the process. The slight alkaline soils and sediments might more likely have more bioavailable $Cr^{6+,}$ mobile Cr^{3+}, chromates and dichromate ions associated with oxygen. This might explain the 100% remediation under aerated conditions in S-BMF which had a sandy texture. Additional mechanism pointed out by Learman et al., [22] was the efflux pump associated with chrA, chrR and yieF genes in bacteria connecting to Cr^{6+} [23]. chrR gene in *Pseudomonas putida* promoted the reduction of Cr^{6+} to Cr^{5+} while yieF gene in *E.coli* catalyse the reduction of Cr^{6+} to Cr^{3+}. Kermani et al., [24] pointed out that both living and non-living cells of the strain *Pseudomonas aeruginosa* could eliminate Cd^{2+} from contaminated solutions. Also, cadmium metal and its oxides were reported to be insoluble in water, some salts are hydrophilic by interactions with oxygen [25] implying the crucial role of implementing mechanical aeration in the current experiment on the absorption mechanisms of Cd by bacteria. Copper conversely exerts a homeostasis control on Cu^{2+} all through the bacteria to prevent toxicity [26]. It was additionally emphasised by Cornu et al., [27] that bacteria used active and passive pathways to mobilise or immobilise copper in soils and sediments due to their high chemical reactivity but required deepened analysis for enhanced site remediation. It was reported that Mn bio-oxidation followed concomitantly two distinct pathways: (1) direct, which is governed by cellular components like enzymes [28] and (2) indirect, Mn^{2+} oxidation occurs as a result of alterations in pH and redox conditions of the atmosphere caused by bacterial metabolites and microbial growth [29]. Similar pathways by Ni^{2+} which is moderately soluble reached a maximum of 33.7% remediation in the form of Ni-sulphate when assessed with 3 bacteria species (*Stenotrophomonas* spp, *Pseudomonas* spp and *Sphingobium* spp) [30]. In line with the current experiment, Fan et al., [31] stated that *R. sphaeroides* bacteria could not remove entire concentration of lead in soil, however it could change its speciation and was reported to be less effective compared to Cd. The principle mechanism used was the precipitation formation of inert compounds such as lead sulphide and lead sulphate [32]. Lastly, zinc is absorbed in bacteria and is used as metalloenzymes, playing essential roles in survival [33]. Zinc in the form of Zn^{2+} followed similar pathways as those above but was found to compete with Cd movement and bio-accumulation [34]. Nevertheless, these remediation efficiencies are also influenced by biotic and abiotic factors.

In line with the results of Kermani et al., [24] abiotic factors including pH were favourable for the growth of bacteria. The findings were further supported by Li et al., [32] whereby the latter mentioned optimum pH for R. sphaeroides was 7 and temperature 30–35° C for bioremediation of heavy metals. Mechanical aeration of the soil initiated the incorporation of oxygen and water which helped either the bacteria or the metal to react. Examples include Mn(III) and Mn(IV) which are prevalent in occurrence with oxygen and high pH values compared to Mn(II) which is thermodynamically stable [35]. Similarly, a plausible explanation suggested that S-BMF with a sandy soil and having a lower bulk density than S-SJD (loamy soil), implied that more pores were available between soil particles in S-BMF and hence could retain more air and water. Results indicated a retention potential of 2% more by S-BMF compared to S-SJD soil which might help reaction of Cr which might complex readily with organic matter and utilised by bacteria. The outcomes were in agreement with Evanko and Dzombak [36] and Garbisu and Alkorta [37]. These mentioned that microorganisms utilised bioavailable heavy metals in their catabolic processes to derive energy, which sequentially detoxified the soil.

5. Conclusions

Despite increasing effectiveness of bioremediation is still being explored with several stimulation techniques reported by various authors. However, no experiment was testified so far on using mechanical aeration to enhance the bioremediation rate. Results of implementing mechanical aeration for coastal sediments showed an increase in bioremediation rate by 60% for Cd, 14% Cr, 25.8% Cu, 1.8% Mn, 38.1% Zn, 47% Ni and 24% Pb as well as for soils the rate were increased by 63.1% Cd, 26.7% Cr, 7.3% Cu, 9.2% Mn, 11.6% Ni, 24.5% Pb and 34.1% Zn. Regardless whether the experiment was conducted under natural and atmospheric conditions, abiotic factors were favourable for the growth and development of bacteria hence for the remediation process. Conclusively, the current proposal seemed promising and mechanical aeration showed to be an efficient, greener and user-friendly approach for increasing the rate of heavy removal in soils and sediments.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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