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 fluorescens* 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 °C and initial pH of 8.0. However it was also pointed out that the temperature range for remediation could vary from 25 °C to 40 °C 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 Adiloglu [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 °C 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 HNO3 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 subtilis*) were inoculated in Muller-Hinton broth and allowed to grow for 24 hours at 37 °C. 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, 100% Ni, 50.3% Pb and 59.6% Zn 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       | $37.8 \times 10^7$ | ± $1.4 \times 10^7$ | count | $23.4 \times 10^7$ | ± $1.4 \times 10^7$ | 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/cm³| 1.45       | ± 0.2    | g/cm³|
| 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 × 10⁷   | ± 1.9 × 10⁷ | count | 55.6 × 10⁷ | ± 1.1 × 10⁷ | count |
| Microbial resp. rate  | 0.658      | ± 0.010  | mg/hour | 0.568      | ± 0.002  | mg/hour |
| Microbial biomass 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 Mean Concentration (ppm) | C-PAS Mean Concentration (ppm) | S-SJD Mean Concentration (ppm) | S-BMF Mean Concentration (ppm) |
|------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Percentage       | 0.076A ± 0.005                  | 7.620A ± 0.020                | 129.73A ± 7.96                 | 0.721A ± 0.088                 |
| remediation      | achieved (%)                   |                                 |                                | Before                         |
| remedi agosto    | 5.3                            | 7.597A ± 0.132                | 126.34A ± 1.71                 | 0.517B ± 0.023                 |
| 21.1             | 0.060A ± 0.015                  | 3.082C ± 0.168                | 65.42G ± 1.93                  | 0.468B ± 0.128                 |
| 13.2             | 0.066A ± 0.003                  | 5.980B ± 0.112                | 82.00F ± 0.80                  | 0.580AB ± 0.047                |
| 15.8             | 0.064A ± 0.003                  | 5.403B ± 1.250                | 90.74E ± 0.73                  | 0.470B ± 0.019                 |
| 7.9              | 0.070A ± 0.000                  | 6.012B ± 0.148                | 98.22DE ± 1.20                 | 0.544B ± 0.040                 |
| 15.8             | 0.064A ± 0.005                  | 4.053C ± 0.349                | 102.98CD ± 1.67                | 0.253C ± 0.110                 |
| 7.9              | 0.070A ± 0.000                  | 6.230B ± 0.152                | 110.82BC ± 0.12                | 0.499B ± 0.094                 |
| 17.1             | 0.063A ± 0.007                  | 4.116C ± 0.036                | 105.72BCD ± 1.93               | 0.433B ± 0.031                 |
| 21.1             | 0.060A ± 0.006                  | 6.366B ± 0.108                | 111.21B ± 1.10                 | 0.589AB ± 0.054                |

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

### Table 6. Bioremediation of copper.

|                  | S-BMF Mean Concentration (ppm) | S-SJD Mean Concentration (ppm) | C-PAS Mean Concentration (ppm) | C-GPS Mean Concentration (ppm) |
|------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Percentage       | 6.281A ± 0.380                  | 7.642A ± 0.345                 | 6.802A ± 0.074                 | 8.074A ± 0.43                  |
| remediation      | achieved (%)                   |                                 |                                | Before                         |
| remedi agosto    | 5.399B ± 0.051                  | 7.150B ± 0.207                 | 6.861A ± 0.112                 | 7.150B ± 0.207                 |
| 1.898CD ± 0.023  | 69.8                           | 3.675D ± 0.058                 | 0.841C ± 0.035                 | 54.5                           |
| 2.340C ± 0.010   | 62.7                           | 4.373C ± 0.023                 | 2.687B ± 0.037                 | 62.8                           |

Continued on next page
| S-BMF          | S-SJD          | C-PAS          | C-GPS          | Cu (ppm) |
|----------------|----------------|----------------|----------------|----------|
| Mean Concentration (ppm) | Mean Concentration (ppm) | Mean Concentration (ppm) | Mean Concentration (ppm) |          |
| 1.911 ± 0.070 | 3.844 ± 0.081 | 0.814 ± 0.062  | 3.843 ± 0.081  | 52.4     |
| 2.387 ± 0.027 | 4.440 ± 0.020 | 2.627 ± 0.041  | 2.700 ± 0.050  | 66.6     |
| 1.723 ± 0.236 | 3.103 ± 0.040 | 0.840 ± 0.073  | 3.102 ± 0.040  | 61.6     |
| 2.180 ± 0.020 | 4.692 ± 0.021 | 2.558 ± 0.012  | 3.005 ± 0.250  | 62.8     |
| 1.517 ± 0.621 | 3.810 ± 0.081 | 0.820 ± 0.020  | 3.810 ± 0.081  | 52.8     |
| 2.220 ± 0.044 | 4.401 ± 0.079 | 2.660 ± 0.500  | 3.100 ± 0.050  | 61.6     |

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) | Mean Concentration (ppm) | Mean Concentration (ppm) | Mean Concentration (ppm) |          |
| 0.150 ± 0.000 | 24.99 ± 0.91  | 1.686 ± 0.065  | 7.379 ± 0.261  | Before   |
| 0.121 ± 0.245 | 25.32 ± 2.13  | 1.752 ± 0.123  | 7.589 ± 3.250  | Ctrl     |
| -0.021 ± 0.563 | 17.52 ± 3.25 | 1.326 ± 1.247  | 6.171 ± 3.685  | PA       |
| 0.040 ± 0.020 | 23.36 ± 0.01  | 1.562 ± 0.002  | 7.508 ± 0.082  | PA(N)    |
| 0.120 ± 0.122 | 21.77 ± 3.25  | 1.397 ± 0.684  | 6.880 ± 0.055  | PF       |
| 0.050 ± 0.005 | 23.20 ± 0.00  | 1.505 ± 0.500  | 7.265 ± 0.005  | PF(N)    |
| -0.092 ± 2.011 | 23.19 ± 2.13 | 1.610 ± 0.246  | 6.880 ± 1.230  | BS       |
| 0.045 ± 0.015 | 24.05 ± 0.05  | 1.590 ± 0.045  | 7.257 ± 0.843  | BS(N)    |
| -1.101 ± 2.132 | 21.77 ± 2.13 | 1.468 ± 0.000  | 6.880 ± 3.254  | BC       |
| 0.050 ± 0.010 | 24.35 ± 0.30  | 1.598 ± 0.028  | 7.302 ± 0.068  | BC(N)    |

Note: A.B: Tukey’s test at 95% confidence interval; mean ± standard deviation values.
### Table 8. Bioremediation of cadmium.

|          | S-BMF | S-BMF | S-SJD | S-SJD | C-PAS | C-PAS | C-GPS | C-GPS | Cd  |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| S-BMF    | Mean  | Percentage | Mean | Percentage | Mean | Percentage | Mean | Percentage | (ppm) |
| Mean Concentration (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-BMF | S-SJD | S-SJD | C-PAS | C-PAS | C-GPS | C-GPS | Mn  |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| Mean Concentration (ppm) | Mean Concentration (ppm) | Mean Concentration (ppm) | Mean Concentration (ppm) | Mean Concentration (ppm) | (ppm) |
| 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\(^A\) ± 0.079 | 10.2 | 40.92\(^A\) ± 3.56 | 12.7 | 1.700\(^A\) ± 0.045 | 7.3 | 1.329\(^A\) ± 0.094 | 11.6 | Ctrl |
| 1.352\(^A\) ± 0.509 | 27.4 | 18.09\(^A\) ± 2.62 | 61.4 | 1.226\(^A\) ± 0.065 | 33.1 | 0.827\(^A\) ± 0.149 | 45.0 | PA |
| 1.640\(^A\) ± 0.010 | 11.9 | 22.40\(^B,C,D\) ± 0.05 | 52.2 | 1.380\(^B\) ± 0.120 | 24.7 | 0.920\(^B\) ± 0.080 | 38.8 | PA(N) |
| 1.101\(^C\) ± 0.129 | 40.8 | 19.23\(^D\) ± 1.71 | 59.0 | 1.135\(^D\) ± 0.017 | 38.1 | 0.787\(^C\) ± 0.112 | 47.7 | PF |

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|          | S-BMF       | Percentage remediation achieved (%) | S-SJD       | Percentage remediation achieved (%) | C-PAS       | Percentage remediation achieved (%) | C-GPS       | Percentage remediation achieved (%) | Mn (ppm) |
|----------|-------------|-------------------------------------|-------------|-------------------------------------|-------------|-------------------------------------|-------------|-------------------------------------|----------|
| Mean     |             |                                     |             |                                     |             |                                     |             |                                     |          |
| Concentration (ppm) | 1.520±0.020 | 18.3                                | 23.15±0.05  | 50.6                                | 1.245±0.005 | 32.1                                | 0.813±0.012 | 45.9                                | PF(N)    |
|          | 1.107±0.064 | 40.5                                | 23.23±4.94  | 50.4                                | 1.329±0.295 | 27.5                                | 0.873±0.084 | 42.0                                | BS       |
|          | 1.550±0.050 | 16.7                                | 26.44±0.02  | 43.6                                | 1.324±0.034 | 27.8                                | 0.810±0.290 | 46.1                                | BS(N)    |
|          | 1.170±0.129 | 37.1                                | 19.80±2.62  | 57.8                                | 1.101±0.107 | 39.9                                | 0.873±0.183 | 42.0                                | BC       |
|          | 1.720±0.010 | 7.6                                 | 27.20±0.10  | 42.0                                | 1.305±0.015 | 28.8                                | 0.996±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       | Percentage remediation achieved (%) | S-SJD       | Percentage remediation achieved (%) | C-PAS       | Percentage remediation achieved (%) | C-GPS       | Percentage remediation achieved (%) | Ni (ppm) |
|----------|-------------|-------------------------------------|-------------|-------------------------------------|-------------|-------------------------------------|-------------|-------------------------------------|----------|
| Mean     |             |                                     |             |                                     |             |                                     |             |                                     |          |
| Concentration (ppm) | 0.757±0.000 | NA                                  | 0.749±0.013 | NA                                  | 0.720±0.032 | NA                                  | 0.494±0.027 | NA                                  | Before   |
|          | 0.661±0.018 | 12.7                                | 0.651±0.031 | 13.1                                | 0.610±0.064 | 15.3                                | 0.374±0.031 | 24.3                                | Ctrl     |
|          | 0.333±0.018 | 56.0                                | 0.362±0.047 | 51.7                                | 0.240±0.065 | 66.7                                | -0.038±0.117 | 100.0                               | PA       |
|          | 0.412±0.028 | 44.4                                | 0.452±0.010 | 39.7                                | 0.427±0.010 | 40.7                                | 0.210±0.000 | 57.5                                | PA(N)    |
|          | 0.333±0.018 | 56.0                                | 0.363±0.064 | 51.5                                | 0.240±0.047 | 66.7                                | -0.069±0.175 | 100.0                               | PF       |
|          | 0.420±0.120 | 44.5                                | 0.435±0.005 | 41.9                                | 0.445±0.072 | 38.2                                | 0.182±0.002 | 63.2                                | PF(N)    |
|          | 0.374±0.031 | 50.6                                | 0.374±0.031 | 50.1                                | 0.209±0.018 | 71.0                                | 0.034±0.031 | 93.1                                | BS       |
|          | 0.428±0.028 | 43.5                                | 0.442±0.024 | 41.0                                | 0.412±0.031 | 42.8                                | 0.171±0.000 | 65.4                                | BS(N)    |
|          | 0.363±0.035 | 52.0                                | 0.363±0.035 | 51.5                                | 0.209±0.065 | 71.0                                | -0.151±0.000 | 100.0                               | BC       |
|          | 0.430±0.097 | 43.2                                | 0.465±0.025 | 37.9                                | 0.429±0.000 | 40.4                                | 0.232±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 Mean Concentration (ppm) | S-SJD Mean Concentration (ppm) | C-PAS Mean Concentration (ppm) | C-GPS Mean Concentration (ppm) | Pb (ppm) |
|----------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|----------|
|          | Percentage remediation achieved (%) | Percentage remediation achieved (%) | Percentage remediation achieved (%) | Percentage remediation achieved (%) |          |
| 1.210A ± 0.000 | NA                              | 2.94A ± 0.06                     | 1.22A ± 0.02                   | 1.71A ± 0.01                   | Before   |
| 1.175AB ± 0.043| 2.9                             | 2.85A ± 0.11                     | 1.03B ± 0.09                   | 1.22B ± 0.01                   | Ctrl     |
| 0.706F ± 0.043| 41.7                            | 1.99B ± 0.04                     | 0.78D ± 0.04                   | 0.88D ± 0.04                   | PA       |
| 0.940BCDE ± 0.060| 22.3                           | 2.20B ± 0.05                     | 0.96D ± 0.02                   | 1.13C ± 0.03                   | PA(N)    |
| 0.731DE ± 0.043| 39.6                            | 2.11B ± 0.04                     | 0.81D ± 0.04                   | 0.93D ± 0.04                   | PF       |
| 0.930BCDE ± 0.070| 23.1                           | 2.11B ± 0.04                     | 0.95D ± 0.03                   | 1.23B ± 0.01                   | PF(N)    |
| 0.805BCDE ± 0.043| 33.5                           | 1.99B ± 0.19                     | 0.83CD ± 0.07                  | 0.90D ± 0.00                   | BS       |
| 1.001ABC ± 0.174| 17.3                           | 2.18B ± 0.12                     | 0.99D ± 0.01                   | 1.22B ± 0.01                   | BS(N)    |
| 0.805ABCDE ± 0.114| 33.5                           | 1.62C ± 0.37                     | 0.78D ± 0.04                   | 0.85D ± 0.04                   | BC       |
| 0.970ABCD ± 0.130| 19.8                           | 2.34B ± 0.02                     | 0.95D ± 0.01                   | 1.26B ± 0.01                   | 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 Cr⁶⁺, mobile Cr³⁺, 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 Cr⁶⁺ [23]. chrR gene in Pseudomonas putida promoted the reduction of Cr⁶⁺ to Cr³⁺ while yieF gene in E.coli catalyse the reduction of Cr⁶⁺ to Cr³⁺. Kermani et al., [24] pointed out that both living and non-living cells of the strain Pseudomonas aeruginosa could eliminate Cd²⁺ from contaminated solutions. Also, cadmium metal and its oxides were reported to be insoluble in water, some salts are hydrophilic with 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 Cu²⁺ 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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