

Assessing the potential of mechanical aeration in soil phytoremediation

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Abstract—

Increasing effectiveness of remediation is still being explored. Mechanical aeration was instigated along with phytoremediation using *Helianthus annuus* and *Brassica juncea* for a period of 90 days. Among seven land sites impacted by heavy metals selected, two most contaminated soils were selected and remediation experiment was conducted. The outcome under aeration treatment with *H.annuus* showed a reduction in metal concentrations upto 30.7% Zn (See Abbreviations), 71.7% Cu, 68.8% Cr, 80.2% Cd, 58.8% Mn, 54.9% Ni and 70.7% Pb as compared to *B.juncea* which were 40% Zn, 73.2% Cu, 67.3% Cr, 80% Cd, 43.1% Mn, 53.4% Ni and 24.5% Pb within the same time frame. Notably, implementing mechanical aeration enhanced phytoremediation efficiency significantly upto 50.7% compared to phytoremediation solely. Nonetheless, other physiochemical parameters in particular pH, EC, organic matter, total NPK played essential roles in the enhanced-phytoremediation experiment.

Index Terms—*Helianthus annuus*, *Brassica juncea*, heavy metals, time frame, physiochemical.

I. INTRODUCTION

The Earth is confronting colossally extreme impacts due to anthropogenic activities such as industrialization, modern practices and undesirable competition for supremacy. These activities gave rise to emerging pollutants in nature which are connected to different intense and persistent detriments in biotic components and hence deteriorate the environment with serious hazards [1]. In the wide sense, extensive researches have been effected on phytoremediation technology with regards to degradation of organics, however for certain contaminants this is relatively new [2] and strategies for increasing effectiveness and time frame are still being explored. While several biostimulation techniques have been reported by various authors, no experiment was testified so far on using mechanical aeration to enhance remediation rate.

Phytoremediation was stimulated with the addition of compost and vermicompost which lead to an enhanced potential of the plant to move and bioaccumulate the metals [3]. Experiment conducted by Saadia and Azka [4], *Z.mays* plant after 1 crop cycle reduced the metal concentration in the soil as for Pb: 66.36%, Cu: 17.65%, Cd: 44.83%, Ni: 29.33% and Cr: 4.526%. When stimulated with EDTA, the remediation potential of Pb metal only increased to 68.43%. *H.annuus* was found to have better remediation potential for Cd 56.03% and Pb 48.86% compared to maize. After stimulation with EDTA, significant removal efficiencies were noted, i.e. 74% for Pb only. In addition, according to the results of Adiloğlu [5] rhizoremediation using hyperaccumulator plants was said 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 study conducted by Shrestha [6] showed that rhizoremediation can be boosted using compost and was found that this stimulation procedure reduced significantly bioavailable fractions of metals. In a soil pH of 7.12 and under-fertilised soil, Patel [7] found that *B.juncea* L. plant could accumulate 0.623 mg/l of Pb and 0.290 mg/l Cu while *H.annuus* could accumulate 2.369 mg/l of Pb and 4.136 mg/l of Cu respectively. In the experiment, the author also made use of EDTA enhancers and ammonium sulphate to rectify the pH so as to boost rhizoremediation. It was reported that *H.annuus* could then accumulate

7.109 mg/l of Pb and 5.063 mg/l of Cu, enhancing remediation upto 3 times.

Hence, it was of utmost importance to identify the sources of heavy metals, above and beyond quantifying their concentrations and spatial variability in the soils and with aim of remediating the land. The objective was to assess statistically the potential of combining phytoremediation and mechanical aeration for enhancing remediation process.

II. MATERIALS AND METHODS

A. Site selection

The study areas were geographically located across Mauritius with soil samples taken from seven sites which were suspected to be impacted by heavy metals originating from anthropogenic wastes. The coordinates and size of contaminated sites were referred using Google Maps (Table 1). A preliminary site analysis was conducted and identified based on their land uses and activities. The two most polluted soil samples (S-SJD and S-BMF) were retained for further investigation and for enhancing phytoremediation experiment.

B. Sampling and treatment allocation

Soil samples (30 independent samples) were taken using the 'W' method covering maximum of the sites [8]. These were collected up to a depth of 30 cm then mixed thoroughly to ensure uniformity and homogeneity of the land under investigation. A pot experiment having each 5.0 kg of soils were used for the investigation. Three seeds of *Helianthus annuus* and *Brassica juncea* were sown in each pot for phytoremediation. The treatments were summarized in table 2. Parameters assessed in laboratory were done in three replicates.

- Enhanced-phytoremediation was used for the experiment which consisted of aerating the soil mechanically. Aeration involved making "tiny holes" of 1 cm diameter and 20cm depth in the media every week to keep them aerated and reduced compaction. Each treatment and parameters assessed had three replicates with 3 plants in each pot.

- Phytoremediation treatments were carried out for 90 days and the contaminant residues were tested again in the soils and coastal sediments.

Table 1 Polluted sites under study

Sites	Activities	Coordinates	Pollution Area (Ha)
S-M1	Motorway Road	20°11'06.8"S, 57°28'51.7"E	7.4
S-BMF	Agricultural Site (Intensive)	20°11'60.0"S, 57°46'50.4"E	0.68
S-SJD	Petrol station neighbouring	20°13'49.4"S, 57°38'16.4"E	0.27
S-UOM	Agricultural experimental site	20°14'08.2"S, 57°29'26.3"E	0.5
S-LCC	Waste compost industry	20°13'57.6"S, 57°25'50.7"E	7.4
S-MCL	Sanitary Landfill station	20°23'21.1"S, 57°37'50.5"E	8.4
S-AIR	Airport vicinity	20°25'32.7"S, 57°40'17.5"E	16

Table 2 Allocation of treatments for soil remediation

Treatment code	Treatments details allocated
Initial	Initial condition prior to treatment
Control	No plants and no aeration
Trt B	<i>Brassica juncea</i> + mech. aeration
Trt B(N)	<i>Brassica juncea</i> + No mech. aeration
Trt H	<i>Helianthus annuus</i> + mech. aeration
Trt H(N)	<i>Helianthus annuus</i> + No mech. aeration

C. Soil physical parameters analysis

Moisture content (Oven dry method, [9])

100 g of soil/sediment were measured in a pre-weighed envelope. It was then placed in oven at 110 ± 5 °C overnight and the mass was recorded every 24 hours until a constant mass was recorded after being cooled in a desiccator. 3 replicates were done. Measurement was recorded upto to 2 decimal places.

Bulk density (Iron core-ring method, [10])

A core ring of known diameter and height was inserted into the soil/sediment using a hammer till completely immersed. The core ring was insulated on the underside to prevent soil loss. It was then inserted into a pre-weighed envelope, measured and placed into the oven at 110 ± 5 °C overnight and the mass was recorded every 24 hours until a constant mass was recorded after being cooled in a desiccator. 3 replicates were done. Measurement was recorded upto to 2 decimal places.

Soil texture (Stokes' Law and Textural triangle, [11])

20 g of air-dried soil sieved at 2 mm was measured in a cylinder of 100 ml. Deionised water was added to make up the mark and then shaken vigorously for 5 minutes. It was allowed to stand for 48 hours. 3 replicates were done. The different layers after settling could easily be identified and measured after which the percentage of each textures were calculated and determined using the texture-triangle.

D. Soil biological parameters analysis

Bacterial count (Plate Count Method) prior to experiment and after enhanced-phytoremediation.

1 g of soil was put in a 50 ml measuring cylinder and shaken vigorously for 5 minutes. Serial dilutions were then made with factors 10^2 , 10^4 , 10^6 , 10^8 . Under sterile conditions, 0.2 ml of the solution was poured on to nutrient agar and spread. It was then incubated for 24 hours. 3 replicates were done for each and number of colonies were counted and calculated.

E. Soil chemical parameters analysis

pH (Probe method [12])

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.

Electrical conductivity (Probe method [12])

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.

Soil Organic Matter (Colorimetric method)

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 absorption of the solution at 660 nm was measured using a photospectrometer. Accuracy of measurement was of order $\pm 1\%$.

Total Nitrogen (Kjeldahl method [12])

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 $\pm 1\%$. Three replicates were done.

Total Phosphorus ([12])

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 %.

Total Potassium ([12])

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.

Heavy Metals using AAS ([12])

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 125rpm 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 %.

F. Plant parameters analysis

The measured plant parameters included germination rate as per Ranal and Santana [13], plant height measured weekly from the ground to the apex, and the number of true leaves formed on a weekly basis, concentrations of heavy metals in the root, stem and leaves of the plants. The shoot and roots tissues were cut in tiny pieces and oven-dried at 60 °C for 48 h. Using a grinding machine, the root, stem and leaves were ground separately into powder. Placed in crucibles, these were then ashed in a furnace at 550 °C for 6h followed by digestion process. Nitric acid followed by hydrochloric acid were added to the ash and were heated at 70 °C for 30 minutes until a light-coloured solution were observed. After filtration and dilution, the absorbances were read using an AAS spectrometer. Detection limit was of order ± 0.1 %.

G. Statistical analysis

Complete randomized design (CRD) was used at 95% confidence interval to statistically analyse the data. Tukey's test was also performed at 5 % error to evaluate the significance of the difference in the data after experiment as compared to that of prior to experiment. Correlation using Pearson's coefficient tests were also carried out to investigate any

relationship between the parameters assessed linking with phytoremediation efficacy.

Table 3 Soil parameters under remediation treatment

Parameters	S-BMF Site	S-SJD Site	Unit
Moisture content	9.73 ± 0.01	23.37 ± 0.89	%
Bulk density	1.09 ± 0.35	1.11 ± 0.39	g/cm ³
Texture	Sandy	Loamy	-
pH	8.21 ± 0.02	7.75 ± 0.02	-
E.C	360 ± 10	327 ± 1	μS/cm
Organic matter	15.56 ± 0.07	38.61 ± 0.01	ppm
Total nitrogen	0.17 ± 0.01	0.32 ± 0.01	%
Total phosphorus	0.548±0.003	0.129±0.002	ppm
Total potassium	5.3 ± 0.0	44.9 ± 0.3	ppm
Bacterial count (x10 ⁷)	37.8 ± 1.4	23.4 ± 1.4	count

III. RESULTS AND DISCUSSIONS

A. Heavy metals quantification and special variability

The actual findings could be explained due to the different land uses background and the diverse sources of anthropogenic heavy metals probabilities. S-SJD was found to be the most contaminated due to its location and dumping of various materials containing oils, petroleum derivatives which in turn potentially had heavy metals in their compositions. This could be elucidated due to the heavy metals such as Cu, Pb, Cd, Cr and Zn found in the petroleum products and crudes according to Akpoveta and Osakwe [14]. It was pointed out by Yao *et al.*, [15] that this HM also acted as catalyst in the conversion of organic matter to petroleum.

HM were reported to affect growth, morphology and metabolisms through various processes (functional disturbance, protein denaturation and destruction of integrity of cell membranes) [16]. Along with the experiments of Diaz-Ravina and Baath[17] in laboratory and on-field conditions the trends were more or less similar, i.e. soils having high metal concentrations contained lower number of microbes and had lower respiration rates than uncontaminated habitats [18]. In parallel with the actual findings, microbial parameters mostly were negatively correlated with HM concentrations (as bioavailability of HM increases, inhibition increases), which suggested that the metals affected microbial biomass and activities by behaving synergistically or additively with each other [19].

Soil physical properties such as texture, structure, aeration and water status are among the factors affecting root-organism activity and performance. It is also the root exudates that cause the soil to become firm resulting in, movement of oxygen into deeper soil layer, higher root growth, more micro-organisms' activity hence increasing degradation [20]. Soil characteristics and plant-microbe interaction, significantly affect soil nutritional status, the quality and quantity of root exudates and consequently on bioavailability-remediation of heavy metals at the rhizosphere area [20].

B. Remediation of zinc metal in soil

The more consequent decrease in Zn level was under treatment Trt B at S-BMF (from 0.615 ± 0.006 ppm to 0.368 ± 0.074 ppm accounting upto 40% remediation) compared to Trt B(N) which reached only 24% while Trt H reached 30.7% compared to non-aerated treatment amounting to 21%. The decrease in Zn concentration after phytoremediation compared to initial Zn concentration were all statistically significant at 95% confidence intervals (CIs) for S-BMF. However, while comparing the efficacy of the use of mechanical aeration, only Trt B significantly reduced the concentration of zinc metal using Tukey's method at 5% error.

In line with the statement of Hajabbasi [21], the soil type played an essential role in phytoremediation processes. Similar trends were observed for S-SJD, however both *Brassica juncea* and *Helianthus annuus* significantly ($P < 0.05$) reduced the zinc concentration with the use of mechanical aeration. These were ameliorated by 4% and 8.4% respectively. It could be depicted from the perusal of the elemental data that both metallurgical plants successfully remediated Zn from the soil and were ameliorated using the current biostimulation technique. Carpio *et al.*, [22] also performed biostimulation using glucose solution to boost microbial consortium for zinc remediation. The findings showed enhanced remediation accounting for upto 50% metal removal which was analogous to current findings.

C. Remediation of copper in soil

Both plants were reported to have the ability to remediate Cu in literatures. De Bernardi [23] mentioned that *Brassica* spp., mainly *B.juncea*, *B.napus* and *B.Rapa*, were the most effective species responded to the phytoremediation of Cu however, the current experiment showed that *H.annuus* had even superior remediation capacities than *B.juncea* under biostimulation hence making it a promising phytoremediator. Fundamental outcomes suggested that mechanical aeration improved remediation effectiveness for S-BMF by 34.5% under treatment Trt B compared to non-aerated treatment (Trt B(N)) and 29.6% under treatment Trt H while for S-SJD by 12.7% under Trt B and 21.5% under Trt H only. Based on the result of Mahardika *et al.*, [24] the highest elimination percentage of copper reached up to 85.56% with the exposure time for 9 weeks. In line with the present results, phytoremediation of Cu using *Helianthus annuus* was significantly decreased ($P < 0.05$) by upto 71.7% with the exposure time of 15 weeks. Other factors such as pH, bioavailability of the HM, nutrients present etc...might have influenced the phytoremediation process [25].

Even under natural attenuation treatment, a significant decrease ($P < 0.05$) in level of Cu were noted. The decrease might be attributed to the increased number of bacterial count which might have fed on these contaminants as alternate source of energy, hence causing a decrease in the concentration of Cu.

D. Remediation of chromium in soil

It was mentioned by Revathi *et al.*, [26] that higher the concentration of Cr exposure, the more these plants could accumulate the Cr compared to lower concentration. This complex mechanism, involved chelating and isolating metals ions ligands such as phytochelations and metallothionins might had been developed by plants to control and take up HM [27]. In the experiment of Revathi *et al.*, [26], extraction of Cr metals in all samples was upto 50% which was quite similar to the actual findings whereby normal phytoremediation treatments (Trt B(N) and Trt H(N)) could reach upto 41.3% and 47.8% respectively under sandy soil conditions while 8.6% and 16.6% respectively under loamy soil texture within the same time frame.

Using mechanical aeration combined, the remediation efficacy increased by 26.3% and 19.8% for *Brassica juncea* and *Helianthus annuus* correspondingly for S-BMF however these increase were not significantly different when tested at 95% confidence interval ($P > 0.05$).

Similar trends were observed also for S-SJD whereby remediation processes were improved by 4.8% and 8.4% but changes were statistically insignificant ($P > 0.05$) at 5% error. Factors such as nutrients contents play a vital role for enhancing phytoremediation capacities of soils [28] which could probably explain the variation in results for these two soils. In addition, soil physical characteristics, as explain in the sub-sections, alters the remediation capabilities of the soil [29] which was in parallel to current findings. Additionally, experiment conducted by Saadia and Azka [4], showed that *Z.mays* plant after 1 crop cycle reduced the metal concentration in the soil for Cr by 4.526% and stimulation using EDTA showed no significant response. Key findings suggested hereby that the package combination of aeration with phytoremediation using *H.annuus* and *B.juncea* was way off better than the treatment of Saadia and Azka [4].

E. Remediation of cadmium in soil

Cadmium, being more soluble than other metals such as Ni, Zn, Cu etc...was reported to be a more frequent contaminant [30]. Results obtained were in agreement to Kathal *et al.*, [31] which indicated that *B.juncea* could be classified as a successful phytoremediator of Cd. The latter also mentioned that *B.juncea* was able to uptake significant Cd in his experiment accounting for 60% reduction in soil after phytoremediation while the current experiment reached upto 66.7% under treatment Trt H(N) and 60% under treatment Trt B(N) in agreement. The findings were further supported by Khalid *et al.*, [32], who reported *H.annuus* showing high tolerance to heavy metals and its use as phytoremediator. Abdullah *et al.*, [33] mentioned that Cd concentrations decreased by 58%-72% when using *Helianthus annuus* without any stimulation in agreement to current results. Fasih *et al.*, [34] explained that the remediation might be attributed to the uptake, translocation and metabolism mechanisms of *Helianthus annuus* and the interaction with the soil pollutants. Key findings clearly portrayed that mechanical aeration in combination with phytoremediation using both metallurgical plants increased the efficiency of remediation capacity by 13.5% under Trt H and 20.2% under Trt B.

F. Remediation of manganese in soil

Limited studies had been focused on remediation of Mn. It was mentioned that *Brassica juncea* could be classified as a hyperaccumulator plant [23] and could remove upto 24% Mn in 60 months [35]. Using similar plants, Mn was remediated upto 27.7% in 90 days experiment for sandy soil and using *H.annuus*, remediation could reach upto 40% for loamy soil within the same time frame. Enhanced phytoremediation procedures proved to be more efficient to remediate Mn with improved removal efficiency for S-BMF by 6% for Trt B and 16.9% for Trt H while for S-SJD by 25.5% for Trt B and 19.7% for Trt H respectively which were all significantly different at 5% error using Tukey's method of comparison. Several factors might also have influenced the phytoremediation process. These include: nutrients contents of soils [28], solubility of the metal form [30], uptake, translocation and metabolism mechanisms of the plants and interactions with the contaminants [34], the soil type [21] amongst others. Key findings from the experiment exhibited *H.annuus* to be more efficient for degradation and removal of Mn in soils under the mentioned package treatment.

G. Remediation of nickel in soil

In the experiment of Kathal *et al.*, [31] it showed that Ni uptake by *B.juncea* from contaminated soil proved that it was a good hyperaccumulator and that *B.juncea* revealed to have extracted Ni and reduced its concentration in soil by 60.13% which was quite in line with the current findings. For both S-BMF and S-SJD, *B.juncea* could extract Ni 33.9% and 34.1% under non-aerated conditions while it improved significantly by 19.6% and 15.6% using mechanical aeration respectively. The uptake, translocation and metabolism mechanisms of

the plants and interactions with the contaminants [34] might be one of the factors affecting remediation of nickel in soil. Also, the pH within the rhizosphere and other soil chemical parameters could be another factors affecting the uptake of metal ions [36] and hence affecting phytoremediation process.

Treatment Trt H and Trt H(N) significantly reduced Ni concentration after the experiment ($P < 0.05$) for both soils. The maximum success rates were 54.8% and 45.5% and improved remediation efficiencies were of 18% and 7.6% under aerated conditions. Mukhtar *et al.*, [37] mentioned that *Helianthus annuus* could have accumulated a total of 12 ppm Ni and was categorized as a good phytoremediator of Ni. It was mentioned by Tangahu *et al.*, [38] that the metal movement was affected by its chemical extractions forms and the capacity to combine with organic and inorganics in the soil. The present result also showed that enhanced phytoremediation was a good and effective way to remediate Ni metal from the soil. Fundamental findings that both plants possessed the ability to remediate soils contaminated with Ni which was further supported by the authors mentioned above.

H. Remediation of lead in soil

According to Cui *et al.*, [39], the authors reported that to remediate Pb in highly contaminated soils, it would take 100 years which was impractical; hence the phytoremediation process was enhanced using biostimulation (aeration). Lead was reported to have very limited solubility in soils and its properties such as complexation with SOM, sorption on oxides, clay and minerals, precipitates of phosphates, hydroxides and carbonates made it difficult for plant uptake [40] and breakdown, which might be an explanation to the results of natural attenuation (no significant change in Pb concentration from prior to experiment and after). In agreement with the mentioned reasons, the current findings showed that generally the percentage of remediation of lead was lower compared to the other metals assessed. Nonetheless, the most significant decrease was under Trt H which accounted upto 70% decrease at S-SJD. Saadia and Azka [4] mentioned that after 1 crop cycle, *H.annuus* reduced Pb concentration to 66.36%, and when stimulated with EDTA, the remediation potential of Pb metal only increased to 68.43% using *Z.mays*. Moreover, *H.annuus* was found to have remediation potential for Pb 48.86%. Based on the perusal of data analysis, it could be inferred that the use of *H.annuus* in combination with the current biostimulation strategy was found to be more efficiency in remediating Pb in soil. The phytoextraction coefficient for *Brassica juncea* was reported to be 1.7 and it was found that a lead concentration of 500 mg/L was not phytotoxic to this *Brassica* species [41]. It was also indicated that *Brassica juncea* was capable of removing 1,550 kg of lead per acre [42] which also made *B.juncea* a good plant for phytoremediation purpose which was in agreement with the actual findings. *B.juncea* had the potential as well to remove Pb but not as efficiently as *H.annuus*.

IV. CONCLUSION

Above and beyond quantifying the concentration of heavy metals and spatial variability in sites resulting from anthropogenic activities, the statistics indicated that current method is promising. This is the first report on the use of mechanical aeration in combination with phytoremediation using metallurgical plants. The study reveals the potential and the efficiency to clean up heavy metals in the contaminated soils though several physical, chemical and biological factors that might influence the process from optimization.

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ABBREVIATIONS

AAS: Atomic Absorption Spectrometer
Cd: Cadmium
Cr: Chromium
CRD: Completely Randomised Design
Cu: Copper
EC: Electrical conductivity
EDTA: Ethylenediaminetetraacetic acid
HCl: Hydrochloric acid
HM: Heavy metals
HNO₃: Nitric acid
K: Potassium
Mn: Manganese
N: Nitrogen
Ni: Nickel
P: Phosphorus
Pb: Lead
SOM: Soil organic matter
Zn: Zinc