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Biomineralization of Iron ore Tailing soil with Iron-oxidizing bacteria and *Sesbania sesban* plant

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ABSTRACT
Deterioration of soil quality induced by soil salinization and heavy metal contamination in combination is an ever-growing global problem due to climate change and the rapid development of irrigated agriculture, posing a threat to both environmental and agricultural sustainability in both developing and non-developing countries. The current study aims that the Sesbania sesban was able to tolerate and grow in the co-contaminated soil, especially bio augmented with iron-oxidizing bacteria. Flowering pots and soil were artificially contaminated with iron ore 50g were mixed in 1.5kg of soil. The soil sample was inoculated with an enhanced culture prepared in 3 pots for plant growth. The analysis took place on days 0, 15, 30, 45, and day 60, respectively. The yielded results were shows that the texture of soil was 40% sand, 20% clay, and 40% silt. The initial concentration of nitrogen was 0.76, and yielded concentration was 1.74, phosphorus before treatment was 0.034, and after treatment, it becomes 0.56. The highest microbial count and the microbial load were at day 15 (7.1x109) in bioaugmented soil. The highest iron dissolution percentage was found at day 60 (83.3%) in bioaugmented soil. The current study aims that treatment with bioaugmentation has more impact than treatment with plants. To recognize the responsible bacteria species that dissolve the iron is still not fully understood.
Keywords: Bioaugmentation; mineralization; tailing soil; irrigation

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INTRODUCTION

Due to environmental pollution, elevated mortality rates are reported in humans [1], compared to advanced countries, developing countries have a high level of pollution. Due to the waste disposal sectors, the metropolitan region has an elevated level of pollution relative to the rural areas. The farmers used polluted water in irrigation in developing countries [2], where 70-80% of individuals depend on agriculture for their livelihood. The mining industries also play a major role in polluting our natural environmental equilibrium by grinding large particles into the small granules, some of which have escaped our environment and thus disturbing our food chain by consuming effective plants and agricultural products [3]. Large-scale pollution and pollution lead to industrialization and natural resource extraction. Heavy metals and noxious chemicals contaminate the soil, underground water, and air that are the primary globe issue. New techniques can be used to protect these assets, i.e., air, soil, due to their potential for entering the food chain [4]. Different techniques (microbial remediation, bioventing, biosparging, In-Situ, bioaugmentation, biopiling, bioremediation, ex-situ bioremediation, bioreactors, have been developed to regulate the pollutants of the various sectors to reduce the risk to the population.
The wealthy element in the earth is iron (5%) in the lithosphere, which ranks next (after aluminum) among all components [5]. They occur in a wide variety of mineral stages, including sulfides, carbonates,

silicates,and oxides [6], which has iron (by weight) of 28%. Moreover, iron oxides are most important such as cobalt and nickel [7] and act as the main element for all types of life, except for Lactobacillusspp. Mostly iron is needed in less amount because it is micronutrient, but in some rare cases such as magnetotactic bacteria, the iron amount in cells is up to 11.5 fold more significant than in any other typicalmicroorganism[8]. Earlier, it was believed that life on earth is oxygen-free and that iron plays a vital role. It is still expected that the earliest type of all living cells could be descended from iron-Sulphur metalloclusters, which could have created a closed section needed to expand various metabolic procedures. Thus, iron plays a leading role in cellular skeleton life derivation [9]. Natural iron ores are reported to have low mechanical power. Therefore, iron ore lumps should be more prone to break-up and dust formation in the reduction furnace during the phase of processing and descent.

Consequently, the void age of the blended bed and the penetration of the reduction of gas through the bed is significantly reduced. Also, thermal decrepitation, disintegration,and swelling occur at temperatures between 300-1000°C, which also limits the immediate use of natural iron ore lumps in reduction procedures [10]. Around 1000 million tons of iron ore have been discovered in Pakistan. These reserves have been found in Kalabagh (350 million tons), Dilband (Kalat division), Nizampur (Peshawar division) 10 million, Pezu, Galdanian (Abbottabad), Langrial (Abbottabad) Pachin Koh Nokkundi, Chilgazi, Langrial town (Tehsil Haripur) 30 km south of Abbottabad has 28 million tons of iron, while Hazara division has more than 100 m of iron[11]. Biomineralization of iron ore tailing soil was the main objective of this research, with the use of magnetite tailing soil with iron-oxidizing bacterial culture. Comparison of soil mineralization of natural and bioaugmented magnetite and Phytoremediation by *Sesbania sesban* of magnetite contaminated soil.

MATERIAL AND METHODS

The soil was artificially contaminated with iron ore, and the pot experiment was conducted. 1.5 kg of soil and 50 g of iron ore were gently mixed (maintained 40-50% moisture content) and transferred into each pot, One-pot was used as a control without treatment while,and1 pot was used as a blank pot for sterilized soil. In 3 pots soil sample was inoculated with microbial culture prepared iron-oxidizing,and 3 pots were used to grow in each plant and analysis was conducted on days 0, 15, 30, 45 and 60 respectively.

Preparation of iron-oxidizing consortium

The iron-oxidizing consortium was prepared in the mineral salt medium (MSM) that served as an essential growth medium for iron-oxidizing bacteria. [12].The mineral salt medium was prepared and autoclaved at 121^0C for 20 minutes. 20gm of soil samples were introduced in triplicate in 250ml of the flask and kept at 30^0C for 3 days in the shaking incubator. 100ml of ancient MSM media was added in 250ml of fresh MSM media after 3 days and incubated for 48 hours and then checked the count of bacteria. FeCl3 media has been introduced as a substratum in MSM media 0.5gm. The culture was then centrifuged, and pellet moved to new 250 mL MSM media and used in soil samples for inoculation [13].

Plant cultivation and analysis

In the laboratory experiment, *Sesbania sesban* was chosen for phytoremediation. Firstly, the seeds were collected and left to remove all microorganisms for 30 minutes in 10% hydrogen peroxide ($H_2O_{2)}$. Seeds have been held overnight in a saturated $CaSO_4$ solution. Sowing seeds and analyses were done at 0,15,30,45 and 60 days.

Physicochemical properties of oil

Analysis of soil texture

One-pot was picked up with a cover and semi-filled with soil where the soil was soaked to make the soil more stable. The soil level was labeled, adding water to the pot top and vibrating until the soil was blended with water [14]. Mixing with a plunger for 1 minute, reading the hydrometer for percentage sand after 40 seconds and then for percentage clay after 2 hours. Reading has been fixed by subtracting from the blank, and the formula has calculated the proportion of sand, silt, and clay as follows:

- Gravimetric soil water content= Air dry soil-oven dry soil/oven-dry soil
- Percentage of sand= Original concentration-corrected 40 second readingx100/original concentration
- Percentage of clay = Corrected 2 hours readingx100/original concentration
- Percentage of silt = 100 – (sand + clay)

Soil texture was estimated by using the table below			
Soil classification	**Clay Soil**	**Loam soil**	**Sandy soil**
Percent clay	40-100%	7-27%	1-10%
Percent silt	0-40%	28-50%	1-15%
Percent sand	0-45%	23-52%	85-100%

Sterilization of soil for control

One sample of the soil section was autoclaved in triplicate (three after a 24-hour interval of sterilization at 100^0C for one hour) and soil moisture was controlled with sterilized distillation water if necessary. According to AFNOR French typical method NFX31-103(1988): soil pH was calculated in water. 10 g (dry weight) of the soil was 2 mm sieved, 25ml of water placed and 1 hr stunned in the dark. After 2hr sedimentation, the pH was calculated in 1:2.5 combination of soil, water (0.01 M $CaCl_2$ solution) by a pH electrode. Soil sample temperature was evaluated by a potable thermometer before and after treatment [15].

Analysis of water holding capacity

Empty pot (Wp) was weighted, then filled with soil and weighed (WPS) again. Subtracting WP from WPS calculated the total soil weight. Then the pot was placed in a container filled with water, andtheabsorbent paper was put on the surface and left for 4-8 hours until absorbent paper showed humidity then it was again weight (WSW). The proportion of WHC was calculated as indicated by the formula as follows:

WHC =WSW-WS

Analysis of the moisture content of the soil

Weighed 10-20gm of soil and dried for 8 hours at 150°C and weighed again. Two weights discrepancy indicates humidity content.

Moisture (%)= loss in mass of aeration(g)x100/original sample mass (g)

Analysis of total carbon in the soil

Organic matter in soil was analyzed using Walkley-Black modified method, 1gm of soil was collected in a flask,and 10 ml of $K_2Cr_2O_7$ (1mol L^{-1}) was added and thoroughly homogenized. 20 ml of concentrated sulphuric acid was added and agitated by rotation for one minute (the reaction temperature was approximately 120°C) for better homogenization and was put on the insulating plate. It was held for oxidation for 30 minutes then added 3-4 drops of indicator along with 200ml of distillation water. For the same blank soil-free procedure.0.5 MFe $(NH_4)_2$ $(SO_4)_2$ solution (Mohr salt) was packed in a desk up to O mark, the flask was put on a magnetic stirrer and titration continued until the colour of the solution changed from orange to green and brownish-red. The following formulas were used to calculate organic Carbon and organic matter:

Organic C (gm kg-1) = c(v-v) x100-3x3x1.33/weight of soilx1000

Organic matter (gm kg-1) =Organic C x 1.724

Where

C = Concentration of Fe (NH4)2 (SO4)2 =0.5 % sol

V = Volume of Fe (NH4)2 (SO4)2 for the samples

Vo= Volume of Fe (NH4)2 (SO4)2 for control

C: N: P ratio was maintained to 100: 10: 1 after preliminary soil analysisandNH_4NO_3 and K2HPO4 were mixed in the soil sample due to amendments.

Analysis of total phosphorus in soil

Preparation for sampling and control: 1 gm of 0.25 mm sieve soil was drawn in a 100 ml volumetric flask, and a few drops of distillation water were added, then 8 ml of concentrated H_2SO_4 and 10 drops of perchloric acid were added. The sample was heated at 320°C for 2 hours until its colour changed to grey-white. Heating persisted for 20 minutes longer, and the sample was cooled down, and up to 50 ml was produced with distillation water quantity and retained for settlement overnight. Control was used soil-free. Although, dinitrophenol indicator was added to all pipes including standard and sample tubes, and 50%NaOH was added for yellowish colour. 5ml of anti-reactive molybdenum antimony was added into 50ml of distilled water, the solution was kept for 30 minutes. The absorbance was noted by using UV-sample spectroscopy at 700 nm. A standard curve was obtained finally, andtheconcentration of P (mg L^{-1}) of samples wasderived from this curve. The sample has been diluted to the spectroscope measuring boundaries (absorbance below 1).

Analysis of total nitrogen in the soil

This technique includes the transformation of nitrogen from biological materials to $(NH_4)_2SO_4$ by digestion with H_2SO_4 followed by NH_3 distillation in an alkaline medium, which is suitable for soil, plant products, and active nitrogen fixers. The digestion mixture consists of 100g K_2S0_4, 10g $CuSO_4.5H_20$ and 1 g Se which was mixed with 1gm soil sample together with 8ml H_2SO_4 and was softly heated on the hot

plate for 1.5 hr or until all the water was removed and charring finished, and all nitrogen could be transformed into $(NH_4)_2S0_4$. The flask might be cool.

Hoskins steam distillation device was commonly used for distillation. Digested material (10-25 ml, depending on the material N_2 content) was drawn in the distillation flask, and 10-15 ml of 40% NaOH was added by alkali inlet to the sample. The flask containing 20 ml of 2% boric acid and indicator solution (0.099 g of green bromocresol and 0.066 g of methyl blue in 100 ml of water) was held under the distillation device condenser. Heating was strictly controlled to avoid sulfuric acid from sucking back. Titrated with conventional 0.1 N HCl, 150 ml distillate was collected. The colour was changed from green to purple. The quantity of HCl was recorded, and the completeamount of Nitrogen was calculated using the formula below:

Formula : $$N(g/Kg) = \frac{(V - V_0) \times C_{HCl} \times 14}{m}$$

Here in the formula;

V- the volume of standard hydrochloric acid when titrated with sample (ml)

V0- the volume of standard hydrochloric acid when titrated with control (ml),

CHCl -Concentration of hydrochloric acid (mol/L),

m- Weight of soil sample (g).

Total viable count by counting chamber

The counting chamber of Petroff-Hausser was washed with 70% alcohol and allowed to dry for air. The culture was well blended, and the counting chamber with Pasteur pipette was implemented with a single drop. Using high energy, oil immersion target, the counting chamber was examined. Five different fields were observed, and theaverage value was calculated[15]. The overnight culture concentration of the cells was estimated using the formula:

Total cellsx2.5x10^5xdilution factor=cells/ml by [16].

Analysis of Iron (Fe_2O_3) concentration in soil

0.3gm of soil samples were collected and put on a hotplate. For each sample, 20ml of 12M (HCl) concentration was dissolved. The sample was gently heated.5ml of 6MHNO_3 was added to the sample and boiled for a few minutes to ensure that all iron was oxidized to Fe_3. The sample was diluted to 200ml with distilled water and added 2M ammonia with continuous stirring. Boiled for 5 minutes, then left to settle. There was a solution poured into the funnel. Precipitates were cleaned and transported to the beaker with NH_4NO_3 and added a few drops of 0.1 $AgNO_3$. It was repeated until the colour of the white became faint. The solution was carried through the filter paper, and the filter paper was folded into a crucible and put on a spirit lamp and burned for 15 minutes until dark red steel grey colour emerged. Weight-based measurement of iron concentration[17].

RESULTS

An analysis of iron ore biomineralization was performed for 2 months. Before and after treatment, various physicochemical parameters were analyzed. The iron-oxidizing bacterial load in soil has been calculated as follows for bioaugmentation:

Total number of bacteria present in soil = 6.0x10^8

Required inoculum size = 1x10^9

Bacterial number in culture= 3.6x10^9CFU/ml

The total amount required =Required-Present/culture

$$= \frac{10X10^8 - 6.0X10^8}{3.6X10^9}$$

$$= \frac{4X10^9}{3.6X10^9}$$

=1.11ml

For 1.5kg (1500gm) of soil =1.11ml/1500gm

The complete soil bacteria count was 6.0x10^8. The bacteria required inoculum size was 1x10^9. In culture, the number of bacteria was 3.6x10^9CFU/ml. The total amount required is equal to the presence required minus divided by the culture. Results showed that 1.11ml/1500gm was needed for 1.5 kg of soil.

Gravimetric soil water content:

Soil moisture content was calculated as follow:

$$= \frac{\text{Air dry soil} - \text{oven dry soil}}{\text{oven} - \text{dry soil(g)}}$$

$$= \frac{6-7}{7}$$

= 0.142

Percentage of sand, silt and clay in the soil sample

Air-dry soil weight added to cylinder = 10gms

Air dry Gravimetric water content = 0.142gm

Calculated oven-dry soil weight = 10gm

OD soil weight $= \frac{\text{AD soil weight}}{(1+\text{GWC})} = 10$ gm

Original concentration of soil in-cylinder = 10gm/L

$= \frac{10 \text{ gm OD soil}}{100\text{ml}}$

Correct hydrometer readings from soil cylinder

Table 1. Gravimetric water content			
Reading time	**B**	**Soil**	**Soil – BL g/L**
40 sec	1	7	6
2 hours	1	3	2

Analysis of soil texture % sand, silt and clay were calculated as follow:

$$\% \text{ Sand} = \frac{\text{original concentration} - \text{corrected 40 sec reading x 100}}{\text{original concentration}}$$

$$= \frac{10 - 6X\ 100\%}{10} = 40\%$$

$$\% \text{ Clay} = \frac{\text{corrected 2 hr reading X100\%}}{\text{orignal concentration}}$$

$= \frac{2}{10}$x 100% = 20%

% Silt = 100 – (%sand + % clay)

=100 – (40%+20%)

= 100 – 60%

= 40%

The regarding data showed that 40% were sand and silt while 20% was clay.

Therefore, no texture amendments were required.

Soil physicochemical analysis before and after treatment

Different physicochemical parameters in soil were analyzed that include PH, WHC, temperature, concentration of nitrogen, phosphorus and carbon.

Table 2. Soil Physico-chemical analysis before and after treatment			
S.NO	**Parameter**	**Initial values**	**Final values**
1	pH	7.3	7
2	WHC	2.6	2.6
3	Temperature	19	20
4	TN(g kg^{-1})	0.76	1.74
5	TP (g kg^{-1})	0.034	0.56
6	IML (CFU/ml)	$6.0x10^8$	$4.5x10^9$
7	TC (g kg^{-1})	13.5	14.1

WHC: (Water holding capacity), TN:(Total nitrogen (g kg^{-1}), TP:(Total phosphorus (g kg^{-1}), IML: (Initial microbial load (CFU/ml)), TC: (Total carbon (g kg^{-1})).

Different soil Physico-chemical parameters were evaluated and yielded pH before therapy was 7.3, while after treatment, even because iron-oxidizing bacteria could survive and demonstrate better outcomes in neutral pH. The rest of the parameters, including WHC 10% before and after treatment, initial nitrogen concentration was 0.76 and increased to 1.74. Therefore, the content of nitrogen and phosphorus for nitrogen NH_4NO_3 and phosphorus $(NH_4PO_4)_2$ was initially low. The temperature was 19 in the early stages and 20 in the final stages. The initial load was $6.0x10^8$, up to $4.5x10^9$. Initial concentrations of carbon were 13.5 and yielded 14.1, respectively.

Calculations:

Weight of pot = 1.5kg

Weight of pot + Weight of soil = 1.5 + 1.5 3kg

Water holding capacity =Final weight -initial weight

=3,100gm-3000gm

$= \frac{100gm/L}{1000}$

WHC= 0.1gm/ml

WHC= 0.1X100

WHC= 10%

WP is weight of empty pot WS is the weight of soil.

Table 3.Nutrient addition (C/N/P ratio)				
S.NO	**The ratio used in soil**	**C**	**N**	**P**
1	High value ratio	100	25	10
2	Low value ratio	1.25	1.25	0.125
3	Or mg/kg	12500	1250	125
4	In sample soil (g/kg)	10700	519	29

For nitrogen 1250-519=731
For 1.5 kg =731/1.5kg=0.751g/1.5kg
Amount of ammonium nitrate (NH_4NO_3) was added as follows:
Molecular weight = 80.04
0.751x = 28:80
X= 0.537g/1.5kg
For phosphorus
K_2PO_4 x = 0.8g/1.5kg

Table 4. Average of iron-oxidizing microbial count in microbial bioremediation						
S.NO	**Days**	**B1**	**B2**	**B3**	**Average**	**STDEV**
1	0	3.6×10^9	3.5×10^9	3.6×10^9	3.6×10^9	±0.05
2	15	7×10^9	6.9×10^9	7.1×10^9	7×10^9	±0.081
3	30	5.2×10^9	5×10^9	5.2×10^9	5.133×10^9	±0.094
4	45	3.9×10^9	3.7×10^9	3.6×10^9	3.733×10^9	±0.124
5	60	4.5×10^9	4.2×10^9	4×10^9	4.233×10^9	±0.251

B: Bioaugmented soil

The maximum microbial count was reported on day 15, which was 7.1x109, while the minimum count was founded on day 45 (3.6x109 cells/ml) in (table 4).

Table 5. Average microbial load in *Sesbania sesban* bioremediation experiment						
S.NO	**Days**	**P1**	**P2**	**P3**	**Average**	**STDEV**
1	0	1.5×10^9	1.4×10^9	1.5×10^9	1.46×10^9	±0.057
2	15	1.7×10^9	1.7×10^9	1.7×10^9	1.7×10^9	±0
3	30	1.6×10^9	1.5×10^9	1.4×10^9	1.5×10^9	±0.1
4	45	1.2×10^9	1.2×10^9	1.2×10^9	1.2×10^9	±0.629
5	60	1.4×10^9	1.3×10^9	1.2×10^9	1.3×10^9	±0.1

P: Planted soil

The overall average of the microbial load was observed to be high in bioremediation on day 15 ($1.7x10^9$) compared to phytoremediation.

Table 6. Average microbial count in bacterial and *Sesbania sesban* bioremediation experiment						
S.NO	**Treatment**	**0 day**	**15day**	**30day**	**45day**	**60day**
1	Bioaugmented	3.6×10^9	7×10^9	5.133×10^9	3.733×10^9	4.23×10^9
2	Planted treatment	1.4667×10^9	1.7×10^9	1.5×10^9	1.2×10^9	1.3×10^9
3	Sterilize	$1.8x10^8$	$2.0x10^8$	$4.0x10^8$	$5.0x10^8$	$7.0x10^8$
4	Control	$7.5x10^8$	$8.5x10^8$	$9.0x10^8$	$8.5x10^8$	$8.3x10^8$

The highest average of microbes was $7x10^9$ in bioaugmented soil on day 15, and the lowest count was $3.7x10^9$, while the highest average was 1.7x109 cells/ml in-plant treatment, and the lowest count was 1.2x109 cells/ml.

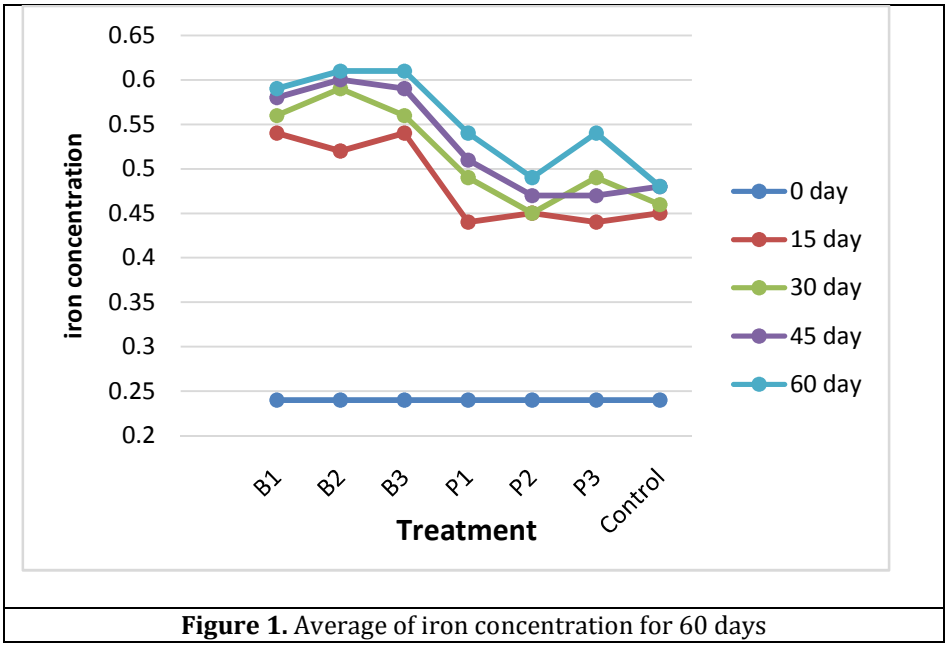

Figure 1. Average of iron concentration for 60 days

The highest iron concentration of 0.833, indicating peak mineralization was discovered in bioaugmented soil on day 60, whereas the lowest was discovered at day 0 (0.24).

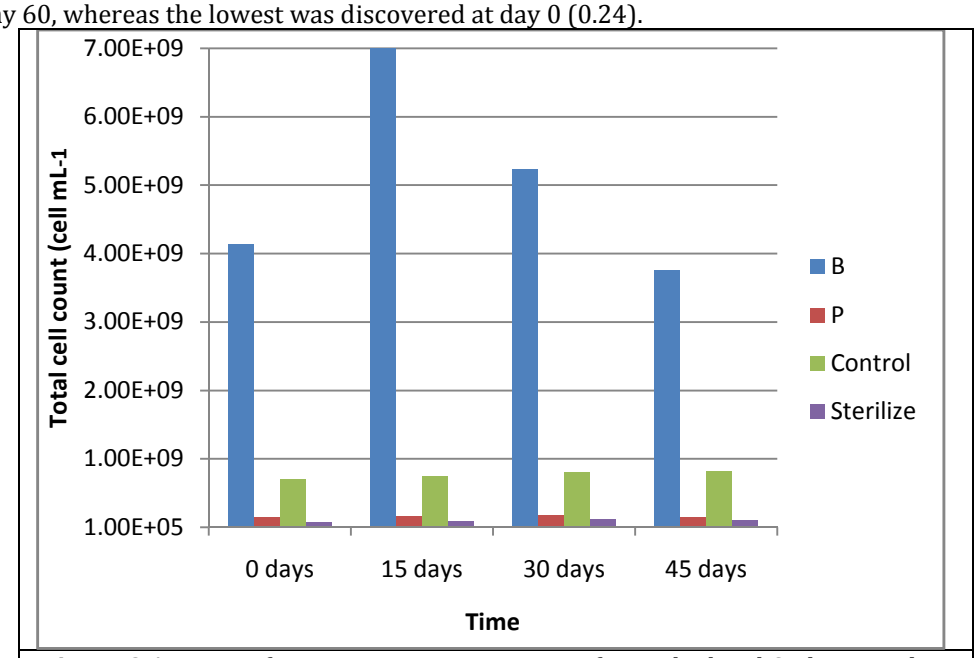

Figure 2. Average of iron concentration in case of microbial and *Sesbania sesban* bioremediation experiment

The figure demonstrates the distinct soil iron concentration, for all microbial bioremediation, phytoremediation, and control conditions on day 0 iron initially present in the soil (24%). The iron dissolution rate at day60 was (83.30%) in bioaugmented soil.

DISCUSSION

During the study, different Physico-chemical parameters were investigated for the microbial population (pH, nitrogen, phosphorus, and carbon analysis, WHC, and temperature). It demonstrated that the microbial population is affected by the soil environment [18]. These variables can improve soil contaminant development and degradation. If these variables are at the optimum stage, the microbial activity will be more significant, and the rate of decomposition will be higher as well [19]. Soil texture assessment in the current research showed 40% sand and silt, while 20% clay respectively. It was observed that clay soil had a higher physicochemical contaminant attenuation capacity than coarse sands[20]. Severalstudies indicated that there is an approximate linear rise in the number of iron-oxidizing bacteria with height.The content of soil moisture could be one of the significant environmental

factors that regulate the bacterial community's circulation. With height, soil moisture content decreases, the availability, and depth of oxygen dispersed into the soil is likely to increase, which should help in the iron-oxidation of bacterial[21]. However, as soil oxygenation increases, chemical iron oxidation can consume more ferrous iron. The decrease in iron is lower in the content of soil moisture and slows down the iron response. Researched microbes being able to move a lot in coarse-textured soil and then fine-textured soil. Before soil pH treatment was identified, 7.3 and after treatment, it changed to 7, which is neutral, showing consistency with Emerson group studies [22] [23]. They investigated the latest development of neutrophilic bacteria's novel iron-oxidizing strains, distantly linked to classical iron oxidizers and morphologically distinct. Soil pH can influence contaminant solubility, mobility, and ionized forms. In the event of neutrophilic iron oxidizers such as Leptothrix and Gallionella, the polysaccharides around the cell protect it from iron damage [24]. It is reported that the neutral pH of microbes can oxidize iron in the environment. On G. ferruginea stalks, highly reactive (and poisonous) oxygen species are produced [25]. The localized iron oxidation on the stalk protecting the cell against damage. The research demonstrates that the original nitrogen level was 0.76, which rose to 1.74, phosphorus concentration before therapy was 0.034, and after treatment, it becomes 0.56. Initially, a small content of nitrogen and phosphorus was added for nitrogen (NH4NO3) and phosphorus (NH4PO4)2 [26].Previous studiedconducted the need for additional nutrients if the ratio of nitrogen and carbon, and phosphorus are below the required, extra nutrients should be supplied [27].Bacteria initial load was 6.0x108CFU, finally reaching 7.1x109 CFU, due to the addition of iron culture and supplement to the soil, which resultant the increase of the microbial count. Results showed that the highest microbial count was at day 15 (7.1x109CFU) followed by day 30, the percentage of iron dissolution was at least 0 day (24%), and the maximum percentage at day 60 (83.3%) in bio-treated soil. The iron dissolution showed consistency with the previous studies of [28-29], their findings showed high efficacy of bacteria to dissolve metals like iron. The results of the soil acquired from *Sesbaniasesban*origins in the current research also showed consistency with the outcomes of [30-31] as iron-oxidizing bacteria in the mentionedplant. The findings also showed consistency with [32]outcomes, which showed this plant assistance in protecting the environment by oxidizing iron ore.

The current research showed 83.3% iron dissolution by iron-oxidizing bacteria that showed consistency with [22] finding, that 50-80% of the iron-oxidizing activity is due to iron-oxidizing bacteria. It has demonstrated that, under favourable conditions, 18-53% of total Fe (II) is oxidized by iron-oxidizing strain[33]. It has been revealed that up to 90% of the iron oxidation in the environment that resembled the present study was represented by a pure culture of a Fe-oxidizing bacterium[34].It was detected that broad range of microbes at neutral pH. Can oxidized iron at optimum temperature 18-19 ºC; Results can be enhanced by changing parameter like temperature up to 45ºC to 50ºC[22]. Pb/Zn mine tailings compared growth output, metal accumulation of Vetiver (Vetiveriazizanioides) and two legume species (*Sesbania rostrate* and *Sesbania sesban*) cultivated on tailings modified with domestic waste and fertilizer[35]. The observation showed that artificial fertilizer enhanced the survival rate and development of V. zizanioides and two species of Sesbania, showing reliability with the current research. Fertilizer has been introduced in the currentstudy to improve *Sesbania sesban* development. An experiments were performedfor a copper mine in Ecuador (Mina San Bartolome) in Peru (Mina Turmalina and Copper Mine in Chile (Mina El Teniente) for the screening of plant species from three distinct mining fields in South America. The highest concentration was observed among the grass species (Poaceae) in the shoots of Paspalum species (> 1000 mg kg−1) and Eriochloaramosa (460 mg kg−1) from the Cu mine in Peru, and in Holcuslanatus and Pennisetumclandestinum (> 200 mg kg−1) from the silver mine in Ecuador[36].Metal uptake patterns study from the pyrite chalcopyrite mining area of Vigonzano (Northern Apennines, Italy). *Silenearmeria* (Caryophyllaceous) plants were collected, Salix species (Salicaceae) and dumping material from *Populusnigra* (Salicaceae) and mine spoil[37].

Consequently, the observation showed that the leaves had the highest concentration. Astudy conductedin Noamundi, Tata-Steel that could survive the tailings in which they grow nine plant species on Fe-tailings. The highest accumulation of Fe was discovered in Oxalis (7442 mg kg-1), while the highest accumulation of Mn and Zn was found in Blumealacera (88 mg kg-1) and Avera Aspera(109 mg kg)[38]. The phyto stability study of materials produced by Calophylluminophylum during mining and iron processing. Plant growth, chlorophyll content,and Calophylluminophylum metal uptake pattern were studied. Results showed that plant survival in all treatments was 100%. In Calophylluminophyllum, metal accumulation was high, suggesting that this plant could be an outstanding implement for phytoremediation. Maximum accumulation in plant tissues of Pb (1662 µgm/gm) and Fe (2313 µ/gm) followed by Cu, Zn, Cr, and Ni was observed.Most heavy metals have been removed up to 30% like Fe, Pb, and Cu and Zn during one

year of observation in all treatments[39]. The research showed that phytoremediation can be helpful in removingcontaminants from mine soil.

CONCLUSION

Some bacteria are helping to clean up our environment from heavy metals such as iron ore in the soil. The dissolution of iron ore induced by iron-oxidizing bacteria is a highly efficient, time-consuming, and eco-friendly process. The addition of microbes to the soil was better than *Sesbania sesban* phytoremediation. The higher microbial concentration in bioaugmented soil at day 15 was 6.9x109 CFU / ml, while the greater microbial count in cultivated soil was less than 1.7x109 on day 15. In conclusion, iron-oxidizing bacteria are responsible for oxidizing metals such as iron.

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CONFLICT OF INTEREST

There is no conflict of interest.

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