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***Research Article** (Open Access)*

A PRELIMINARY APPROACH TOWARDS BIOREMEDIATION OF LEADPOLLUTED SOIL IN MANALI USING *PSEUDOMONAS AERUGINOSA* IMMOBILIZED IRON NANOPARTICLES

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ABSTRACT

The extensive use of chemicals like pesticides, herbicides, insecticides have caused the accumulation of chemicals in the soil and further cause soil pollution. This will ultimately have a tragic effect on human life in the near future. Soil pollution could also be caused due to increasing industrial activities, agricultural runoff, improper waste management and so on. This results in the aggregation of pollutants like contaminated organics and heavy metals in the soil which leads to secondary effects like decrease in land productivity, water scarcity and climate change. As a solution for this problem, this study focuses on exploiting the microbial metabolism of *Pseudomonas sp.,* immobilized on the surface of superparamagnetic iron nanoparticles in order to remove the heavy metal lead from the soil. Iron magnetic beads are synthesized using co-precipitation method and freshly cultured *Pseudomonas Sp.* is immobilized on the surface of the magnetic beads by adsorption accordingly to remove a targeted heavy metal from the soil. The maximum removal efficiency of lead from the soil using the synthesized magnetic nanoparticles was observed in the pH range of 7.6 to 8 and in the temperatures 24℃ to 28℃. The molecular identification of *Pseudomonas Sp.* was carried out and the species was found to be *Pseudomonas aeruginosa*. Lead is one among the useful heavy metals that has been in use since the dawn of civilization. Soil pollution due to accumulation of lead can have an enormous impact on the planet, one good example could be biomagnification of lead in humans that causes neurological disorders. Therefore,it is necessary to rectify the damage caused to the soil so that the after effects can be minimized to a greater extent. The present study will thereby help us to understand the removal efficiency of *Pseudomonas aeruginosa* immobilized iron nanoparticles as a potential bioremediation material in the long run which could be used as a sustainable solution for soil pollution.

Keywords: *Soil Pollution, Iron Nanoparticles, Pseudomonas aeruginosa, Bioremediation, Sustainability, Molecular Identification, Bacteria Immobilization*

INTRODUCTION

Soil is an important component for the survival of human life. Soil plays a major role in Earth's ecosystem. It helps in anchoring the roots of plants and trees, it houses billions of microorganisms which are beneficial to human survival. It displays a broad array of diversity. It is also one of the major carbon sinks which helps in sequestering organic carbon. It is also necessary for food and biomass production. Soil has the ability to combat and mitigate climate change (Rodríguez-Eugenio *et al.*,2018).

Soil is healthy is important for human wellbeing. But due to increasing urbanization and industrialization, we have been causing enormous amounts of pollution to the soil. Soil pollution causes a negative impact on its physical, chemical and biological aspects of soil and lowers its productivity(Mishra *et al.*, 2016). Soil pollution can lead to many secondary effects like decrease in land productivity, food security, water scarcity and climate change. There is a high probability of these events cascading over each other and ultimately have

a tragic effect on the ecosystem.

Soil pollution greatly impacts the entire planet and also the successive generations to come. Therefore, it is important to rectify the damage that has been caused to the soil so that the after effects can be minimizedto a greater extent. Soil pollution occurs due to accumulation of pollutants like heavy metals such as arsenic, antimony, mercury, lead, zinc, cadmium, selenium, beryllium, thallium, chromium, copper , nickel, Polycyclic aromatic compounds(PAH), industrial waste, pesticides, insecticides, herbicides, fungicides, agricultural fertilizers, antibiotics and fossil fuels.

Soil pollution is a major environmental concern that needs immediate attention. Some of the main causes of soil pollution would be excessive and improper use of pesticides and fertilizers, excessive industrial activities and poor management of inefficient disposal of waste from industries and manufacturing factories.

Extensive works are done to decontaminate the soil. But some of the pollutants are resistant to physical, chemical and biological degradation which presents a greater burden to the environment (Mishra *et al.,* 2016). Due to this more than one cleanup setups are required to efficiently remove the contaminants from the soil. One such method could be bioremediation in combination with nanotechnology.

Various microorganisms could be used to remove heavy metals from the soil. Bioremediation is an environmentally safe and low-cost approach to remove organic pollutants from the soil (Zhuang *et al.,* 2015). A combination of endophytic bacterial strains *Aeromonas salmonicida*, *Pseudomonas indoloxydans*,*Bacillus cereus*, *Pseudomonas gessardii* and *Rhodococcus sp* were seen as efficient in removing trace elements like Fe, Mn, Ni. Pb, and Cr (Shahid *et al.,* 2020). *Pseudomonas aeruginosa* also has the potential to degrade diazo dyes (Nadi *et al.,* 2018).

It is the need of the hour to develop remediation technologies comprising a mixture of both nanomaterials together with biologic agents to clear the contaminants from the soil. Due to the abundance, easy access and cost-effectiveness, nano zero valent iron has been widely used in developing remediation technologies (Araújoa *et al*., 2015). Nanomaterials are exploited because of their functional properties like large specific surface area, improved chemical reactivity, different nature at nanoscale level and their wide availability in nature. However, their use also faces several challenges (Fang *et al.*,2012 and Fan *et al*., 2013).

In this study, the microbial metabolism of *Pseudomonas aeruginosa* and the superparamagnetic properties of the iron nanoparticles are exploited to remove the metal contaminant lead from the soil. The bacteria *Pseudomonas aeruginosa* is immobilized on iron nanoparticles to increase the removal efficiency of lead from the soil.

METHODOLOGY:

The study took place in four different stages as depicted below in the flowchart.

1. COLLECTION OF SOIL SAMPLE

2. SYNTHESIS OF IRON NANOPARTICLES

3. IMMOBILIZATION OF BACTERIA ALONG WITH NANOPARTICLES

4. ADSORPTION ASSAY

SELECTION OF *Pseudomonas aeruginosa* STRAIN:

The pure culture of ATCC Strain of *Pseudomonas aeruginosa* was procured with the help of HetroGeneBiotech Pvt., Ltd. The procured strain was subcultured and used for further downstream processes.

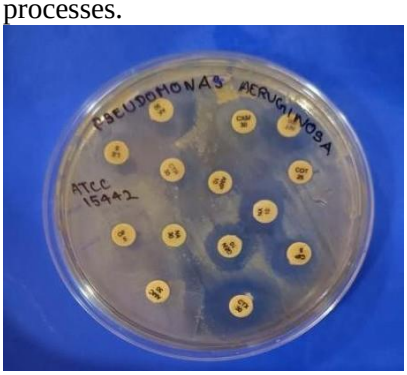

Fig. 1 Procured *Pseudomonas aeruginosa* culture plate

SYNTHESIS OF MAGNETIC NANOPARTICLES:

The magnetic iron nanoparticles are synthesized using a co-precipitation method. In this method, 4.46 grams of ferric chloride and 1.6 grams of ferrous chloride were dissolved in 80 ml of distilled water at room temperature. The beaker containing the above prepared mixture is kept at a magnetic stirrer. 10% ammonia solution is added dropwise to the beaker and stirred for 10 minutes. Then, it was followed by heating for 1 hour at 90℃. The formed iron nanoparticles were cooled down to room temperature and were washed with 70% ethanol solution three times. The collected iron nanoparticles were kept incubated at 60℃ overnight for dehydrating and the dehydrated nanoparticles were scraped off and redissolved in distilled water.

Fig. 2 Magnetic nanoparticles was synthesized using co-precipitation method

CHARACTERIZATION OF SYNTHESIZED MAGNETIC NANOPARTICLES:

Field Emission Scanning Electron Microscopy (FE-SEM) was used for morphological characterization of the synthesized magnetic iron nanoparticles. For the FE-SEM measurements, the magnetic nanoparticle suspensions were dropped on carbon-coated copper grids and then they were dried at room temperature overnight.

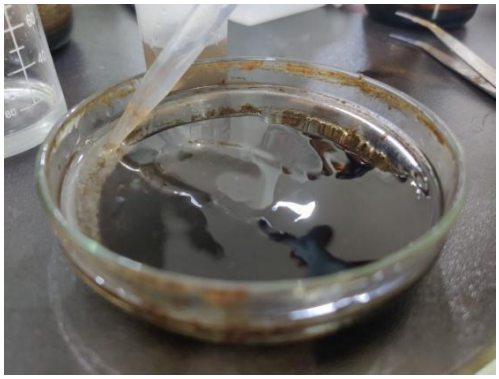

Fig.3 Synthesized iron nanoparticles washed and resuspended in distilled water

IMMOBILIZATION OF *Pseudomonas aeruginosa* ON MAGNETIC NANOPARTICLES:

The procured strain of *Pseudomonas aeruginosa* was subcultured in LB broth. The 24 hours culture of *Pseudomonas aeruginosa* was centrifuged for 10 minutes at 7000g. The bacterial pellets were suspended in saline and synthesized iron nanoparticles were added to the suspension. The beaker containing the above mixture was kept in a magnetic stirrer at 150 rpm at 37℃ for 30 minutes for immobilization. Then the

immobilized iron nanoparticles were collected after washing it with distilled water and removing it with the help of a permanent magnet to remove the unbound *Pseudomonas aeruginosa.*

*Fig.*4 Centrifugation of 24 hours culture of *Pseudomonas aeruginosa*

COLLECTION OF SOIL:

About 1 kilograms of soil were collected from Manali-a metal contaminated industrial area by adopting standard protocols. The soil was collected by removing the surface litter and digging a depth of 15 to 20 cm deep using a shovel. The collected soil was then transferred to a sterile laboratory environment.

Fig. 5 Soil collected from Manali

TESTING OF BASIC PARAMETERS OF SOIL:

SOIL COLOR:

The soil sample is layed out uniformly over a cardboard sheet. The soil particles are then compared with the Munsell's soil color chart to determine the three different properties of color namely hue, chroma and value. Each value is noted down.

SOIL TEXTURE:
The collected soil sample is tested for soil texture within a few days since the soil collection.The soil sample is seen under hand-lens in two different states such as dry and wet or moist state by squeezing between the thumb and fingers. The texture can be identified and classified as one among the following. They are sandy, sandy loam, loam, silt loam, clay loam and clay.

SOIL pH: For testing the pH of the soil, a small amount of soil is taken in a test tube and to the test tube 15 ml of distilled water is added. The solution is left undisturbed till a clear supernatant is formed. The pH of the soil is tested using an electric pH meter,

SPIKING OF THE SOIL WITH LEAD ACETATE:
The collected sample after transferring it to a sterile laboratory environment, about 500g of soil is measured using a weighing machine. It was transferred to a glass bowl. 500 ml of distilled water was added to the soil and it was left undisturbed for half an hour. The filtrate was collected and to it 0.1ppm of lead acetate was added. Then the water was treated with the synthesized *Pseudomonas aeruginosa* immobilized iron nanoparticles for the removal of lead acetate.

Fig. 6 Soil along with 500 ml of distilled water

Fig. 7 Spiking of 0.1 ppm of lead acetate

ADSORPTION ASSAY - DETERMINATION OF THE AMOUNT OF LEAD (Pb) PRESENT IN VARIOUS SAMPLES TAKEN FROM DIFFERENT CONTACT TIMES
The effect of contact time on heavy metal lead removal was studied by conducting experiments under shaking conditions of 300 rpm. The initial concentration of *Pseudomonas aeruginosa* immobilized

nanoparticles of 30 mg/L, temperature (T) of 24°C, and initial concentration of heavy metals of 48 mg/L was present. Samples were collected at different time intervals and analyzed after filtration for assessing the removal efficiency of the nanoparticles. Significant percentage removal (%R) of about 55% of removal of lead was observed to occur during the first 130 minutes, due to the presence of high active sites. ICP-OES (inductively coupled plasma spectrometer) is carried out initially and after the adsorption assay to determine the removal efficiency of lead by the synthesized *Pseudomonas aeruginosa* immobilized iron nanoparticles. Determination of Pb ions attached to the surface of the *Pseudomonas aeruginosa* immobilized iron nanoparticles was done. The removal efficiency of lead from the soil was calculated from the results of the ICP-OES.

Fig. 8 Inductively coupled plasma optical emission spectrophotometry

RESULT AND DISCUSSION

CHARACTERIZATION OF SYNTHESIZED MAGNETIC NANOPARTICLES:

Characterization of the synthesized nanoparticles was done using scanning electron microscopy. The morphology and size distribution of iron magnetic nanoparticles was determined. The morphology of the iron nanoparticles is of irregular circular shape. The size of the nanoparticles ranges from 0.1nm to 28.6 nm.

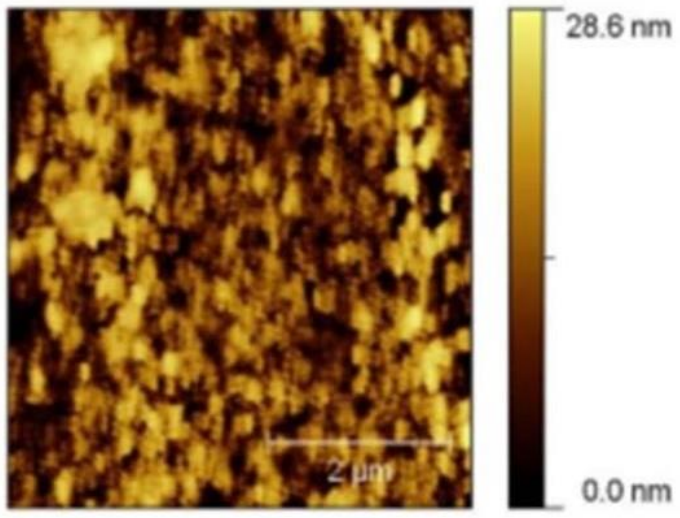

Fig. 9 SEM image of the synthesized iron magnetic nanoparticles

IMMOBILIZATION OF *Pseudomonas aeruginosa* ON MAGNETIC NANOPARTICLES:

During the immobilization, the 24 hours culture of the *Pseudomonas aeruginosa* was kept in the centrifuge for 10 minutes at 7000 g. The bacterial pellets resuspended in normal saline along with the synthesized iron nanoparticles were kept in a magnetic stirrer for 1 hour at 150 g.

Fig. 10 Bacterial pellet formation after centrifugation

Fig. 11 Bacterial pellets along with iron nanoparticles kept in a magnetic stirrer

TESTING OF BASIC PARAMETERS OF SOIL:

SOIL COLOUR:

According to Munsell's soil colour chart, the soil colour was dark reddish brown with value 3 and chroma 4 (3/4).

SOIL TEXTURE:

According to crude estimation, the texture of the sample soil was found to be fine grained clay soil.

SOIL pH:

The pH of the soil was found to be slightly acidic with a pH of 6.2.

ADSORPTION ASSAY - DETERMINATION OF THE AMOUNT OF LEAD (Pb) PRESENT IN VARIOUS SAMPLES TAKEN FROM DIFFERENT CONTACT TIMES

ICP-OES (Inductively coupled plasma optical emission spectrophotometry) was done for samples taken at different contact time during the adsorption assay. The samples were taken at 0 minutes, 15 minutes, 30 minutes, 60 minutes and 90 minutes). Using ICP-OES the amount of lead present in the samples was

determined. The tests were done in triplicates. The removal efficiency of the synthesized *Pseudomonas aeruginosa* and iron nanoparticles complex was then calculated.

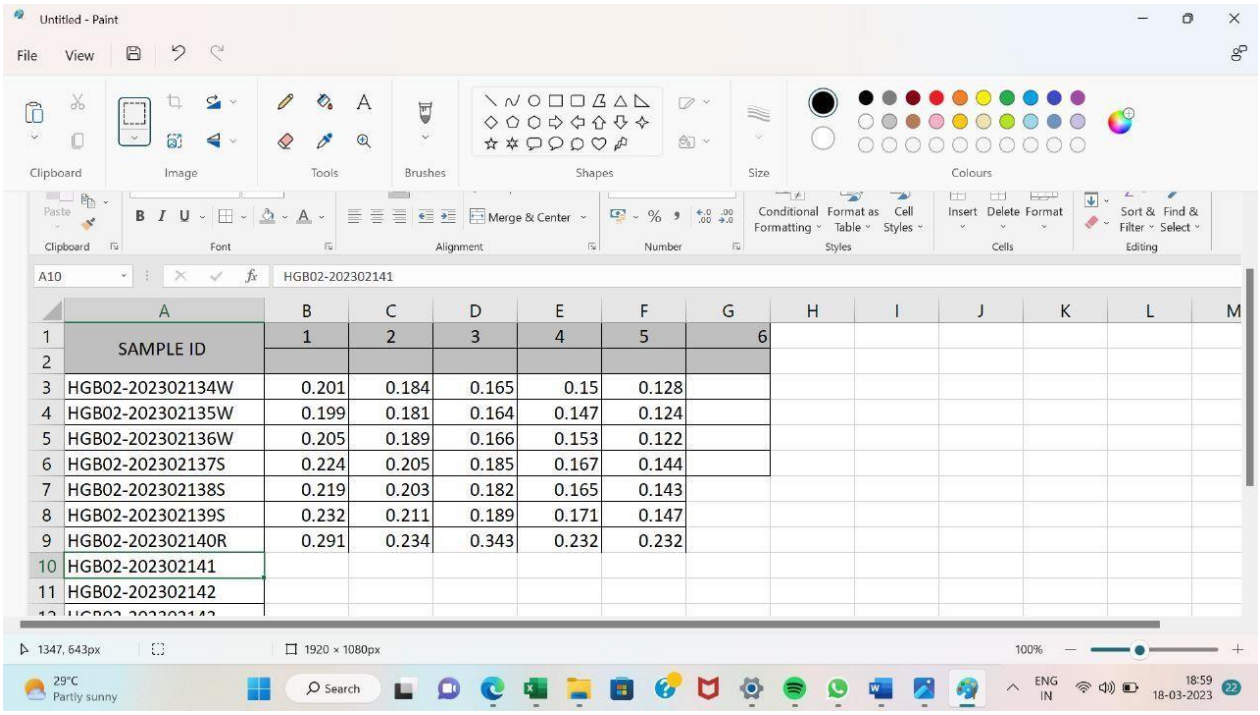

Fig. 12 Snapshot of the ICP-OES results for the samples taken at various contact time during adsorption assay (0,15,30,60,120 minutes)

Table 1: Amount of lead (Pb) present at different contact time during adsorption assay

CONTACT TIME/ TRIALS	0 minutes	15 minutes	30 minutes	60 minutes	120 minutes
TRIAL 1	0.224	0.205	0.185	0.167	0.144
TRIAL 2	0.219	0.203	0.182	0.165	0.143
TRIAL 3	0.232	0.211	0.189	0.171	0.147

Table 2: Removal efficiency of *Pseudomonas aeruginosa* immobilized iron nanoparticles at different contact time during adsorption assay

REMOVAL EFFICIENCY/ TRIALS	0 minutes	15 minutes	30 minutes	60 minutes	120 minutes
TRIAL 1	100%	8.48%	17.41%	25.45%	35.71%
TRIAL 2	100%	7.3%	16.89%	24.66%	34.70%
TRIAL 3	100%	9.05%	18.53%	26.29%	36.64%
AVG	100%	8.28%	17.61%	25.47%	35.68%

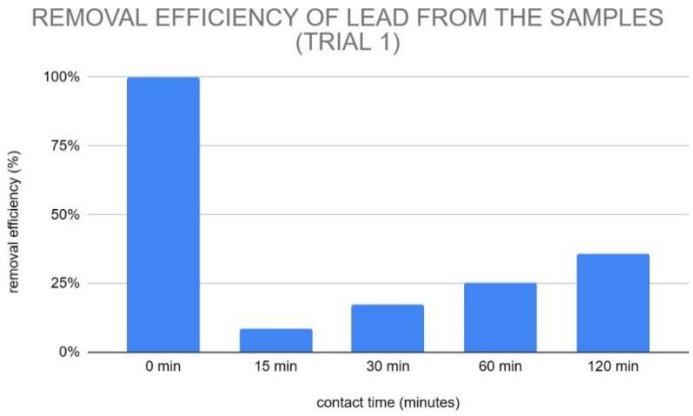

Fig. 13 Trial 1- Removal efficiency of lead from the samples at different contact times

During trial 1, initially 0.224 mg/L of lead was present. After 15 minutes, the amount of lead in the sample reduced by 8.48%. After 120 minutes of contact time, the iron nanoparticles showed removal efficiency of 35.71%.

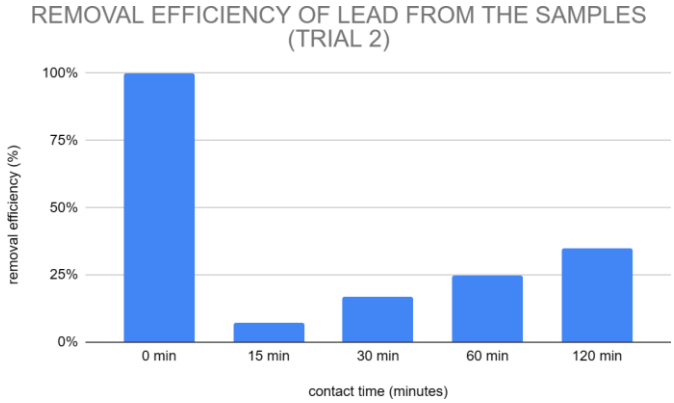

Fig. 14 Trial 2- Removal efficiency of lead from the samples at different contact times

During trial 2, initially 0.219 mg/L of lead was present. After 15 minutes, the amount of lead in the sample reduced by 7.3%. At 30 minutes, the amount of lead was found to be 0.182 mg/L. After 120 minutes of contact time, the iron nanoparticles showed removal efficiency of 34.70%.

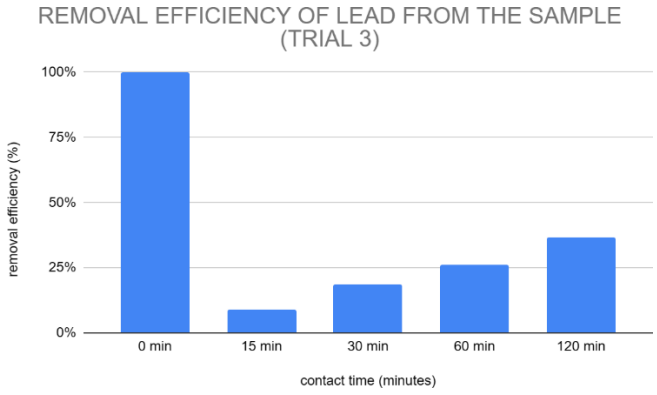

Fig. 15 Trial 3- Removal efficiency of lead from the samples at different contact times

During trial 3, initially 0.232 mg/L of lead was present. After 15 minutes, the amount of lead in the sample reduced by 9.05%. At 60 minutes, the amount of lead was found to be 0.171 mg/L. After 120 minutes of contact time, the iron nanoparticles showed removal efficiency of 35.68%.

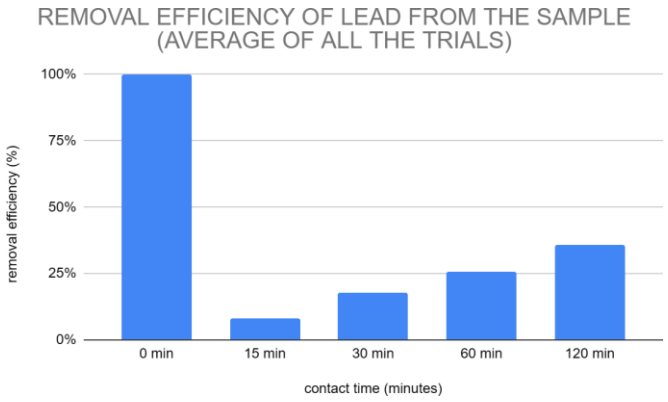

Fig. 16 AVERAGE - Removal efficiency of lead from the samples at different contact times

Overall, the removal efficiency of lead from samples at 15 minutes was 8.28%. After the initial 30 minutes of contact time the removal efficiency was found to be 17.61%. The removal efficiency of lead at the end of 60 minutes was 25.47%. The *Pseudomonas aeruginosa* immobilized iron nanoparticles has a removal efficiency of 35.68% at the end of 120 minutes.

The bacteria-nanoparticle complex showed a significant removal rate of 35.68% at the initial contact time of 120 minutes. This shows that this complex has the potential to remove a significant amount of lead from the samples. By standardizing the bacterial strain, size of the nanoparticle and the surface to volume ratio of the bacteria- nanoparticle complex there is a possibility to achieve an even higher rate of removal efficiency.

There were many limitations encountered during the study. The adsorption assay was carried out at a fixed *Pseudomonas aeruginosa* immobilized iron nanoparticle concentration of 30 mg/L. The effect of different concentrations of the complex was not tested out during the study. The effects of temperature and pH were also not tested out during the study. The temperature was fixed at 24°C during the entire assay. The test was carried out in a temperature controlled environment.

In future, the effect of various concentrations of the complex per liter of the sample can be studied. The effect of temperature and pH on the removal efficiency of the complex could also be studied. The effect of nanoparticle removal from the samples after the treatment was also not studied.

This shows that this combination of bioremediation with biologic agents such as microbes along with nanotechnology can be a potential solution for the removal of contaminants from the soil samples. By exploring this field of solution, we can create a more pollution free environment. By removing the lead in the soil, about 96% of lead in the soil could be removed which in turn reduces the after effects of lead accumulation in the soil. The soil's natural properties can be retained and maintained and thereby promotinga high yield of plant growth. A number of diseases caused due to accumulation of lead in the food chain could also be prevented by removing the lead from the lowest trophic level. Synthesizing this nanoparticle complex is eco-friendly and cost-effective which can be adapted by developing countries.

CONCLUSION

During the study, It was found that the synthesized *Pseudomonas aeruginosa* immobilized iron nanoparticle has the ability to remove 35.68% of lead from the sample during a 120 minutes of contact time. In this study, the bacterial metabolism of *Pseudomonas aeruginosa* and the superparamagnetic properties of the iron nanoparticles are exploited to create a solution for the removal of lead from the soil. More work has to be done to standardize the iron nanoparticle complex to make it more viable to use in the practical

field. The Concentrations of the nanoparticle complex, effect of temperature and pH are factors to be standardized. This solution could be a future eye opener in the field of pollution control.

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