Special Edition on “Frontiers of Biosciences in Sustainable Development (FBSD)”
CIBTech Journal of Zoology ISSN: 2319–3883; http://www.cibtech.org.htm
2023 Vol.12/S1, pp.38-46/Keerthi and Amritha
***Research Article** (Open Access)*

SYNTHESIS OF *PSEUDOMONAS AERUGINOSA* IMMOBILISED IRON NANOPARTICLES FOR THE REMOVAL OF LEAD FROM THE POLLUTED WATERS OF ENNORE, CHENNAI

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ABSTRACT
Ennore Creek, a tidal estuary located in the north-eastern part of Chennai, is the point of confluence of the Kosasthalaiyar river and the Bay of Bengal. It is an extremely sensitive ecosystem that supports a variety of floral and faunal life by acting as a transition zone between inland freshwaters and the sea. Unfortunately, North Chennai for many decades has been the hub of numerous industrial activities. Water pollution due to heavy metals and toxic effluents released by industries and human activities threatens to destroy not just the biodiversity but also the livelihood of local fisherfolk communities whose existence depends on the creek. The present study aims to conduct a preliminary assessment of the current hydrological scenario of Ennore.
Further, the study intends to investigate the efficiency of *Pseudomonas aeruginosa* immobilised iron nanoparticles to remove lead, one of the most common heavy metals found in polluted waters. Majority of the parameters tested proved to be within permissible limits. Maximum removal efficiency of lead was found to occur at a contact time of 120 minutes with the initial concentration of the bacteria immobilised nanoparticles being 30 mg/l.

Keywords: *Ennore, Iron oxide nanoparticles, Lead Pollution, Heavy metals*

INTRODUCTION
Out of the many different natural resources in the world, water truly is nature’s gift to mankind. Numerous scientists from around the world believe that water is the cradle in which life originated, therefore it comes as no surprise that all living beings in some way or form need water for sustenance and survival (Das, 2019).
Surface water resources, all over the world, are being subjected to heavy stress to meet a variety of requirements and hence are greatly susceptible to pollution from numerous sources such as sewage waste, agricultural and industrial waste, thermal power stations etc. Once polluted, it is expensive and cumbersome to restore and regain the original water quality.
Globally, increasing industrialisation and urbanisation have contributed to alarming increase in the levels of heavy metals found in water. Heavy metals continue to persist in the environment because they are not biodegradable (Pinto, 2017). . They have the capacity to accumulate in living organisms which is detrimental to the general health of that particular organism.
One of the most common heavy metals released into soil and water is lead (Pb). WHO regulations find water with lead levels below 10 µg/l to be acceptable. Anthropogenic sources which discharge lead into the ecosystem include metal purification processes, pesticides and fertilisers from agricultural runoff and industrial waste. Acute lead poisoning has catastrophic effects on the body. It results in dysfunctional kidney, causes damage to the nervous system, kidneys often resulting in extreme sickness and in serious cases, death. Exposure of even minimal amounts of lead have been known to have teratogenic effects on pregnant women and leads to the abnormal development of the foetus. In children, vulnerability to lead toxicity is enhanced when calcium and zinc deficiencies co-exist. Children exposed to lead have a poorer intelligence quotient (IQ) compared to other children their age because one of the major detrimental effects that lead has is felt in the CNS and in the development of the grey matter of the brain (Singh and Kalamhad, 2011). Therefore, not

only is lead a potent toxic chemical, which even when present in minute quantities, can have adverse impacts on the kidneys and the nervous system but it is also a powerful carcinogenic agent (Vareda, 2019).

Chennai is no exception, when it comes to facing this globally prevalent environmental challenge. Chennai, formerly known as Madras, was once a land rich in hydrological resources. The landscape of Chennai is adorned with numerous lakes, with the most notable ones being Chembarambakkam lake, Puzhal, Retteri, Kaliveli, Poondi reservoir and the Pulicat lake and estuary in the outskirts of the city. Three magnificent rivers - the Cooum, Adyar and Kosasthalaiyar, which ran through the city are now marked by pollutants from various sources.

The Kosasthalaiyar river, also known as Kortalaiyar river, is the largest of Chennai's three rivers but known to a lesser extent.The river is fraught with problems of encroachment and discharge of untreated industrial effluents because of its passing across Ennore and Manali, the industrial hub of North Chennai.

Ecologically, the Ennore Creek is a novel habitat which supports a vast diversity of floral and faunal life. This is a tidal estuary formed by the confluence of the Kortalayair river and the Bay of Bengal. Mangroves, salt pans, mud flats and fish farms are some of the microhabitats that characterise the uniqueness of the creek. This ecosystem also sustains the lives and livelihoods of several communities. Six fishing hamlets, especially Mugathwara Kuppam, Kaatu Kuppam and Sivanpadaiveethi Kuppam and the community of fisherfolk that live here depend wholly upon the faunal diversity of the creek for survival (The Coastal Resource Center, Chennai).

Over the course of the last decade, the use of nanotechnology for remediating environmental issues has gained tremendous traction. Iron oxide nanoparticles possess powerful magnetic properties which have been made use of to remove heavy metal ions from water. They possess a considerable amount of surface charge, high redox potential, and have the ability to be reused to a certain extent (Kansara, 2018).

Objective

To determine the efficiency of *Pseudomonas aeruginosa* immobilised iron nanoparticles in removing lead from the waters of Ennore.

MATERIALS AND METHODS

Area of Study

Ennore is a highly industrialised coastal area situated in the northern part of Chennai, Tamil Nadu, India. The Ennore Creek(13°13'56.39"N, 80°19'48.26"E) is a tidal inlet that is formed at the point of confluence of the Kosasthalaiyar river and the Bay of Bengal. This unique habitat provides critically needed space to house a diverse variety of flora and fauna. Flanked by the Pulicat lagoon on one side and the Ennore-Manali marshland complexes on the other, this ecologically sensitive region is facing the dire threat of habitat destruction due to water pollution.

Previous studies in the region have documented a good variety of floral and faunal life. About 482 species of aquatic biodiversity with representatives of 135 species of phytoplanktons, 59 species of zooplanktons and 288 species of other estuarine fauna have been documented (Samuel, 2022).

The water quality of Ennore has been a matter of concern for a long time because of the release of industrial effluents, sewage, discharge of thermal power stations, both into the creek itself and the Kosasthalaiyar river which carries these pollutants into the creek.

METHODOLOGY

Sample Collection

Sampling points from the Ennore Creek were selected randomly and samples were collected in 500ml clean, sterile glass containers by adopting the standard protocols. The glass containers were rinsed with the water sample to be analysed to remove traces of any residual impurities before being filled up with the sample. The samples were collected in triplicates to ensure accurate and reliable values during analysis. They were quickly and carefully transported to the laboratory and the preliminary analysis of the samples collected was performed.

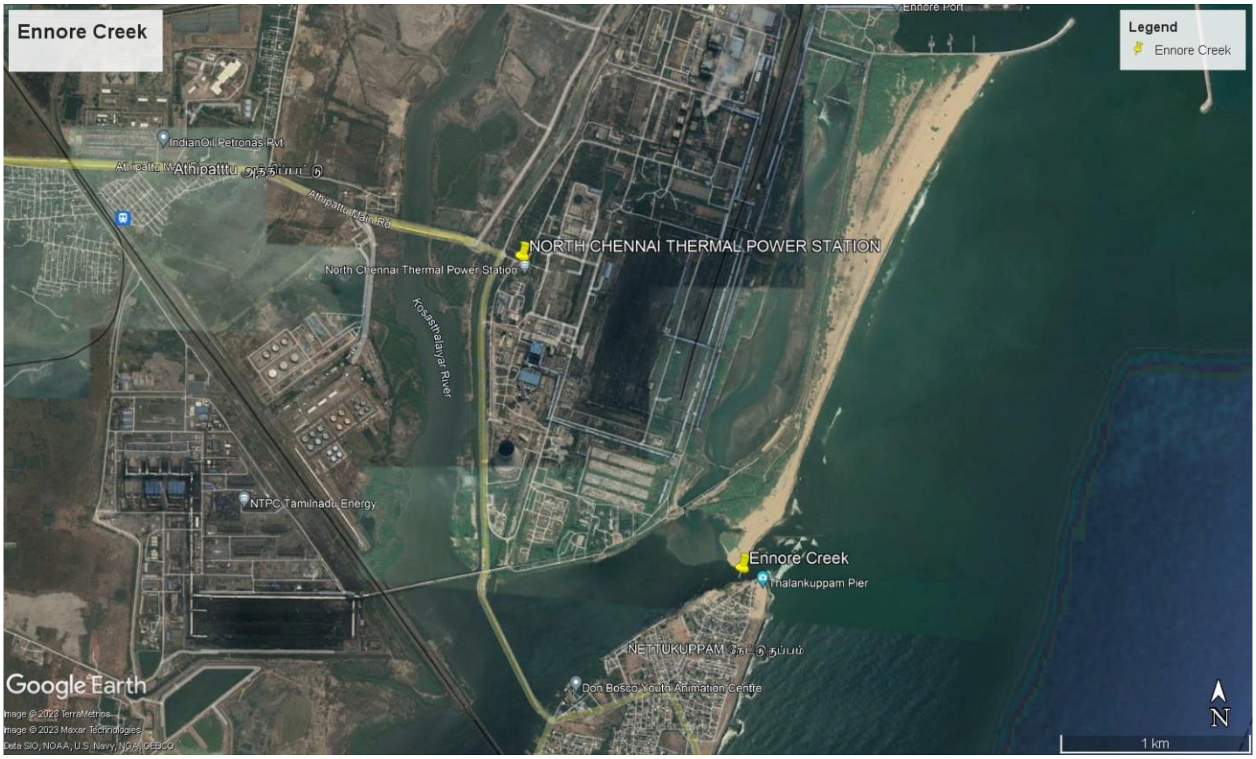


Figure 1: Area of Study, Ennore Creek, Chennai, Tamil Nadu, India

Preliminary Analysis of the Sample

Physical characteristics of the sample - colour and odour were qualitatively estimated while temperature was determined using a thermometer at the study site before transportation to the laboratory. Other physico-chemical parameters were determined soon after transportation to the laboratory. pH and electrical conductivity were estimated using a calibrated pH metre to determine the nature of the water sample. The levels of dissolved oxygen, total alkalinity, total hardness and total Dissolved Solids were estimated using standardised procedures recommended by the Bureau of Indian Standards, IS 10500.

Synthesis of iron oxide nanoparticles

Iron oxide nanoparticles were synthesised using the co-precipitation method (Ali, 2016). Aqueous salt solution containing Iron (III) chloride hexahydrate and Iron (II) chloride tetrahydrate in the molar ratio of 2:1 was prepared. 4.46g (16.6 mmol) of $FeCl_3.6H_20$ and 1.6g (8.1 mmol) of $FeCl_2.4H_2O$ were dissolved in 80 ml of deionised water at room temperature. The solution was degassed by bubbling with nitrogen gas for 5 minutes.

The solution was then placed on a magnetic stirrer. 10 ml of Ammonia was added dropwise while stirring continuously for 10 minutes at room temperature.

The resulting solution was then washed with ethanol and deionised water twice to remove any residual impurities. The iron oxide nanoparticles were separated out by applying a magnetic field. The magnetic nanoparticles were then dried in an incubator for 1 hour at 90 °C.

Procurement of Bacterial Strain and Cultivation

Pseudomonas aeruginosa ATCC 15442 was procured and subcultured in Lysogeny Broth (LB) culture medium. The composition of the medium was as follows: 10g of tryptone, 5g of yeast extract, 10g of NaCl dissolved in 1l of distilled water. The bacteria were cultured overnight in an incubator set to a temperature of 37 °C.

Immobilisation of Pseudomonas aeruginosa on the iron nanoparticles

The preculture containing bacterial cells in their mid-exponential growth phase was centrifuged at 7000 rpm for 10 minutes. The bacterial pellets thus obtained were suspended in a solution of Phosphate Buffered Saline (PBS). Simultaneously, a suspension of Fe_3O_4 nanoparticles in PBS at the concentration of 1mg/ml was prepared which was added to the bacterial suspension at a volume ratio of 1:10. To ensure complete immobilisation of the bacteria onto the nanoparticles, the mixture was placed in a magnetic stirrer and stirred continuously at 150 rpm for 2 hours. This was followed by washing with PBS and ethanol to remove impurities, remnants of the culture medium and unbound cells (Nadi, 2018).

Characterisation of Iron Oxide Nanoparticles

The synthesised iron oxide magnetic nanoparticles were subjected to Scanning Electron Microscopy (SEM) for characterisation. Morphology, size and distribution of the nanoparticles was inferred from the SEM images generated.

Adsorption Assay

The water sample collected from Ennore was spiked with 0.1ppm of lead acetate solution. This step is essential in the creation of a controlled environment, where a known quantity of lead is added to accurately calculate the amount of lead removed. Further, this ensures that the concentration of lead is in the detectable range for analysis using the ICP-OES technique.

To 500ml of water sample spiked with lead add 30 mg of bacteria immobilised nanoparticles. The mixture is then placed on a magnetic stirrer and stirred continuously for 120 minutes. The sample was analysed at different time intervals after filtration to study the levels of lead. The concentrations of heavy metal ions were measured using an Inductively Coupled Plasma-atomic Emission Spectrometer (ICP) (Model AA 100, Perkin-Elmer).

Statistical analysis

Statistical analysis was performed using Microsoft Excel, Version 2007.

RESULTS AND DISCUSSION

Water Quality Parameters

The results obtained from the testing of the water samples for basic parameters are listed out in Table 1.

TABLE 1: Environmental parameters to assess the water quality in Ennore

S.No	**Parameters**	**Specifications as per IS 10500 - 2012**		**Result**
		Acceptable limit	**Permissible Limit**	
1	Appearance	-	-	Clear colourless liquid
2	Odour	Agreeable	Agreeable	Agreeable
3	Temperature	-	-	29°C
4	pH	6.5-8.5	No relaxation	8.36
5	Conductivity	-	-	850.1
6	Dissolved Oxygen	-	-	5.4ppm
7	Total Dissolved Solids	500	2000	506 mg/l
8	Total Hardness	200	600	341 mg/l
9	Total Alkalinity	200	600	105 mg/l

The colour and the odour of the water sample collected was found to be agreeable implying that the region is free from contaminants impacting these parameters. The temperature of the water collected was found to be 29°C. This is an important physical parameter that has a great effect on several other biological, physical and chemical properties of water such as the nature of the aquatic organisms that the water body can support, water flow and the chemistry of the water (solubility and reactivity of many chemical compounds) (Vincy MV, 2012). Another important parameter which influences the quality of surface waters is the pH of the water body. Water collected from the Ennore estuary was found to be slightly alkaline, with a pH of 8.36. The alkaline nature of industrial wastewaters and components of household sewage such as detergents could contribute to the alkalinity of the waters (Mohanraj, 2013).

Dissolved oxygen levels were calculated to be 5.4 ppm. This is one of the most crucial parameters that determines the health of a water body. Low levels of dissolved oxygen could be an indication of the presence of organic contaminants that might have entered the water system along with domestic and industrial effluents. Organic pollutants deplete the oxygen content of the water body and have the capacity to drastically alter the distribution and the amount of life found in the ecosystem (Bozorg-Haddad, 2021).

The conductivity values and the levels of total hardness and total dissolved solids indicate that the water has a moderate level of dissolved salts and minerals which was found to be within the permissible limits. However, the alkalinity, which is a measure of the buffering capacity of the water (ability to resist fluctuations in pH) was found to be lower than the range recommended by IS 10500 standards set by the Bureau of Indian Standards. Low alkalinity could lead to fluctuations in the pH, which has negative implications on the health of the water body. This also increases the difficulty of subjecting the water to various treatment processes (Mohanraj V, 2013).

Characterisation of Iron Oxide Nanoparticles

SEM images revealed that the synthesised iron oxide nanoparticles were found to be within the size of 28.6nm. Even distribution of slightly globular nanoparticles were observed.

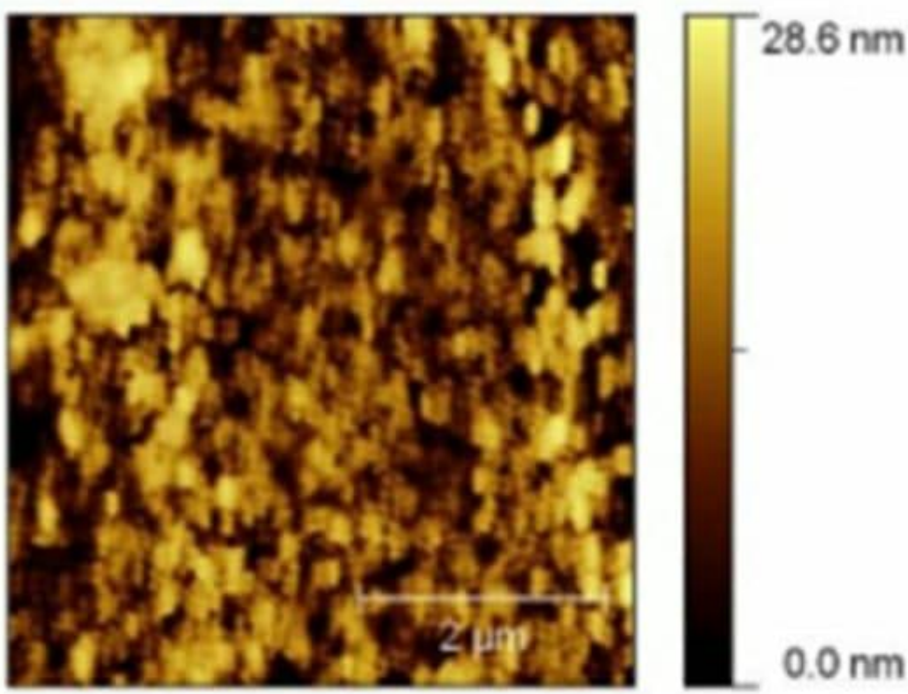


FIGURE 2: SEM Image of the Synthesized Iron Oxide nanoparticles

Adsorption Assay

TABLE 2: Trial 1 - Efficiency of Lead Removal by *Pseudomonas aeruginosa* immobilised iron nanoparticles

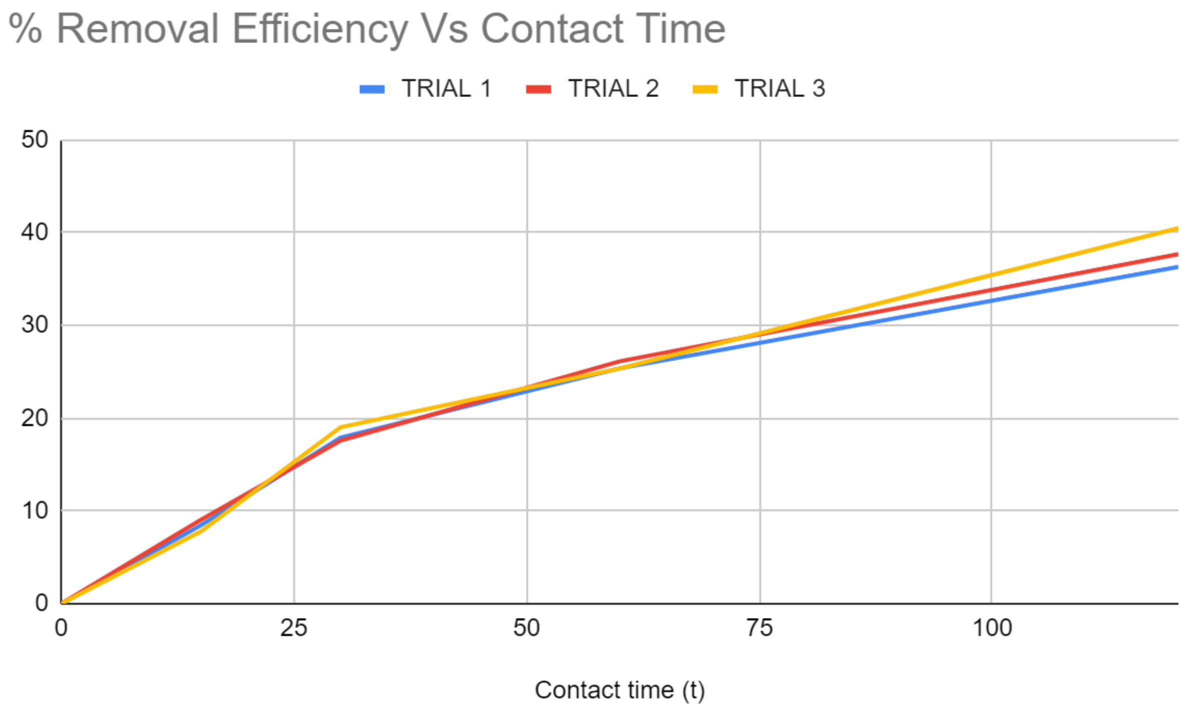
Contact time (t)	Concentration of Lead	% Efficiency in Lead Removal
0	0.201	0
15	0.184	8.457711443
30	0.165	17.91044776
60	0.15	25.37313433
120	0.128	36.31840796

TABLE 3: Trial 2 - Efficiency of Lead Removal by *Pseudomonas aeruginosa* immobilised iron nanoparticles

Contact time (t)	Concentration of Lead	% Efficiency in Lead Removal
0	0.199	0
15	0.181	9.045226131
30	0.164	17.5879397
60	0.147	26.13065327
120	0.124	37.68844221

TABLE 4: Trial 3 - Efficiency of Lead Removal by *Pseudomonas aeruginosa* immobilised iron nanoparticles

Contact time (t)	Concentration of Lead	% Efficiency in Lead Removal
0	0.205	0
15	0.189	7.804878049
30	0.166	19.02439024
60	0.153	25.36585366
120	0.122	40.48780488



GRAPH 1: % Removal Efficiency Vs Contact Time.

The effect of contact time (tcontact) on lead removal was studied by conducting experiments under shaking conditions of 300 rpm at room temperature. It is evident from the graph above that the efficiency of adsorption of lead by the *Pseudomonas aeruginosa* immobilised iron nanoparticles increases with contact time. Maximum adsorption of 40.48% was found to occur at 120 mins in the third trial. The initial concentration of lead was 48 mg/l and the initial concentration of the magnetic nanoparticles- *Pseudomonas aeruginosa* complex was 30 mg/l.

Although the results of the preliminary study to test the efficiency of Pseudomonas aeruginosa immobilised iron nanoparticles is promising, it is important to note that a removal efficiency of 40.48% is still rather low. The adsorption capacities of nanoparticles largely depend on their physical properties which in turn depend upon their method of preparation. Therefore it is important to perform further studies to optimise the production process. Also, the concentration of the nanoparticles could be varied to determine if higher efficiencies are obtained at higher concentrations. Detailed analysis of the sample to understand the nature of other contaminants and organic pollutants is of paramount importance to understand the efficiencies of the treatment process. This however is beyond the scope of the present study.

Nonetheless, this research work has laid the foundation for future studies aiming to optimise and improve the procedures for remediation of contaminated waters using *Pseudomonas aeruginosa* immobilised iron nanoparticles. Improvements in the adsorption efficiencies would make this a viable, cost-effective treatment process for the large-scale removal of heavy metals from industrial waste waters.

CONCLUSION

The present study was successful in synthesising *Pseudomonas aeruginosa* immobilised iron oxide nanoparticles. A removal efficiency of 40.48% was achieved after 120 minutes of treatment of lead contaminated water samples with an initial lead concentration of 48 mg/l. The potential of integrating nanotechnology and bioremediation of water bodies is quite evident from the nature of the data generated. The results of the study suggest that *Pseudomonas aeruginosa* immobilised iron nanoparticles can be a promising material to remove lead from polluted water samples, which can be applied in the development of new water treatment technologies. However, further studies are required to optimise the process conditions and scale-up the technology for practical applications. Conservation of our existing water resources is the need of the hour. Integrating the use of sustainable technologies and development is the only way forward if we, human beings, want to continue to live on a planet that is habitable and hospitable.

ACKNOWLEDGEMENT
The authors would like to express their sincere gratitude to the Head and the faculty of the Department of Zoology, Stella Maris College (Autonomous), Chennai whose support and feedback has been pivotal in the successful completion of this work. They would also like to Dr. Thooyavan George and Ms. Vaishali Srinivas, Hetero Gene Biotech Pvt Ltd, for their guidance and for providing the necessary laboratory facilities to conduct the research.

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