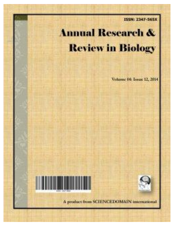

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Evaluation of Metal Bioleaching Property of *Actinomyces* sp. in Waste Foundry Sand by *In-vitro* Methods

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Authors' contributions

This work was carried out in collaboration between both authors. Author SV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EP managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Metals can be leached either directly (physical contact between microorganisms and solid material) or indirectly. The removal of metals from these industrial wastes brings out detoxification of the residues and thus improves the quality of the environment. The waste foundry sand was analyzed for the presence of toxic metals, as the plant uptakes these toxic metals through their food chain which in turn may be harmful to the human beings. In this study Hibiscus was grown on sand blends containing 50% of waste foundry sand (WFS) to assess the availability of Sio_2, Al, Ca, Mg, Pb, Cu and Zn. The chemical properties of treated and untreated waste foundry sand were observed. The analysis shows the level of untreated WFS Fe (76.36%), Ca (43.65%) and K (37.49%). *Actinomyces sp.* was isolated and identified from WFS and was used to bioleach the sand (treated) and was observed to reduce the level of metals present in WFS [Fe (26.54%), Ca (27.67%) and K(5.84%)] and untreated foundry sand had metal levels of [Fe(49.82%), Ca (15.98%)

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and K(31.65%)]. The treated and untreated sand was later used for growing Hibiscus plant sapling under controlled conditions and was analyzed for the traces of metals absorbed by the plant. The presence of metals was calculated by Atomic Emission Spectroscopy technique that can determine the concentration of trace to major elements. Our observations provide a supportive document on bioleaching of WFS by *Actinomyces* sp. was adequate in the growth of ornamental plant *Hibiscus rosa-sinensis*.

Keywords: Foundry sand; bioleaching; Hibiscus rosa-sinensis; ICP-OES; heavy metals.

1. INTRODUCTION

Waste foundry sand (WFS) is a byproduct of the casting industry obtained from the molding and core making processes. In the mineral industry, sludge, dust and other waste generated by the metallurgical industries should require another way to extract metals. Some time precious metals like gold and silver and few rare earth elements are mined based on its composition, nature and primary ores used [1]. *Actinomyces* species are mesophilic, grow optimally at neutral pH and gram-positive organisms [2,3]. Bioleaching is a process of extraction of metals from their ores through the use of living organisms. The application of bioleaching form mining ores to industrial wastes, as increasingly waste quantities of hazardous industrial waste (e.g. fly ash, waste foundry sand, slag and filter dust etc.,) are suggested to be devoid of metal before discarding. Microbial leaching methods are being increasingly applied for metal recovery from low-grade ores and concentrates that cannot be processed economically by conventional method. However, it has been known only for about 50 years that bacteria are mainly responsible for the enrichment of metals in water from ore deposits and mines. The solubilization process is called bioleaching and occurs in nature wherever suitable conditions are found for the growth of the ubiquitous bioleaching microorganisms. Bioleaching mechanisms are based more or less exclusively on the activity of *T. ferroxidans*, *Actinomyces* which convert heavily soluble metal sulfides via bioleaching oxidation reaction into water-soluble metal sulfates in principle metals can be released from sulfide minerals by direct and indirect bacterial leaching. Indirect bacterial leaching, there is physical contact between the bacterial cell and the mineral sulfide surface, and the oxidation to sulfate takes place via several enzymatically catalyzed steps. Investigations by Torma A. E. [4] have shown that the following non-iron metals sulfides can be oxidized by *T. ferroxidans* and *Actinomyces* through direct interaction.

The bacteria do not attach to the whole mineral surface but prefer specific sites of crystal imperfection, and metal solubilization is due to electrochemical interaction [5]. The objective of this study was to assess the uptake of several metals, both essential (i.e. B, Co, Cr, Cu, Fe, Mg, Ni, and Zn) and non- essential (i.e. Al, Be, Ca, Pb, and V) by hibiscus (*Hibiscus rosa-sinensis*.) which is capable of growing on sands from aluminum, iron, and steel foundries. The *Actinomyces sp.* was isolated from wasted foundry sand and is harvested in the laboratory and used for bioleaching process. The treated and untreated WFS by using Hibiscus *(Hibiscus rosa-sinensis*) plant. Under green house condition showed that inoculation of sand treated with *Actinomyces* for bioleaching improved the quality of sand increased the leaf area, plant height, and root-shoot biomass. Using plants to assess the availability of metals in WFS is an important step in analyzing the environmental risks of these materials before they are beneficially used in soils.

2. MATERIALS AND METHODS

The waste foundry sand was collected from Coimbatore district, Tamil Nadu. About one gram of sand sample was dissolved in 100 ml of distilled water and vigorously mixed for two minutes. Then the suspension was serially diluted from 10^{-1} to 10^{-6} and spread plated on potato dextrose agar (PDA), rose bengal agar and starch casein agar medium (HiMedia, India) and incubated at 25°C- 35°C for 48 hours. Identification was carried based on colony are vegetative hyphae are relatively smooth surfaced but later develop a granular, powdery and velvety. Morphology, colour, texture, and appearance and growth pattern of colonies on agar medium. The biochemical analysis showed indole, methyl red, citrate, catalase, TSI and carbohydrate fermentation test was carried out for the isolates, according to Cappuccino J. C. and Sherman N. [6].

2.1 Analysis of Total Metals

The total metals present in the waste foundry sand were analyzed following the procedure [7]. 1.0g of WFS sample was digested according to United States Environmental protection Agency (US EPA) method 3050B. The Fe, Al, and Mg contents were then analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES), where the other 16 metals were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS). Four replicated measurements were conducted for each treated and untreated sand samples.

2.2 Selection of Biological Plant

Hibiscus is a genus of flowering plant in the mallow family Malvaceae. The genus is quite large, containing several hundred species that are native to warm-temperate, subtropical and tropical regions throughout the world. *Hibiscus rosa-sinensis*, known colloquially as Chinese hibiscus, China rose, Hawaiian hibiscus and shoeblack plant, is a species of tropical hibiscus, native to East Asia. *Hibiscus rosa-sinensis* grows in summer and autumn. It is widely grown as an ornamental and medicinal plant. Its flower has been reported to have beneficial effects in heart diseases, emollient, refrigerant, aphrodisiac, anti-inflammatory, demulant, aphrodisiac, refrigerant, menorrhagic, anticomplementary activity, antifertility [8]. The phytochemical substances reported in Hibiscus are steroids, flavonoids, tannins, biochemical substances, alkaloids, resins, vitamin B complex, terpenes etc. Flavonoids give a bitter taste to leaves and used as anti-corrosive and anti-microbial activities which are equal to Neem [9].

2.3 Bioleaching of WFS Metals Using Microorganisms

- WFS were spread in plastic tray in layers. Broth cultures for the growth of *Actinomyces* sp., was prepared using Actinomyces Broth (HiMedia M233, India). Cultural characteristics observed after incubation at 25-30°C for 40-72 hours.
- 1 cm thick layer of waste foundry sand was made as bottom layer and it was drenched with water.
- Middle layer of waste foundry sand was sprayed with broth culture. These plastic trays were then placed at room temperature for 22°C-40°C for 30 days.
- After 30 days, the samples were considered as treated foundry sand and are used for cultivating plants. the chemical properties of treated and untreated WFS were analyzed by ICP-MS and ICP-AES methods.

2.4 Growth of Flowering Plant in Waste Foundry Sand under Controlled Conditions

Each plastic pot (15 cm and dia.) was filled with 1.5 kg of the foundry sand blend. The drainage holes were covered with nylon mesh to help retain the sand in the pots. There were four replicates of each treated, untreated and control. Water was added to the pots 48 h before sowing to thoroughly wet the sand. Pots were sown with, Hibiscus plant. The pots were kept in a growth chamber under a light-dark cycle.

2.5 Determination of Total Metal Concentration Present in Plant Parts

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) analysis was performed to identify and measure a range of chemical, trace to major elements necessary for the analysis of metal samples can detect most elements in periodic table. The plant leaves were digested to determine total metal concentration following the method [10]. Quality control was maintained with reference materials of Hibiscus leaves to calculate qualitative and data that can be included in an ICP test report [11].

3. RESULTS AND DISCUSSION

3.1 Isolation of Actinomyces

The growth on agar plates showed colonies with vegetative hyphae are relatively smooth surfaced but later develop a granular, powdery and velvety. The strains were observed as long purple colour gram positive rods in oil immersion and long vegetative hyphae with branches in lacto phenol cotton blue staining.

3.2 Biochemical Analysis

The biochemical analysis showed indole positive, methyl red negative, citrate positive, catalase negative and the carbohydrate fermentation test like sucrose, maltose, glucose were utilized and lactose was not reduced, TSI (K/A) according to Guney et al. [12].

3.3 Analysis of Total Metals in WFS

Total metal concentrations of the WFSs such as SiO_2, Cu, Pb, Fe, Ca, Al, Mg and Zn were presented in Table 1.

3.4 Analysis of Total Metals

Table 1. Represents the percentage of total metals in untreated and treated waste foundry sand

Metals	Untreated (%)	Treated (%)
SiO_2	1131(11.31%)	719(7.19%)
Cu	231(2.31%)	130(1.3%)
Pb	30(0.3%)	DL (0.1) (0.0001%)
Fe	76364(76.36%)	26542(26.54%)
Zn	696(6.96%)	184(1.84%)
Ca	43651(43.65%)	27672(27.67%)
Al	36(0.36%)	DL (0.1) (0.0001%)
Mg	28550(28.55%)	14655(14.65%)
K	3749(37.49%)	584(5.84%)

Dl- Detected limit; X = Untreated, Y = Treated; At 5% LOS (level of significance); (8, 6) = 4.1468; Here Fc=FT; 4.146=4.546;
Apply F test for this Table 1 conceding Untreated as X variable and Treated as Y variable. The result is consisting of Null hypothesis is accepted

Null hypothesis (HO):

Two populations have the same variance.

Alternative hypothesis (H1):

Two populations do not have same variance.

3.5 Analysis of Waste Foundry Sand in Flowering Plant under Controlled Growth Conditions

The plant hibiscus was grown on treated, untreated and control sand (fertile soil). It was observed that the control pot (without waste foundry sand) having hibiscus plant measured 20 cm initially and after 90days showed 90 cm in length and similarly, the treated pot containing WFS sand that was bioleached by *Actinomyces* sp. was observed for growth of hibiscus plant showed a growth 20 cm initially and after 90 days was observed to have increased upto 108 cm in length and its size of leaves was larger than control and also had increased flowering capacity. Whereas, the untreated pot containing waste foundry sand without *Actinomyces* sp. measured 20 cm initially and after incubation upto 90 days the plants grew 98 cm and its flowering was less and the leaves were smaller compared to other pots (Fig. 1).

Null hypothesis (HO):

There is no significant difference in the days and measurement.

Alternative hypothesis (H1):

There is a significant difference in the day and measurement.

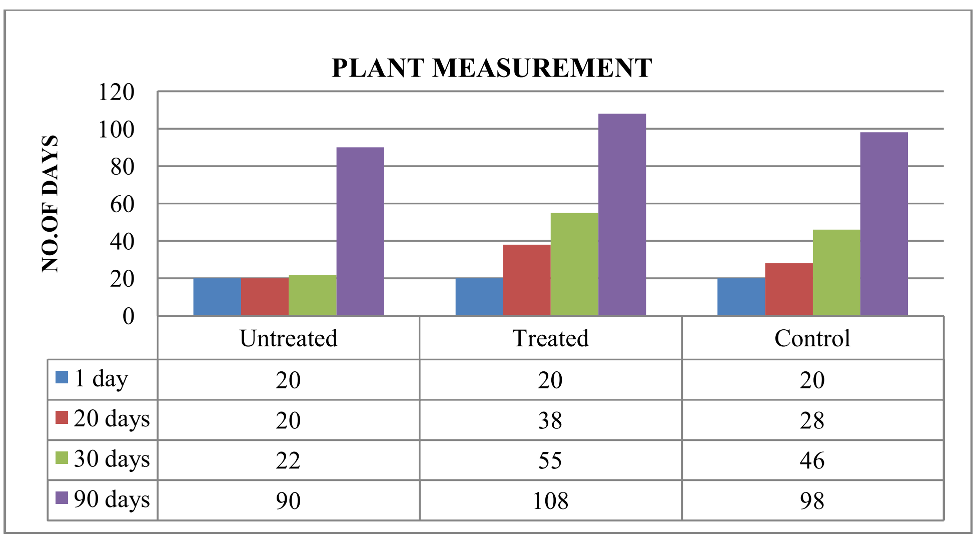

Fig. 1. Growth rate of *Hibiscus rosa-sinensis* in WFS is shown in bar diagram (in cm)
X_1= Untreated; X_2= Treated; X_3 = Control

Table 2. Represents the statistical analysis using ANOVA for growth rate of Hibiscus plants

Source of variance	Source of squares	D.O.F	Mean sum of squares	Variance ratio
Between column	800.22	3-1=2	MSC=800.22/2 =400.11	MSE/MSC =1414.11/400.11 =3.53
Error	8484.67	9-3=6	MSE=8484.67/6 =1414.11	

D.O.F = Degree of freedom; At 5% LOS (Level of significance); (6, 2) = 19.330 (Ft); As per calculation FC=3.53 Here FC<FT; Therefore 19330>3.53; Apply ANOVA for Fig. 1 considered X_1, X_2, and X3. The result consist of Null hypothesis is accepted

Table 3. Shows the results of metal extractions

Sample ID	Line	Concentration
150218-22-UT-1	Ca 393.366r	3.5067
150218-22-UT-1	Mg 279.553r	2.5739
150218-22-UT-1	Fe 259.90	0.1800
150218-22-UT-1	Zn 213.856	0.0199
150218-22-UT - 1	Pb 220.353	< 0.0000
150218-22-UT - 1	Cu 324.754	< 0.0000
150218-22-T – 1	Ca 393.366 r	2.4737
150218-22-T – 1	Mg 279.553 r	1.0914
150218-22-T – 1	Fe 259.940	< 0.0000
150218-22-T – 1	Zn 213.856	< 0.0000
150218-22-T – 1	Pb 220.353	< 0.0000
150218-22-T – 1	Cu 324.754	< 0.0000

UT-Untreated plant, T-Treated plant

3.6 Metal Extractions

The treated and untreated plants were analysed for the presence of metals absorbed and showed bioleaching had greatly reduced the levels of metals from the soil which intern had reduced the absorption by plants. Hence this will help in the safety of cultivation of ornamental plants nor can food crops in waste foundry soils be achieved in near future.

4. SUMMARY AND CONCLUSION

Foundry sand is used to make mold and cores. The waste generated from the industries cause environmental pollution and hence, the reuse of this waste material can be emphasized. Foundry sand is high quality silica sand that is a byproduct from the production of both ferrous and nonferrous metal casting industries. Waste foundry sand in this study was a by-product of metal foundries which contained heavy metals like Ca, Cr, Pb, Hg, Li and other toxic substances. Moisture content was found to be observed from 0.95-0.95. The pH of waste foundry sand was 9.09-9.10. Untreated WFS contained high content of Fe (76.36%), Ca (43.65%) and K (37.49%). WFS was bioleached the metal level content Fe (26.54%), Ca (27.67%) and K (5.84%). The samples were detected for the presence of metal degrading bacteria, *Actinomyces*. The *Hibiscus rosa-sinensis* sapling grew well under controlled conditions in bioleached WFS and control sand. The concentration of Fe in untreated soil was found to be 76% whereas after treatment with *Actinomyces,* it was rapidly reduced to 26.5%, similarly, K was 37.4% in untreated sand and 5.8% in treated sand, Ca was reduced from 43% to 27.6%, Mg was 28.5% and was reduced to 14.6%. In untreated sand, the plant leaves were small and growth was slow. The metal degrading ability of *Actinomyces* sp. helps in converting useless WFS sand to fertile soil rich to grow seeds or saplings. Our observations provide supportive document on bioleaching of WFS by *Actinomyces sp.* was adequate in the growth of *Hibiscus rosa-sinensis*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sethurajan M, Lens PN, Horn HA, Figueiredo LH, Van Hullebusch ED. Leaching and recovery of metals. In Sustainable Heavy Metal Remediation. 2017;161-206.
2. Franco-Correa M, Quintana A, Duque C, Suarez C, Rodríguez MX, Barea JM. Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. Applied Soil Ecology. 2010;45(3):209-17.
3. El-Tarabily KA. Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing. Streptomycete *Actinomycetes*. Plant and Soil. 2008; 308(1-2):161-74.
4. Torma AE. The role of *Thiobacillus ferrooxidans* in hydrometallurgical processes. In Advances in Biochemical Engineering, Springer, Berlin, Heidelberg. 1977;6:1-37.
5. Bosecker K. Bioleaching: metal solubilization by microorganisms. FEMS Microbiology Reviews. 1997;20(3-4):591-604.
6. Cappuccino JC, Sherman N. Microbiology: A laboratory manua Ed. Benjamin/ cummings Pub. Co., New York. 1992;125-179.
7. Dungan RS, Dees NH. The characterization of total and leachable metals in foundry molding sands. Journal of Environmental Management. 2009; 90(1):539-48.
8. Sethi N, Nath D, Singh RK. Teratological study of an indigenous antifertility medicine, *Hibiscus rosa-sinensis* in rats. Arogya J Health Sci. 1986;12:86-8.
9. Dwivedi RN, Pandey SP, Tripathi VJ. Role of Japapushpa in the treatment of arterial hypertension. A trial study. Journal of Research in Indian Medicine, Yoga and Homeopathy. 1977;12:13-36.
10. Kukier U, Chaney RL. In situ remediation of nickel phytotoxicity for different plant species. Journal of Plant Nutrition. 2004; 27(3):465-95.
11. Uden PC. Element-specific chromatographic detection by atomic emission spectroscopy. Washington, DC: American Chemical Society; 1992.
12. Guney Y, Sari YD, Yalcin M, Tuncan A, Donmez S. Re-usage of waste foundry sand in high-strength concrete. Waste Management. 2010;30(8-9):1705-13.

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