Microbial Treated Waste Foundry Sand and its Metal Leachate Analysis

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Abstract— *As the quantity of Waste Foundry Sand (WFS) is generated in huge amount, only a part of WFS is considered as the hazardous waste and the rest of the part is considered as non-hazardous industrial waste foundry sand. The leachate from WFS may contain hazardous compounds, which may probably effect the environment. The random release of heavy metals into the soil and waters is a major health alarm worldwide, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem. Leaching characteristics are essential in understanding the environmental impact or toxicity, dumping and potential development of beneficial applications of WFS. This study investigates that a trial was done to find out a fungal species from dumped site of a local foundry located at Coimbatore (Tamilnadu, India). Isolation of different fungal species from soil sample was carried out. All the isolated fungal species were then screened for their organic acid production by using HPLC. The molecular characterization of 18S rRNA for the isolated fungus was matched with R. oryzae. Physiochemical Characterization of WFS was done. Assessment of impacts of using fungal treated WFS its leachate quality was done using atomic absorption spectroscopy.*

Keywords— Waste Foundry Sand, Isolation, organic acid, molecular characterization, leachate.

I. INTRODUCTION

Increasing awareness on the environment has dramatically contributed to the concerns related with disposal of the wastes generated and discharged. Industrialization is considered to be a significant factor for the development of a country's economy. But the plants and factories from this industrialization process which discharges wastes and byproducts causes severe disastrous to the environment contaminating the surface water, ground water and soil. There are a number of reasons the waste are not safely treated [1]. Waste foundry sand (WFS) is one of such industrial by-product or considered as waste discarded material coming from ferrous and nonferrous metal-casting industry. It contains high content of silica sand with uniform physical characteristics which is used by the foundry industry to create metal casting molds [2]. WFS contain different heavy metal-leachate which needs to be treated before discharged. The leachate from such materials may contain dangerous compounds, which may possibly affect the atmosphere [3]. But with the scarcity of space for land filling and due to its ever increasing cost, waste utilization from these industries has become an attractive alternative to disposal. Research is being carried out on the utilization of waste products in concrete as a replacement of natural sand. Discarded tyres, plastic, glass, burnt foundry sand, and coal combustion byproducts were considered to be as such waste products, provides specific effect on the properties of fresh and hardened concrete. The use of waste products in concrete not only makes it economical, but also helps in reducing disposal problems. Thus the reuse of bulky wastes is considered as the best environmental alternative for solving the problem of disposal [1]. The high cost of land-filling and the potential uses of WFS in construction purposes have driven research into their beneficial reuse [2].

Bhat and Lovell [4] estimated that for every ton of metal castings produced and shipped that a typical foundry generates approximately one ton of waste sand. After molding is completed, the sand is discarded and generally land-filled. The rate of land-filling may vary from country to country. These costs are generally more in developed countries in measurement to developing and under-developed countries. According to Winkler and Bol'shakov [2] metal casting foundries in US, disposed of approximately 9 million metric tons of waste foundry sand in landfills. The annual cost of WFS disposal was around US$ 135–675 million which inclusive of storage, transportation and labor costs. This issue is gradually more addressed by alternate scenarios of beneficially reusing WFS. Thus WFS was considered to be a potential alternative in a range of applications including construction materials such as Controlled Low-Strength Material (CLSM) and concrete. According to Bhat and Lovell [4] foundry sand has become a viable candidate for use in Controlled Low Strength Materials because of cost effective, increasing availability, and satisfactory performance.

The use of recycled product has been increased worldwide due to conserving resources as well as reduction in fund available for the concrete of construction. The upcoming days make challenges for civil engineers for the utilization of the recycled solid waste and by-products for the basic properties of concrete and its materials. For high-performance construction materials microbial (bacteria/fungi) modified concrete has become an important area of research [2]. Fungi are ubiquitous in natural environment and more than 70,000 species of fungi have been described. Some estimates suggest that 1.5 million species may exist [5]. The filamentous fungi isolated from heavy metal contaminated soil such *Aspergillus* and *Pencillium* has high level of resistance to a number of metals which makes them attractive potential candidates for further investigations regarding their ability to remove metals from contaminated waste water [6]. Asokan et al. [7] reported that the microbial treatment of waste foundry sand was analyzed for its metal leachate property.

Mineralization was biologically induced and significant geological process. The use of microorganisms in the concrete leads to the process of biomineralization which is considered to be a potential field of research in concrete technology [8]. Bio mineralization is a general complex phenomenon by which organisms form minerals, occurring in diverse systems. The process creates heterogeneous materials composed of biologically organic and inorganic compounds like carbonate, phosphate, oxalate, iron, silica, or sulfur-containing minerals, with in homogeneous distributions that reflect the environment in which they form [9]. The objective of this study was to isolate and identify the fungal cultures from foundry sand and characterization of waste foundry sand. Mainly this study investigates metal leachate analysis of microbial modified WFS.

II. EXPERIMENTAL DESIGN

2.1 Materials

All chemicals (99% purity) used in this study were purchased from Hi-Media Laboratories (Mumbai, India). Waste foundry sand (WFS) sample for fungal isolation was collected in sterilized bags from the dumped site of a local foundry located at Coimbatore (Tamilnadu, India). Sieve analysis of all fine aggregates & coarse aggregate was carried in the laboratory.

2.2 Physical analysis of WFS (ASTM C128, 2001)

Physical analysis including specific gravity, density, fineness and absorption were determined by ASTM C128 [10]. Pycnometer was used to analyze the physical analysis. The specimen was dried at 110^{o} C. It was then allowed to cool and covered with water by immersion. Then it was allowed to stand for 24 hours. Excess water was decanted off. Sample was spreaded on non-absorbent surface. The pycnometer was partially filled with water. Immediately 500 g of saturated-surface-dry (SSD) specimen (WFS) was introduced. The weight of this SSD sample was placed in the pycnometer was recorded. The pycnometer was filled to 90% of its capacity. The pycnometer was rolled, inverted, and agitated to eliminate all air bubbles (this can take 15 to 20 min). The pycnometer was calibrated. The total weight of the pycnometer, specimen, and water was determined. Then the specimen was removed from the pycnometer and dried to constant weight in an oven at 110^{o} C for 1 hour, then cooled to room temperature, and weighed. The weight of the pycnometer filled to its calibration capacity with water was also determined. Calculations were made on the basis of the following equations:

Specific Gravity = A/ (B+S-C)

Bulk Specific Gravity = S/ (B+S-C)

Absorption (%) = 100 [(S-A)/A]

Where, A = weight of oven dried test sample in air, (in gms)

B = weight of pycnometer filled with water to calibration mark, (in gms)

S = weight of saturated surface dried (SSD) sample in air, (in gms) (prior to placement in pycnometer)

C = weight of pycnometer with test sample and water to calibration mark, (in gms)

2.3 Chemical analysis of WFS (Gurdeep 2013)

Chemical analysis of WFS was carried out by using Energy Dispersive spectroscopy (EDS). For this WFS sample was grinded and percent oxide forms of metals present in WFS samples were obtained [11].

2.4 Isolation of fungi (Warcup, 1950)

Soil samples were collected for the isolation of fungi. One gram was transferred to aliquots of 9 mL sterile distilled water in test tube. It was shaken vigorously at constant speed for 15 min and plated onto Potato Dextrose Agar (PDA) medium containing (component g/L) potato infusion (infusion from 200 g potatoes), 4; dextrose, 20; agar, 15. The soil suspension was then subjected to serial dilutions upto 10^{-6}. After sterilization, the medium was supplemented with 30 µg

streptomycin/ml (Himedia, Mumbai, India) to inhibit bacterial contamination. The medium was incubated at 35 °C for 72 h. One plate was kept as an uninoculated control. Pure colonies were isolated by subculturing on PDA. Stock cultures were maintained at 4°C. Colonies having zone formation were subcultured in potato dextrose broth. The spore morphology was determined by light microscopy [12].

2.5 Organic acid production by fungal cultures

Fungal cultures were analyzed for organic acid production by using High Performance Liquid Chromatography (HPLC) technique. For this, fungal cultures were allowed to grow in minimal medium for 15 days at 28^{o} C. Fungal cultures were inoculated in both minimal medium containing WFS and without WFS. After 15 days of incubation the samples were allowed to filter through ordinary filter paper and then the extracts were filtered through 0.45 micron filter assembly in sterilized vials. For HPLC analysis, samples were analyzed by using model Shimadzu LC 20AD HPLC. The mobile phase used for this was 100 mM Phosphate buffer at 2.5 at a flow rate of 0.5ml/min. The operation column for HPLC was C 18 column. Operation was carried out at temperature of 30^{o} C at 254 nm. Standard solutions of citric acid and oxalic acid were prepared along with samples.

2.6 Molecular Characterization

2.6.1 Extraction of Fungal Genomic DNA

Conidia of fungal isolates were inoculated in MEA broth and incubated at 25 °C in the dark. The fungal mycelium were harvested after 36 h of incubation and transferred into sterile freeze dry bottles. After 2 days of freeze dried, the dried mycelium was ground using liquid nitrogen into fine powders. In this study, extraction of DNA of fungal samples was performed using the fungal genomic DNA isolation kit RKT13 (Chromous Biotech, Bangalore).

2.6.2 Classic and molecular identification of fungal strain

The most powerful tool to identify the unknown microorganism is to sequence the gene (DNA) coding for 18S rRNA .This was carried out by standard protocols as follows (Chromous Biotech pvt Ltd, Bangalore, India) (William et al, 2000 and E O Wiley et al, 1991); DNA: 1 µl (100 ng), forward primer: 400 ng, reverse primer: 400 ng, dNTPs (2.5 mM each) 4 µl, 10× Taq DNA polymerase assay buffer 10 µl, Taq DNA polymerase enzyme (3 U/µl) 1 µl, water X µl to make up the total reaction volume: 100 µl. The following cycle times were set for the different processes: Initial denaturation at 94°C for 5 minutes, followed by a 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 mins, and a final extension at 72°C for 7 minutes. The PCR amplified product was subjected to 1.2% agarose gel (with ethidium bromide) electrophoresis for bp size analysis (Chromous Biotech, Bangalore).

2.6.3 Sequence and phylogenetic analysis

Sequencing of the PCR amplified product was performed on ABI 3500 × L Genetic Analyzer of Applied Bio system Micro Amp, USA, using cycle sequencing kit and using Big Dye Terminator Version 3.1. 10 µl of the sequencing analysis mixture contained 4 µl of Big Dye Terminator Ready Reaction Mix, 1 µl of PCR amplified product (100 ng/µl), 2 µl primer (10 pmol/λ) and 3 µl Milli-Q Water. Analysis conditions for sequencing was programmed to - denaturation at 96°C for 1 minutes, followed by 25 cycles of denaturation at 96°C for 10 seconds, hybridization at 50°C for 5 seconds and elongation at 60°C for 4 minutes. The resultant nucleotide sequence was analyzed using the software Seq Scape version 5.2, which follows a analysis protocol of BDTv3-KB-Denovo_v 5.2. Jukes–Cantor corrected distance model was used to generate a distance matrix. A minimum comparable position of 200 ignoring alignment insert was used. The phylogenetic tree was created using Weighbor with alphabet size 4 and length size 1000 using utilizing the sequences aligned with a system software aligner Seq Scape_v 5.2.

2.6.4 Metal leachate analysis (EPA, 1996)

For Fungal treatment, WFS was spread in plastic tray in specific manner (layers) for proper spread of fungal mycelium with optimized conditions done by previous study [7]. For this, Fungal treated WFS collected every 10 days interval was analyzed. For determining the total metal concentration in the soil samples, EPA method 3050B was used (EPA, 1996)[13]. This method is not a total digestion technique; instead it will give environmentally available metals. For the digestion of the samples a representative sample of 1 gram, dry weight, was mixed with 10 ml 1:1 nitric acid, heated on a hotplate located in a fume hood and refluxed for 15 minutes at a temperature of 95°C ± 50°C. This was followed by digestion of samples with repeated addition of 5 ml of concentrated nitric acid, which was added until no further reaction occurred with the nitric acid.

Absence of brown fumes from the solution indicates the completion of nitric acid digestion. Then the sample was digested with 30% hydrogen peroxide. Hydrogen peroxide was repeatedly added (1 ml each) to the sample until the sample appearance was unchanged. Finally, the sample was digested with 4 ml concentrated hydrochloric acid for 15 minutes. The digested sample was then filtered through Whatman No. 40 filter 28 paper and collected in a 100 ml volumetric flask and made up to 100 ml with distilled water. Proper dilutions of filtered sample were prepared and analyzed by Atomic Absorption (AA) Spectrometry (Shimadzu AA700). Digestion of samples were done in triplicate and performed under a hood to ensure safety.

2.7 Statistical analysis

The results of all experiments performed were expressed as Mean ± SD of three determinations, the test of significance was applied wherever necessary and values obtained as $p<0.05$ were considered as statistical significance.

III. RESULTS AND DISCUSSION

3.1 Physical analysis

Physical characteristics of WFS were analyzed and Table 1 presents the physical properties of waste foundry sand. Specific gravity was observed as 1.576 and bulk relative density was 1.574 kg/m^3. The pH of waste foundry sand was 9.87. The typical specific gravity of foundry sand varies between 2.39 and 2.55 and bulk relative density was 2589 kg/m^3 [14]. Dayton et al. [15] notified the specific gravity of WFS varies between 2.39 and 2.55. Obtained value (2.16) of specific gravity was ranged between the values as mentioned by Javed and Lovell [14]; Dayton et al.[15].

TABLE 1
PHYSICAL PROPERTIES OF WASTE FOUNDRY SAND

Physical properties	values
Specific gravity	1.576
Bulk relative density (kg/m^3)	1.574
pH at 30^o C	9.87

3.2 Chemical properties of WFS

Based on type of metal molded, type of binder and combustible used at the foundry, Chemical composition of the WFS is varied. Waste foundry sand was analyzed for percent metal oxides present in it. Table 2 presents data regarding the chemical composition of WFS. Silica and Calcium oxide shows the maximum value of 98300 and 26807 mg/L respectively. Titanium and sulphur was observed as 1% and 0.05 % respectively. Manganese was found to be negligible in waste foundry sand. Alumina (Al_2O_3), Magnesium Oxide (MgO), Sodium Oxide (Na_2O), Potassium Oxide (K_2O), Sulphur Trioxide (SO_4) Titanium Dioxide (TiO_2) and Manganese (Mn_3O_4) were found in little quantities. Earlier, Guney et al.[16], Etxeberria et al. [17], Siddique et al. [18] and American Foundry men Society[19] reported that percent silica was found to be higher than the rest of metal oxides. Results show iron oxide (3473 mg/L) is present in WFS, as the foundry sand obtained from the ferrous based foundry.

TABLE 2
CHEMICAL COMPOSITION OF WASTE FOUNDRY SAND

Constituents	Values
Silica (SiO2)	98300 mg/L
Alumina (Al2O3)	268 mg/L
Titanium Dioxide (TiO2)	1%
Calcium Oxide (CaO)	26807 mg/L
Magnesium Oxide (MgO)	3856 mg/L
Iron Oxide (Fe2O3)	3473 mg/L
Sodium Oxide (Na2O)	19901 mg/L
Potassium Oxide (K2O)	1447 mg/L
Sulphur Trioxide (SO4)	0.05 %
Manganese (Mn3O4)	0.01 %

3.3 Screening

A total of 5 strains were screened from the soil around a foundry on PDA for the production of organic acid. The cultures were inoculated on broth containing Bromocresol blue and incubated at 28 °C for 2-3 days. To investigate the production of organic acids, flasks containing PDB medium were inoculated with isolated cultures and sample were analyzed by high performance liquid chromatography (HPLC) after 15 days. Samples (Figure 1b, 1c, 1d, 1e and 1f) were compared with the standard peaks of citric acid and oxalic acid (Figure1a). Retention time, peak height and peak area of standard organic acids are shown in Table 3. It was observed from the HPLC data that major acid produced by isolates was oxalic acid followed by citric acid. These acids are good metal chelators which can form complex with metal ions. Organic acid is called chelator if it has two or more electron donor groups. Hence one or more rings are formed and then the organic acid can be termed as chelating agent and resulting complex is termed as metal chelator [20].

TABLE 3
RETENTION TIME, PEAK HEIGHT AND PEAK AREA OF STANDARD ORGANIC ACIDS

Organic acids	Retention Time (min)	Peak area (%)	Peak height (%)
Citric acid	12.35	84.87	90.06
Oxalic acid	24.50	12.06	5.87
Isolate 1	12.30	18.98	17.00
	24.09	36.94	12.71
Isolate 2	12.14	35.75	45.45
	23.80	48.55	28.67
Isolate 3	12.30	43.83	52.43
	24.56	37.59	19.44
Isolate 4	12.49	38.21	46.94
	25.25	40.25	22.05
Isolate 5	12.35	33.02	34.55
	24.18	20.24	9.61

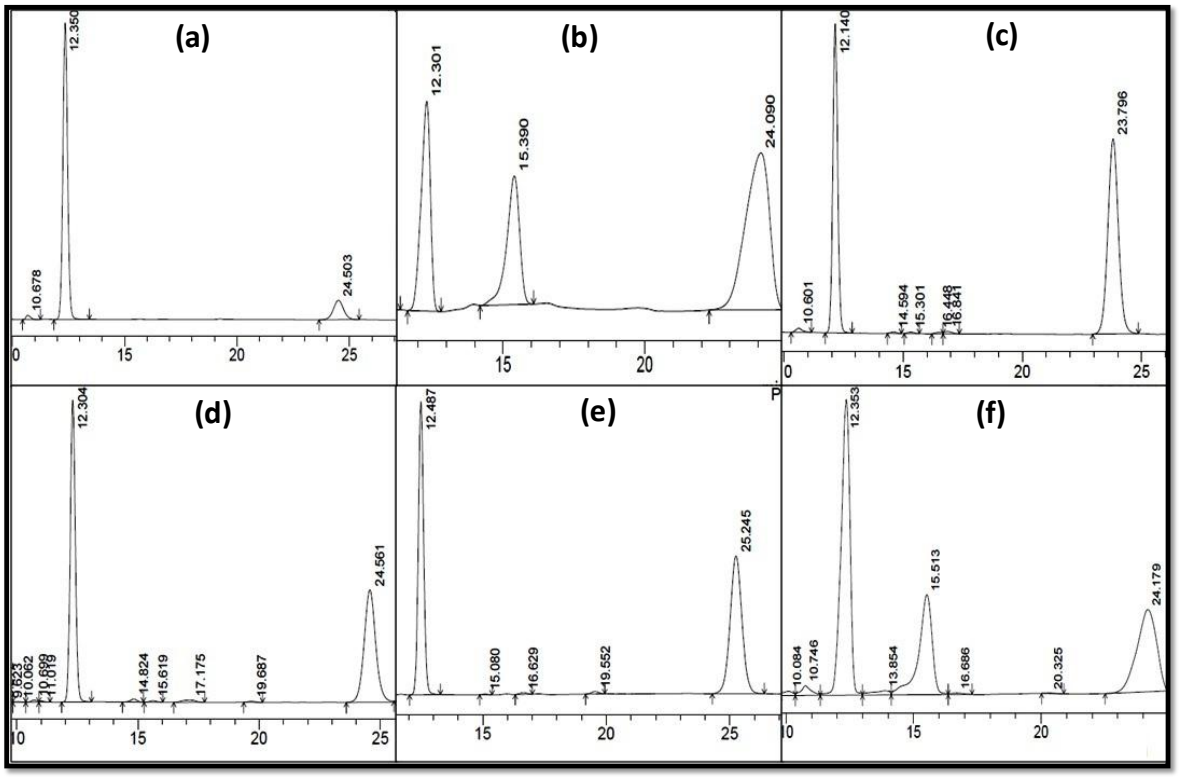

FIGURE 1 PEAKS SHOWN BY STANDARDS AND ISOLATED ORGANISM PRODUCED ORGANIC ACIDS BY HPLC (A):STANDARDS OF OXALIC AND CITRIC ACID ; B): ISOLATE 1; C): ISOLATE2; D):ISOLATE 3; E): ISOLATE 4; F):ISOLATE 5

3.4 Morphological Characteristics

Morphological features of fungal isolates of 3-5 days growth were studied using light microscopy after staining with lactophenol cotton blue. Lactophenol cotton blue stain was placed in the centre of a clean slide. A small clump of the fungus was transferred into the drop using an inoculation teasing needle and teased gently. Preparation was examined under high magnification for the presence of characteristic mycelia and fruiting structure whereas the conidiophores aggregations and pustles developed later. Figure 2 shows the morphological structure of the isolated fungi. The species encountered were identified in accordance with Cheesbrough [21]. The microscopical observations were found to be the similar with Kwon et al. [22].

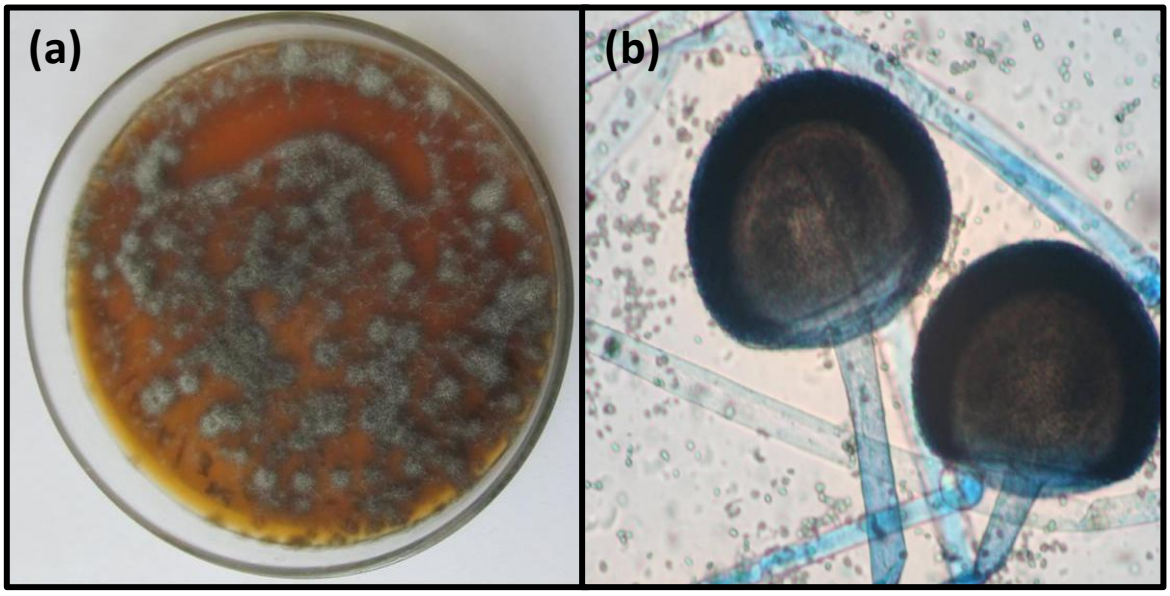

FIGURE 2. COLONY MORPHOLOGY AND MICROSCOPICAL OBSERVATION OF *R.ORYZAE*

3.5 Identification of fungal genome

Identification of genome was done 18s rRNA sequencing, and the sequence was obtained. This was blasted against the microbial genome database in National Center for Biotechnology Information (NCBI). The BLAST result showed only 98% similarity with other existing fungal species and thus was found to be *Rhizopus oryzae* (NCBI accession number: gb|KJ417528.1).Phylogenetic tree was constructed from neighbor-joining program, using bootstrap consensus test with 100 in Phylogenetic Tree Builder and the branch lengths are in the same as those of the evolutionary distances used to infer the phylogenetic tree. The isolated fungal strain was closely related to R. oryzae with 98% similarity. Based on this similarity the isolated new fungal strain was identified as a *Rhizopus oryzae*.

3.6 Metal leachate analysis

Soil was treated with isolated fungi *R. oryzae* with optimized conditions of 6% of fungal inoculum, 3% 0f waste foundry sand and 0.6% of additional nutrient (glucose) for 7 days. The total metal concentrations in the soils were measured using the conventional digestion procedure described in EPA Method 3050B [13] followed by analysis in an atomic absorption spectrophotometer. The analysis was carried out on WFS at regular interval of treatment with fungal mycelium to determine the concentration of six metals (Cu, Co, Fe, Mg, Ni and Zn). The concentration of each metal was detected by inductively coupled plasma mass spectrometry (ICP- MS). Table 4 presents a comparison of concentrations of critical trace metals in leachate from WFS of untreated and fungal (*R. oryzae*) treated. The leachate extracted from the treated WFS using Fungal contains the concentrations of heavy metal, the examined concentrations when compared to the untreated WFS. The heavy metal concentrations of Cu-94.5 mg/L, Co-0.5 mg/L, Fe-3473 mg/L, Mg-122669 mg/L, Ni-0.2 and Zn-103 mg/L are extremely high in their values from the leachate extracted from untreated WFS. It was also observed that the amount of metals measured at 30 days interval of fungal treatment in WFS showed to be with a reduction of than the value (Cu-<0.5 mg/L, Co-0.2 mg/L, Fe-741 mg/L, Mg-3856 mg/L, Ni-<0.02 and Zn-0.2 mg/L) obtained from control. These metals can be

harmful to human and animal health if found at prominent concentrations in the environment [23]. Decline of metal leaching is attributed due to the uptake or absorption of metals by fugal mycelium for its metabolic activity.

TABLE 4
METAL LEACHATE ANALYSIS

Metal	Control Soil	Fungal Treated WFS (10 days)	Fungal Treated WFS (20 days)	Fungal Treated WFS (30 days)
Cu	94.5	0.5	<0.5	<0.5
Co	0.5	0.2	0.2	<0.2
Fe	3473	1361	1015	741
Mg	122669	156852	79188	3856
Ni	0.2	0.2	0.16	<0.02
Zn	103	51	43.7	0.2

IV. CONCLUSION

Waste foundry sand is waste material from foundries which exhibits lower unit weight, higher water absorption and higher percentage of void compared to regular sand. This study aimed to present the utilization of fungal treated WFS in concrete (eco-friendly). The ability of producing organic acid from the fungi *R.oryzae* was selected by HPLC. From the previous optimization study concludes 6% of fungal inoculum, 3% 0f waste foundry sand and 0.6% of additional nutrient (glucose) gives maximum organic acid production in 7 days of incubation. Study also included leachate analysis obtained from the concrete mixes made with fungal treated WFS and untreated WFS. Results showed the metal concentration of Cu, Co, Fe, Mg, Ni and Zn were reduced to significant levels. Reduction of metal leaching is attributed due to the uptake or absorption of metals by fungal mycelium for its metabolic activity. Its shows the reduction in the metal concentration in leachate obtained from fungal treated concrete. The beneficial use of such by-products in construction materials results in reducing the cost of construction materials' ingredients and also helps in reducing disposal problem. It could be conveniently used in making good quality concrete, white ware bodies, construction materials, soil amendments, flowable fills and embankment. Strength properties of concrete mixtures increase with the increase in foundry sand content.

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