Bioremediation of Heavy Metals' using Mixed Bacterial Culture from contaminated Soil of Abandoned Kolar Gold Mine Residue

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***Abstract*: An attempt has been made in the laboratory to study the applicability of microbial culture for heavy metals removal from mining residues and simulated soil residue to develop bioremediation process. Firstly, the heavy metals concentration in the groundwater samples and the soil samples were analyzed. Later, mixed bacterial culture was prepared from the soil collected from the Kolar Gold Mine. To assess the heavy metals removal capacity of the bacteria, the mixed bacterial strains were added to water with the Initial concentrations of the heavy metals Cu, Cr, Ni, As and Pb and assessed for the uptake in 24 hrs and 48 hrs respectively. The initial concentrations used range is 100ppm for different heavy metals. Standard Experimental conditions were followed for this study. There was a significant uptake of heavy metals by indigenous bacteria after 24 hrs of incubation. The uptake or in other words removal was over 99% for all the metals considered. Further, the studies are contemplated on the heavy metals found in mining area soil samples. The groundwater and soil samples were collected from Kolar Gold Fields (KGF), also called as Kolar Schist Belt of South India, which were mined for several decades leaving behind huge deposits of residues and were assessed for heavy metals' contamination. The results showed high presence of heavy metals in the groundwater and soil samples from the depths of 1.00m of soil residue. Later, the soil was simulated with known concentrations for the selected heavy metals and was subjected to bioremediation process. Bioremediation studies were conducted by two techniques: (i) addition of only microorganism; (ii) addition of micro organisms and organic amendment. The simulated soil study showed that the two techniques use for Bioremediation was effective method for the reduction of heavy metals.**

Keywords: Mining Residue, Bioremediation, Mixed culture, Heavy metals, Organic Amendments

1. INTRODUCTION

Presence of heavy metals in soil has become a major environmental problem due to their potential of entering in to the food chain. Past evidences have shown that uncontrolled mining can lead to large and long lasting impact on environment. However localized impact can be highly visible, if the contamination has not been occurred often in the past due to which depression can occur over hundreds of kilometers. Modern gold mines discharge voluminous tailings that are formed due to extraction of small amounts of gold from large volumes of rock. Tailings are generally impounded behind large dams and when mining ceases, these impoundments are capped, revegitated and left as a permanent features of the landscape.

Gold was first mined in the area prior to the 2nd and 3rd century AD (golden objects found in Harappa and Mohenjo-daro have been traced to KGF through impurities - analysis - the impurities include 11% silver concentration, found only in KGF ore. The precious metal continued to be mined by the eleventh century kings of South India, the Vijayanagar Empire from 1336 to 1560 and later by Tipu Sultan, the king of Mysore state and the British. It is estimated that the total gold production from Kolar,Karnataka to date is 1000 tons. Renewed interest in the Kolar Gold fields occurred towards the end of the nineteenth century. During all these years of exploitation, tailings from the underground mines were dumped on the surface without any proper treatment. Around 32 millions tones of sand, which make up 15 mine dumps are spread out along 8km distance in the mine area. The impoundments were disposed off in slurry form comprising spent ore. Spent ore is mainly composed of quartz with lesses amount of calcite, feldspar, chlorite, mica, amphiboles and pyrite. The dumps are left exposed to the environment without any re-vegetation. Many technologies are currently used to clean up heavy metals contaminated soils. The most commonly used ones are soil removal and land filling, stabilization/solidification, phytoremediation, physic-chemical extraction, soil washing, flushing, in situ soil remediation techniques, such as excavation, transport, land filling of contaminated soils, acid leaching, chemical stabilization, and electro reclamation. None of these techniques are completely accepted because they are associated with high cost, low efficiency, temporary solutions and are environmentally destructive. Various microorganisms have a high affinity for heavy metals. Although the toxic metals remain in the soil, once they are bound to the microorganisms, the metals are less likely to be taken up by plants or animals living in the soil. So an attempt is done to clean up soil residue by bioremediation process. Bioremediation techniques use microorganisms to treat contaminants by degrading compounds to less toxic materials such as carbondioxide, methane, water and inorganic salts. An attempt has been made in the study the applicability of microbial culture for heavy metals removal from mining residues.

STUDY AREA

The KGF mines are spread in an area of about 20 sq. km, bounded by latitude 12°54'- 13°00 and longitudes 78°13'-78°17' in the Kolar District of Karnataka State, India.it is located 100 km east of banglore and about 300km west of Chennai (Figure 1). The study area is composed of hornblendic rocks bounded by granites and conglomerates and ferrugenous quartzites which include significant quantities of magnetite, ilmenite, graphite, pyrrhotite, chalcopyrite, spharelite, and arsenopyrite. The principal soil types include clay soil and loamy soil. The Kolar schist belt has been mined systematically for gold over ten decades. KGF mines occur in semi-arid region in southern plains of Karnataka. The region is characterized by annual rainfall of 740 mm spread over 45 days in a year. The day temperature over most part of the year is around 27° – 35 ° C. fig 1.1 shows thw study area kolar gold mine.

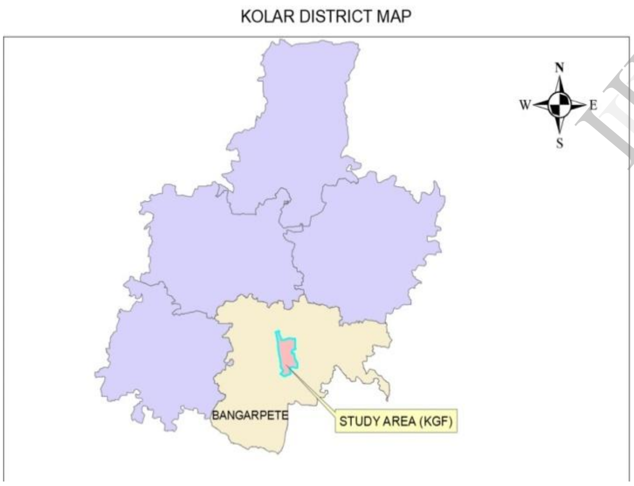

Fig1.1 : Kolar Gold Mine

GEOLOGY OF THE AREA

The eastern Dharwar Craton of southern India includes at least three schist belts. One of them is the Kolar Schist Belt, trending north-south. The width of the belt is about 4 - 6 km with the rocks having a general westerly dip. They are 2.7 Ga old, and have been alterated by mesothermal, quartz-carbonate vein gold mineralization. The host rocks are amphibolites and the ore veins are flanked by only a thin zone of biotitic alteration. It consists predominantly of tholeiitic amphibolites, in minor amounts komatiitic amphibolites (BIF), graphitic schists and felsic schists (Champion Gneiss). The gold-quartz vein mineralization is most intense in the central zone, along a tectonic contact zone between two suites of metavolcanics. All the above formations occur within the regional "Peninsular gneissic complex" and are metamorphosed. The longitudinal dolerite dykes which intrude the formations, are post-metamorphic in age. Pegmatites as flat and irregular fracture fillings are seen in the deeper parts of the mines. Diagonal faults trending NW-SE have dislocated the rock formations. The major faults are:- (i) The Balaghat north fault, which defines the northern limit of the ore bodies in Nundidoorg area. (ii) The Mysore north fault in the Champion Reef – Mysore Mine area and, (iii) The Gifford's system of faults in champion Reef Mine area.

2. MATERIALS AND METHODS

Standard procedure was adopted for the collection of groundwater and soil from study area.

CHARACTERIZATION OF THE GROUND WATER SAMPLES.

Surface water bodies were not to be found in the vicinity of the study area. Total 15 groundwater samples were collected from the study area. Standard procedure was adopted for the collection of groundwater samples in the study area. Groundwater samples were collected from the borewells and shafts of KGF.

BIOREMEDIATION STUDY WITH SIMULATED METAL-CONTAMINATED WATER SOLUTION

A trial test was conducted to find out the uptake of heavy metals by indigenous microbes based on ground water samples concentrations. A loop ful culture was inoculated in 250-ml conical flask containing heavy metals with a minimum concentration 100mg /L of Chromium, Copper, Nickel, Arsenic and Lead. The conical flasks were kept in rotator shaker for 24h and 48 h. Then the cells were harvested at mid-exponential growth phase by centrifugation at 4,000 rpm. The supernatant was tested for the heavy metal removal efficiency by the added indigenous bacteria by ICP method.

SOIL SAMPLE COLLECTION

The soil samples were collected from ten different locations of the contaminated site located at Kolar, Karnataka, India. The contaminated soil area of about 1m x 1m was marked, debris was cleaned and soil sample was collected using tools to a depth of 1m. The soil samples were collected in a thick quality self – locking polythene bag and transferred immediately to the laboratory and stored in the freezer.

PREPARATION OF SOIL SAMPLES AND CHARACTERIZATION

The samples were air dried for two days. The dried soil sample is then ground using mortar and pestle and sieved through 2mm mesh sized sieve. The sieved soil sample is then analysed for heavy metal concentrations. The soil was also characterized for the presence of heavy metals' such as Chromium, Copper, arsenic, cyanide, Nickle, manganese, Zink, Iron and Lead based on the presence of heavy metals in groundwater. The total heavy metal content in the soil was determined by acid digestion Method. The digested liquid was filtered and the filtrate was analyzed for the heavy metals content using an atomic absorption spectrophotometer. Pb, Ni, As, Cu, Zn, Cr were found to be in the higher concentrations and initially As, Ni, Pb, Cu and Cr were studied for the bacterial uptake in a simulated sample.

PREPARATION OF INDIGENOUS BACTERIAL CULTURE

Bacterial Strains were prepared from soil samples collected in and around the contaminated site. One gram of soil sample was taken for serial dilution. Different dilutions of extracts were inoculated in nutrient agar contained in Petri plates by using standard spread plate method. The inoculated plates were reversed and incubated at 38◦C for 48 h. The indigenous bacterial colony was used for bioremediation studies.

BIOREMEDIATION STUDY OF METAL-CONTAMINATED SOIL RESIDUE

In this study 5kg of soil was mixed with 50 mg/L concentration of copper sulphate solution, potassium di-chromate solution, nickel sulphate, Lead Nitrate and arsenic sulphate two set of reactors set in lab. Bioremediation studies were conducted by the following two techniques: (i) addition of only microorganism (ii) addition of organic amendment. Two set of reactors were set in lab to check the heavy metal reduction efficiency by indigenous bacteria and organic amendment. The reactors were added with 50mg/L concentrations of selected heavy metals individually and in combined form. The first set of reactors consisted of 5 kg of soil which were incubated with 1ml of indigenous bacteria. The bacteria's was supplemented with 1% of nutrient media and 1% of minimal media initially for their growth in each reactor. The second set of reactors consisted with 5kg of soil simulated with different heavy metals. The second set of reactors was inoculated with 5% of amendment (cowdung) only. Bacterial uptake of heavy metals in each of reactors was analyzed by collecting the samples from each reactor at an interval of 24hours for 5days.

3. RESULTS AND DISCUSSION

CHARACTERIZATION OF GROUNDWATER SAMPLES

The surface water bodies were not to be found in the study area. The ground water samples were collected from the bore wells and shafts that are present in the study area KGF. Most of the bore wells were dry and there were presence of water in the shafts which was stagnant. The groundwater samples results showed high concentration of heavy metals and Sodium adsorption ratio, % of sodium, EC results are as shown in table 1.1 respectively. Refer table 1.1.

TABLE 1.1: SAR, %NA, EC

Sl No	SAR	% Na	EC	Remarks
1	10.51	17.8	4875	Unsuitable
2	10.04	17.61	4640	Unsuitable
3	10.7	17.64	4980	Unsuitable
4	6.84	17.13	2050	Doubt full to unsuitable
5	12.08	19.52	5010	Unsuitable
6	8.99	18.78	2915	Doubt full to unsuitable
7	5.5	15.29	1680	Good to permissible
8	4.04	12.18	1500	Good to permissible
9	5.9	14.09	2280	Doubt full to unsuitable
10	6.78	16.04	2380	Doubt full to unsuitable
11	6.46	15.34	2325	Doubt full to unsuitable
12	5.92	18.17	1380	Good to permissible
13	5.6	16.73	1480	Good to permissible
14	5.3	14.78	1760	Good to permissible
15	5.25	17.67	1130	Good to permissible
SAR < 3 – Suitable 3-9 – Restriction >9 Unfit				

Most of the samples from the shafts have high SAR value which makes it unsuitable for irrigation purpose. But sample from borewells in and around mining area has relatively lower SAR value.

CHARACTERIZATION OF KOLAR GOLD MINE SOIL SAMPLES

The samples were air dried for two days. The dried soil sample is then ground using mortar and pestle and sieved through 2mm mesh sized sieve. The sieved soil sample is then analyzed for heavy metal concentrations. The soil was also characterized for the presence of heavy metals' such as Chromium, Copper, arsenic, cyanide, Nickle, manganese, Zink, Iron and Lead based on the presence of heavy metals in groundwater. The total heavy metal content in the soil was determined by acid digestion Method. The digested liquid was filtered and the filtrate was analyzed for the heavy metals content using an atomic absorption spectrophotometer. Table 1.2 shows the heavy metals concentration which was found to be very high and initially As, Ni, Pb, Cu and Cr were studied for the bacterial uptake in a simulated sample.

BIOREMEDIATION STUDY OF METAL-CONTAMINATED SOIL RESIDUE

In this study consist of 5 kg of soil and was mixed with 50 mg/L concentration of copper sulphate solution, potassium di-chromate solution, nickel sulphate, Lead Nitrate and arsenic sulphate. Bioremediation studies were conducted by the following two techniques: (i) addition of only microorganism (ii) addition of organic amendment. Two set of reactors were set in lab to check the heavy metal reduction efficiency by indigenous bacteria and organic amendment. The reactors were added with 50mg/L concentrations of selected heavy metals individually and in combined form. The first set of reactors consisted of 5 kg of soil which were incubated with 1ml of indigenous bacteria. The bacteria's was supplemented with 1% of nutrient media and 1% of minimal media initially for their growth in each reactor. The second set of reactors consisted with 5kg of soil simulated with different heavy metals. The second set of reactors was inoculated with 5% of amendment (cow dung) only. Table 1.3, 1.4, 1.5, 1.6, 1.7, 1.8 shows the bacterial uptake of heavy metals in each of reactors was analysed by collecting the samples from each reactor at an interval of 24hours for 5days. The results reveal that the bacterial uptake in mixed conditions is more than the individual conditions

TABLE 1.2: CHACTERIZATION OF K.G.F SOIL SAMPLE

Concentration in mg/L	24 hrs	48hrs	72hrs	96hrs	120hrs
Chromium	11.304	10.741	10.428	8.86	6.686
Copper	0	0	0	0	0
Nickel	3.66	0.21	0.157	0.132	0.112
Lead	0	0	0	0	0

TABLE 1.3: BACTERIAL UPTAKE OF INDIVIDUAL HEAVY METALS BY INDIGENIOUS BACTERIA

Concn in mg/l	24 hrs	48hrs	72hrs	96hrs	120hrs
Chromium	0	0	0	0	0
Copper	0.036	0	0	0	0
Nickel	0.442	0.122	0.059	0.051	0.047
Lead	0.001	0	0	0	0

TABLE 1.4: BACTERIAL UPTAKE OF HEAVY METALS WITH AMMENDMENT

S.No	pH	Zn	Fe	Cu	Mn	Cr	As	Cn	Ni
FAO STD (mg/kg)	**6-8.**	**150-200**	**3500-4000**	**500-550**	**150-200**	**0.1**	**50-55**	**0.01**	**0.1**
SS1	8.24	1435	8156	1734	109	--	4826	--	1800
SS2	8.14	1368	9652	2123	203	--	4367	--	1876
SS3	8.02	1283	8029	1903	1364	--	3876	--	1860
SS4	4.01	2044	2352	1111	2637	45	5396	--	1100
SS5	3.39	685	115	2583	141	--	3826	--	1870
SS6	3.68	28	2376	2141	2632	--	3987	--	1900
SS7	6.02	1185	9075	2828	1617	158	4286	--	1213
SS8	6.15	902	225	119	4228	--	2146	--	1730
SS9	6.2	104	1937	112	3944	--	2010	--	1692
SS10	6.1	904	2038	1019	4323	--	2190	--	1772

TABLE 1.5: BACTERIAL UPTAKE OF HEAVY METALS IN MIXED CONDITION BY INDIGENIOUS BACTERIA

Concn mg/L	24 hours	48 hours	72 hours	96 hours	120 hours
Chromium	9.934	6.667	4.149	3.981	3.504
Copper	0	0	0	0	0
Nickel	0.269	0.072	0.061	0.06	0.058
Lead	0	0	0	0	0

TABLE 1.6: BACTERIAL UPTAKE IN MIXED CONDITION (INDIGENIOUS- KGF)

Concn in mg/l	24 hrs	48hrs	72hrs	96hrs	120hrs
Chromium	0.673	0.524	0.318	0.254	0.087
Copper	0	0	0	0	0
Nickel	1.579	0.088	0.067	0.061	0.054
Lead	0.04	0	0	0	0

TABLE 1.7: BACTERIAL UPTAKE IN MIXED CONDITION (AMMENDMENT)

Concentration in mg/l	24 hrs	48hrs	72hrs	96hrs	120hrs
Chromium	0.037	0.034	0.017	0	0
Copper	0.007	0	0	0	0
Nickel	1.749	1.599	1.2	1.105	1.028
Lead	0.042	0.038	0.017	0.014	0.012

TABLE 1.8: BACTERIAL UPTAKE IN MIXED CONDITION (KGF - AMMENDMENT)

Concn in mg/l	24 hrs	48hrs	72hrs	96hrs	120hrs
Chromium	0.004	0	0	0	0
Copper	0	0	0	0	0
Nickel	0.542	0.522	0.421	0.391	0.329
Lead	0.04	0.035	0.033	0.033	0.033

CONCLUSIONS

1. Bacterial uptake of copper and lead was 100% in 24 hours. Bacterial uptake of chromium and nickel were found to be 77.4% and 92.68% respectively in 24 hours, however at end of 120 hours the concentration of chromium and nickel was reduced to 86.6% and 99.7% respectively as depicted in table 4.
2. The second set of reactors which was amended with 5% cow dung. Bacterial uptake of lead was 100% in 24 hours. Bacterial uptake of chromium and nickel were found to be 80.132% and 99.46% respectively in 24 hours, however at to 92.99% and 99.88% respectively. This shows the bacterial uptake was much efficient in amended conditions.
3. Bacterial uptake in KGF soil was more efficient as it achieved 99% in initial 24hours whereas in simulated soil 97% was achieved.
4. The results reveal that the bacterial uptake of chromium in mixed conditions is more than the individual conditions. This is because the bacteria might have used the other heavy metals and its compounds as additional nutrient for their growth and have uptake chromium much more efficiently

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