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BIOREMEDIATION OF HEAVY METAL NICKEL IN SOIL USING NATIVE STRAINS OF BACTERIA

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ABSTRACT

The appraisal of the study was to investigate the bioremediation potential of bacteria on heavy metal nickel (Ni) in soil. Treated sewage water and soil samples were collected and heavy metal analysis showed increased levels of Ni in the soil. Nickel tolerant bacteria were identified from treated sewage water irrigated soil samples and were characterized based on morphological, biochemical and DNA sequencing protocols. An initial study in nutrient broth exhibited their nickel reduction properties. The consortium of bacteria was then subjected to a soil study along with amendments such as vermicompost and biochar. The nickel removal by the bacterial consortium under sterile and non sterile soil conditions was studied. The results revealed that application of the consortium along with biochar as an amendment increased the removal of nickel from the soil by 36.5 % in non sterile condition than in sterile condition (27.10%). Thus from this study it is evident that application of bacterial consortium along with organic amendments such as biochar could effectively reduce heavy metal concentration in soils and render them safe and useful for agricultural activities.

KEY WORDS
Sewage water
Soil
Nickel, Bioremediation; Amendments

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INTRODUCTION

Environment pollution is a byproduct of development and in fact a price for progress. The increasing world population and annual metal usage per capita both lead inevitably to ecological problems because of wide dispersal of potentially toxic metals into the natural environment (Sherene, 2012). Naturally heavy metals are available in soil, water and living organisms in a minimum quantity and they are crucial for healthy life. Some of these metals like Zn, Cu, Mn, Ni, and Co are micronutrients which necessitate the plant growth, while others have unknown biological function (Akpor and Muchie, 2010). As these metals pass from one trophic level to the next higher one, they are concentrated and may become a dominating and sinister stress at a considerable ecological distance from the point or trophic level of its initial introduction into the biosphere (Roy, 2010). Nickel is one among the many trace metals widely distributed in the environment, being released from both natural and anthropogenic activity. Its corrosion resistance, better toughness, better strength at high and low temperatures and a range of special magnetic and electronic properties make it more applicable in industries. Nickel above its permissible limit is a human carcinogen causing allergy, tumor, respiratory cancer, nasopharyngeal carcinoma, chronic bronchitis, etc. In the aquatic ecosystems, heavy metals are prominently known to accumulate in fishes like *Channa Punctatus* (Bloch). In a study by Mahananda et al. (2013), the fishes were exposed to different concentrations of nickel and at 40 ppm, it lead to significant, progressive accumulation in the various tissues of the fish (Gill> Ovary> Muscle> Kidney> Liver> Testis). Upon soil accumulation, it adversely affects the soil ecology, agricultural production quality, ground water quality which in turn results in increased uptake by plants ultimately affecting animals and humans. These metals can enter the soil environment from a variety of sources like industrial wastes, fertilizers, vehicle emission, domestic and urban wastes (Wuana and Okieimen, 2011). Jorgensen (1979) reported the global emissions of Pb and Ni into atmosphere, water and soil. Singh *et al*. (2000) indicated that higher concentrations of Pb and Ni in ground water may be due to various galvanizing, electroplating and other industries which surely reached upto underground sources due to leaching from dumping sites. Rattan et al. (2005) reported the buildup of DTPA- extractable Zn (208%), Cu (170%), Fe (170%), Ni (63%) and Pb (29%) in soils irrigated with sewage for 20 years. Similarly, sewage- irrigated land recorded significantly higher concentrations of heavy metals (Cr, Ni, Pb and Cd) than bore well water- irrigated land due to gradual accumulation of heavy metals in sewage- irrigated soils (Salakinkop and Hunshal, 2014). Heavy metals occur in typical background concentrations in the ecosystems. However anthropogenic releases can result in higher concentrations of these metals relative to their normal background values. When these happen, heavy metals are considered serious pollutants because of their toxicity, persistence and nondegradable conditions in the environment, thereby constituting threat to human beings and other forms of biological life (Tam and Wong, 2000; Yuan *et al*., 2004; Nwuche and Ugoji, 2008; Aina *et al*., 2009; Mohiuddin *et al*., 2010).

The threat of heavy metal pollution to public health and wildlife has led to an increased interest in developing systems that can remove or neutralize its toxic effects in soil, sediments and wastewater. Unlike organic contaminants, which can be degraded to harmless chemical species, heavy metals cannot be destroyed. Remediating the pollution they cause can therefore only be envisioned as their immobilization in a non-bioavailable form, or their re-speciation into less toxic forms. Microorganisms have been successfully exploited to deal with heavy metal pollution in a variety of schemes (Valls and Lorenzo, 2002). The goal of microbial remediation of heavy metal contaminated soils and sediments are to immobilize the metal insitu or to remove the metal from the soil. Metal remediation strategies using microorganisms can minimize bioavailability and biotoxicity of heavy metals (Gadd, 2000). There are a number of microorganisms that can be used to remove metal from the environment, such as bacteria, fungi, yeast and algae (Davis *et al*., 2000). Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards. Husain et al. (2013) showed that bacterial biosorbents can be used for heavy metal removal. 95 % nickel uptake upto 1000 ppm concentration was recorded when *Pseudomonas fluorescence* cells were subjected for bioremoval of nickel ions. In a study by Basha and Rajaganesh (2014) four microbial strains viz., *Escherichia coli*, *Salmonella typhi*, *Bacillus lichenformis* and *Pseudomonas fluorescence* reduced maximum of 98.34% of Cd, 94.83% of Pb and 96.14 % Zn from the effluent samples. Similarly, in a work on identification of heavy metal tolerant bacteria from the Panteka stream, four bacterial strains were isolated and identified to be *Staphylococcus epidermidis, Serratia marcescens, Proteus mirabilis* and *Escherichia coli* which proved to be effective in removing lead (100%), nickel (100%), cadmium (90.29%), zinc (84.95%) and iron (54.82%) (Nwagwu *et al*., 2017). In view of such successful studies on the use of bacteria for the removal of heavy metals from wastewaters, the present study is focused on the application of one such technique for the bioremediation of heavy metal nickel (Ni) from contaminated soils. The objective of the current work was to study the potential of heavy metal tolerant bacteria in remediation of nickel contaminated soil substrates through a laboratory scale experiment.

MATERIALS AND METHODS

Sampling location and collection

The treated sewage water and treated sewage water irrigated soil samples were collected from The Municipal Sewage Treatment Plant located at Avaniyapuram, Madurai, Tamil Nadu (Fig.1).

Treated sewage water and treated sewage water irrigated soil samples were collected from the Municipal Sewage Treatment Plant situated at Avaniyapuram, Madurai district, Tamil Nadu which has a treatment capacity of 125MLD. The treated wastewater is being used for irrigating gardens established with flowering crops and fruit trees such as banana, sapota, moringaa and other shade trees such as pungam, etc. The samples were collected in polypropylene containers rinsed with distilled and sampling water and stored at 4°C for further analysis. The soil samples were collected from 0-15 cm depth (surface) from 4 locations where continuous irrigation using treated sewage water was done and crops such as neem, pungam and Cumbu Napier grass (var. CO2) were raised. A separate set of soil samples (1 kg) was collected in air tight polyethylene bags for microbial isolation purposes. It was stored in 4ºC for further use.

Physicochemical characterization of treated sewage water and treated sewage water irrigated soil samples

Characterization of water samples was done by following the APHA standard methods (2005) and the soil samples were analysed for various parameters such as pH and EC (Jackson 1973), Organic Carbon (Walkley and Black, 1934) and Heavy metals (Ni, Cr, Pb and Cd) (USEPA. 1979) (Table 1&2).

Isolation of bacteria and determination of Maximum Tolerance Limit (MTL)

The bacteria from the heavy metal contaminated soils were isolated through serial dilution technique recommended by Prescott *et al.* (2002). The bacterial tolerance to nickel was done by assessing the Maximum Tolerance Limit (MTL) (Hookoom and Puchooa, 2013). The isolated bacteria were subjected to various concentrations of nickel (as $NiSO_4$). Three out of ten bacteria showed tolerance to nickel up to 150ppm. They were subjected for removal studies.

Molecular analysis

Isolates showing high tolerance to nickel were sent for 16S rDNA analysis to Hyderabad, Andhra Pradesh for molecular analysis.

Nickel removal by bacteria in soil

To study the nickel removal in soil by the bacterial isolates, the procedure followed in Ghazali et al. (2004) was followed with the few modifications. The experiment soil was divided into two batches. One batch was autoclaved (sterilized) at 121ºC for 30 min twice (24 h apart) to remove the indigenous microbial population. The other batch was left non-sterilized. The soil was spiked with $NiSO_4$ at the concentration of 150

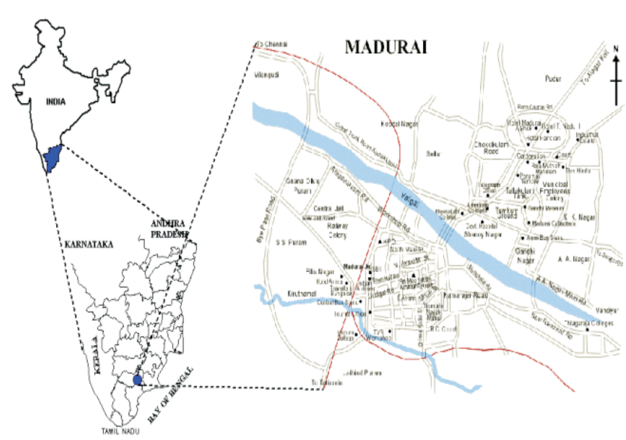

Figure 1: Sampling Location

mg kg^{-1} dry weight soil. 10% (v/w) culture inoculum was introduced into the soil in liquid form. As controls one set of sterile soil and non- sterile soil were left un-inoculated. Organic amendments such as vermicompost and biochar were added to the soil based on their recommended dose (5t/ha and 2t/ha respectively).

Two microbial inoculants were chosen based on the results obtained in broth study. In order to obtain a standard inoculum, individual bacteria and the prepared consortia were grown for 12-18 h in nutrient broth at 37ºC in an orbital shaker at 150 rpm. The absorbance reading was adjusted to 0.5 at 540nm. When used as an inoculum at 10% (v/w) the resulting colony forming unit (cfu)/ g of soil was between 4.9 to 5.2 x 10^6 and 8.2 to 8.5 x10^6 cfu /g.

The study was conducted at room temperature and the moisture content was maintained according to the water holding capacity (field moisture capacity) of the soil. Monitoring of pH, EC, organic carbon, microbial count and heavy metal content was performed on days 0, 5, 10, 15, 20, 25 and 30. To monitor cell numbers, 1 g of soil sample was taken and resuspended in 10 mL of sterilized distilled water. Serial dilutions were made and 0.1 mL of 10^{-6} and 10^{-7} dilutions were plated for CFU counts.

RESULTS AND DISCUSSION

Physico-chemical characterization of treated sewage water

The treated sewage water collected from Avaniyapuram, Madurai district was analysed for its physico-chemical properties (Table 1). It showed an alkaline pH of 9.08 and an electrical conductivity of 2.36 dSm^{-1}. BOD and COD were found to be 360 and 690 mg L^{-1} respectively. High BOD reflects the high organic load in the water.

Physico-chemical characterization of treated sewage water irrigated soils

The physico-chemical properties of the soils are enlisted in Table 2. The soils collected from treated sewage water irrigated lands were alkaline and saline in nature. Long term irrigation with sewage water had led to the buildup of organic carbon in the soils (0.97 %). Next to soil pH, Soil Organic Carbon (SOC) is the most important indicator of soil health. Moreover, intense cultivation of fodder grass and other crops in the lands had restored the soils as crops continuously add plant residues and litter to the soil which is considered as a significant resource of soil organic matter. Similarly, the Cation Exchange Capacity (CEC) of the soils was found to be 15.37 meq/ 100g of soil which were in correlation with the high concentration of soil organic matter. Heavy metal analysis revealed that nickel (Ni)

Table 1: Characteristics of treated sewage water sample

S.No	Parameters	Unit	Treated sewage water	References
I.	Physico-chemical properties			
1.	pH	-	9.08	Jackson (1973)
2.	EC	dSm^{-1}	2.36	Jackson (1973)
3.	BOD	mg L^{-1}	360	Anon, 1989
4.	COD	mg L^{-1}	690	Anon, 1989
5.	Na	meq L^{-1}	1.74	Jackson (1973)
6.	K	meq L^{-1}	0.69	Jackson (1973)
7.	Ca	meq L^{-1}	6.10	Jackson (1973)
8.	Mg	meq L^{-1}	1.70	Jackson (1973)
9.	CO_3	meq L^{-1}	0.67	Piper (1966)
10.	HCO_3	meq L^{-1}	3.85	Piper (1966)
11.	Cl	meq L^{-1}	16.93	Jackson (1973)
12.	SO_4	meq L^{-1}	11.64	Jackson (1973)
13.	Nickel	mg L^{-1}	37.2	USEPA (1979)
14.	Chromium	mg L^{-1}	3.42	USEPA (1979)
15.	Cadmium	mg L^{-1}	BDL*	USEPA (1979)
16.	Lead	mg L^{-1}	BDL*	USEPA (1979)
II.	Biological properties:			
17.	Bacteria	x10^6 cfu/ mL	34	Waksman and Fred (1922)
18.	Fungi	x10^4 cfu/ mL	27	Waksman and Fred (1922)
19.	Actinomycetes	x10^3 cfu/ mL	8	Waksman and Fred (1922)

Table 2: Physico-chemical properties of treated sewage water irrigated soil samples

Parameters	Sampling location				Mean	References
	S1	S2	S3	S4		
pH	8.59	9.06	8.51	8.15	8.58	Jackson (1973)
EC (dSm^{-1})	2.04	2.11	2.07	2.75	2.24	Jackson (1973)
Organic Carbon (%)	0.26	0.77	2.10	0.74	0.97	Walkley and Black (1934)
CEC (meq/ 100 g of soil)	19.56	11.04	12.84	17.9	15.34	Saxena *et al.* (1978)
Nickel (Ni) (mg/kg)	176.4	180.5	153.8	179.4	172.5	USEPA (1979)
Chromium (Cr) (mg/kg)	12.8	19.1	14.7	16.2	15.7	USEPA (1979)

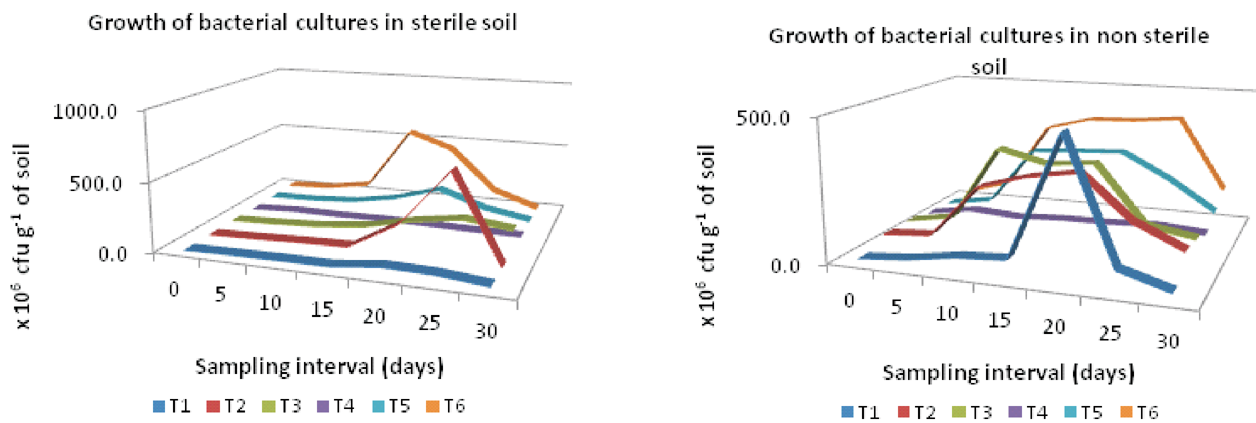

Figure 2: Growth of bacterial cultures during nickel removal

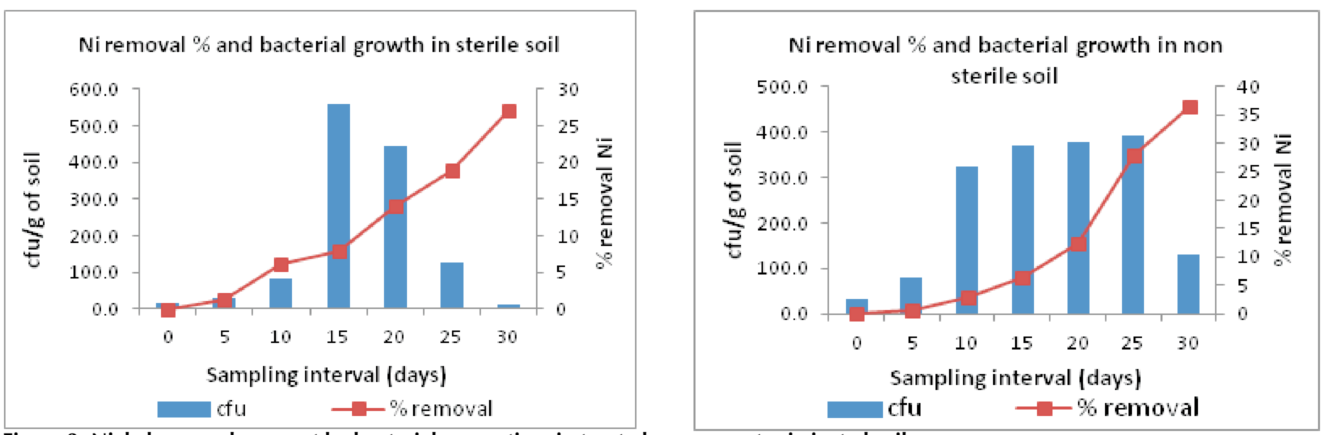

Figure 3: Nickel removal per cent by bacterial consortium in treated sewage water irrigated soils

content was above the permissible limit of 100 mg/kg for soils (172.5 mg/kg), which might be due to the accumulation over a period of time.

Ni removal in soil

Growth of bacterial cultures during nickel removal

The bacterial count was found increased until the 25th day and decreased thereafter in all the treatments. Giller *et al*. (2009) postulated that increasing metal stress in soils may lead to an increase or decrease in microbial diversity, depending on the initial state of the system. Similarly, decrease in the bacterial count in the soil after 25th day may also be contributed to depletion of nutrients in the substrate. In soils amended with biochar + consortium (T_6) the bacterial count was found to be increasing till the 15th day and then decreased by the 30th day in both sterile and non sterile soils. Simultaneously, in sterile soil + biochar (T_4 control), the mean bacterial count was only 11.32 x 10^6 cfu/ g of soil whereas in sterile soil + biochar + consortium (T_6), it was 181.28 x 10^6 cfu/ g of soil. This shows the abundance of the inoculated bacteria in the soil. Similarly in non sterile soil + biochar (T_4) showed 52.76 x 10^6 cfu/ g of soil whereas non sterile soil + biochar + inoculum 7 showed 243.05 x 10^6 cfu/ g of soil. Thus increased bacterial count in soils treated with biochar as an amendment and consortium was observed by 78% in sterile soils and 93% in non sterile soils on comparison with control (Fig. 2). The bacteria took time to establish i.e. low population until 5-10 days in the soil. The extended lag phase of growth due to Ni stress might be attributed to the requirement of time for buffering the stress. Similar results have been documented by Das *et al*. (2014). Many studies show an increase in microbial abundance in soil on addition of organic amendments. Improved microbial abundance after addition of biochar to the soil is reported by Abujabhah *et al*. (2016), Pandian *et al*. (2016) which correlate with the results from present findings.

Nickel removal in soils

The microbial inoculum was seen to have a significant effect ($p < 0.05$) on the nickel removal in both sterile and non-sterile soils. The soils were spiked with 150mg of nickel per kg of soil, and on the 0th day a mean of 150 mg/kg of nickel was found in all treatment soils (both sterile and non sterile). The highest nickel removal per cent was obtained in T_6: Soil + Biochar + inoculum 7 in both sterile and non sterile soil (27.10 % and 36.50 % respectively) (Figure 3). Nickel removal per cent was 9.4% higher in non sterile soil than in sterile soil. This indicates that the consortia of bacteria works well as a bioaugmentor rather than a inoculant since its efficacy in nickel removal is more in non sterile soil where a least microbial count is already present. In a similar work by Samal and Kotiyal

(2013), copper resistant bacteria were isolated from an industrial area and subjected for bioremediation studies. Out of the 10 bacterial isolates, 2 strains showed considerable reductions in the copper levels in the soil under study. Heavy metal bioremediation by a multi-metal resistant endophytic bacteria L14 (EB L14) isolated from the cadmium hyperaccumulator *Solanum nigrum* L. showed uptake of 75.78%, 80.48%, 21.25% of Cd (II), Pb (II) and Cu (II) under the initial concentration of 10 mg/L (Guo et al., 2010). Biosorption of Ni and Cd studies over a range of metal ion concentrations with *Escherichia coli* WS11 both in single and bi-metal systems showed that after 2 h of incubation in a single metal solution the biosorption of Ni increased from 6.96 to 55.31 mg/g of cells (Ansari and Malik, 2007). And also, the consortium remediates heavy metal nickel more in the presence of biochar than vermicompost. The porous structure of biochar gives high internal surface area which aids in adsorption of heavy metals. Abundant surface functional groups (mainly oxygen containing groups, -COOH, -OH) exist on the surface of biochars, which have strong interactions with heavy metals such as electrostatic attraction, ion-exchange and surface complexation. These effects can be evidenced by the changes in functional groups of biochar before and after the metal adsorption (Khare *et al*. 2013). In addition, the mineral components of biochar play a crucial role in the adsorption process. Biochars have various magnitudes of surface areas and pores within the particles, which enable them easily accessible to metals (Khare *et al*. 2013). Another contribution to Ni removal in soils is the amendments added. On comparision between amendments applied to the soil, higher nickel removal from the soil has taken place in soil with biochar as amendment. In both sterile and non sterile soil, the second best removal of heavy metal has taken place in soil amended with vermicompost. Thus, both biochar and vermicompost have a significant impact on heavy metal removal from the soil, but the rate of Ni removal in soils amended with biochar is 0.93% and 2.54% higher than in soils applied with vermicompost. Similarly, a decrease in bacterial population is seen after 25th day in the soils whereas the nickel removal per cent keeps increasing. From this it can be inferred that, the bacterial inoculum viably reduced nickel only until 25th day in non sterile soils. The removal of nickel later on in the soil might be due to the amendments added to the soil. Thus, application of consortia with biochar as an amendment to the soil at the recommended dose was found to reduce the heavy metal content of the soil up to 36.5%.

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