Isolation And Characterization of Heavy Metal Resistant Bacteria From Petroleum Soil of Kanpur Region

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Abstract- *Isolation and identification of heavy metal resistant bacteria from petroleum soil in Kanpur region, determination of the heavy metal resistant bacteria. Heavy metal resistant bacteria were isolated from the soil samples of petroleum storage site and the samples were processed by serial dilution method on Nutrient Agar . Then bacteria were identified on the basis of morphology by Gram staining preparation. On Nutrient Agar Plates P. fluorescence produced initially white color colony and later appears slightly yellowish. When the plates were placed inside UV light it produces greenish color florescence. The growth of the bacteria was observed in the maximum concentration respectively of Calcium, Manganese, Lead, Mercury . Such strains of bacteria can be useful in the remediation of heavy metals in soil and water. The results of inhibitory activity of different antibiotics on the bacteria strain will also be presented.*

Keywords- Heavy metals, Pseudomonas fluorescence, Ultra violet

I. INTRODUCTION

Heavy metal pollution of soil is one of the great consequences of industrialization in the sector of mining, petroleum refining, automobiles, paints etc. [(2)] The associated anthropogenic have often resulted in environmental pollution. Heavy metals such as Cu, Ni, Zn, Pb, Cr, Hg, Cd, etc. are prominent components of industrial effluents which are discharged into the soil, consequently polluting the ecosystem [(5)]. The presence of these heavy metals in the soil has been a subject of great concern due their toxicity and non – biodegradable nature.

Bacteria are recognized for their superior ability to produce a wide variety of extracellular proteins, organic acids and enzymes etc. Their waste biomass may be used as effective bio sorbent for removal, reduction, and detoxification of industrial effluents. However, these effluents contain high concentrations of heavy metals which may enter into human and animal's population through food chain, resulting in many metabolic disorders in the affected person [(3)]. Therefore, it is necessary to remove the heavy metals from soil and wastewater through low cost technology such as reverse osmosis, solvent extraction, lime coagulation, ion exchange etc. Chemical precipitations for removal of heavy metals are inefficient or very expensive especially when the concentration of the heavy metal ion is low in the range of 1 mg/l to 100 [(2)]. The heavy metals are non- biodegradable pollutants and for this reason they can be transformed only through absorption, methylation, complexion and changes in valency state. These transformations affect the mobility and bioavailability of metals. At low concentrations, metals can serve as important components in life processes, often serving important functions as co-factor of the enzymes for better enzyme activity. However, such metals at certain threshold concentrations may certainly be toxic to the biological system.

Introduction of heavy metal compounds into the environment generally induces morphological, cytological and physiological changes in the microbial communities. This exerts a selective pressure on the microorganisms. Generally, the sites contaminated with heavy metals are the sources of metal resistant microorganisms. Fortunately, microorganisms can affect the reactivity and mobility of metals and thus can be used to detoxify some metals and can be used to detoxify some metals preventing further metal contamination. There is a wide variety of microorganisms (fungi, yeast, algae, bacteria, etc.) which are capable of uptaking pollutant. *E.coli* has a great potential for removing Ni and Cu especially under low concentrations of these metals in the external environment.[(1)] Removal of heavy metal ions using bioaccumulation is a promising technology. As a result, the interest in this area has grown substantially during recent years. Removal of heavy metals contamination by using bacteria, fungi, algae and yeasts has been reported by several workers.

Certain species Staphylococcus, Bacillus, Pseudomonas, Citrobacter, Klebsiella, Rhodotorula and Pichia are the commonly used microorganisms in bioremediation

processes which include bio-augmentation wherein microbes and nutrients are added to supplement the intrinsic microbes of the site .These metal contaminants pose adverse health effects to those who live in the vicinity near the polluted sites. Metal waste is commonly found in soil, sediments and water. Breathing, drinking, feeding and skin contact are the possible routes of exposure for metal contaminations. Metals such as mercury, lead and arsenic may potentially be toxic to the kidney and decrease mental capabilities and also cause weakness, headaches, abdominal cramps, diarrhea and anemia. Chronic exposure to these pollutants can cause permanent damage to the kidney and brain.

In-situ bioremediation is a technique that can be used to reduce the spread of metal contamination by applying biological treatment to hazardous chemicals in soil and ground water [(6)]. In-situ bioremediation has the ability to transform contaminations to less toxic compounds, making it a promising tool for environmental clean-up. It accelerates the desorption and dissolution of the contaminants by treating pollutants close to their sources. Amongst all toxic heavy metals contaminating the aquatic environment, those particularly lead, chromium, cadmium and mercury are of great concern for human beings as well as for the environment because of their acute toxicity and high mobility in food chain. Due to rapid industrialization and urbanization, pollution of heavy metals has become a matter of global concern. Cadmium, copper, arsenic, chromium, lead, mercury, nickel and zinc are considered the most hazardous heavy metals and are included on the EPA's (Environmental protection agency) list of priority pollutants. Sources of metals include domestic and industrial effluents, the atmosphere, runoff and lithosphere. Once heavy metals are allowed to pass through the municipal waste treatment facility, they return to the environment where they are persist, cannot biodegraded. The metals can absorb onto the soil, runoff into rivers or lakes or leach in the ground water, an important source of drinking water. Exposure to the heavy metals through ingestion or uptake of drinking water particularly where water is reused and foods can lead to accumulation in animals, plants and humans. Among heavy metals, Pb, Cd and As are considered potentially important environmental pollutants due to their trends to accumulate on vital organs of humans and animals. The most common metals found at contaminated sites in the increasing order are: lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu) and mercury (Hg).The soil-plant system is the fundamental constructive unit of the geo-sphere and biosphere.

Therefore, heavy metal pollution of soil has an important influence not only on the yield and quality of crops, but also on the quality of atmospheric and aquatic environment, and even on the health of human beings via food chains. Heavy metals are among the pollutants that need to be removed from such contaminated sites. Several heavy metals such as Cd, As and Cr are considered hazardous waste metals that can accumulate in the human body with a relatively longer half-life. It has been stated for example, that Cd has half-life of 10 years once in the human body.

Additionally, some species of Cd, Cr and Cu have been associated with health effects ranging from dermatitis to various types of cancers The heavy metal lead group (II) of periodic table is highly toxic as its presence in drinking water above the permissible limit (5μg/ml) causes adverse health effects such as anemia, encephalopathy, hepatitis and nephritic syndrome

A large number of food and natural products have been found to be contaminated with lead. As specified in the European Union (EU) has already banned the import of Indian honey alleging that consignments of natural nectar from the country are contaminated with lead.

Recently, micro-organism have been reported as biological adsorbent for the removal of heavy metals at low cost and in an eco-friendly way. The potential use of micro-organism in the treatment of heavy metal- contaminated wastewaters and recovery of metals in mining wastes or in metallurgical effluents is of special importance.

Bacteria, fungi, yeast and algae can remove heavy metals and radionuclides from aqueous solution in substantial quantities.

Bio sorption as "non-directed physic-chemical interaction that may occur between metal/radionuclide species and the cellular compound of biological species"(.[3]) Fungi and yeast accumulate micronutrients such as Cu, Zn, Mn and non-nutrients metals like, U, Ni, Cd, Cr and Hg in amounts higher than nutritional requirement. The potential of bacterial biomass as biosorbent has been recognized for the removal of heavy metals and radionuclide's from polluted waste materials.

Bacterial cell walls and their components have major role in the sequestering of metals because they contains the different functional groups i.e. carboxyl, hydroxyl, sulfhydryl, amino and phosphate groups which help them in binding of the heavy metals.

Bacterial biomass can also take up considerable quantities of heavy metals from aqueous solution by adsorption or related process, even in the absence of

physiological pH, temperature, and availability of nutrients [(4)]. Many micro-organisms such as Bacillus sp., Pseudomonas sp., have ability to accumulate the metal nutrients in higher amounts. Bacteria is constitutes a versatile group which can adapt and grow under various extreme conditions.

Bioremediation is the most promising and cost effective technology widely used now a days to clean up both soils and wastewaters containing organic or inorganic contaminants. Bio absorption is essentially a non-directed physico-chemical complication reaction between dissolved metal species and charged cellular components, which involves absorption of metals to living or dead cells. This process may simply be defined as utilization of inexpensive dead or alive microbial biomass to sequester metals from industrial effluents. It has gained importance during recent years due to their good performance, low cost, specificity, minimum sludge generation and amenability for repeated use.

II. METHODOLOGY

A study of three month duration (Dec-March 2018) was conducted in Uttar Pradesh, India at Kanpur in Microbiology laboratory of Saaii college of medical sciences and technology. Out of 5 samples 3 isolates recovered from soil sample collected from 2 different site .

Sample collection: The soil samples from petroleum sites were collected after digging 20 cm deep in soil and were collected in sterilized container .These container are sealed and brought up in microbiology laboratory of Saaii college of medical sciences and technology ,Kanpur, India & the sample were storage at 4°C.

Staining: The isolates were identified by microscopic examination with Gram Stain.

Cultural isolates: Bacterial strain were isolated by using serial dilution method. Soil suspensions were prepared in sterile water by serial dilution up to ten to the power four and spread over Nutrient agar. The plates were incubated for overnight at 37°C and observed for the development of colonies, isolated colony were transferred to freshly prepared Nutrient Agar in order to obtain pure cultures .

The purified bacterial strain were screened for determining their tolerance limit to Mg, Ca++,Mn, Hg.CuSO4 . The resistant level were identified by the growth of bacteria over nutrient agar. For determining the resistant level ,The bacterial strain were inoculated on Nutrient agar having metals of different molarity ranging from 0.1-0.5mm.The colonies were picked and inoculated on the Nutrient agar for 24 Hours.The minimum concentration of the isolated were determined at the lowest conc. of each metal the show visible growth of bacteria.

Disc diffusion method : Kirby baur method was used to determine susceptibility to the bacterial isolates and resistant to heavy metal to antibiotics as per CLSI guidelines: Chloramphenicol, Amoxicillin, Norfloxacin, Erythromycin, Gentamicin.

III. RESULT

A total of 5 isolates recovered from soil sample collected of 2 different site .The isolates were identified on their morphology on Nutrient Agar and Microscopic examination with Gram Stain. They were identified as *Pseudomonas fluorescens*. The confirmatory test is done by biochemical test such as Catalase ,Coagulase, Citrate ,Oxidase etc.

On Nutrient Agar Plates P. fluorescence produced initially white color colony and later appears slightly yellowish. When the plates are placed inside UV light it produces greenish color florescence.

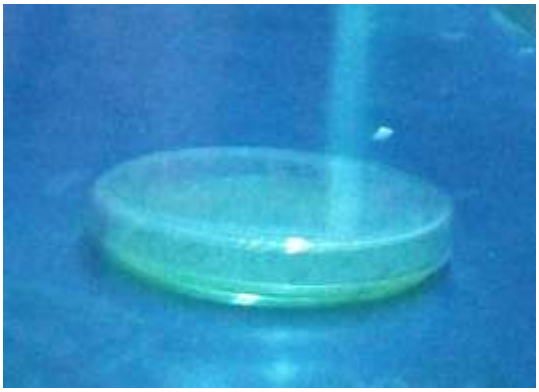

Fig 1.1 Fluorescence under U.V light

Table(3.1) Biochemical test:

Biochemical test	Positive	Negative
Catalase	Positive	----------
Coagulase	----------	Negative
Citrate	Positive	----------
Oxidase	Positive	----------

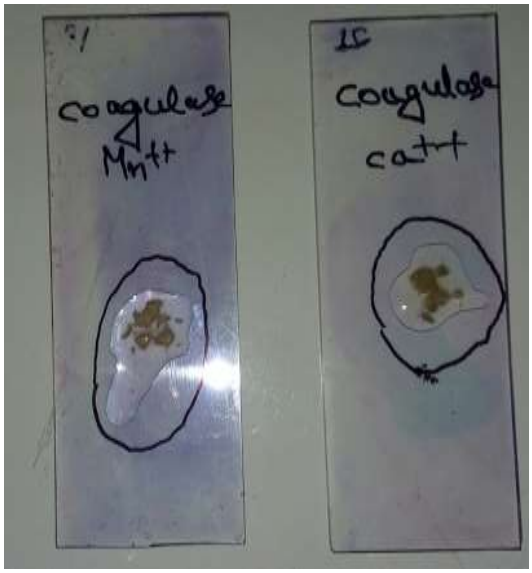

Fig 3.1 : Coagulase test

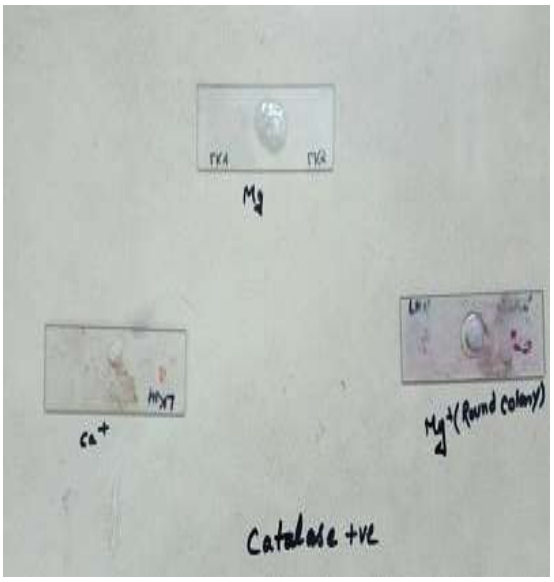

Fig 3.2: Catalase test

Fig3.3: Oxidase test

Fig3.4: Citrate test

Table 3.2 Antibiotics property against the bacterial isolates (Resistant ,Intermediate Sensitive and zone sizes measurement.

Antibiotics	PM1(mm)	PM2(mm)	PM3(mm)
Gentamycin	**15(Sensitive)**	**21(Sensitive)**	**17(Sensitive)**
Norfloxacin	**21(Intermediate)**	**14(Resistant)**	**18(Resistant)**
Chloramphenicol	**06(Resistant)**	**07(Resistant)**	**21(Sensitive)**
Erythromycin	**06(Resistant)**	**13(Intermediate)**	**08(Resistant)**
Amoxicillin	**16(Intermediate)**	**06(Resistant)**	**12(Resistant)**
Pencillin G	**06(Resistant)**	**07(Resistant)**	**06(Resistant)**

Fig 3.5: Antibiotic sensitivity testing

IV. DISCUSSION

As a consequence of industrial progress and urbanization, heavy metal pollution has become one of the most serious environmental problems world over during recent years. In nature, heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd), nickel (Ni), zinc (Zn), copper (Cu), chromium (Cr) are present in very small amounts in soil and

water bodies. But at certain concentration, they can be toxic to plants, animals, humans and aquatic life. Pollution of heavy metals is important because they are non-biodegradable and indestructible. It is therefore, necessary to remove such metal from the environment .As the heavy metals have toxic effects on human, animal and plant health as discussed in the section of introduction and review of literature it is essential to minimize heavy metal conc. The microbes play a major role in the bio-geochemical cycling of toxic heavy metals and also in cleaning up or remediating metal-contaminated environments. The improvisation of effective and cheaper methods for the remediation of heavy metals is therefore, warranted. There are several approaches for heavy metal removal from the environment such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation, and sorption for heavy metal removal techniques because they can have lower cost and higher efficiency at low metal concentrations microbial remediation or bioremediation has the advantages of removal of large amounts of heavy metal efficiently at a low cost. The microbe related technologies provide an alternative to conventional method of removal of the heavy metals as well as its recovery.

There are a number of microorganisms that can be used to remove metal from waste water, agricultural soils, dumping sites etc such as molds, yeasts, bacteria, and seaweeds. The ability of microbes to grow in the presence of heavy metals has been found useful in the waste water treatment where microorganisms are directly involved in decomposition of organic matter for waste water treatment.

Microorganism are of primary importance in bioremediation of contaminated soils and wastewater, essentially because of their ability to alter the chemical nature of the metal ions and in turn metal ions mobility through processes such as reduction, bioaccumulation, mobilization and immobilization .Among the microorganisms, bacteria are very important for bioremediation due to their plasmid containing metallo theonin enzyme and well documented ability to accumulate metals of all kinds . Bacterial resistance to heavy metals develops through various mechanisms such as active transport of metal ions inside the plasmid, masking metals by chelation, enzymatic transformation of metal ions, creating vacuoles in which metal ions are gathered and immobilization in the form of polyphosphates, increased production of metallotheonin and other pigments, and production of specific metal binding compounds inside the cell .The removal of metals from soil bodies by bacteria thus has industrial relevance as this process not only cleans the environment and protects its biodiversity, but it also allows the recovery of the metals and their subsequent reuse .Considering the importance of bacteria in bioremediation, we attempted to identify certain bacteria from the petroleum industry .The majority of the isolates belonged to genus Pseudomonas. The purified colonies of the bacteria were screened for their tolerance indices on potato dextrose agar which contained 0.5 -0.7 Mm concentration of each heavy metals, Cd, Zn, Co, and Pb separately. The maximum tolerance indices was observed in case of pseudomonas florescence. out of 5 isolates shown maximum tolerance index of Cd, Zn, Ca and Hg heavy metals.The highest tolerance level was observed in case of Pseudomonas fluorescence.

In the present study the heavy metal tolerant bacteria recovered from different sources further tested for their susceptibility to most commonly used antibiotics agents. The antifungal commonly used to treat human bacterial infections are: Gentamycin ,Amoxicillin, Chloramphenicol, Erythromycin, Penicillin G.

Conflict of Interest: None.

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