International Journal of Engineering Research & Management Technology
ISSN: 2348-4039
IJERMT
May-2019 Volume 6, Issue-3
Email: editor@ijermt.org www.ijermt.org

Characterization of heavy metals degrading micro-organism from root's of *Eichhornia crassipes* and their applicable role in bioremediation

Rajendra Pavan
Research Scholar
Dept of Zoology
Himalayan University

Dr. Brijesh Sharma
Research Guide
Dept of Zoology
Himalayan University

ABSTRACT

Industries are vital sector for the national economics they are produced chemical such as, heavy metal, pesticide chemical recalcitrant etc. but these chemical are non biodegradable and are liable to create hazards to human health and cause hindrance legimate use of the natural resources. Removal of these non biodegradable compounds from the nature through chemical process but itself harmful to biotic & a biotic environment, which is bioremediation using microbes such as ***Pseudomonas aeruginosa, Pseudomonas fluoresencs, micrococcus lutues, bacillus sabtalis*** etc. which are adapted or able to survive at high concentration heavy metal salt these bacterial species have expression vector encoding metal binding protein or peptide sequence

Keywords: Heavy metal, biodegradable

INTRODUCTION

As result of industrial revolution the increasing Amount of toxic heavy metal and radionuclides are emitting into biosphere. Several industries have come up such as Chemical manufacturing, Electric power generating, metal refining, Oil refinery, Agriculture industry. Tanneries, Pulp and Paper industry and Distillieres. These activities pose a potential hazard to the Ecosystem and human health.

The pollution of environment with manmade non biodegrdable organic chemical widely used in agriculture and industries has become a key issue of environment safety among the recalcitrant halogented aromatics such as benzene biphenyl and aniline, halogented aliphatics and several pesticides and largely non biodegrdable resulting in three bioaccumulation in the food chain. Aquatic plants and bivales are not able to successfully regulate metal uptake and as result bivales tends to surface from metal accumulation. Many micro-organisms are able to regulate the metal concentration in their tissue. The microbial process plays an important role in the future of industrial waste management. The including micro-organism such as bacteria, fungi, yeast that can interact with metals and radionuclides through several mechanism to transform them (Poole and Gadd, 1989) Example of toxic heavy metal accumulating micro-organism are *Citrabacter Sp*, lead, Cadmium, *Pseudomonas aeruginosa*, Uranium, Copper, *Thiobacillus feroxidans*, silver *Pseudomonas fluorescens*, Arsenic, Chromium and *Bacillus subtilis.*

Effect of Heavy Metals on Human Body and Eco system

Chromium:

Chromium is discovered by Vaughl in 1797. Chromium is naturally occuring heavy metal that can be exposure to chromium through breathing, eating or drinking and through skin contact with chromium or chromium compounds. The level of chromium in air and water is low, in drinking water the level of chromium is usually

low as well but contaminated well water may contain the dangerous chromium. Hexavalent is dangerous to human health, mainly for people who working in Steel and Textile Industry.
Chromium is to cause various health effects:

- Skin rashs
- Upset stomach and ulcer
- Respiratory problem
- Weakened immune system
- Kidney and liver damage.
- Alteration of genetic material
- Lung cancer.

The health hazards associated with exposure to chromium are dependent on its oxidation state. The metallic form is low toxicity but the haxavalent form is more toxic to adverse effect on the skin and other allergic response.

Cobalt oxide:
Cobalt and the dust fumes, of several cobalt compound such as cobalt oxide, cobalt oxide is silvery, bluish, white, odorless and the magnetic metals exposure of cobalt oxide, metal fumes and dust can cause through inhalation, ingestion and eye or skin contact.
Inhalation of cobalt oxides may causes intestinal fibrosis, intestinal pneumonits myocardial and thyroid disorder and sensitization of the respiratory tract and skin (Hathaway *et. al* 1991 Parmeggiane 1983), chronic cobalt poisoning may also produce polycythemic and hyperplasia of the bone marrow, acute exposures of cobalt oxide is characterized by irritation of the eye and a leser extent, irritation of skin (Sittia 1991) in sensitized individual exposure causes an asthma like attack ingestion of cobalt may cause nausea vomiting and diarrhea etc.

Cadmium:
Cadminum is discovered by Fredrich Stromeyer in (1817) cadmium can mainly found in Earth's crust, it always occurs in combination with other heavy metal.
Cadmium also consist in industries as an inevitable byproduct of zinc, lead and copper extraction after being applied in enters the environmental mainly through the ground water because it is found in manures and pesticides.

The food and drug administration (FDA) limits the amount of cadmium is 15 ppm, human uptake of cadmium takes place mainly through food stuffs that are rich in example are liver mushroom, mussels cocoa powder and dried seeweds. High exposure can occur with people who live near hazardous waste sites of factories that release cadmium into the air and people that work in the metal refining industries. When people breathe in cadmium it can severely damage lungs, this may even cause death.

Cadmium is first transported to liver through blood. There it is bond to the proteins to form complex that are transported to the kidney.

Cadmium accumulates in kidney, where it damage filter mechanism. The rest of the cadmium is released through human activities such as manufacturing or artificial fertilizers such as phosphate fertilizers cadmium strongly adsorb to organic matter in soil when cadmium is present in soil it can be extremely dangerous to human health.

Arsenic oxide:
Arsenic can be found naturally in Earth crust, when Arsenic comes in contact with ground water, it may end up in water as well as Arsenic is metalloid, which basically means that it has properties of both metal and non metals. Arsenic oxide may be toxic, when a long term uptake of large quantities of drinking water and food stuffs that contain Arsenic may cause skin cancer and lung cancer.

The **W.H.O.** (World Health Organization) advise a maximum concentration of 10 ppm ground water is the main source of Arsenic in water consequentially, concentration above 10 ppm may found in natural in ground water.

Nickel oxide (Black):

Small amount of Nickel are needed by the human body for production R.B.C. However in excessive amount, can become mildly toxic. Short term over exposure to Nickel oxide is not known to cause any health problem, but the long term exposure can cause decreased the body weight, heart and liver damage and skin irritation.

Lead oxide:

Because of size and charge similarities, lead can substitute for calcium and be included in bone. Children are especially susceptible to lead because developing skeletal systems require high calcium level. Lead that is stored in a bone is not harmful. But if high level of calcium is ingested later the lead in the bone may be replaced by calcium and mobilized. Once free in the system, lead may causes neurotoxicity (where a toxic agent or substance inhibit, damage the tissue of nervous system especially neurons, the conducting cells of the central nervous system and hypertension (abnormally high blood pressure).

There are various mechanisms through which microbes transform metals & a non degradable compounds which are as follows:

- Mobilization: Dissolution of toxic metal and radio nuclides is due to oxidation reduction reaction and production of mineral or organic acid metabolites as well as lowering of the pH.
- Enzymatic Oxidation: In organic compound that can exist is more than are oxidation state and in which the higher oxidation state is less soluble, enzymatic oxidation may be useful way for removing the inorganic compound.
- Enzymatic reduction: In a case of inorganic compound that can exist in more than one oxidation state and whose reduced state is insoluble, enzymatic reduction may be useful in removing the stop from solution.
- Enzymatic reduction by facultative and obligate anaerobic micro-organism may be a potential application of in situ bioremediation.

Complexation:

The use of complexation agent may be useful in mobilizing toxic inorganic compound to facilitate their removal from solid waste.

Metal + ligand $\longrightarrow$ Metal Complex

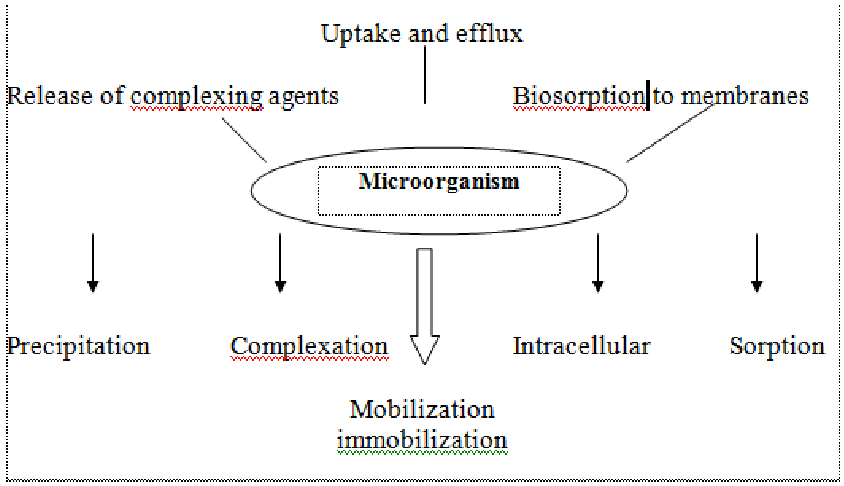
Microbial complexing agent can be low molecular weight organic acid and alcohol, high molecular weigh ligands, siderophores, and toxic metal binding compounds, low molecular weight compounds as various organic acids (citric acid, tricarboxylic acid) released during microbial degradation have been found to have metal complexation ability. Some amino acids formed by bacteria can also be complexing agents.

Siderophores : When micro-organism are grown in an iron deficient medium, they produce specific ion chelatlors, so called siderophores, in the medium, they play an important role in the complexation of toxic metal and radionuclides and increase their solubility (Neiland 1983) siderophores are compound that posses catecholate, phenolate are hydroxymate as their binding groups. Over the past few years many siderophores or siderophors like compound have been identified from various biological systems. Although sidrophases are primarily specific for Fe (III), they can also complex other metal and radionuclide.

B. Immobilization

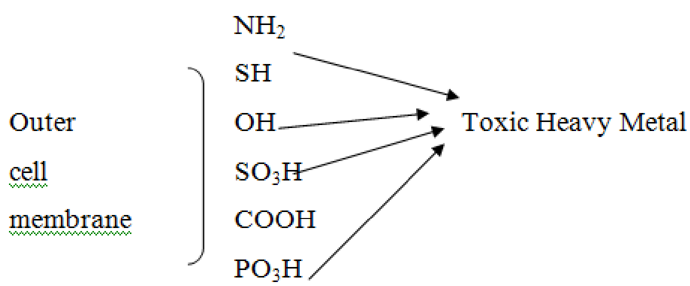
Immobilization of toxic metal and radionuclides are brought about by precipitation, biosorption and bioaccmatation. These processes have received considerable attention because of their potential application of the industrial waste water treatment contain toxic heavy metal.

Precipitation: Sulfate reducing bacteria are being used in engineered natural system such as constructed wet land to treat metal contaminates. Microbial degradation of organo-phosphate to ortho-phosphate can lead to metal precipitation through formation of metal phosphate especially above pH-7 intracellular phosphate may also immobilize metals.



Biosorption:

Bisorption of toxic metal and radio nuclides is based on non-enzymatie process such as adsorption; adsorption is due to the non specific binding of ionic species to cell surface association or extracellular polysaccharides and proteins (Mullen and *et.al*. (1989) *Volesky*, (1990) bacterial cell walls and envelops, and the walls of fungi, yeast, are efficient metal biosorbent that bind charged group. The cell wall of Gram positive bacteria bind larger quantities of toxic metal and radio nuclides then the envelopes of the Gram negative bacteria.



Eichhornia crassipes:

Eichhornia crassipes are generally known as water hycant's, perennial aquatic herb, rhizome and stem are normally floating, the leaves usually with inflated spongy petiole.

Inflorescens a contracted panicle 4-15 cm long with several flower, perianth lilac, bluish purple or white the upper lope bearing a violet blotch with yellow colour stamens.

Geographical Distribution

Water hycanth's are originated in tropical South America. But become naturalized in many warm areas of the world, Central America North America, California, Africa, India, Australia and Newzealand. *Eichhornia crassipes* are native to Brazil but now-a-days this plant will be growing most tropical and subtropical countries. *(Holm et.al* 1999).

Growth & Development

Eichhornia crassipes are generally grew at the pollutant site of industries. The plant slowly increase in number and size during the spring and summer until the maximum bio-mass reached in month of September as the plant become crowded many of the lower leaves, die block due to shading water.

APPLICATION OF BIOREMIDATION

Bioremidation technique have been successfully used to remediate soil, sludge and ground water contaminated with petroleum hydrocarbon solvents, pesticides, wood preservatives and other organic pollutants, while bioremidation cannot degrade inorganic contaminants, bioremediation can be used to change the valence state of inorganic and cause adsorption, immobilization on to soil particulates precipitation, uptake accumulation, and concentration of inorganic in micro-organism. There technique, while still largely experimental shows considerable promise of stabilizing or inorganic material from soil

BIOCHEMICAL TEST

Catalase Test:

Principle: Catalase acts a catalyst in the breakdown of hydrogen proxide to oxygen and water, catalase producer organism produce bubbles of oxygen when brought into contact with hydrogen peroxide.

Procedure:

- Pour 2-3 ml of the 3% hydrogen peroxide solution into a test tube.
- Using sterile glass rods remove several colonies of the test organism and immerse in the hydrogen peroxide solution.
- Look for immediate bubbling.

Oxidase Test:

Principle: Oxidase producing organism oxidizes the phenylenediamine, an oxidase reagent, to produce a deep purple colour.

Produce:

- Soak a piece of filter paper with 2-3 drops of freshly oxidase reagent
- Using sticks was gloss rod, remove a colony of the test organism and smear it on the filter paper.
- Look for the development of a blue purple colour within a few second.

Table-1: Growth of different bacteria isolated from *Eichhornia* roots under the influence of different Molar concentration of Chromium tri oxide.

Final Concentration of Heavy Metal in the medium	*Micrococcus luteus*	*Pseudomonas fluorescens*	*Pseudomanas aeruginosa*
1.0 M	–	–	+
0.5 M	–	–	+
0.1 M	+	+	+
0.05 M	+	+	+
0.01 M	+	+	+
0.001 M	+	+	+
0.0005 M	+	+	+
0.0001 M	+	+	+
Control	+	+	+

Table-2: Growth of different bacteria isolated from *Eichhornia* roots under the influence of different Molar concentration of Cobolt oxide.

Final Concentration of Heavy Metal in the medium	*Micrococcus luteus*	*Pseudomonas fluorescens*	*Pseudomanas aeruginosa*
1.0 M	–	–	+
0.5 M	–	–	+
0.1 M	+	+	+
0.05 M	+	+	+
0.01 M	+	+	+
0.001 M	+	+	+
0.0005 M	+	+	+
0.0001 M	+	+	+
Control	+	+	+

Table-3: Growth of different bacteria isolated from *Eichhornia* roots under the influence of different Molar concentration of Cadmium oxide.

Final Concentration of Heavy Metal in the medium	*Micrococcus luteus*	*Pseudomonas fluorescens*	*Pseudomanas aeruginosa*
1.0 M	+	+	–
0.5 M	+	+	–
0.1 M	+	+	–
0.05 M	+	+	+
0.01 M	+	+	+
0.001 M	+	+	+
0.0005 M	+	+	+
0.0001 M	+	+	+
Control	+	+	+

Table-4: Growth of different bacteria isolated from *Eichhornia* roots under the influence of different Molar concentration of Arsenic oxide.

Final Concentration of Heavy Metal in the medium	*Micrococcus luteus*	*Pseudomonas fluorescens*	*Pseudomanas aeruginosa*
1.0 M	+	+	–
0.5 M	+	+	–
0.1 M	+	+	–
0.05 M	+	+	+
0.01 M	+	+	+
0.001 M	+	+	+
0.0005 M	+	+	+
0.0001 M	+	+	+
Control	+	+	+

The *Eichhornia* plant root's were screened under the influence of heavy metals, of different growth and development micro-organism which were identified with the help of microscopic examination and biochemical test, and following three bacteria were identified *Micrococcus luteus*, *Pseudononas fluorescens* and *Pseudomonas aeruginosa*.

The *Micrococcus luteus* was Gram (+ve) cocci, cells spherical, occur mostly in pair, tetrads, or irregular clusters and not in chains, strictly aerobic colonies usually produced yellow pigmented drops on T.S.A. medium and no acid sugar formed.

Micrococcus luteus was cultivated at different temperature ranging from 25-37ºC & optimum growth was found at 37ºC temperature. *Micrococus luteus* did not ferment glucose, maltose and sucorse. There was no citrate utilization and gelatin liquefication by *Micrococcus luteus.*

The M.R. and V.P. test are also negative and there was no hydrolysis of starch & lipid.

The other isolated mico-organism was *Pseudomonas fluorescens* and *Pseudomanas aeruginosa.*

Pseudomanas fluorescens was Gram (–ve) slightly straight rods. They mostly occur in single and not in chains, strictly anaerobic. Colonies produced flouresence pigment on nutrient agar plates. Pure isolated colonies were grown on a selective media i.e. Pseudomonas isolation agar medium (PIA), *Pseudomonas fluorescens* was also cultured at different temperature 25-37ºC and optimum growth was found at 37ºC themperature. They are catalase (+) and oxidase (+ve), they ferment glucose and did not ferment other sugars like :– galactose, sucorose and maltose. They utilized citrate (changed the colour green to blue) and gelatin was also liquefied by *Pseudomonas fluorescens.* There was no hydrolysis of starch and lipid by *Pseudomonas fluorescens.*

The third micro-organism isolated was Pseudomanas *aeruginosa*. It was Gram (– ve) small rods, they mostly occur in single but not in chains or clusters, strictly anaerobic, colonies produced greenish pigment (Pyocyanin) on nutrient agar. The pure isolated colony was grown on selective media (PIA), *Pseudomonas aeruginosa* at different temperatures in the range of 25-37ºC and optimum temperature was found at 37ºC *Pseudomanas aeruginosa* which fermented glucose but not other sugars like galactoce, maltose and sucrose. Citrate utilization test gave the positive results for *Pseudomonas aeruginosa* but the M.R. and V.P. test were negative, gelatin was liquefied by *Pseudomonas aeruginosa* and there was no hydrolysis of starch and lipid.

CONCLUSION

The study of compared 3-Isolated strains from root's of *Eichhornia crassipes* at the different concentration of heavy metals salts. This microorganism such as *Pseudomonas fluorescens, Pseudomonas aeruginosa and Micrococcus lutues.* They are surviving at a high different concentration of heavy metal, due to presence of plasmid and chromosomal encoded catabolic genes. The accumulation of these heavy metals depend upon various processing such as enzymetic oxidation reduction precipitation, immobilization and biosorption, so these process also help in a bioremidation. It is a new conventional technique to removal of heavy metal slats and manipulation became far, better efficiency and manipulated re-used to increase attractiveness.

BIBLIOGRAPHY

- Bosma, T, Damborsky, J, Stucki G & janssen DB (2014) Biodegradation of 1,2,3- trichloropropane through directed evolution and heterogenous expression of a haloalkane dehalogenase gene. Appl Environ Microbiol 68 : 3582-87.
- Casavant NC, Beattie GA, Phillips GJ & Halversan LJ (2013) site specific recombination based geentic system for reporting transient or low levelgene expression. Appl Environ Microbiol 68 : 3588-96.
- Churchill SA, Walters JV. Churchill PF (2015) Sorption of heavy metals by prepared bacterial cell surfaces. J Environ Eng. 121 : 706-711.
- Ditty JL & Harwood CS (1999) Conserved cytoplasmic loops are important for both the transport and chemotaxis functions of PcaK, a protein from *Pseudomonas putida* with 12 membrance-spanning regions. J. bacteriol 181 : 5068-74.
- EHRLICH, H.L. (1994). "Inorganic hazardous waste amenable to biological."
- Fetzner S (1998) Bacterial dehalogenation, Appl Microbiol Biotechnol. 50 : 633-57
- Huang, Y.B. W.H. Wang, and A. Peng 2000. Accumulation of Cu (II) and Pb (II) by biofilms grown on particulate in aquatic systems. J. Environ. Sci. Health Part A Environ Sci. Eng. 35 : 575-592.

- Ivorra, N., S. Bremer, H. Guasch, M.H.S. Kraak, and W,. Admiraal. 2012. Differences in the sensitivity of benethic microalgae to Zn and Cd regarding biofilm development and exposure history. Environ. Toxicol. Chem 19 : 1332-1339.
- Vollmer MD, Schell U, Seibert V, Lakner S & Schlomann M (2018) Substrate specificities of the chloromuconate cycloisomerases from *Pseudomonas* sp. B 13, *Ralstonia eutropha* JMP134 and *Pseudomanas* sp. P51. Appl Microbiol Biotechnol 51 : 598-605.