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Phytoremediation: An Ecological Solution to Heavy Metal Polluted Water and Evaluation of Plant Removal Ability

Jatin G. Italiya[1], Mitali J. Shah[2]

Department of Environmental Engineering, Sarvajanik College of Engineering and Technology
Department of Environmental Engineering, Faculty in Sarvajanik College of Engineering and Technology

ABSTRACT

Increasing urbanization, industrialization and over population is one of the leading causes of environmental degradation and pollution. Heavy metal such as Cu and Fe are one of the toxic pollutants which show hazardous effects on all living livings. The study involves the capacity of spinach to filtrate, absorb and accumulate iron and copper. In the work of Rhizofilteration & Extraction the spinach has planted in different pots and arranged it properly for outlet collection and after arrangement, apply the synthetic wastewater on the spinach as concentration(5,10ppm),time period(30,60,90,120,150min.) and plant size 5.0cm. Here rhizofilteration give 86.5% and 85.9% for Cu and Fe respectively. The results shown that Spinach highest accumulate metals in leaves than roots and stems.

Keywords *– Heavy metals, phytoextraction, plant analysis, rhizofilteration, soil analysis*

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1. INTRODUCTION

Industrialization is the period of social and economic change that transforms a human group an agrarian society into an industrial one. It is a part of wider modernization process, where social change and economic developments are closely related with technological innovation, particularly with the development of large scale energy and metallurgy production[1]. Industrialization has provided humanity with materials and social benefits. It has also brought in its wake up many unwanted substances and social problems. One of these problems is the degradation of the environment. These environmental problems are becoming threats to the very existence of the living beings [2]. Heavy metal concentrations in past few years have reached to a promising toxic level due to consequences of anthropogenic activities and urbanization. Nowadays it is well-known that cities suffer from considerable pollution due to a wide array of substances that contaminate the air, water and soil [3].

Metal persistence in soil for much longer periods than in other compartments of the biosphere is a matter of serious concern. According to Beyersmann and Hartwig [4] heavy metals like Cu and Fe etc, have been classified to be carcinogenic to humans and wildlife. Recently, numerous efforts have been undertaken to find cost-effective technologies for remediation of heavy metal-contaminated soil [5]. Phytoremediation has recently become a subject of public and scientific interest and a topic of many researches [6-8].Copper is a persistent, bio-accumulative and toxic heavy metal which does not break down in the environment, is not easily metabolized and can harm human health [9]. Rivers are depositing sludge on their banks that is contaminated with copper, due to the disposal of copper-containing wastewater. Copper enters the air, mainly through release during the combustion of fossil fuels. Copper in air will remain there for an eminent period of time, before it settles when it starts to rain. The various potential sources of copper pollution are metallurgical and metal finishing, corrosion inhibitors in cooling and boiler systems, drilling mud's catalysts, primer paints, fungicides, copper plating and pickling, corrosion of copper piping, copper releases from vehicle brake pads, architectural copper, Vehicle fluid leaks and dumping, domestic water discharged to storm drains etc[10].Iron is the most commonly available metal on planet earth. The iron content of the water sample is also within the permissible limit of WHO (1.0ppm). The level of iron could be the result of clay deposits in the area and most of from steel or metal plating industries. The high concentration of iron is also of concern as large amount of ground water is abstracted by drilling water wells both in rural and urban areas for drinking and irrigation purposes[11Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water. This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites. Phytoremediation is the name given to a set of technologies that use plants to clean contaminated sites [12].Phytoremediation is the use of green plants to clean-up contaminated hazardous waste sites. The idea of using metal-accumulating plants to remove heavy metals and other compounds was first introduced in 1983, but the concept has actually been implemented for the past 300 years on wastewater discharges [13]. Phytoremediation has the potential to clean an estimated 30,000 contaminated waste sites throughout the US according to the EPA's Comprehensive Environmental Response Compensation Liability Information System (CERCLIS). Sites included in this estimate are those that have either been owned or contaminated by: battery manufacturers, electroplating, metal finishing, and mining companies [14].

Ex-situ method

It requires removal of contaminated soil for treatment on or of site, and returning the treated soil to the resorted site. The conventional ex-situ methods applied for remediating the polluted soils relies on excavation, detoxification and/or destruction of contaminant physically or chemically, as a result the contaminant undergo stabilization, solidification, immobilization, incineration or destruction.

In-situ method

It is remediation without excavation of contaminated site. Reed et al. defined in-situ remediation technologies as destruction or transformation of the contaminant, immobilisation to reduce bioavailability and separation of the contaminant from the bulk soil[15].

1.1Various Phytoremediation processes

(a)Phytoextraction

Phytoextraction is the uptake of contaminants by plant roots and translocation within the plants. Contaminants are generally removed by harvesting the plants. Phytoextraction is primarily used for the treatment of contaminated soils. To remove contamination from the soil, this approach uses plants to absorb, concentrate, and precipitate toxic metals from contaminated soils into the above ground biomass (shoots, leaves, etc.)It is the best approach to remove contaminants from soil, sediment and sludge.[16,12].

Advantages

-Cost of phytoextraction is fairly inexpensive.
- The contaminant is permanently removed from the soil (Henry, 2000).

Disadvantages

-Metal hyperaccumulators are generally slow-growing with a small biomass and shallow root systems.
-Plant biomass must be harvested and removed, followed by metal reclamation or proper disposal of the biomass. Hyperaccumulators may accumulate significant metal [12].

(b)Phytostabilization

Phytostabilization, also referred to as in-place inactivation, is primarily used for the remediation of soil, sediment, and sludges. It is the use of plant roots to limit contaminant mobility and bioavailability in the soil.Phytostabilization can occur through the sorption, precipitation, complexation, or metal valence reduction. It is useful for the treatment of lead (Pb) as well as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu) and zinc (Zn)[17].

Advantages

-Soil removal is unnecessary.
-It has a lower cost and is less disruptive than other more-vigorous soil remedial technologies.
-Disposal of hazardous materials or biomass is not required [12].

Disadvantages

-The contaminants remain in place. The vegetation and soil may require long-term maintenance to prevent rerelease of the contaminants and future leaching.
-Application of extensive fertilization or soil amendments, mandatory monitoring is required.

(c)Rhizofilteration

Rhizofilteration is primarily used to remediate extracted groundwater, surface water, and wastewater with low contaminant concentrations. It is defined as the use of plants, both terrestrial and aquatic, to absorb, concentrate, and precipitate contaminants from polluted aqueous sources in their roots. Rhizofilteration can be used for Pb, Cd, Cu, Ni, Zn, and Cr, which are primarily retained within the roots[18,19].

Advantages

-Either terrestrial or aquatic plants can be used. Although terrestrial plants require support, such as a floating platform, they generally remove more contaminants than aquatic plants. This system can be either in situ (floating rafts on ponds) or ex situ (an engineered tank system).

-An ex situ system can be placed anywhere because the treatment does not have to be at the original location of contamination [12].

Disadvantages

-The pH of the influent solution may have to be continually adjusted to obtain optimum metals uptake.

-A well-engineered system is required to control influent concentration and flow rate

(d)Phytovolatilization

Phytovolatilization involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and transpiring them into the atmosphere. This method is that the contaminant, mercuric ion, may be transformed into a less toxic substance (i.e., elemental Hg.

Phytovolatilization occurs as growing trees and other plants take up water and the organic and inorganic contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations. Phytovolatilization has been primarily used for the removal of mercury, the mercuric ion is transformed into less toxic elemental mercury [19].

Advantages

-Contaminants could be transformed to less-toxic forms, such as elemental mercury and dimethyl selenite gas.

-Contaminants or metabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation [12].

Disadvantages

-The contaminant or a hazardous metabolite might accumulate in vegetation and be passed on in later products such as fruit or lumber.

- Low levels of metabolites have been found in plant tissue [12].

(e)Phytodegradation

In phytoremediation of organics, plant metabolism contributes to the contaminant reduction by transformation, break down, stabilisation or volatilising contaminant compounds from soil and groundwater. Phytodegradation is the breakdown of organics, taken up by the plant to simpler molecules that are incorporated into the plant tissues [20]. Plants contain enzymes that can breakdown and convert ammunition wastes, chlorinated solvents such as trichloroethylene and other herbicides. The enzymes are usually dehalogenases, oxygenases and reductases.

All phytoremediation technologies are not exclusive and may be used simultaneously, but the metal extraction depends on its bio available fraction in soil [17].

Advantages

-Plants are able to grow in sterile soil and also in soil that has concentration levels that are toxic to microorganisms [12].

Disadvantages

-Toxic intermediates or degradation products may form.

-The presence or identity of metabolites within a plant might be difficult to determine; thus contaminant destruction could be difficult to confirm [12].

(f)Phytostimulation

Using plants to stimulate bacteria and fungi to mineralize pollutant using exudates and root sloughing. Some plants can release as much as 10-20% of their photosynthesis in the forms of root sloughing and exudates (pilon smits, 2005).
Phytostimulation, also referred to as enhanced rhizosphere biodegradation, rhizodegradation, or plant-assisted bioremediation/degradation, is the breakdown of organic contaminants in the soil via enhanced microbial activity in the plant root zone or rhizosphere.

Advantages

This method is useful in removing organic contaminants, such as pesticides, aromatics, and polynuclear aromatic hydrocarbons (PAHs), from soil and sediments. Chlorinated solvents also have been targeted at demonstration sites [21].

Disadvantages

Locations at which phytostimulation is to be implemented should have low levels of contamination in shallow areas. High levels of contaminants can be toxic to plants [22].

EXPERIMENTAL

Material And Methods

2.1 Introduction

This chapter presents the materials used, methods employed and analytical techniques adopted. To have a better understanding of the Phytoremediation method and its efficiency to degrade the heavy metals in wastewater, experiments were conducted on a laboratory scale model. The whole experiment work was carried out in our college laboratory. The experiments were conducted by providing batch feed of wastewater to the reactor. The pollutant concentrations at outlet were checked for every retention time. An extensive sampling and analysis was carried out in the laboratory for all samples collected from the experimental setup according to different sets using procedure of "Standard Method of Examination of Water and Wastewater".
The purpose of the experiment is to investigate the applicability of using spinach for the removal of Cu and Fe in the laboratory by preparing synthetic wastewater.

2.2 Chemicals and Materials used

The following chemicals and reagents were used in the experiments.

For the Copper

Stock copper solution, 1% $NH_2OH.HCL$, 40% Sodium citrate, 0.1% Neocuprine solution. chloroform, sulfuric acid, acetic acid

For the Iron

Hydrochloric acid, 10%Hydroxyl amine hydrochloric acid solution, Ammonium acetate buffer solution, 0.25% 1, 10 phenanthroline solution. Iron stock solution

2.3Method of Analysis

a) For the Copper

Spectrophotometrically Method (APHA)

Series of standards were prepared from stock solution. Followed by addition of 5 ml $NH_2OH.HCL$, 10 ml 40% Sodium citrate, and 10 ml 0.1% Neocuproine solution. Shaken for 30 sec with 10 ml chloroform and allowed for layers separation. Organic layers was collected in 25 ml volumetric flask and made up to mark with distilled water. Blank was prepared by addition of all the reagents. Absorbance was read at 450 nm. Calibration curve of Concentration Vs absorbance was drawn.

b) For the Iron

Spectrophotometrically Method (APHA)

From intermediate iron solution prepare a series of standard as follows from intermediate iron solution pipette 3ml, 6ml, 9ml, 12ml, 15ml as per respectively in mg/l.Then add 1ml NH2Oh.HCl solution and 4ml phenonthroline solution and 10ml ammonium acetate buffer solution and dilute upto 50ml with distilled water. Mix thoroughly and allow at least 10 to 15 min for colour development. Read the absorbance at 510nm.draw the graph of absorbance vs. concentration and calculate iron.

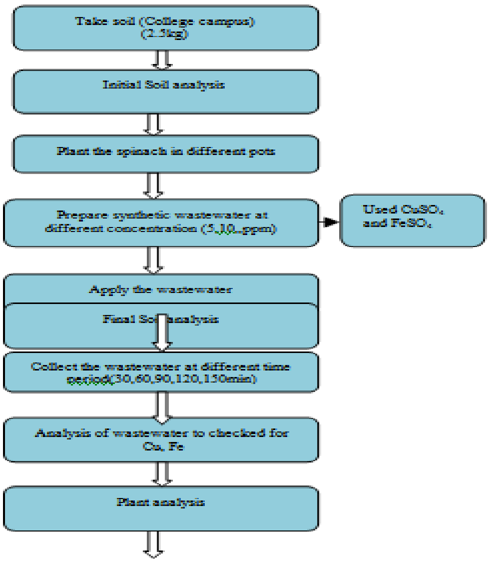
2.4 Methodology of Treatment

Here at the starting of process in India in Surat city near Athwagate at college campus we take a soil sample for the practical purpose and measured parameters like N, P, K and Cu and Fe. The dried soil are similar to natural one, sandy clay, but with metal concentration. Here very small amount of Cu and Fe was present. Then we dried soil for the 2 days then take a 2.5 kg soil in a different pots and make a small hall below the pots then plant a spinach with seeds and put for the grow. After a 25 days when height of the plant become a 5 cm then we prepared a synthetic wastewater by using hydrated copper and iron sulphate of definite concentration like a 5,10 ppm. Adjusted the pH of wastewater to neutral.

In a different pot according to the concentration applied the wastewater then at a decided time interval collect the wastewater from bottom of the pots. Here because of the roots of the spinach rhizofilteration process takes place.

The water which collected from bottom was analyzed and measured amount of Cu and Fe removed and it will be give amount of removal efficiency by spinach. It adsorbed the heavy metal on its roots that's why metal will be removed from the wastewater. After completion of rhizofilteration process some amount of water will inside the pots at now after a some time period as plant grow its roots extract the metal from soil and then comes to stems and then finally at leaves. It was measured with the below process and found out the phytoextraction capacity of spinach. Still some amount of heavy metals are present in soil that will analyzed by soil analysis.

2.4.1Flow Diagram of Experimental Process



2.5 Initial Soil Analysis Report

Parameter	Value
Fe w/w%	0.12
Cu w/w%	2.50
N mg	1.484
P µg/gm	2.98

K mg/lit	7.8

2.6 Plant Analysis

The samples were brought in plastic bags laboratory and they were cleaned with deionized water repeatedly. These were later dried in an oven at 65°C for about 2 days and were ground. After then, 0.5 g of ground plant sample is digested with 5 ml of nitric acid and 3 ml of hydrogen peroxide. The digestion temperature was about 160°C. The samples were then analyzed with the spectrophotometer with their respected wavelength [23]. Guidelines for maximum limit (ML) of metals in vegetables was adopted from FAO- WHO [24, 25].

2.7 **Experimental Setup at different Stages**




RESULT AND DISCUSSION

Table 3.1 Removal of the Copper and Iron from wastewater at
Plant size: 5.0 cm
Concentration: 05 ppm

Time (minute)	Copper concentration in ppm	Iron Concentration in ppm	% Removal of Copper	% Removal of Iron
0	5	5	0	0
30	1.83	1.89	63.4	62.2
60	1.52	1.58	69.5	68.4
90	1.27	1.24	74.6	75.2
120	0.91	1.08	81.7	78.4
150	0.78	0.94	84.3	81.2

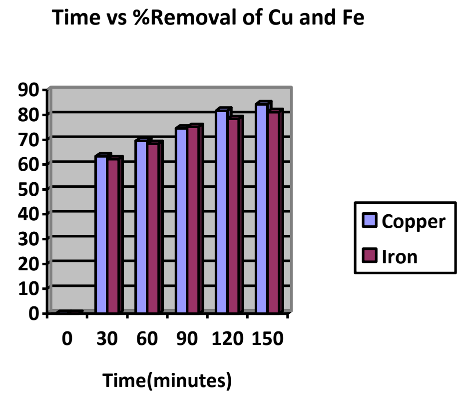


Fig 3.1 Removal of the Copper and Iron from wastewater at Plant size: 5.0 cm Concentration: 5 ppm
From the graph we can see that % removal of the Cu and Fe in wastewater is increase with time and we can get 84.2 and 81.2% for the copper and iron respectively by spinach olaracea.

Table 3.2 Removal of the Copper and Iron from wastewater at
Plant size: 5.0 cm
Concentration: 10 ppm

Time (minute)	Copper concentration in ppm	Iron Concentration in ppm	% Removal of Copper	% Removal of Iron
0	10	10	0	0
30	3.59	3.68	64.1	63.2
60	3.31	2.92	66.9	70.8
90	2.62	2.12	73.8	78.8
120	1.74	1.69	82.6	83.1
150	1.35	1.41	86.5	85.9

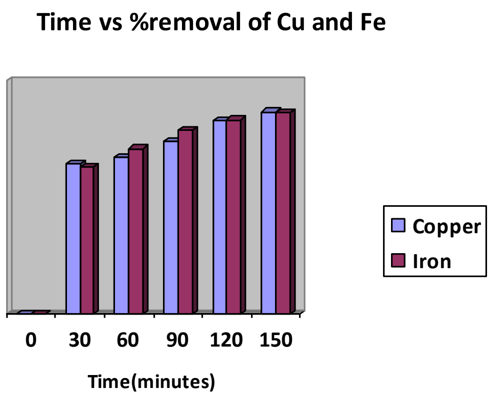


Fig 3.2 Removal of the Copper and Iron from wastewater at Plant size: 5.0 cm Concentration: 10 ppm

From the graph we can see that % removal of the Cu and Fe in wastewater is increase with time and we can get 86.5 and 85.9% for the copper and iron respectively by spinach olaracea.

PLANT AND SOIL METAL COMPOSITION

Heavy metals contamination of arable soil showed several problems, including phytotoxic effects of certain elements such as Fe,Cd,Pb and Cu, which are well known as micronutrients and cause several phytotoxicities if critical endogenous levels are exceeded [26,27].

Another and even a more serious problem is posed by the up taking of potentially noxious elements through food or forage plant species and their being transferred to the food chain and, finally, to humans [28]. All heavy metals at high concentrations have strong toxic effects and are regarded as environmental pollutants [28].

The use of plants for environmental restoration is an emerging technology. In this approach, plants capable of accumulating high levels metals are grown in contaminated soils [29]. Interest in phytoextraction has significantly grown following the identification of metal accumulator plants.

Copper (Cu) is an essential element for plants and animals. However, excessive concentrations of this metal are considered to be highly toxic. Generally, roots of most attained higher Cu concentrations than other organs, with maximum value of 741 µg.gG1 d.w attained by Phragmite australis root. Cu concentrations in plants above 10-30 µg.gG1 d.w are regarded as poisonous [30]. Within roots, Cu associated mainly with cell walls and is largely immobile. However, higher concentrations of Cu in shoots (leaves and stems) are always in phases of intensive growth and at the luxury Cu supply level [31].

Iron (Fe) is an essential micronutrient for plants and animals [32]. However, excessive Fe uptake can produce toxic effects. Fe is the most abundant metal in the area. Results obtained from plant analysis asserted that roots of all seven plants are found to be highly capable of Fe accumulation.

Fe concentrations above 40-500 $\mu g.g^{-1}$ d.w are considered as toxic to plants. As indicated by Tiffin [31], roots tend to absorb Fe^{+2} cation more than Fe^{+3}. The ability of roots to reduce Fe^{+3} to Fe^{+2} is believed to be fundamental in the absorption of this cation by most plants [33]. Moreover, some bacteria species (e.g. Metallogenium sp.) are involved in Fe, Zn reduction and are known to accumulate this metal on the surface of living cells [34]. Higher concentrations of Fe the roots of the investigated species could be due to its precipitation in iron- plaque on the root surface [35, 36].

METAL UPTAKE BY PLANTS

Plants possess highly specialized mechanisms to stimulate metal bioavailability in the rhizosphere, and to enhance uptake into their roots (Romheld and Marschner, 1986). Root exudates have an important role in the acquisition of several essential metals. For example, some grass species have been documented to exude from their roots a class of organic acids called siderophores (mugeneic and avenic acids), which were found to significantly enhance the bioavailability of soil-bound iron and possibly zinc (Cakmak, 1996 a, b).

Metal bioavailability may also be affected by various plant and/or microbial activities. Some bacteria are known to release biosurfactants (e.g., rhamnolipids) that make hydrophobic pollutants more water-soluble (Volkering et al., 1998).). Plants growth promoting rhizobacteria (PGPR) and arbuscullar mycorrhizal fungi (AMF) has been shown to reduce the toxicity of heavy metals by decreasing the bioavailability of toxic heavy metal or increasing the availability of non-toxic heavy metals (Denton, 2007).

The uptake of the metal ions has been shown to take place through the action of some secondary transporters such as channel proteins and/or H+ ion coupled with carrier proteins (Ghosh and Singh, 2005). Once inside the plant, most metals are too insoluble to move freely in the vascular system, they therefore usually form carbonate, sulphate or phosphate precipitates immobilizing them in apoplastic (extracellular) and symplastic (intra cellular) compartments in the plant roots (Salt et al., 1995).

ROOT-TO-SHOOT TRANSPORT

Subsequent to metal uptake into the root, three processes govern the movement of metals from the root into the xylem: sequestration of metals inside the root cells, symplastic transport into the stele and the release of the metals into the xylem (Gaymard, 1998; Bubb and Lester, 1991). The transport of heavy metals from root to shoot has been observed to primarily take place through the xylem via a specialized membrane transport processes (Salt et al., 1995).

This membrane, which usually has a large negative resting potential, provides a strong electrochemical gradient for the inward movement of the metal ions. For example, the xylem loading of Ni may be facilitated by binding of Ni to free histidine (Kramer et al., 1996). Since xylem cell walls have high cation exchange capacity (CEC), non-cationic metal-chelate complexers may also be transported across the plasma membrane via such a specialized carrier, as is the case for Fephytosiderophore transport in graminaceous species (Cunningham and Berti, 1993) [37].

Table 3.3 Plant Analysis

Spinach	Copper		Iron	
Applied on plant	5 ppm	10 ppm	5 ppm	10 ppm
Accumulation by leaves	1.67	3.32	1.50	3.14
Accumulation by stems	0.36	0.87	0.34	0.77
Accumulation by roots	0.67	1.17	0.59	1.08

From the above table we can see that as par the time permits leaves of the spinach accumulate highest metal than roots and stems.here the accumulation of copper is higher than the iron in all plants. Accumulation takes some time as metal taken by roots after some time it's transfer to the stems and from the stems it's transfer to the leaves.

As total heavy metal concentration of soils is poor indicator of metal availability for plant uptake, accumulation factor was calculated based on metal availability and its uptake by a particular plant (Brooks et al., 1977). The whole experiment was divided into three categories: Level 1 (Soil-Roots), Level 2 (Roots-Stems) and Level 3 (Stems-Leaves)[38].

Accumulation Factor for plants was calculated as:

$$AF = \frac{\text{Metal plant Conc.}(\mu g\ g^{-1})\ (\text{roots+stems+Leaves})}{\text{Mean Soil available } (\mu g\ g^{-1}) \text{ Concentration}}$$

Mobility Index (MI) was calculated for each level by using the formula:

$$(MI) = \frac{\text{Conc. of Metal } (\mu g\ g^{-1}) \text{ in the receiving level}}{\text{Conc. of Metal } (\mu g\ g^{-1}) \text{ in the source level}}$$

Table 3.4 Soil Analysis

Species	spinach	
Total applied	5 ppm	10 ppm
Cu	1.45	3.11
Fe	1.48	3.31

CONCLUSION

In the course of this study, we have conclude that:

1) Spinach is capable of removing heavy metal from wastewater with the help of rhizofilteration.
2)In a pot-1 at dosing of 5ppm as per the time increase removal of heavy metals are also increase and it's gives highest efficiency at 150min. for the both the metal.
3) At 5 ppm and 150min rhizofilteration could give us a 84.3% for Cu and 81.2 % for the Fe.
4)In a pot-2 at dosing of 10ppm as per the time increase removal of heavy metals are also increase and it's gives 86.5% for Cu and 85.9% for Fe.
Here spinach gives less efficiency of Iron compared to Copper.
5) Spinach is a hyperaccumulater of copper and iron and it's extract a heavy metal.

It is eco-friendly and cost effective method and also it do not cause the any type of environment pollution.

It may takes more time for extraction of metals so that process is slow compared to the other conventional treatment methods.

The all process is based on adsorption and absorption so at some time clogging may occurs.

The present study shows that some plant species can be suitable option for phytoextraction and rhizofilteration.

There is a need for field trial experiments, which have become more realistic and helps to incorporate the knowledge on metal uptake, transfer and distribution.

It can be implemented at large scale with special modification in processes for the different industrial effluent discharge.

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