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BONAFIDE CERTIFICATE

Certified that the project report "**SOLAR CELL INDUCED ELECTRO-KINETIC ENHANCED PHYTO-REMEDIATION OF TOXIC HEAVY METAL POLLUTANTS FROM WATER USING HYDROPHYTIC PLANTS**"

is the bonafidework of

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MART Specialities Lab LLP

Memorandum of Understanding (MoU) between
MART Specialities Lab LLP, Hyderabad
&
Department of Chemistry, TARA Govt. College, Sangareddy (Autonomous)

MART Specialities Lab LLP, Hyderabad and **Department of Chemistry**, TARA Govt. College, Sangareddy(A) agree on the importance and the usefulness of establishing scientific and academic links, in order to assert and to consolidate the ties of friendship between two institutions. The subject to mutual consent, the area of cooperation will include the following aspects;

1. Design and developments of Student research projects
2. Participation in Research and academic meetings.
2. Student Training Programmes.
3. Joint Research activities and publications.
4. Providing Analytical services to the research projects.
5. Support in Patent Filing procedures.
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1. Details of the implementation of any particular exchange resulting from this MoU shall be negotiated between the two organizations.
2. This MoU becomes effective on the day it is signed and remains valid for THREE years.
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4. Any amendment or modification to the present text shall be submitted for review to the competent authorities, and shall not binding unless reduced to writing and signed by both the organizations.
5. This MoU does not bind either of the two parties legally or financially. Its aim is to promote relations that will mutually benefit each organization, this being the primary aim of academic collaboration.

In witness whereof, the organizations hereto have offered their signatures.

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Date: 31.12.2021

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SOLAR CELL INDUCED ELECTRO-KINETIC ENHANCED PHYTO-REMEDIATION OF TOXIC HEAVY METAL POLLUTANTS FROM WATER USING HYDROPHYTIC PLANTS

INTRODUCTION

Environmental contamination and pollution by heavy metals is a threat to the environment and is of serious concern [1-2]. Rapid industrialization and urbanization have caused contamination of the environment by heavy metals, and their rates of mobilization and transport in the environment have greatly accelerated since last century [3-4]. These inorganic pollutants are being discarded in our waters, soils and into the atmosphere due to the rapidly growing agriculture and chemical industries, improper waste disposal, fertilizers and pesticides. Heavy metals are persistent in the environment, contaminate the food chains, and cause different health problems due to their toxicity. The trophic transfer of these elements in aquatic and terrestrial food chains/webs has important implications for wildlife and human health. This occurs due to bioaccumulation in the food chains as a result of the non-degradable state of the heavy metals. It is very important to assess and monitor the concentrations of potentially toxic heavy metals and metalloids in different environmental segments and in the resident biota. A comprehensive study of the environmental chemistry and ecotoxicology of hazardous heavy metals and metalloids shows that steps should be taken to minimize the impact of these elements on human health and the environment. Chronic exposure to heavy metals in the environment is a real threat to living organisms [5].

The most common heavy metal pollutants are lead and mercury. The Institute for Health Metrics and Evaluation (IHME) estimated that in 2019, lead exposure accounted for 900 000 deaths and 21.7 million years of healthy life lost (disability-adjusted life years, or DALYs) worldwide due to long-term effects on health. The highest burden was in low- and middle-income countries. Once lead enters the body, it is distributed to organs such as the brain, kidneys, liver and bones. The body stores lead in the teeth and bones, where it accumulates over time.

Lead stored in bone may be released into the blood during pregnancy, thus exposing the fetus. Undernourished children are more susceptible to lead because their bodies absorb more lead if other nutrients, such as calcium or iron, are lacking. Lead exposure can have serious consequences for the health of children. At high levels of exposure lead attacks the brain and central nervous system, causing coma, convulsions and even death. Children who survive severe lead poisoning may be left with intellectual disability and behavioural disorders. Similarly mercury poisoning causes peripheral neuropathy, presenting as paresthesia or itching, burning, pain, or even a sensation that resembles small insects crawling on or under the skin (formication); skin discoloration (pink cheeks, fingertips and toes); swelling; and desquamation (shedding or peeling of skin).

Mercury irreversibly inhibits selenium-dependent enzymes and may also inactivate *S*-adenosyl-methionine, which is necessary for catecholamine catabolism by catechol-*O*-methyl transferase. Due to the body's inability to degrade catecholamines (e.g. adrenaline), a person suffering from mercury poisoning may experience profuse sweating, tachycardia (persistently faster-than-normal heart beat), increased salivation, and hypertension (high blood pressure).

Phytoremediation is a sustainable process in which green plants are used for the removal or elimination of contaminants in soils. Both organic and inorganic contaminants can be removed or degraded by growing plants by several mechanisms, namely phytoaccumulation, phytostabilization, phytodegradation, rhizofiltration and rhizodegradation. Phytoremediation has several advantages: it can be applied in situ over large areas, the cost is low, and the water bodies do not undergo significant damages. However, the restoration of a contaminated site by phytoremediation requires a long treatment time since the remediation depends on the growth and the biological cycles of the plant. It is only applicable for shallow depths within the reach of the roots, and the remediation efficiency largely depends on the physico-chemical properties of the water and the bioavailability of the contaminants. The combination of phytoremediation and electrokinetics has been proposed in an attempt to avoid, in part, the limitations of phytoremediation. Basically, the coupled phytoremediation–electrokinetic technology consists of the application of a low intensity electric field to the contaminated soil in the vicinity of growing plants. The electric field may enhance the removal of the contaminants by increasing the bioavailability of the contaminants. Variables that affect the coupled technology are: the use of AC or DC current, voltage level and mode of voltage application (continuous or periodic), soil pH evolution, and the addition of facilitating agents to enhance the mobility and bioavailability of the contaminants. Several technical and practical challenges still remain that must be overcome through future research for successful application of this coupled technology at actual field sites.

In the current project we have focused on phytoremediation of Hg and Pb from sample solutions of using Citrate as a Chelating agent under the reduced pH and Electro-kinetic induction of 6V voltage with help of light weight solar panels which acts as renewable energy sources. The chemistry behind this project based on concepts Coordination complexes and Electrochemistry. The Chelated heavy metal complexes are stable and mild on plant cell hence the Phytoremediation process prolonged and maximum amount of heavy metal was removed from sample solution. The Modified Ek-PR in which small amount of current was passed through plants directly can enhance the heavy metal pumping into the plant roots due to electrostatic charges developed in the both Xylem and Phloem of the plants. Selection of Citrate as a Chelating agent based on its chemical tendency to form stable charged M-L complex which can be absorbed by plants under Ek-PR. Reduced pH is useful to increase the mobility of heavy metal complexes and leach them from sediments and plant surfaces.

RESEARCH PROBLEM

The conventional methods for treating metal-contaminated waters are ions exchange, coagulation, flocculation, chemical precipitation and adsorption. These traditional technologies for cleaning contaminated waters have been proved to be efficient, but not cost-effective. Phytoremediation, a plant-based green technology, has appeared as a promising alternative and cost-effective method for metal removal from moderately contaminated waters [6]. This technology is based on the ability of plants to absorb and accumulate metal contaminants in their tissues and eliminate high amount of these elements from water. The process of Phytoremediation requires metal absorption by roots and its translocation to shoots and leaves. Since biosorption of aquatic macrophytes is an fast, reversible, metal-binding process, while bioaccumulation is a slow, irreversible, ion-sequestration step [7], among various plant groups used for phytoremediation, aquatic macrophytes attain the most important position.

Several species of aquatic macrophytes such as water lettuce (*Pistia. stratiotes*), water hyacinth *Eichhornia. crassipes*), and duckweed (*Lemma. gibba*) have been used for the removal of heavy metals from waste water[8]. The aquatic macrophytes are free floating aquatic plants, with entire root system of these plants submerged in water [9]. In addition, free-floating aquatic plants can be more easily removed from polluted water than submerged macrophytes after phytoremediation. Among the free floating species, *Pistia stratiotes* is widely distributed and highly productive in Patancheru and Sangareddy industial areas. *Pistia stratiotes* is well known to accumulate a wide range of heavy metals [10]. For this study *Pistia stratiotes* was selected to test its metal removal potential from Hg and Pb-contaminated water.

Even though PR is a sustainable and green method of remediation of heavy metals but its efficacy is very low and time consuming process. Hence to enhance the capacity and rate of remediation process PR is coupled with Electro-Kinetic setup. The combination of phytoremediation and EKR has been proposed in an attempt to avoid, in part, the limitations of Phytoremediation (Bedmar et al., 2009) [11]. Basically, the coupled phytoremediation–EK technology consists of the application of a low intensity electric field to the contaminated soil in the vicinity of growing plants. The electric field may enhance the removal of the contaminants by increasing the bioavailability of the contaminants by desorption and transport of the contaminants, even over short distances. Some significant variables that affect the coupled technology are: the use of AC or DC current, the voltage level, the mode of voltage application (continuous or periodic), the evolution of the soil pH by the electrolysis of water at the electrodes, and the possible addition of facilitating agents to enhance the mobility and bioavailability of the contaminants.

The major problem faced with EK-PR is the oxidative stress in plant body caused by the formation ROS owing to EK-flux which pumps the high concentration of heavy metal ions into the plant cells with faster rates which consequently causes cellular damage and also suppress the EK-PR process with in the shorter period of the times. To increase the effective working period of EK-PR setup by controlling cellular damage we need to block heavy metal-plant peptide association by applying “Chelation technology” using suitable natural ligands. The chelated heavy metal complex will be stabilized and the toxic impact will also be reduced which certainly increases the viability of plant cells and heavy metal tolerance of the plant, consequently the life span of the plant which is used for Ek-PR also enhanced.

Another research problem associated with Ek-PR is the difficulties in the remediation of heavy metals which ‘locked up’ in bottom sediments. To tackle this issue we need to increase the mobility of heavy metals by decreasing pH of the contaminated water and also select the chelating agents which form water mobile soluble complexes which bear enough charge to get influenced by applied Electro kinetic force.

Another crucial problem might arise when approaching for the large scale remediation plans like cleaning up the contaminated ponds and lakes where you need large quantity of electricity will be needed for generation of electric field in the water body with effective level of voltage. This problem can be solved by using renewable energy sources like solar energy and strategically designing the micro-electro zones around plants to minimize the energy wastage.

OBJECTIVES

The purpose of this research project was to evaluate the removal of Hg and Pb from the predesigned water sample using modified green Ek-PR technology in the presence of Citrate as a natural chelating agent under the reduced pH with the help of lemon extract using *Pistia stratiotes*.

REVIEW OF LITERATURE

EK-PR (Electro Kinetic enhanced Phytoremediation) [12] is a bio electro restoration technique strategically designed and developed for in situ treatment of contaminated soils. The combination of EKR (Electro Kinetic Remediation) and phyto remediation enhances the effectiveness of remediation process and overcome the limitations of phyto remediation. (Hodko *et.al.* 2000; Bedmar *et.al.,* 2009) [13,11]. This process involves action of electrolysis using low intensity electric field potential and phyto absorption of contaminants from soil. EKPR consists of application of low intensity electric field in the vicinity of plant grown in the contaminated soil.

Effects of Electric field on plants characteristics was reported first by Lemstorm(1904) [14]. He stated that plants tolerate electric field, stay greener and shows an increase in yield. This experimental finding inspired to develop newer strategies of combining EKR with phytoremediation for effective environmental restoration. In the EK-PR cleaning up process of contaminants performed by plants and this process is enhanced by electric field by increasing bioavailability of contaminants.

Factors affects the efficiency of EK-PR are;

1) Use of AC/DC current

2) Voltage level

3) Frequency of voltage (continuous or periodic)

4) pH level of soil/water

5) Addition of Facilitating/ Chelating agents

In the EK-PR technology, due to applications of electric field which effectively brings large amount of soluble heavy metal ions towards plant's root system, this sometimes result stress conditions for the plants. Thus plants with high tolerance level and hyper accumulating capacity are selected for EK-PR application (Bedmar *et.al*., 2009) [11].

The coupled electrokinetic phytoremediation of of Pb, As and Cs in contaminated paddy soil under an Ek-PR were studied by Mao *et al.* [15] The results show that the EKF treatment is effective on lowering soil pH to around 1.5 is beneficial for the dissolution of metal(loid)s, thus increasing their overall solubility and translocation of Pb, As and Cs from plant roots to shoots were enhanced by Ek-PR. However, this study indicated the overall low phytoextraction efficiency of these plants.

Several studies have demonstrated that water lettuce was able to accumulate and remove heavy metals from contaminated water. According to the study investigated by Tewari et al. [16], The maximum decrease rate was observed after day 15 in the amount of Pb (97.6%) succeeded by Ni, Cu,Cr, Mn and Zn (66.94%, 95.59%, 62.9%, 74.11%, and 67.57%, respectively.

Yuanqing *et al.*, have conducted phytoremediation experiments using *Pistia stratiotes* L. (water lettuce) for removal of two important heavy metals chromium (Cr) and lead (Pb) from metal solution. It has been reported that *Pistia stratiotes* L. has efficiently removed up to 80% of Cr and 93% of Pb after 10 days [17]. The accumulation of heavy metals was more obvious in the roots as compared to leaves. These findings contribute to the application of aquatic *Pistia stratiotes* L. to lead and chromium removal from moderately contaminated waters.

Role of Chelating Agents in EK-PR

To increase mobility and bioavailability of heavy metals chelating agents are commonly used in EK-PR.(Reddy and Cameselle, 2009) [18]. Usually polydentate ligands like EDTA are extensively used in EK-PR (Lim et al., 2004 and Lim et al., 2012) [19-20]. EDTA forms soluble metal complexes in soil with heavy metals like Pb, Cu, Zn etc.

Zhou et al., (2007) [21] studied the impact of the chelating agents on EK-PR of a soil contaminated with high doses of Zn (1200-1550mg Kg^{-1}) and Cu (1200-1550mg Kg^{-1}). The investigations mainly focused on effect of EDTA and EDDS (Ethylendiamine disuccinic acid) on efficiency of EK-PR using rye grass column by vertical application of electric field. The results of this experiment showed that higher uptake of Cu and Zn by ryegrass compared with the test with no chelating agent.

Although efficiency of EK-PR increases by using chelating agent like EDTA but at the same time these chelating agents (EDTA) shows negative impact on plants growth and induces environmental issues, like contamination of ground water due to lixivation of soluble heavy metal complexes, so the chelating agents should be carefully selected to minimize the retarding effect on plant's growth and hazardous consequences to environment.

Many reports suggested that heavy metal complexes with citrate as a chelating reagent can greatly enhance their mobility and leaching from sediment particles. Moreover these complexes are more stable under and less toxic compared to their ionic moieties.

RESEARCH METHODOLOGY

Phytoremediation technologies use living plants to clean up soil, air, and water contaminated with hazardous contaminants [22]. It is defined as "the use of green plants and the associated microorganisms, along with proper soil amendments and agronomic techniques to either contain, remove or render toxic environmental contaminants harmless"[23]. Phytoremediation may be applied to polluted soil or static water environment. This technology has been increasingly investigated and employed at sites with soils contaminated heavy metals like with cadmium, Mercury, lead, aluminum, arsenic and antimony. These metal can cause oxidative stress in plants, destroy cell membrane integrity, interfere with nutrient uptake, inhibit photosynthesis and decrease plant chlorophyll [24].

Phytoremediation has been used successfully include the restoration of abandoned metal mine workings, and sites where polychlorinated biphenyls have been dumped during manufacture and mitigation of ongoing coal mine discharges reducing the impact of contaminants in soils, water, or air. Contaminants such as metals, pesticides, solvents, explosives,[25] and crude oil and its derivatives, have been mitigated in phytoremediation projects worldwide. Many plants such as mustard plants, alpine pennycress, hemp, and pigweed have proven to be successful at hyper-accumulating contaminants at toxic waste sites.

For the modified Ek phyto-remediation we have selected postea plants as they were predominantly found in the lakes of the Patancheru and Sangareddy industrial corridor and literature survey also strengthen our views as pistea has high phyto-remediation efficiency compared to other hydrophytes. The present research project confined on Ek phyto-remediation of Lead (II) and Mercury (II) from their aqueous samples under reduced pH conditions [26]. Further the Ek-PR process is facilitated by 'Citrate' as a chelating agent. Citrate moiety is an important polydentate ligand which easily forms complexes with these heavy metals which increases their stability and mobility in the aqueous samples which is a favorable move towards Ek-PR [27]. The heavy metal-citrate complexes are mild on plant cells due to retardation heavy metal-peptide bonding hence Ek-PR process prolonged due to extended plant viability for longer periods.

Acidic conditions improves electrolytic ambience by spreading charged H^+ ions. Due this heavy metal ion's transportation increases and protic conditions also elevate the leaching of trapped heavy metal ions from sediments.

In the present study, Lemon juice extract was strategically used to attain both reduced pH and to provide chelating agent i.e. citrate ion. Lemon juice extract is an eco-friendly and economical substance that doesn't shown any retarding impact on plant viability.

The novelty of the project count on its innovative approach of strategic development of micro-electric fields around the plants rhizome to minimize the energy wastage. In this methodology we inserted micro-electrodes into the lower shoot of the plants which are connected to the light weighted solar panels which provides sufficient voltage around plant area under irradiation of sunlight. These inserted micro-electrodes initiate electrostatic charges on both phloem and xylem effectively pump the ions into the plant tissues *via* plasma membrane.

MATERIALS AND METHODS

Plant material and culture conditions

Water lettuce used in our experiments was obtained from Sangareddy Lake. The plants were rinsed gently with distilled water to remove debris and approximately the same size and weight (34g of biomass) were transplanted in a borosilicate beaker containing a 300 mL of 100ppm heavy metal solution.

Experimental design

The 100ppm concentrated solution of Hg and Pb prepared in distilled water selected to assess the ability of water lettuce for Ek-PR. All of these metals were added as single metal solution. These heavy metals were added as Mercuric chloride and lead nitrate ($Pb(NO_3)_2$). Experimental setup was categorized into 5 different sets namely;

1. Control plants in plain distilled water,
2. Plants in 100ppm Hg and Pb solutions separately subjected for normal Phytoremediation (PR),
3. Plants in Hg and Pb solutions along with 3ml of Lemon juice extract subjected for normal Phytoremediation (PR),
4. Plants in 100ppm Hg and Pb solutions separately subjected for Electro-kinetic Phytoremediation (Ek-PR),
5. Plants in Hg and Pb solutions along with 3ml of Lemon juice extract subjected for Electro-kinetic Phytoremediation (Ek-PR).

Ek-PR experimental design

6A-60mA voltage light weighted solar panels were connected to micro-electrodes made up of stainless steel as shown in the **Figure 1**. These electrodes were coated with saturated KCl-NAM gel and then inserted carefully into the plant's lower shoot area. The saturated KCl-NAM is used to increase plants elctro-conductivity and restore the plant wound and minimize the mechanical stress on the plant.

All the replications were elaborated in triplicate and experiments were carried out up to plant's considerable viability. Solution samples were collected at the end of experiment and evaluate the metal accumulation by plants.

All the plants were incubated under 8/16 hour (light/ dark cycle) photoperiod using natural sun light. At the end of the experiment plants were thoroughly cleaned with distilled water and dried in hot air oven at 60^{o}C for 48 hours to prevent any enzymatic activity. The plants were tested for metal absorption and accumulation using AAS.

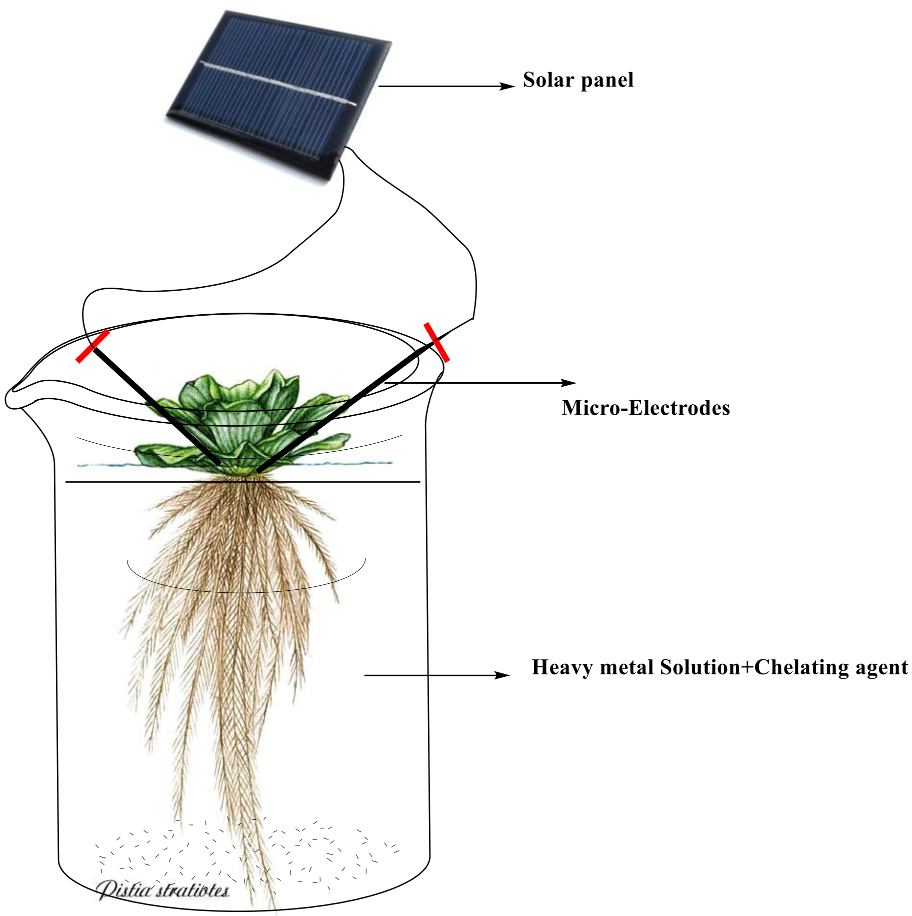

Figure 1: Experimental design for modified Ek-PR

Estimation of Hg and Pb

Vitro plants were washed with deionised water, and dried in oven at 60ºC for about 48hours to stop any kind of enzymatic activity and to obtain a constant dry weight, then ground to fine powder for analysis. The Hg contents in both sample solution and plant material were analysed by applying CV-AAS using Shimadzu AA-7000 Atomic Absorption Spectrophotometer, whereas, the Pb contents were determined by using flame AAS analysis using Shimadzu AA-6300 Double Beam Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

Viability of Plants

Ek-PR in generally reduces plant viability due to electric-stress. To minimize this effect we have used a short electric voltage input of 6V to the plants using micro-electrodes with the help of solar cells. These electrodes were coated with saturated KCl-NAM gel to cut down the mechanical stress on the plants and also heal the plant area where electrodes were inserted. But the crucial aspect of maintaining plant viability in heavy metal stress is the "Chelating Technology" where metals were allowed to form complexes with green chelating agent Citrate due to which plant viability protected. Plant viability was greatly increased in the presence of Citrate when treated with metal solutions. Approximately 60% of viability of the plants under Ek-PR was increased due to Citrate supplementation. Plants with Citrate supplementation showed considerable viability upto 96 hours where as plants treated with heavy metal solutions without Citrate supplementation showed reduced phyto-cell viability due to ROS stress because of free metal ions which have high efficacy towards metal-peptide bonding which leads to metal toxicity. The viability of plants was calculated by correlating plant's biomass (wet weight) in the due course of the experiment. Plants without treatment of Citrate lose biomass in the form of water content and bio fluids up to a great extent due to direct metal stress on the plant cell which causes phyto-cell necrosis. Whereas, Plants treated with Citrate retain their biomass considerably due to metal-citrate complex which is less toxic towards plant cells. The plant viability trends were depicted in **Table 1**. The viability trends displayed in **Figure 2.**

Table 1: Viability of Plant under different PR-conditions

Treatment	Conditions	Wet Weight of Plants (in g)			
		24-hour	48-hour	72-hour	96-hour
Hg	Normal PR (pH=7.8)	30.7	25.3	20.4	17.0
	Ek-PR (pH=7.8)	29.1	24.7	18.6	16.5
	PR with Citrate as Chelating agent (pH=5.2)	32.2	28.1	25.2	22.8
	Ek-PR with Citrate as Chelating agent (pH=5.2)	32.0	27.5	24.1	20.8
Pb	Normal PR (pH=7.8)	30.1	24.8	20.2	16.1
	Ek-PR (pH=7.8)	28.5	22.5	16.7	15.5
	PR with Citrate as Chelating agent (pH=5.2)	31.7	26.1	24.3	22.9
	Ek-PR with Citrate as Chelating agent (pH=5.2)	31.1	26.7	22.2	20.2

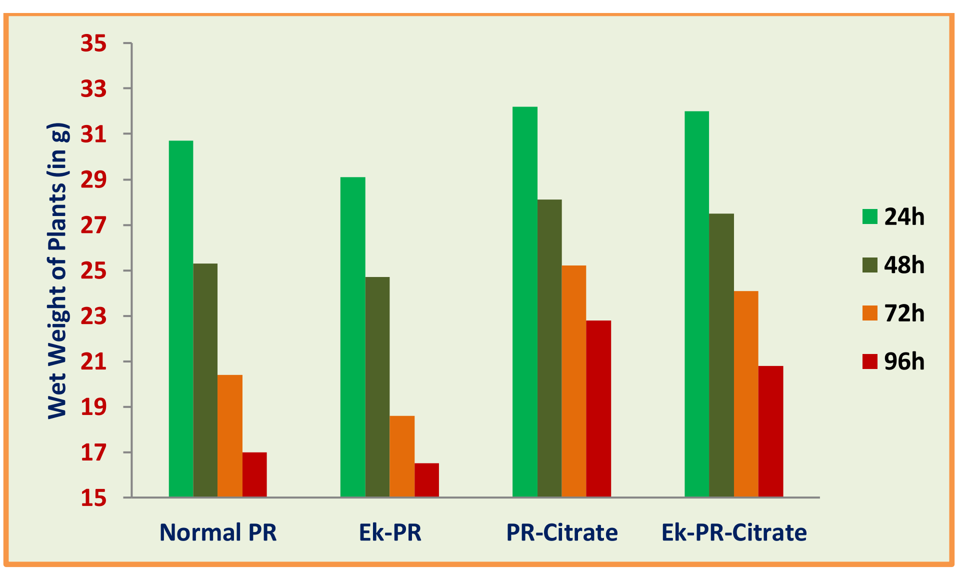

Figure 2: Viability of Plant under different PR-conditions

Heavy metal removal from water

Table 1 displayed the amount of Hg and Pb removed by *Pistia stratiotes* were observed at 200ppm concentration under different conditions. The removal efficiencies for Hg and Pb varied with varying conditions of these heavy metals solutions and PR methodology. Plants placed in metal salt solution which is treated with Citrate(Lemon juice extract) along with Electrokinetic attachment showed maximum metal absorption in both Hg and Pb sample solutions. Water lettuce was able to remove the 82 % Hg and 83% Pb in 96 hours incubation period in heavy metal-Citrate sample solution in Electrokinetic coupled phase, whereas normal phytoremediation in heavy metal-Citrate sample solution without Electrokinetic coupling showed 62%Hg and 71%Pb absorption in 96 hours, which is 20% lower in case of Hg and 12% lower in case of Pb compared Ek-PR approach.

In the absence of chelating reagent i.e. citrate the metal removal from sample solutions very low compared to the heavy metal-Citrate sample solutions. Water lettuce can only removes 42% of Hg and 67% of Pb from corresponding solution samples under normal phytoabsorption process in 96 hours incubation period. While the Ek-PR showed 54%Hg and 70%Pb absorption in 96 hours which is considerably low compared to Metal-Citrate complex solution sample with Ek-PR method.

Accumulation of heavy metals in plant

The Hg and Pb concentrations in *Pistia stratiotes* increased under Electrokinetic induction in the presence of acidic conditions (pH=5.5) along with chelating agent, Citrate (**Table 2**). To quantify metal accumulation in plant biomass, the bioconcentration factor (BCF) is more significant than the amount accumulated in plants since it provides an index of the ability of the plants to accumulate metal element with respect to the element concentration in water [28]. The bioconcentration factor (BCF) was calculated as follows:

$$\text{BCF}= \frac{\text{Hg/Pb in Plant Biomass (mg/kg in dry weight)}}{\text{Hg/Pb in Solution (mg/L)}}$$

Table 2: BCF and removal of Hg and Pb in *Pistia stratiotes* for different conditions of PR

Treatment	Conditions	Initial Concentration (mg/L, Estimated by AAS)	Final Concentration (Estimated by AAS)		BCF	Removal %
			In sample Solution (mg/L)	In plant material (mg/g dry weight)		
Hg	Normal PR (pH=7.8)	98.766±1.537	57.133±0.208	10.666±0.152	187	42
	Ek-PR (pH=7.8)		45.833±1.059	10.866±0.057	237	54
	PR with Citrate as Chelating agent (pH=5.2)		37.400±0.360	12.400±0.100	332	62
	Ek-PR with Citrate as Chelating agent (pH=5.2)		17.466±0.472	18.200±0.556	1042	82
Pb	Normal PR (pH=7.8)	99.933±0.152	32.533±0.152	12.666±0.251	389	67
	Ek-PR (pH=7.8)		29.566±0.577	12.866±0.057	435	70
	PR with Citrate as Chelating agent (pH=5.2)		28.500±0.400	14.833±0.115	520	71
	Ek-PR with Citrate as Chelating agent (pH=5.2)		16.833±0.115	19.600±0.435	1164	83

According to Zayed *et al*.[27], any sort of Phytoremediation plan must have BCF over 1000and also considered as worthy for large scale modifications. In the present project BCF value ranged between 187 and 1042 for Hg and between 389 and 1164 for Pb, respectively. The highest values were attained under acidic and Electrokinetic conditions along with the treatment of Citrate as a metal chelating agent. It is evident from the study that heavy metals were more accumulated in root parts compared leaves. But the difference is decreased in Ek-PR (**Table 2**). In Ek-PR in metal-citrate solution, metal accumulation by plant was found to be highest (**BCF Hg: 1042; Pb: 1164**) compared to other conditions of phytoremediation that are metal-citrate solution (without Ek-PR) and Metal solution (with and without Ek-PR).

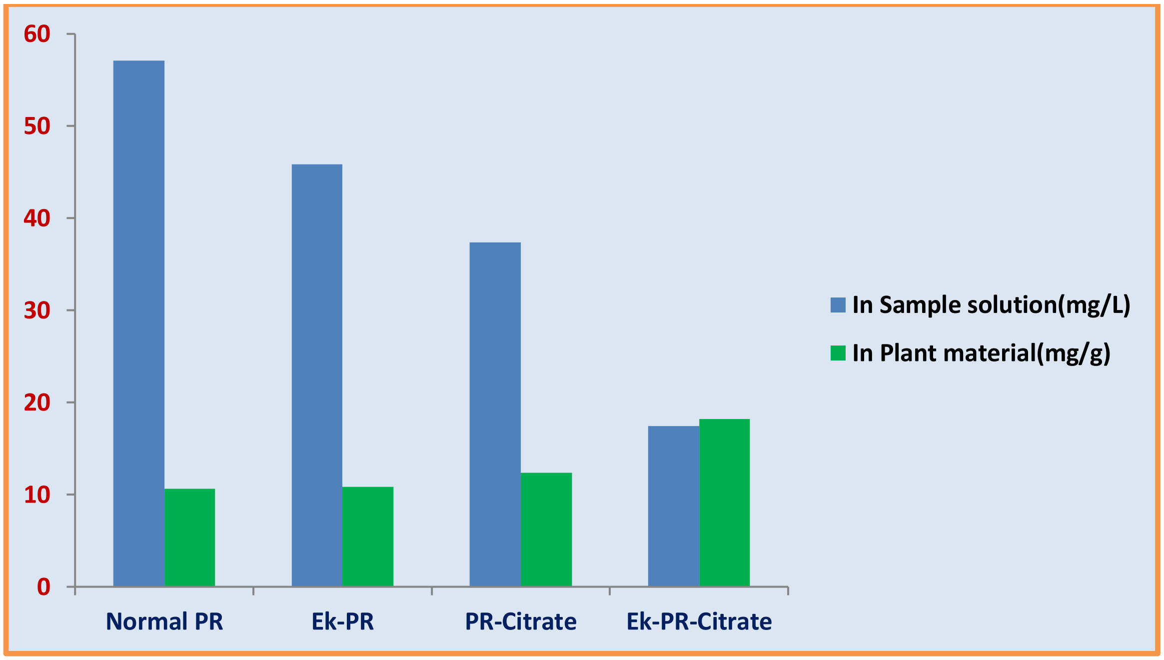

Figure 3: Phyto-accumulation of Hg by Pistia stratiotes under different conditions

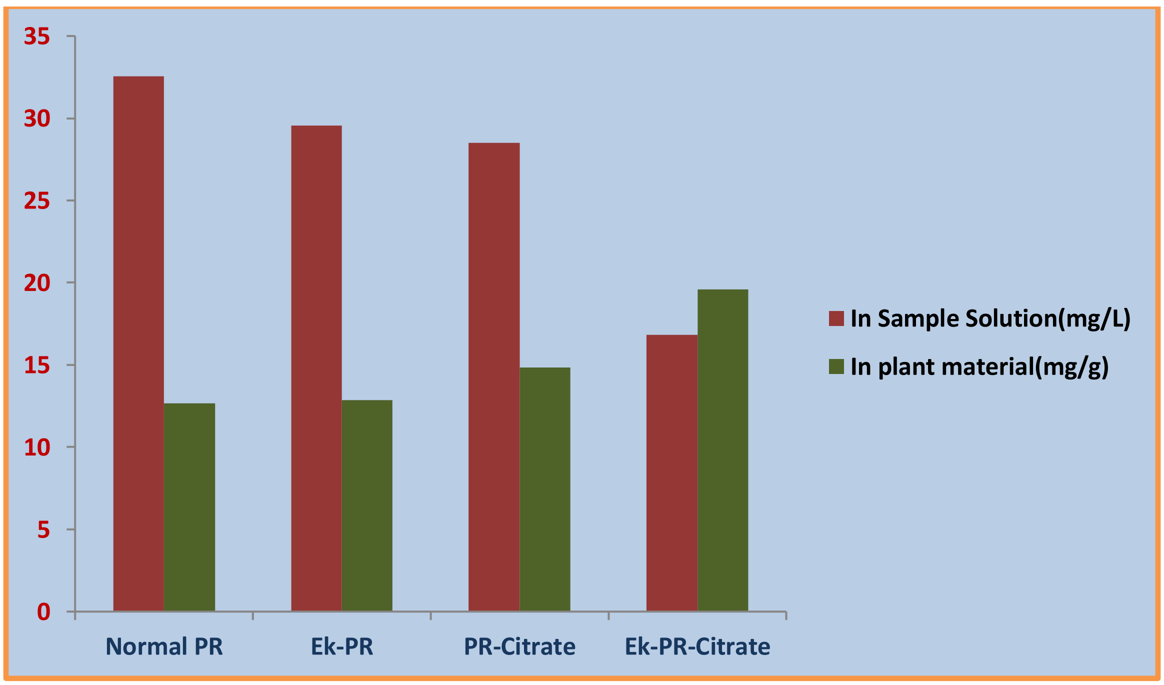

Figure 4: Phyto-accumulation of Pb by Pistia stratiotes under different conditions

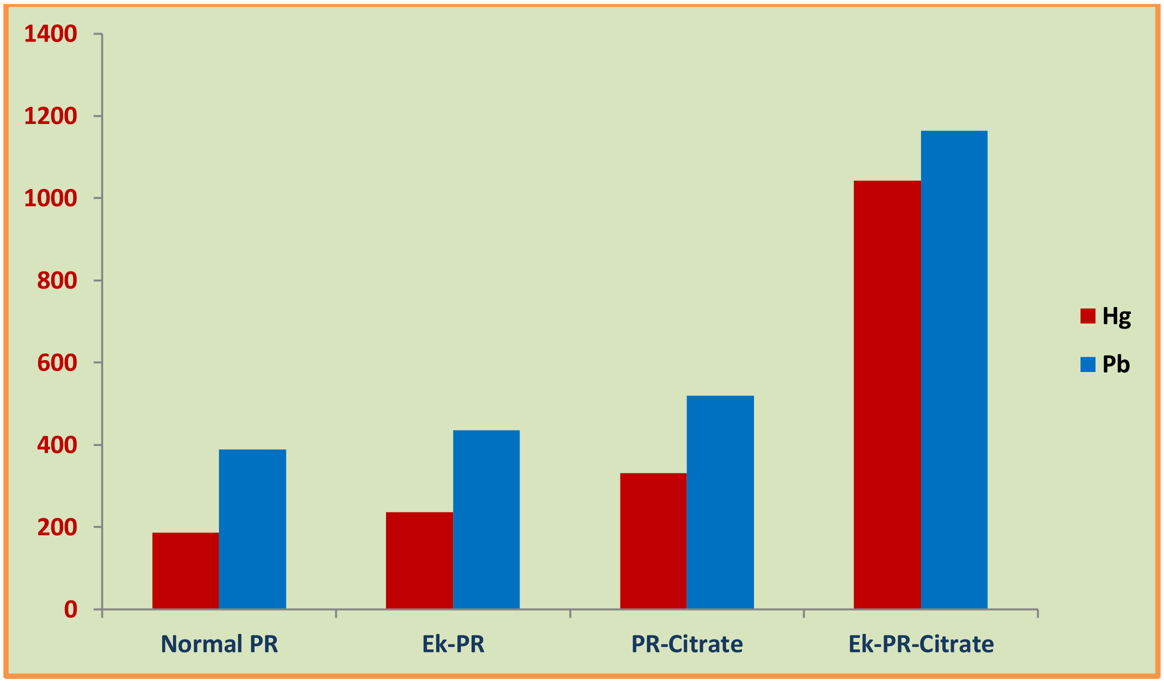

Figure 5: BCF of Hg and Pb in Pistia stratiotes under PR-different conditions

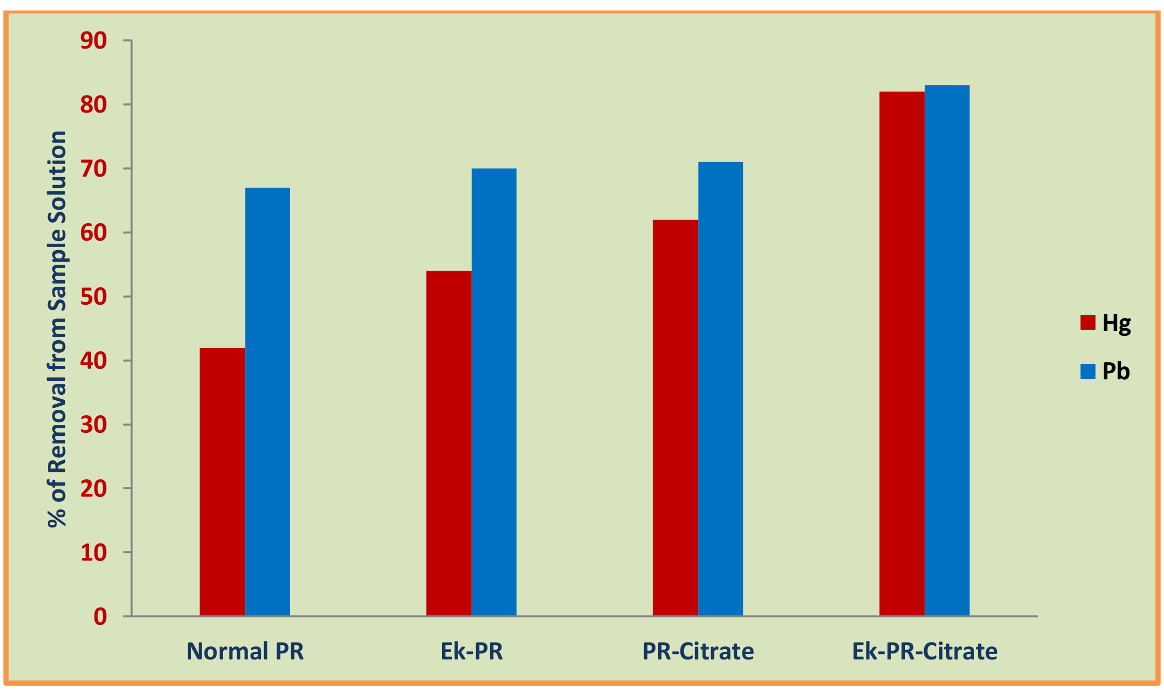

Figure 6: Phyto-removal of Hg and Pb by Pistia stratiotes under PR-different conditions

Distribution of Heavy metals in Plants

Plants accumulated heavy metals more in their roots compared to their leaves, which indicates restricted movement of absorbed heavy metals in the plant body. In the Ek-PR system, this trend was further enhanced and showed 20 to 22% of increased accumulation of Heavy metals in the roots due to insertion of micro-electrodes in the lower shoot area which will not allow the metal movement beyond that area.

Table 3: Mean concentration of heavy metals in leaves and roots of *Pistia stratiotes*

Treatment	Conditions	Accumulation of Heavy metal in Plants (Estimated by AAS)		RSI
		In leaves (mg/g)	In roots (mg/g dry weight)	
Hg	Normal PR (pH=7.8)	3.89	6.78	1.7
	Ek-PR (pH=7.8)	2.87	8.00	2.8
	PR with Citrate as Chelating agent (pH=5.2)	3.99	8.41	2.1
	Ek-PR with Citrate as Chelating agent (pH=5.2)	2.92	15.28	5.2
Pb	Normal PR (pH=7.8)	4.12	8.55	2.1
	Ek-PR (pH=7.8)	2.17	10.7	4.9
	PR with Citrate as Chelating agent (pH=5.2)	2.87	11.96	3.9
	Ek-PR with Citrate as Chelating agent (pH=5.2)	2.19	17.41	7.9

Further the Chelating agent increases the concentration of the heavy metals in the root area. The selective phyto-accumulation of heavy metals has been expressed in terms of Root Selectivity Index (RSI). As the RSI value increases this indicates the concentration of heavy metals in the root area.

$$\text{RSI}=\frac{\text{Concentration of Heavy metals in Roots(mg/g in dry weight)}}{\text{Concentration of Heavy metals in Leaves(mg/g in dry weight)}}$$

RSI value of Ek-PR along with Chelating agent is 5.2 for Hg and 7.9 for Pb which is approximately three(3) times more than normal PR of Hg and 3.8 times superior to normal PR of Pb.

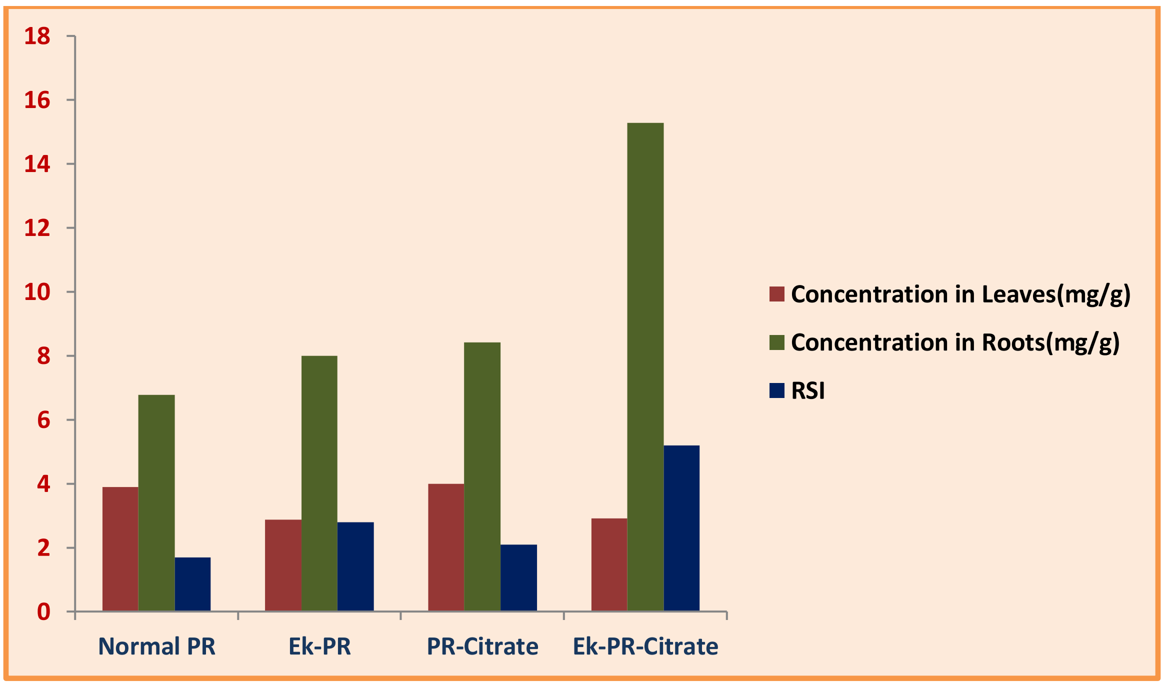

Figure 7: Mean concentration of Hg in leaves and roots of Pistia stratiotes

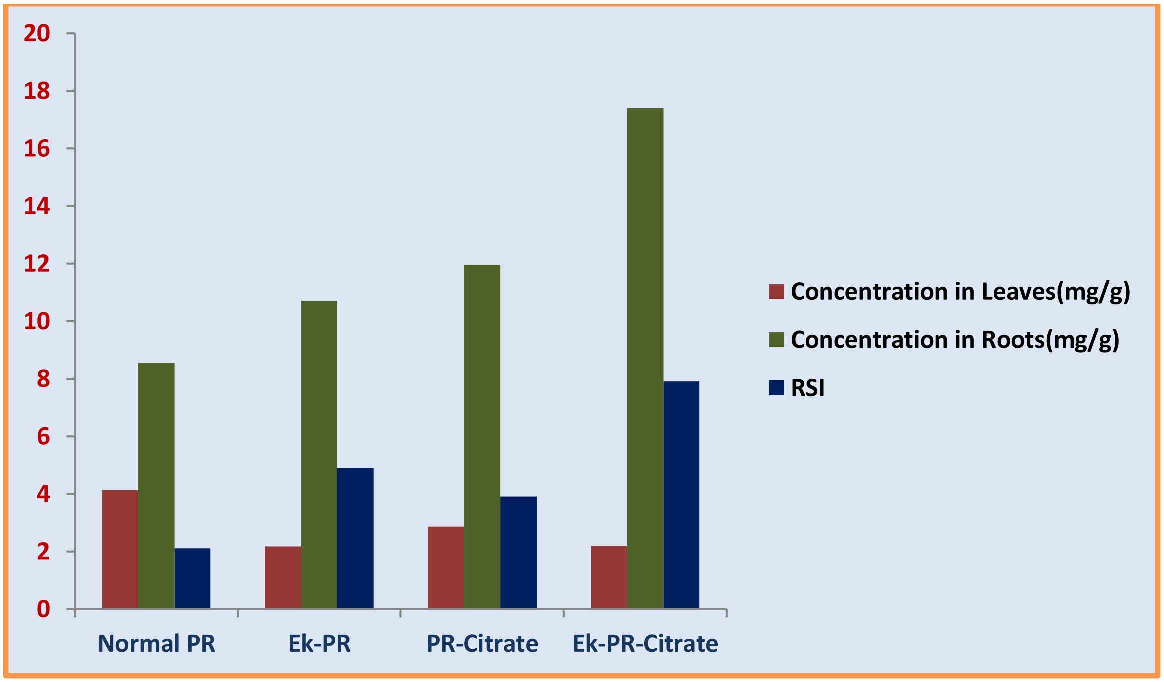

Figure 8: Mean concentration of Pb in leaves and roots of *Pistia stratiotes*

CONCLUSION:

From the current project it is clear that Electro-kinetic induction and Citrate as a Chelating agent has played a vital role in phytoremediation of Hg and Pb using hydrophytic plant, ***Pistia stratiotes*** from sample solutions. The Chemistry behind this concept is Electrochemical pumping of charged heavy metal-Citrate complexes into plant roots with a steady rate. In addition to this the charged heavy metal-Citrate complexes plausibly mild on plant cells due their inability to form chemical bonds with plant's essential components like proteins owing to strong coordination bonding between heavy metal and Citrate as a bidentate ligand. These M-L complexes are stable and possess enough charge to drive into the plant tissues under Electro-kinetic potential. Another merit of this project is the usage or renewable energy source i.e. solar panels for Electro-kinetic potential. The pH factor is also an important aspect for removal of heavy metal from sample solution because of the ionic ambience created in the solution can leach the deposited and adsorbed heavy metals and make them free for phyto-absorption. This Modified Citrate coupled Ek-PR method has an effective method to remove heavy metals like at higher concentrations.

Even though the Electro-kinetic Phytoremediation (Ek-PR) has been proved as an effective method of Phytoremediation compared to normal Phytoremediation method, the efficacy further increased when the experiment conducted in the presence of suitable Chelating agent. It is evident from the results of the research project is that the Citrate coupled Electro-kinetic Phytoremediation (Ek-PR) removes 82% of Hg and 83% of Pb from approximately 100ppm of heavy metal solutions at 5.2 pH which is nearly 40% more in case of Hg under normal sort of Phytoremediation. Similarly Citrate coupled Ek-PR of Pb showed 16% of increased Phytoremediation compared normal conditions.

The Stretegic development of Phytoremediation plan depends on Plant's Bio-concentration Factor (BCF) value which must be greater than 1000. In the current experiment BCF value for Hg is 1042 and for Pb it is 1164 under the Ek-PR-Citrate model. It is observed that absence of chelating agent drops the BCF value below 1000. Hence the Chelating agent is an essential aspect of Ek-PR.

The outlook of the projects also opens new avenue for Environmental protection especially in the restoration of polluted lakes and ponds using natural flora present in them like Pistia, Icornia etc. This process is very convenient, ecofriendly and economical to scale up for bulk usage. In the Current situations where Industrialization is inevitable which consequently leads to pollution of natural resources, it is necessary to design environmental protection strategies using simple natural processes by applying concepts of 'Science and Technology' for the benefit of the society as whole.

SUGGESTIONS:

Further studies should investigate the combination of several nature originated Green Chelating agents in Phytoremediation of toxic heavy metals from polluted ecosystems using mild Electro-kinetic induction using renewable Solar panels.

The following suggestions were made based on this project:

- This novel Lab experimental set up of Ek-PR can be modified into large scale model for remediation of ponds and lakes by using light weighted solar panels which can be floating on the hydrophytic plants.

- Natural green Chelating agents like fruit carboxylic acids and several amino acids can be strategically used for environmentally benign phytoremediation techniques as these Bio-Chelating agents can easily be decomposed naturally and non-hazardous.
- The hydrophytes used for PR can easily replace and discarded after metal accumulations.
- Small scale domestic set ups can be designed based on this concept to purify the drinking water using variety of aquatic plants and small battery system using Bio-chelating agents.
- Identical approach can be used to design hydroponic culturing methods for efficient nutrient uptake.

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