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ELECTRIC CURRENT; A BETTER ALTERNATE COMPARED TO EDTA IN RAPID ACCUMULATION OF HEAVY METAL IN *TAGETES ERECTA*

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ABSTRACT

Electrokinetic remediation is an innovative technique to clean spiked soils, in the experiment undertaken effect of current and chemical chelators have been studied and discussed on marigold in potted plants, 15 d(days) old plant were transferred to pots followed up by discrete treatment with 3 milimolar(mM) ethylenediaminetetra acetic acid (EDTA) and electric current. Increasing voltage of DC current (i.e. 1V, 2V, 3V and 4V) was applied for 15 min (minute) for 30 days (d). The findings unveiled that the shoot of physically amended plant was capable of accumulating higher values of metal on 30thd initially, however on 45thd the accumulation was almost same for chemically and physically amended plants. The faster accumulation of heavy metal was aided by the intermediate values of current which helped the plant to withdraw maximum heavy metal on 30th(d) day which proves electrokinetic remediation to be a rapid and better alternative compared to EDTA which shows retarded remediation initially because of damage caused by it to the root hair and has potential threat to contaminate the underground water due to leaching of heavy metals.

Keywords : EDTA, Heavy metal and *Tagetes erecta*

Introduction

Heavy metals are elements mostly found in fourth period of periodic table, they have high atomic weight and density five times higher than water. Upshot of geological processes like volcanic eruptions, weathering, spring waters and bacterial activity are responsible for heavy metal concentration but some anthropogenic activities such as rapidly expanding industrial area, automobile exhaust, use of fertilizers, mining trails, waste water, combustion of fossil fuels and metallurgical processes are prime cause of adding heavy metals in the ecosystem (McGrath *et al.*, 2001; Schalscha *et al.*, 1988 and Nriagu *et al.*, 1998). In comparison with other organic contaminants heavy metals being not decomposed by microorganisms persist in the surroundings for a very extended span of time hence causing a threat to mankind and its surroundings (Mulligan *et al.*, 2001). Ex-situ remediation of contaminated soil being expensive and tedious task has not been used extensively to overcome the problems of ex-situ remediation of heavy metal contaminated soils; phytoremediation has been adopted widely in past few decades.

Phytoremediation is an age- old technique used to mitigate toxic effect of heavy metals, it accomplishes the criteria of being clean, green solar driven, eco-friendly and sustainable hence phytoremediation has emerged as a popular technology to reduce the organic and inorganic pollutants present in soil, water and air (Salt *et al.* 1998). Phytoremediation being dependent on plant's physiology totally depends upon the strategy and resilience of the plant to thrive in unfavorable conditions therefore alone phytoremediation consumes a long period to fulfill the purpose for which it is planted. To enhance phytoaccumulation efficiency a number of amendments have

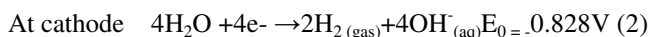
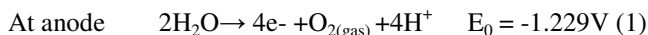
been introduced out of which electrokinetic remediation has made significant stride in the recent past (Cameselle *et al.*, (2013) and Cameselle (2015)). Electrokinetic field (EKF) indulges in the application of direct or alternating current in the spiked soil via electrodes (X Mao (2016); Bi *et al.* (2011,2012) and Aboughalma *et al.* (2008)) Electrokinetic remediation is a directional environmental technique developed on purpose for the removal of a range of contaminants present in the soil, sludge and sediments, this technique can be applied to any solid porous material or surface (Reddy and Cameselle (2009)), the technique is premised on the exertion of the DC electric current of low intensity to a cavernulous matrix which needs decontamination. The contaminants move out of the porous matrix (soil) under the influence of electric field which can be collected and treated after pumping out. Application of electric field near to the plants develop voltage gradient which drives soluble pollutants out of the soil by electromigration, electro osmosis and electrophoresis (Acar *et al.*, 1993 and Yang *et al.*, 1990).

Electromigration is the movement of ions in solution of interstitial fluid and in the soil matrix towards the electrode of opposite charge. Cations move towards the negative electrode cathode whereas anions towards anode. Factors such as charge carried by ion and strength of electric field affect the electromigration rate.

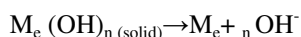
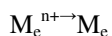
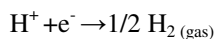
Electro-osmosis is over all flux of water or interstitial fluid instigated by applied electric field. It being a complex process depends on a number of contributory factors such as electric characteristics of soil surface, synergy between soil surface and the components in the soil solution. Soil and sediment particles carrying negative charge tend to move towards the cathode in case of positive soil matrices the

osmotic flow reverse their direction i.e. towards a node (Probstein *et al.*, 1993)

Electrophoresis is defined as the movement of charged particles under the influence of electric field (Mitchell, 1993). Electrophoresis in electrokinetic remediation comes into play only when the soil or the decontaminated matrix is supplemented with surfactants to form micelles which transport on the application of electric current (Acar *et al.*, 1993). Application of electric field to soil leads to electrolysis of water molecule converting it into hydroxyl (OH⁻) and hydrogen ion (H⁺) leading to change of pH of the soil. Electrolysis of water generates oxygen gas and hydrogen ions at anode and hydrogen gas at cathode.



The central reaction of electrokinetic remediation is electrolysis of water where E_0 is standard reduction in electrochemical potential; it is a measure of propensity of a reactant at its standard state to proceed to give the product. Secondary reactions too can take place depending upon the ionic species present in the vicinity of other charged chemical species as follows:



Where M_e refers to metal ions present in the soil.

At early stage because of reaction 1 an acidic medium develops at anode and alkaline at cathode, pH drop (up to 2) occurs at anode due accumulation of H⁺ ions and elevated pH at cathode (up to 12) depending upon the value of DC current voltage applied Acar *et al.* (1988, 1989, 1990 and 1990). In the next stage the acid front developed at anode will make advances towards cathode induced by a number of factors like (i) migration induced by electro chemical gradient (ii) by pore fluid advection caused by electro osmotic flow or any external applied electric field or (iii) internally generated hydraulic potential differences and (iv) diffusion caused by electrochemical gradient (Alshwabkeh, 1992; Acar *et al.*, 1993 and Probstein, 1993). Half cell reactions are supposed to dominate this stage unless they are affected by buffering action of soil promoted as a result of cation exchange capacity of soil along with the presence of organic chemical species and salts such as CaCO₃ which increases the buffering ability of soil and neutralizes acidic front elevated by production of H⁺ ions (Yang *et al.*, 1990). The chemistry of soil bed is considerably affected by the products of electrolysis formed at the boundaries. The movement of hydrogen ions toward the cathode assist in desorption of species from clay surfaces and dissolution of the salts in the soil. The reverse migration and diffusion of hydroxyl ion generated at cathode may lead to premature precipitation of cation moved to this region. Improved techniques are required to overcome this early precipitation. The coherence of transport of a species is directly related to its transference number, which depends on ionic mobility and concentration. As the concentration of the species reduces by transfer across the soil mass, the hike in H⁺ ion concentration in the pore fluid would decrease the transference number of other species, thus decreasing their removal efficiency. All of these transport mechanisms are escorted by sorption in the soil,

precipitation and dissolution and other aqueous phase reactions in pore fluid Acar *et al.* (1993). However ionic migration happens to be the principle component of mass transport in electrokinetic remediation (EKR). Due to accumulation of hydrogen ions at anode the pH drops at anode and increased alkalinity at cathode increases the pH at cathode. Active changes in soil pH occurs due to migration of H⁺ and OH⁻ ions to the soil, as H⁺ moves twice faster than OH⁻ ion the proton front dominates till hydrogen ions meet hydroxyl ion to form water. Thus two zones of high and low pH develop in the soil. The authentic soil pH depends upon the rate of migration of hydrogen and hydroxyl ion along with other geochemical factors (Reddy and Cameselle, 2009).

These electrokinetic amendments can be a better alternative to overcome negative impact of chemical amendments. Moreover movement of charged ions under electric field helps in the transportation of the ions to rhizosphere increasing bioavailability (Hodko *et al.*, 2000) of metals for absorption by the root hair. No substantial comparisons on application of synthetic chelator and electrokinetic remediation has been done so far to explore their effectiveness, therefore a research experiment was planned to study the relative efficiency of physical amendment (electric current) and chemical amendment (EDTA) in enhancing metal accumulation in *Tagetes erecta*.

Materials and Methods

Soil was collected from MJPRU university campus, Bareilly, from a depth of 0-20 cm, after air drying and removing soil lumps, plant debris and small rocks soil was passed through a sieve of 2 mm (millimeter) in order to obtain fine soil. Soil properties of the university are listed in the table (Table 1).

Local variety of plant seeds of *Tagetes erecta* was used for the experiment, plant seeds were sown in well ploughed and drenched nursery prepared by 2-3 harrowing and by mixing field yield manure (FYM) in the month of October with temperature 20⁰ centigrade (C). After two weeks plants were transferred to the potted soil. To carry out the experiments 35 pots were prepared and divided into 7 groups out of which group one (T1) had the standard plants with no heavy metal contaminants, second group (T2) included the pots with 200 mg of heavy metal PbNO₃ (HIMEDIA, Mumbai) group third (T3) had contamination supplemented with synthetic chelators EDTA (3mM) in addition to the lead. Fourth group (T4) till seventh group (T7) had pots contaminated with heavy metal dose along with increasing values of DC current (1V, 2V, 3V and 4V). The experimental set up included a DC current supply of 12V with two electrodes of 15 cm (centimeter) long and 2mm thick kept 10 cm apart and same set up was used to give electric current to the 35 pots. A 15 min current supply was given for 30 days. Chemicals used in the experiment were of analytical grade, and all solutions were prepared in laboratory prepared water. Conventional methods were used to analyze pH of soil, soil organic content (Allison 1965) CaCO₃ (Marr 1909) and electrical conductivity (Cang *et al.*, 2011). The soil samples were digested with HF-HNO₃ (HIMEDIA, Mumbai) HClO₄ (HIMEDIA, Mumbai) after air drying and filtration to determine total heavy metal content of soil. The root and shoot of *Tagetes erecta* were harvested and digested with HClO₄ - HNO₃ (1:5v/v) or assessment of heavy metal

accumulated in respective plant parts by AAS (Analytical Jena).

Statistical analysis

The results obtained were subjected to statistical analysis. Two way ANOVA was applied, 0.5% level of significance between the T1 and T3 and also between T1 and T4, T5 and T6 was observed, whereas T3 and T6 showed 0.1 % level of significance.

Results and Discussion

A variation was observed in the pH and electrical conductivity. The pH decreased around the anode and an increase in pH was observed around the cathode pH increased from 6.98 to 7.39 (0.41 unit pH) was increased in case of lowest current applied i.e. group T4, in case of group T5 pH increased was from 6.98 to 8.23 (1.25 unit pH), from 6.98 to 8.48 (1.5 unit pH) in case of T6 and pH increase was (1.91 pH units) from 6.98 to 8.95 in T7 plants.

Results show coincidence with findings of (Connor, 2003) i.e. higher the voltage applied larger was pH variation, pH variation arose due to DC current that cause acidification at electrodes as a result of electrolysis of water and production of H⁺ and OH⁻ ions and their migration to respective electrodes. Electrical conductivity variation followed the pattern like pH changes, the electrical conductivity of soil in the last group i.e. T7 (0.87 mScm⁻¹) was almost three times higher than T3 (0.25mScm⁻¹) which can be ascribed to the acidification of soil and dissolution of cations in the soil, but the soil EC had no significant changes at the middle of the electrodes.

Plant biomass varied according to the soil composition and applied current, and chelator supplemented, it was found that minimum biomass production was for the pots having only heavy metal treatment (T2), biomass production was affected by the heavy metal contamination and the current applied. The biomass of *Tagetes erecta* was 10.524±0.05 on 30thd which increased upto 18.17±0.05 on 45th day in case of control plants (T1), on 45th d in heavy metal contaminated plants (T2) the biomass was 13.21±0.05, 16.55±0.02 in plants having chelators and heavy metal (T3), 15.35±0.21 in (T4), 15.55±0.06 (T5), 16.92±0.05 (T6), 15.17±0.05 in (T7) on 45th d, similar pattern of reduction in biomass has been reported by (Cang *et al.*, 2011). Reduction in biomass is attributed to decrease in photosynthetic rate causing decrease in cell elongation which ultimately reduces the biomass production the electric current helped in the movement of the ions towards the respective electrodes where they get trapped by the chelators given to the soil. Root biomass too followed the similar pattern like maximum biomass happened to be of the control plants whereas minimum was recorded in pots having only heavy metals followed by pots with heavy metals EDTA and then electric current. The current in increasing order boosted the root biomass similar to earlier observed by (Zohu *et al.*, 2007) however highest voltage applied was not favorable rather low intensity of voltage proved to be more productive analogous to the findings of (Santoyo *et al.*, 2017).

A profound difference was observed between the plants supplemented with EDTA and the treatment assisted by electric current this clear disparity has been accredited to the choice of chelator used in the experiment, EDTA has been reported to cause damage to the root hairs (Prasad, 2007) of

the plants and unable to absorb increasing heavy metals leading the heavy metal draining down. Medium current applied to the plants i.e. group T6 showed maximum and early accumulation than other groups of current supplementation and EDTA application (T3). EDTA too serves the purpose of remediation but being sluggish in action and the propensity of root hair damage it causes doesn't endorse it to fit the criteria of being applauded as a superior amendment in phytoremediation. Marigold has ability to uptake the heavy metal lead in the aerial parts of the plants this characteristic has been exploited in the experiment to enhance accumulation. In controlled plants Lead accumulation in shoot of marigold was found to be 0.119±0.004 mg kg⁻¹ on 30thd (day). Plants in lead spiked soils up took 48.24±0.114 mg Kg⁻¹ in the shoot of the plants, on supplementing soil with EDTA the accumulation increased up to initially 53.18±0.079 which escalated to 70.82±0.0836 on 45thd (day) due to binding ability of the EDTA with the metal ions. The results obtained had higher concentration of Lead in the shoot compared to root which was contrasting with the findings of (Cang *et al.*, 2011) where more accumulation of heavy metals was obtained in the root part.

Application of the electric current increased the metal concentration as expected compared to the group T2 (pots contaminated with lead), highest accumulation was observed in case of intermediate value of current applied (3V) it helped the plants to grow and survive better even in the presence of the stress imposed by the heavy metals, which has also been affirmed by (Cameselle *et al.*, 2019). On 30th day the application of intermediate value of current helped plants to accumulate lead ions rapidly which was 63.18±0.447, higher than accumulated by EDTA (53.184±0.079) which vividly displays intermediate value of current giving quicker results in the first instance, however if values on 45thd (day) are taken into consideration, the EDTA treated plants were capable of accumulating 70.82±0.08366 mg Kg⁻¹ of Lead on 45th d (day) and intermediate value of current being able to garner only 68.4±0.0704 mg Kg⁻¹ of Lead, no significantly divergent results were obtained in both the amendments administered, which was in accord of prior findings by (Huang *et al.* (1997); Zohu *et al.* (2007)), which is a trivial issue, what significant here is the indulgence of intermediate value of current already accumulated 89.21% of heavy metal which was accumulated by EDTA on 45thd certifying intermediate value of DC current to be more effective than chemical amendment. This efficient uptake is time saving, instead of one prolonged duration of crop cycle, many subsequent cycle of 30 d can be used for efficient uptake of heavy metals. Further the demerits of EDTA reported earlier by (Sinha *et al.* 2010; Gupta *et al.*, 2008) made it explicit to cause damage to the plant root hairs and leaching of heavy metals into underground water, these findings in regard to EDTA has relegated it to be an amendment of subsidiary choice in phytoremediation compared to electrokinetic remediation which achieves almost 90% of heavy metals achieved by EDTA two weeks earlier. This superiority of electrokinetic remediation can be speculated it to emerge as a powerful tool in heavy metal decontamination.

Conclusion

This research was conducted to study the effectiveness of electrokinetic remediation and synthetic chelator EDTA on marigold plants to enhance phytoaccumulation potentialities of the plant for lead. The results showed that

compared to the controlled plants, the group T3 was expected to accumulate the higher concentrations of the heavy metals from the spiked soil in accord with the older manuscript by (Shikha *et al.*, 2012) however application of electric field gave somewhat different results compared to prior research done on the EDTA based phytoremediation, application of intermediate current showed rapid accumulation (on 30thd) followed by EDTA treated plants. However on 45thd the accumulation results of both the plants happened to be indistinguishable.

EDTA being a popular and potent chelator boosts metal accumulation; however, the repercussions of the study made clear that electric current too plays significant role in cleansing the spiked soil. Optimum current voltages affect the heavy metal accumulation as it increases pH (in cathode region) and electrical conductivity which increases bioavailability by electroosmosis, migration and precipitation of heavy metals. The electrolysis of water helped in the generation of hydrogen ions which migrated towards the anode resulting in increased acidity, metal ions got solublise near the anode and migrated towards cathode. Electrokinetic remediation compared to use of synthetic chelator shows early significant accumulation of heavy metal by the plants which is limited in case of EDTA. The tendency of EDTA to cause damage to root hair and the inadequacy of EDTA not being able to absorb increasing heavy metal in the soil solution increases the threat of leaching of heavy metals that leads to contamination of underground water leading to metal poisoning after the consumption of contaminated water. Considering the overhead note electrokinetic remediation emerges as a clean, practical, eco-friendly and rapid technology which can be used extensively. Instead of waiting for harvesting of the crops, many cycles of crop can be sown subsequently and simultaneously for efficient remediation. Moreover managing direction of electric current applied, application of current directly to the plant body and better understanding of electroacoustic physical mechanism can be proved beneficial for achieving the objectives of electro remediation.

Moreover consideration of soil electric field, soil pore structure ability, soil –water interaction under the influence of electric field along the use of chelator substituting EDTA might open new avenues overcoming the demerits faced earlier in research.

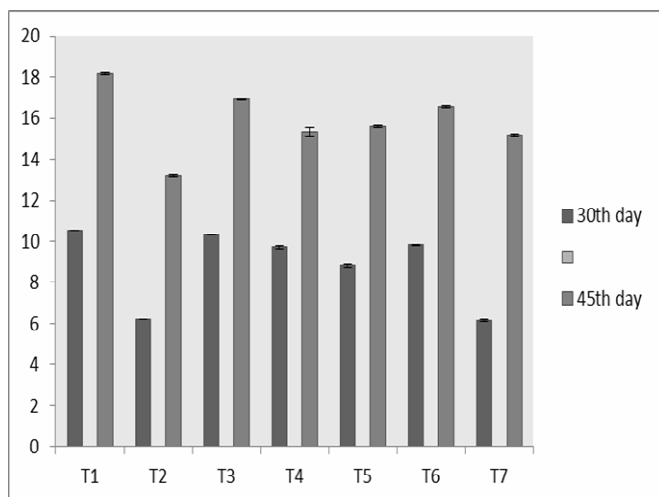


Fig. 1: Biomass on 30th and 45th day.

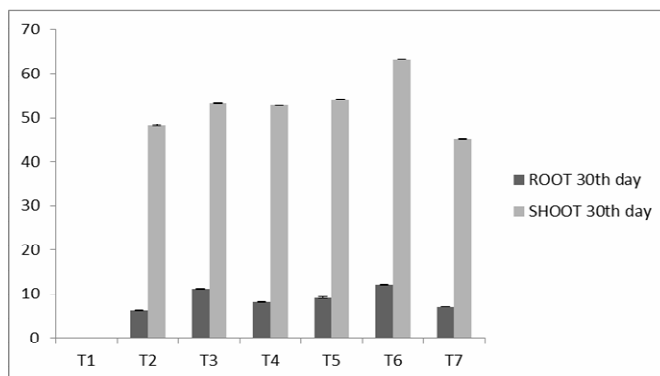


Fig. 2: Lead accumulation in root and shoot on 30th day

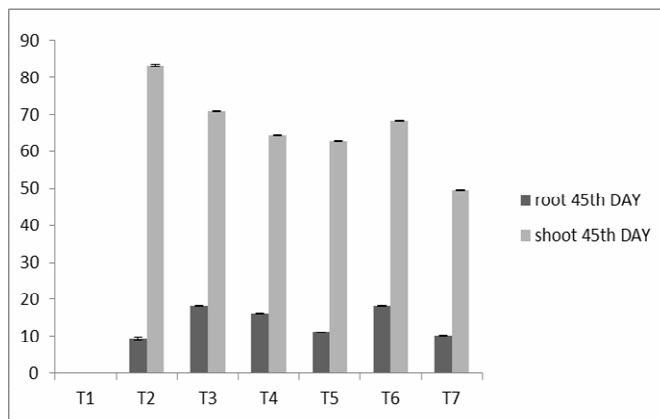


Fig. 3: Lead accumulation in root and shoot 45th day.

Table 1: Soil Characteristics

Sand	35.69%
Silt	36.23%
Clay	27.28%
Soil pH	6.98
Organic C g Kg ⁻¹	27.2%
Total N	1.00%
Total p	0.38
Exchangeable K	0.65

Table 2 : Biomass on 30th day

Treatment	BIOMASS gm on 30 th d (day)	BIOMASS gm on 45 th d (day)
T1	10.524±0.05	18.17±0.05
T2	6.22±0.013	13.21±0.05
T3	10.32±0.05	16.55±0.02
T4	9.71±0.074	15.35±0.21
T5	8.80±0.04	15.55±0.06
T6	9.81±0.014	16.92±0.05
T7	6.17±0.05	15.17±0.05

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