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ELECTRIC CURRENT INDUCED MITIGATION OF LEAD CONTAMINATION IN *VETIVERIA ZIZANIOIDES*

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ABSTRACT

Rapidly expanding industrial areas ensues a negative impact on the soil chemistry, it delivers certain stubborn heavy metals in the soil. Inefficient emission management and pollution alleviation contributes discharge of heavy metals in the surroundings causing a threat to mankind. To subside the effect of heavy metal; most effective, cost free, solar-driven, green technology phytoremediation has been adopted which indulges exploitation of hyper-accumulator plants. Heavy metal toxicity leads to production of reactive oxygen species causing oxidative stress to the plant cell. To mitigate the oxidative stress plant cell initiates production of antioxidative enzymes. The present experiment was setup for analysis and assessment of comparative study of physical (electrokinetic remediation) and chemical (chelators) amendments in enhancing antioxidative enzymes in response to the metal phytoxicity in *Vetiveria zizanioides.*

***Keywords*:** Phytoremediation, electrokinetic remediation, chelators, antioxidants and *Vetiveria zizanioides*

Introduction

Rapidly expanding industrial areas ensues a negative impact on the soil chemistry, it delivers certain stubborn heavy metals in the soil (Aboughalma, 2008). Lack of proper emission management and pollution abatement is vital cause of threat to mankind (Sobhanardakani *et al*., 2018). Agricultural and industrial development plays a pivotal role in the economy of a nation, the desperate urge of development has detoured the environmental protection guidelines to a higher magnitude (Sahu *et al*., 2008). Heavy metal toxicity arising from numerous sources is a pertinent concern of enhanced significance from nutritional, environmental, ecological and evolutionary perspective (Jaishankar *et al*., 2014; Nagajyoti *et al*., 2010). Heavy metals cannot be degraded completely rather transformed from one oxidation state to another (Garbisu and Alkorta., 2001; Gisbert *et al*., 2003).Greater biological half time (BTH) makes heavy metal stubborn and persistent in the soil compared to organic contaminants make it present in the soil for many decades (Giannis *et al.,* 2009). Occurrence of toxic heavy metals becomes inevitable when some indispensible process like application of insecticidal sprays to orchards and crops release heavy metals like arsenic in the soil (USDA NRCS 2000). Persistence (in soil) and leaching down of heavy metals into ground cause contamination of water and crops that were sown in spiked soil moreover food chain bioaccumulation makes it latently menacing (Aycicek *et al*., 2008).In this context phytoremediation happens to be a benign technology for combating heavy metal pollution; literally phytoremediation is a generic term for different plant based physiological processes which uptake heavy metal in the plant system or inhibits the horizontal or vertical metal transfer. Phytoremediation requires lower degree of manpower, is cost effective and can be applied to extensive areas (USPEA2000). The micro floral activity of the soil is enhanced due to presence of phytoremediating plants hence affecting biological properties of the soil (Cameselle *et al*., 2015).However prolonged time taken by hyperaccumulators in heavy metal decontamination and its relevancy for low to moderately contaminated soil are few limitations of the phytoremediation (Cameselle *et al*., 2019) which can be overcome by fortifying phytoremediation with electric current (Aboughalma *et al*., 2008). Application of electric current enhances bioavailability of the contaminants at rhizosphere (Hodko *et al*., 2000), only plants were incapable of withdrawing heavy metals from deeply contaminated sites. Hence phytoremediation coupled with electric current serves the metal decontamination efficiently and effectively. Implementation of low level of current across the electrodes placed at the edges of the soil is termed as electrokinetics (Acar *et al.,* 1993). Generation of reactive oxygen species (ROS) is the inaugural response by the plant cell biochemistry in response of the metal toxicity (Pourrut *et al*., 2011). A number of physiological studies conducted earlier showed enzymes such as APX, CAT, and SOD can remodel ROS into less-toxic products preventing cellular dysfunction and injury (Shahid *et al*., 2014 and Adrees *et al*., 2015). Catalase and peroxidase activity have been reported to increase in wheat against oxidative stress and glutathione reductase within 13 hours of exposure to Pb (Kumar *et al*., 2004). Alteration and oxidation of amino acid sequences, cellular proteins, lipid membranes and nucleic acid occurs due to excess generation of ROS (Adrees *et al* ., 2015). Hyperaccumulators have adopted an alternative path of production of antioxidants such as ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD) to circumvent the cellular distress and damage (Shahid *et al*., 2014). Formation of ROS and alteration of membrane nobility are the paramount key processes at the cellular level under heavy metal stress (Dietz *et al*., 1999). In order to check and choose the better amendment out of physical and chemical amendment in assisting the metal tolerance, the enzymatic antioxidant system in *Vetiveria zizanioides* was investigated by studying their effect on the physiological and biochemical parameters along with metal accumulation efficiency.

Materials and Methods

Soil specimen

The soil used for plantation of Vetiver was alluvial garden soil (used for cultivation of medicinal and ornamental plants) of Rohilkhand University Bareilly U.P. The soil was sampled from a depth of 0 to 0.3 meters (m) after removing top vegetation. Fine textured soil was prepared after discarding the plant debris, lumps, rocks and passing the soil through a mesh of 2 millimeters (mm). The soil was air dried and stored in the plastic bags at room temperature for further analysis. The chief properties of the soil are listed in the Table 1:

Table 1: Properties of Soil

Sand component	35.69%
Silt component	36.23%
Clay component	26.2%
pH	6.98
Organic carbon(g kg^{-1})	27.2%
Nitrogen	1.00%
Phosphorus	0.38%
Potassium	0.65%

Table 1 Soil characteristics

Experimental procedure

For the experimental purpose the plant used was *Vetiveria zizanioides* also known as *Chrysopogon zizanioides* native of tropical and sub-tropical India, belonging to the clade monocots of kingdom *Plantae* family *Poaceae.* Vetiver is a perennial grass reaching up to a height of 5 feet, capable of surviving even in water logged condition. The plant body is differentiated into root, stems (also known as culms) and leaves, the leaves are long thin and ridged due to deposition of silica. The plant has a well-developed dense fibrous root system thriving on a large range of pH (acidic pH=4 to alkaline pH=6) (Aibibu *et al*., 2010; Singh *et al*., 2017; Panja *et al*., 2018; Vimala *et al*., 2021) capable of water conservation and treatment of contaminated water which makes it a suitable plant for phytoremediation. It extracts many heavy metal from the soil like Mn, Zn, Cu, Pb, Pb and Zn from spiked soil and waste water (Roongtanakiat *et al*., 2009; Danh and Veticon., 2010) The Vetiver plants were propagated via culms (stalk or stem of various grasses) the culms were separated from mother plant after uprooting the plant and a 10 centimeter (cm) long culms were prepared and placed vertically in the pots, punctilious irrigation helped the plant culms to flourish above and under the soil. Once the plants were well developed, they were treated with heavy metal Lead chloride (HIMEDIA, Mumbai) 300 mg per Kg and chelator doses i.e., EDTA 50 mM (HIMEDIA, Mumbai) along with the physical amendment. The plants were grouped into seven categories from (Control, C) to T6) with each group having quintuple pots as described in table 2. The experimental setup to provide electric current included a DC current supply of 12V with two electrodes of 15 cm (centimeter) long and 2 mm 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 25 d 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) $CaCO_3$ (Marr 1909) and electrical conductivity (Cang *et al*., 2011). The soil samples were digested with HF–HNO_3^- (HIMEDIA, Mumbai) $HClO_4$ (HIMEDIA, Mumbai) after air drying and filtration to determine total heavy metal content of soil. The root and shoot of *Veteveria zizaniodes* were harvested and digested with $HClO_4$ - HNO_3 (1:5v/v) or assessment of heavy meal accumulated in respective plant parts by AAS (Analytical Jena).

Determination of plant height and dry weight

After growing plants for 45 days 5 plants were randomly selected from each of the group on 30^{th} and 45^{th} day post harvesting their root and shoot length was measured. The fresh weight was measured after cleaning, washing and air drying the plant using a digital balance; however, for dry weight of the plant the paper wrapped plants were kept in an oven for 48 hrs. at 65-75 degree Celsius before weighing

Analysis of antioxidant enzymes

Photochemical NBT (nitro blue tetrazolium) reduction method by Giannopolitis and Reis (1977) for was used for assay of superoxide dismutase activity (SOD). The reaction mixtures along with the blanks were illuminated in glass test tubes of uniform thickness under light intensity of 4000 lux and photo reduction of NBT was measured spectrophotometrically at 560 nm as to cause 50% inhibition in reduction of NBT. Catalase activity was recorded in accord of decrease in absorbance at 240nm following the method of Aebi (1984). Ascorbate peroxidase activity was studied spectrophotometrically adhering the method of Nakano and Asada (1981).The enzyme extract was obtained similar to catalase assay followed by spectrophotometric analysis of reaction mixture (50mM of potassium phosphate buffer of pH 7.0 having 1mM EDTA, 0.5 mM ascorbate, 0.4 mM of H_2O_2 and 0.1ml of enzyme extract) at 290 nm post addition of enzyme extract was recorded.

Results and Discussion

A considerable reduction in the tiller height was observed of vetiver under Pb toxicity. Amendment fortified plants were found to be taller than the heavy metal spiked plants, significant decline in the shoot height was observed in heavy metal contaminated plants compared to the controlled, however reduction in shoot was more compared to the roots similar to the findings of (Prasad *et al*., 2008). Conventionally, heavy metal toxicity triggers decrease in endogenous measure of auxin that is responsible for growth and cell elongation in the plant. In barely root tips IAA homeostasis has been found to get disturb under the brief period of lead toxicity (Zelinova *et al*., 2015). Earlier findings in Arabidopsis have shown primary root elongation suppression due to Pb (Besson-Bard *et al*., 2009). This can be attributed to the Pb toxicity which interferes either with auxin synthesis or mode of action. The plants treated with electric current were found to be much taller compared to the EDTA treated plants on 30^{th} day (7.9%) however on 45^{th} day the difference reduced (3.15%). The reduction in the growth of plant is because of reduced cell division which results after disorganized microtubule structure (Eun *et al*., 2000). Lead causes alterations in tubulin levels and post translational modification critical for cell division and growth (Gzyl *et al*., 2015). A slight decrease in the chlorophyll content was observed only after 30^{th} day and an increase in carotenoid too was observed after 30^{th} day. The drop in the chlorophyll content might have occurred due to enhanced degradation of chlorophyll or retarded rate of chlorophyll formation by heavy metal Pb (Luna *et al* ., 1994). The reduction in the productivity of the plant is related to damage chlorophyll and decreased photosynthetic rate. ALAD (d-aminolevulinic acid) inhibition activity might be responsible for chlorophyll inhibition (Padmaja *et al*., 1990). Carotenoid being involved in protection against high light intensity protects the plant chlorophyll from photo inhibition and photo dynamics that quench and damage chlorophyll (Knox and Dodge, 1995). A similar reduction was observed in chlorophyll pigment accumulation in *Catharanthus roseus* by (Rai *et al*., 2014) which might be the aftermath of Cr treatment stress. Increased concentration of heavy metals may lead to decline in the chlorophyll content in *Pisum sativum* (Gangwar *et al*., 2011).

Table 2 : Biomass of *Vetiveria zizanioides* (gm)

	Biomass	
	30^{th} day	**45^{th} day**
Control(C)	6.492±0.282	7.479±0.28
T1	4.37*±0.373	5.128*±0.447
T2	2.3*±0.122	3.36*±0.11
T3	3.22*±0.350	4.46*±0.114
T4	3.18*±0.130	4.24*±0.194
T5	2.94*±0.122	3.76*±0.151
T6	3.374*±0.429	4.56*±0.089

Table 3 : Plant height of *Vetiveria zizanioides* (cm)

	30^{th} d root length	**30^{th}d shoot height**	**45^{th} d root length**	**45^{th} d shoot height**
Control(c)	45.524±6.799	155.0408±2f.563	53.695±8.157	174.91±2.576
T1	39.126*±0.928	135.086*±5.978	45.173*±2.791	151.286*±1.724
T2	30.9418*±4.119	117.412*±7.0583	38.121*±3.508	126.90*±2.947
T3	33.43*±3.7028	124.174*±4.189	42.593*±3.832	133.356*±1.916
T4	33.218*±2.797	122.126*±3.708	39.132*±1.169	132.102*±1.659
T5	32.3082*±2.313	120.894*±10.83	40.225*±3.059	131.980*±5.00
T6	34.260*±1.932	125.774*±5.114	42.769*±2.031	137.54*±3.892

Table 4 : Antioxidative activities of *Vetiveria zizanioides*

	SOD (units mg^{-1} protein min^{-1})		**CAT (units mg^{-1} protein min^{-1})**		**APX (units mg^{-1} protein min^{-1})**	
	30^{th} day	**45^{th} day**	**30^{th} day**	**45^{th} day**	**30^{th} day**	**45^{th} day**
Control (c)	26.702±2.1498	29.946±1.674	63.3982±1.732	65.412±1.693	1343.932±81.716	1438.568±199.087
T1	34.25*±4.977	37.124*±4.062	53.446*±2.544	56.03*±3.675	1592.978*±43.646	1638.246*±125.845
T2	45.98*±7.24	48.5568*±4.2981	44.208*±2.99	45.15*±4.196	1872.172*±93.152	1885.042*±12.366
T3	36.4766*±0.273	39.3952*±0.402	50.242*±3.869	50.21*±2.973	1611.378*±214.13	1811.008*±185.801
T4	39.128*±2.0122	44.526*±1.651	48.597*±5.761	48.064*±4.808	1763.444*±35.2633	1818.052*±39.708
T5	43.656*±2.7791	46.172*±4.124	46.43*±2.464	47.085*±5.234	1808.822*±68.257	1821.362*±49.17319
T6	34.638*±4959	39.992*±5.585	51.364*±4.021	52.212*±1.969	1640.596*±137.239	1674.596*±85.701

SOD is the introductory line of blockade in opposition to oxidative stress laid by the environment. Enhancement of ROS has also been reported by Mishra *et al.* (2011) during abiotic sress. Various undesirable and growth deterent changes in the plants are there as a result of production of ROS for example hampering of ATP synthesis and DNA blight (Hossain *et al*., 2022). To attenuate the scars of ROS production, plants have choosen a myriad of composite enzyme apparatus known as antioxidant enzyme system. SOD is the first and foremost to start antioxidative activity by following reaction by converting superoxide radical to hydrogen peroxide and oxygen.

$$2O_2{\bullet}- + 2H^+ \rightarrow H_2O_2 + O_2$$

Heavy metals has been reported to elevate SOD activities in water hyacinth (Malar *et al*., 2014. For the present study SOD levels were minimum for the control (C) plants which increased in the Pb treated plants of T1 group. Application of EDTAfurther aggrevated the SOD levels. As EDTA further enhanced lead accessibility and the toxicity to the plants hence higher ROS production (Habiba *et al*., 2015). In *Brassica napus*, where EDTA reduced the SOD and other antioxidant activities. The difference lies in the concentration of Pb adminstered, mild concentration of heavy metals enhances the antioxidant activities however, peaking ones drop the SOD enzyme functioning which decreases the antioxidative activities of the plants. The application of DC current showed the enhanced SOD activity in the plants however results did not cross the values obtained by the chemical chelant.

Loew in 1900 gave the name catalase to the first discovered antioxidant enzyme. Catalase is ubiquitously found in all organisms (Kirkman and Gaetani, 2007) unexpectedly some prokaryotes too are reported to have catalase (Zamocky *et al*., 2008). All organisms perform either photosynthesis, respiration and both with concomitant generation of ROS (Sharma *et al*., 2014). Generation of ROS results when the stable oxygen molecule receives an activation energy (22 Kcal/mol) and ascents to higher energy levels.

A series of reduction reactions ushers to stable intermediates, for complete reduction of stable molecular oxygen and breaking the covalent bond four electrons along with protons are required. Chloroplasts, mitochondrias and peroxisomes are nucleus for the generation of ROS, hence curbing ROS production is inevitable, as they are by-products of vital processes such assuch as photosynthesis and respiration (Ahmad *et al*., 2010). Catalase mitigates the oxidative stress majorly found in peroxisome, H_2O_2 formed in cell as a by-product of different cellular pathways is degraded efficiently by catalase into water (Riaz *et al*., 2021). Basal level concentration was exhibited by untreated plants that spiked by 15.6% and 14% on 30th and 45th day respectively post Pb contamination. The increased CAT values is attributed to the spiked soil as a result of Pb contamination that triggers ROS production which is acted earlier by SOD to convert superoxide ion into hydrogen peroxide followed by CAT. H_2O_2 participates in vital physiological processes as cell signaling, cell growth, development and cell differentiation (apoptosis). Concentration higher than 50µM for H_2O_2 has been reported to be cytotoxic to the cells (Halliwell *et al*., 2000 ; Lennicke *et al*., 2015). Some results documented in past on antioxidative activity showed contradiction with thw outcome of the present study. For example, Verma *et al*., (2003) affirmed reduction in the catalase levels in *Oryza sativa* under lead influence, the disparirty in activities of catalse in both the studies has been attributed to the exceesive H_2O_2 causing delay in dismutation of toxic peroxides hampering the cell membrane.The trend is not confined only to metal stress but water scarcity too was responsible for reduced CAT levels in *Triticum aestivum* (Zhang *et al*., 1992).

Ascorbate peroxidase (APX) is an all-important enzyme of the cell system foraging H_2O_2 and proffering shielding to the chloroplasts against H_2O_2 and OH^- derangement. It contributes electron to reduce H_2O_2 to water along with formation of of monodehydroascorbate (MDHA). The MDHA formed is subsequently disproportionated to ascorbate (AsA) and dehydroascorbate (DHA). APXs have been tracked down in cytosol (cAPX), thylakoid (tAPX), stroma (sAPX), chloroplast (ch APX) and microbody (including glyoxysomes and peroxisome) with inappreciable difference in amino acid composition and sequence (Yamaguchi *et al*., 1995; Yoshimura *et al*., 2000).

Specified APXs operate in response to different environmental cues (Yoshimura *et al*., 2000). Surging concentration $CdCl_2$ shoot the APX activity equated to the control group (Shams *et al*., 2017), similar trends of increased APX values with $PbNO_3$ concentration in group T1 and T2, T3, T4, T5 and T6 (with amendments) was observed, this is attributed to unrestricted reactive oxygen species (ROS) formnation, degeneration of non-specialized enzymes, or competitive inhibition by toxic metals at the site of concerned enzyme's activity (Sieprawska *et al*., 2015). Application of the amendments was beneficial for the enhancement of APX activity as amendment magnified the metal availability by the plants in general.

Conclusion

The present study was taken to compare the utility and efficiency of the chemical and physical amendments by observing the growth parameters and antioxidant enzyme activity in *Vetiveria zizanioides*. The results showed the root and the shoot showed a retarded growth on application of Pb. Growth retardation was attributed to direct and indirect effect of heavy metals (Das *et al*., 1997). Direct effect includes heavy metal toxic effect to the plant and indirect cause non-accession of water and minerals to the plant (Aibibu *et al*., 2010). A decline in biomass was attributed to the accumulation of Pb in the root and shoot of the plants. Accumulation of Pb was recorded to be more in roots compared to shoot. The retention of Pb in the root is a preventive mechanism to avoid diffusion of lead up in the plant (Verklij and Schat, 1990). Higher Pb concentration in roots compared to shoots has been reported in earlier texts (Prasad, 2010). Reduction in biomass is the most important and quantified outcome of heavy metal toxicity, lead affects the cell division and growth off plant along with its development as it targets the meristematic zone (Das *et al*., 1997). Overproduction of reactive oxygen species (ROS) which disrupts antioxidant defense systems and leads to cause oxidative stress. It was observed that EDTA and DC current applied showed the comparable results to enhance the anti-oxidative activity in *Vetiveria zizanioides* however the negative impacts of EDTA such as root damage, water contamination and disruption of micro-habitat can lead to the idea of using DC current to be an alternative for enhancement of metal accumulation by plants.

Besides the direct impact of heavy metals on plants, they can also cause cell toxicity by overproduction of reactive oxygen species (ROS), which impairs antioxidant defense systems and causes oxidative stress to the plant. The amendments used in the experiments were of great significance as they helped in combating the metal stress more over it elevated the accumulation of the toxic metals. The electrokinetic remediation happened to be more rapid and effective amendment in comparison with the chemical chelation as chemical chelation causes root damage and has a threat of leaching on increasing the concentration which affects the underground water quality. Hence electrokinetic remediation can be an effective and better requisition for heavy metal accumulation by hyperaccumulators.

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