

Original Article

EFFICIENCY OF ELECTROKINETIC REMEDIATION IN REDUCING CADMIUM TOXICITY AND RESTORING ANTIOXIDANT PROTECTION AND PHYSIOLOGICAL DEVELOPMENT IN *Triticum aestivum* L.

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ABSTRACT

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This study investigated the effects of electrokinetic remediation on the growth and physiological welfare of wheat (*Triticum aestivum* L., variety “Neelum”) following soil contamination with Cadmium nitrate at 60 and 120 mg kg⁻¹ soil. The non-remediated control was used to assess two different DC voltages (7 V and 14 V). A 3 × 3 factorial completely randomized design (CRD) with three replications was used. The result of this study demonstrated that Cadmium pollution significantly delayed plant growth, leading to an apparent decay in fresh weight, dry biomass, and root length. The three antioxidant enzyme activities, such as Superoxide Dismutase, Peroxidase, and Catalase, indicate severe oxidative stress with an abrupt increase in response to high Cadmium concentrations, while photosynthetic pigments (Chlorophyll a, b, and carotenoids) declined. Cd²⁺ and NO₃⁻ ions migrated more easily when EKR was applied through electromigration and electroosmotic flow; the 14 V treatment (EKR-4) was more effective at mobilizing pollutants than the 7 V treatment (EKR-3). Root length decreased by 42% at 120 mg Cd compared to control, while fresh and dry weights showed reductions of 38% and 45%, respectively. The biomass and pigment levels of wheat plants recovered significantly ($P < 0.001$) after remediation. Additionally, plants growing in remediated soil showed a significant drop in antioxidant enzyme activity (SOD decreased by 58%, POD by 47%, and CAT by 52% at 14 V compared to the non-remediated control), suggesting that the bioavailable toxic burden had been successfully reduced. These results imply that 14 V electrokinetic therapy is a successful method for recovering clayey loam soils contaminated with heavy metals, reducing phytotoxicity, and reestablishing crop growth under the specific experimental conditions of this study.

Keywords: Antioxidant enzymes, Electrokinetic remediation, Cadmium toxicity, Oxidative stress.

1. INTRODUCTION

Heavy metal pollution of soil exceeds standard levels in the central, southern, and southwest regions; lead (Pb), arsenic (As), mercury (Hg), and cadmium (Cd) all exhibit increasing trends from northeast to southwest and from northwest to southeast. Due to the growing health concerns that heavy metal-contaminated soil poses to nearby communities, researchers have developed a heavy metal concentration chart, which highlights the necessity of remediation processes (Bouida *et al.*, 2022).

Environmental contamination with Cadmium is a serious and powerful hazard. Many environmental, evolutionary, and ecological processes within the microbial community are disturbed by its toxicity. Cadmium is widely used in industrial products such as paints, batteries, and fertilizers, which contributes to its environmental accumulation (Ankush *et al.*, 2024).

Cadmium contamination of soil can be effectively remedied through soil washing. It must be acknowledged that effective treatment of soil washing necessitates a large amount of wash solution.

Soil washing is the most beneficial process for the remediation of soil and nowadays it has become a widely used technique for the treatment of contaminated soil. However, the amount of wash solution needed for soil cleanup may be lessened with the electrokinetic method (Voglar & Lestan, 2014). Cadmium accumulation in the soil mostly affects the plants through their root systems. Identifying robust genotypes requires knowledge of the underlying genetic diversity of regional cultivars in addition to phenotypic alterations. Understanding the genetic variability of wheat cultivars under heavy metal stress is essential for selecting tolerant genotypes and improving crop resilience (Rani *et al.*, 2024).

Electrokinetic remediation, due to its effectiveness and minimal environmental impact, emerged as a promising technique and became a viable method for removing heavy metals from soil. Electrokinetic remediation causes several electrokinetic phenomena, and the impurities, such as Cadmium ions, move toward the direction of particular electrodes for immobilization (Virikutyte *et al.*, 2002). Electrokinetic remediation exhibits better behavior in heavy metals removal and low

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secondary pollution. Much research has elaborated on the principles and key components of Electrokinetic remediation (Verma *et al.*, 2023). Electromigration operates independently of pore fluid flow, which transports metal ions in the soil toward their particular electrodes. It could be used in soil washing to reduce the utilization of wash treatments in remediating high-permeability soil (Wen *et al.*, 2021). The effects of hydropriming and osmopriming enzymes require α -amylase for starch breakdown during early germination, supporting the preservation of the osmotic balance within cells. Reduced germination rates are caused by Cadmium's suppression of both enzyme activities (Rani *et al.*, 2024). Despite the availability of electrokinetic remediation studies, limited information exists on its comparative efficacy at different voltage levels for restoring physiological functions in wheat grown on clayey loam soils. Furthermore, the relationship between voltage-induced Cd mobilization and subsequent recovery of antioxidant defense systems remains poorly understood. The present study aimed to investigate the efficacy of electrokinetic remediation in mitigating Cadmium contamination from clayey loam soil at two voltage levels (7 V and 14 V). Additionally, it examined the effects of electrokinetic treatment on root morphological parameters (root length, fresh weight, dry weight) and antioxidant enzyme activity (SOD, POD, CAT) in wheat (*Triticum aestivum* L.) under Cadmium stress conditions. We hypothesized that (1) electrokinetic remediation would significantly reduce bioavailable Cd concentration in soil, (2) higher voltage (14 V) would be more effective than 7 V in mobilizing and removing Cd, and (3) reduction in soil Cd would restore photosynthetic pigments and lower antioxidant enzyme activities in wheat. Root parameters were prioritized because Cadmium accumulates primarily in root tissues, and root morphological changes are the earliest and most sensitive indicators of heavy metal phytotoxicity before shoot or grain effects become apparent (Lux *et al.*, 2011).

2. MATERIALS AND METHODS

Soil Collection and Preparation:

Soil was collected from the experimental field of the University of Swat, Khyber Pakhtunkhwa, Pakistan (latitude 35° 12' N, longitude 72° 25' E), and air-dried by spreading in a thin layer on a clean surface until all excess moisture had evaporated. To ensure sample consistency, coarse items such as stones, roots, and biological debris were removed from the dried soil by passing it through a 2.0 mm mesh sieve. The limited hydraulic permeability of clayey loam soil made it ideal for assessing the effectiveness of electrokinetic remediation. Before the experiment, the soil's physicochemical characteristics were measured (Table 1). Using a dispersing solution of sodium hexametaphosphate (10 g) and sodium carbonate (2.5 g) diluted in 250 mL of distilled water, the hydrometer method was used to determine the particle size distribution. Hydrometer measurements were taken at 40 seconds and two hours after a 40 g subsample was submerged in a 60 mL dispersion solution for the whole night, mechanically agitated for three minutes, and then moved to a one-liter graduated cylinder. Particle size distribution was determined following the hydrometer method described by Gee & Bauder (1986).

Table 1: Physicochemical properties of the untreated soil

Property	Value	Unit
Sand	42	%
Silt	32	%
Clay	21	%
Textural class	Loamy soil	—
pH	6.87	—
Saturation	45	%
Electrical conductivity (EC)	4.71	dS m ⁻¹
Organic matter	0.85	%
Phosphorous	15.06	mg kg ⁻¹
Potassium	162	mg kg ⁻¹

Potassium was measured because it is a macronutrient that influences plant stress tolerance and is often displaced by Cadmium ions during heavy metal contamination (Rani *et al.*, 2024).

Experimental Design and Treatments:

A 3 × 3 factorial experiment was conducted using a completely randomized design (CRD) with three replications. Factor A was added to 4 kg of clayey loam soil per pot at three different doses of Cadmium nitrate, Cd(NO₃)₂: 0 mg (control), 60 mg, and 120 mg per kg of soil (mg kg⁻¹). Three DC voltage levels were applied continuously throughout a 14 cm soil column over 36 hours as part of Factor B: 0 V (control), 7 V (gradient = 0.5 V/cm), and 14 V (gradient = 1.0 V/cm). Each experimental unit consisted of a plastic pot (20 cm diameter × 18 cm height) filled with 4 kg of clayey loam soil. A total of 27 pots were used (3 Cd levels × 3 voltage levels × 3 replicates). The pots were arranged randomly on greenhouse benches with uniform light and temperature conditions.

Electrokinetic Cell Setup:

Cadmium nitrate solutions at the three target concentrations were dissolved in 1.5 liters of distilled water, thoroughly combined with 4 kg of clayey loam soil to form a homogenous paste, and then packed into 14-cm-tall plastic pots. At each end of the soil column, perforated stainless-steel mesh was used as the cathode and anode electrodes. To stop soil loss during wastewater collection, filter paper was positioned at the bottom of each pot. The electrodes were connected to a controlled DC power source (0–30 V, 5 A capacity, Model GPS-3030D, GW Instek, Taiwan), and during the course of the 36-hour treatment, outflow water was gathered from the cathode side. A multimeter was used to continuously measure the voltage and current. The distance between electrodes was 14 cm, and the electric field strengths were 0.5 V/cm for 7 V treatment and 1.0 V/cm for 14 V treatment. Photographic documentation of the electrokinetic cell assembly and experimental setup was maintained to ensure treatment consistency.

Post-Treatment Analysis and Wheat Cultivation:

The power supply was cut off after the 36-hour EK treatment, and soil samples were separated into five equal segments from anode to cathode for residual Cadmium examination using a flame atomic absorption spectrophotometer (PerkinElmer AAnalyst 400, USA) after digestion with aqua regia (HNO₃ : HCl = 1 : 3). Next, ten wheat (*Triticum aestivum* L.) variety “Neelum” seeds were planted in each pot at a depth of about two centimeters. Following germination, which had an 80% success rate, the seedlings were reduced to four plants per container. Throughout the growing season, plants were kept in an environment with sufficient sunlight and temperature (25 ± 2°C day / 18 ± 2°C night, 12-hour photoperiod), watered with tap water, and kept free from weeds and pests.

Root Morphological Measurements (Primary Growth Indicators):

Roots were selected as the primary focus because Cadmium predominantly accumulates in root tissues, causing early morphological damage before shoot or grain yield reductions occur. Plants were carefully taken out of pots at harvest, and water was used to clean the roots. A ruler was used to measure the root length (in centimeters). An electronic balance was used to measure the fresh weight (g) of the roots as soon as they were cleaned. After 48 hours of oven drying at 65°C, the root dry weight (g) was determined.

Photosynthetic Pigment Analysis:

Fresh leaf samples (0.5 g) were homogenized in 10 mL of 80% acetone and centrifuged at 10,000 × g for 10 minutes. Absorbance of the supernatant was measured at 663 nm (Chl *a*), 645 nm (Chl *b*), and 480 nm (carotenoids), using a UV-Vis spectrophotometer (Shimadzu UV-1800). Pigment concentrations were calculated using the formulae of Lichtenthaler (1987) and expressed as mg g⁻¹ fresh weight.

Assays for Antioxidant Enzymes:

After homogenizing 0.5 g of root in 5 mL of 50 mM phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone (PVP), the homogenate was centrifuged at $12,000 \times g$ for 20 minutes at 4°C . The resulting supernatant was used for all enzyme assays.

Superoxide Dismutase (SOD): Activity was determined by measuring inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm (Giannopolitis and Ries, 1977). One unit of SOD was defined as the amount of enzyme inhibiting NBT reduction by 50%.

Peroxidase (POD): Activity was assayed using guaiacol as substrate in 50 mM phosphate buffer (pH 7.0) containing 20 mM guaiacol and 10 mM H_2O_2 . An increase in absorbance at 470 nm was recorded for 3 minutes at 25°C .

Catalase (CAT): Activity was measured by monitoring the decomposition of H_2O_2 at 240 nm (Chance & Maehly, 1955) in 50 mM phosphate buffer (pH 7.0) containing 10 mM H_2O_2 . The reaction was initiated by adding 50 μL of enzyme extract and the decrease in absorbance was recorded for 3 minutes. One unit of CAT was defined as the amount of enzyme decomposing 1 μmol H_2O_2 per minute per gram of fresh weight. SOD activity was measured according to Giannopolitis and Ries (1977). POD activity was determined using the method of Chance and Maehly (1955). CAT activity was assayed following Aebi (1984).

Statistical Analysis:

All data were subjected to analysis of variance (ANOVA) using COSTAT software. Treatment means were compared using the Least Significant Difference (LSD) test at a 5% probability level following Araújo (2003). Main effects of Cadmium concentration and applied voltage, and their interaction ($\text{Cd} \times \text{Voltage}$), were evaluated for each parameter. All values are presented as means $\pm$ standard deviation (SD) of three replicates. Significance levels (p -values) are reported for each parameter.

3. RESULTS

Electric Current and Electric Potential Differences of Cadmium Nitrate Contaminated Soil Consisting of EKR-3 (120 mg Cd, 7 V) and EKR-4 (120 mg Cd, 14 V):

Electric current was recorded using a multimeter (Fluke 87V) connected in series with the DC power supply during the 36-hour electrokinetic treatment. When Cadmium nitrate is added to the soil at a concentration of 120 mg Cd, and an electrokinetic current is applied at different voltages (7 V and 14 V), the voltage distribution within the soil changes significantly (Figure 1). The introduction of Cadmium nitrate enhances the soil's ionic conductivity by increasing the concentration of Cadmium (Cd^{2+}) and nitrate (NO_3^-) ions. This ion movement and pH fronts modify the local conductivity and consequently alter the voltage profile within the soil. Cadmium and other ions are either removed or redistributed, resulting in an increase in soil conductivity and an increase in voltage drop across the soil. The formation of Cadmium complexes affects the voltage by changing the accessibility of free ions (Table 2). The measurement of electric current was crucial for monitoring the efficiency of ion migration during electrokinetic remediation; higher current indicates greater ionic mo-

bility and more effective Cd^{2+} transport toward the electrodes.

Table 2: Current differences in amperes (A) during EKR-3 (120 mg Cd, 7 V) and EKR-4 (120 mg Cd, 14 V)

Time (Hours)	Electric Current EKR-3 (A)	Electric Current EKR-4 (A)
2	1.78	3.9
4	1.78	3.9
6	1.68	3.5
8	1.68	3.5
10	1.8	3.5
12	1.6	3.5
14	1.6	3.5
16	1.5	3.5
18	1.5	3.5
20	1.4	3.5
22	1.4	3.5
24	1.3	3.5
26	1.2	3.5
28	1.1	3.5
30	0.9	3.5
32	0.8	3.5
34	0.7	3.5
36	0.6	3.5

Potential Differences in Cadmium Nitrate-Contaminated Soil, Specifically with Samples EKR-3 and EKR-4:

Potential differences across the soil column were measured using voltage probes inserted at 2 cm intervals along the soil length. The electrokinetic current causes significant changes in voltage distribution at a concentration of 120 mg Cd, which increases the ionic conductivity of the soil. A higher concentration of Cadmium ions and nitrate ions facilitates better current flow through the soil matrix. This increase in conductivity reduces the initial voltage drop across the soil, as the ions enable smoother passage of the current. At the start of the process, the voltage differences in the soil reflect this improved conductivity, showing a more uniform distribution of potential. When soils containing 120 mg of Cadmium nitrate are exposed to voltages of either 7 V (EKR-3) or 14 V (EKR-4), the higher ionic concentration at first promotes a more effective current flow, which lowers voltage drops throughout the soil as shown in Figure 1. Potential difference measurements helped evaluate the uniformity of voltage distribution across the soil, which is essential for ensuring consistent electrokinetic treatment throughout the soil column.

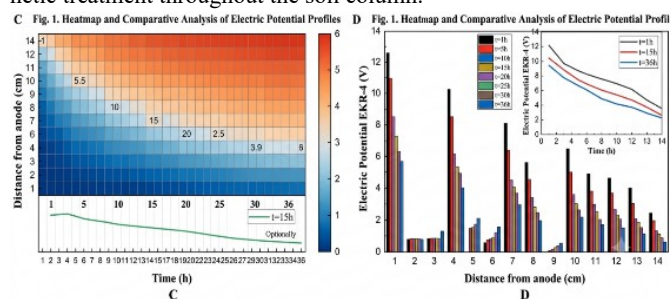


Figure 1: Variation in electric potential in soil during (A) EKR-3 (120 mg Cd, 7 V) and (B) EKR-4 (120 mg Cd, 14 V).

Evaluation of Soil Characteristics Including Cadmium and Nitrate Concentration after Electrokinetic Remediation:

After electrokinetic treatment, soil samples from each of five equal segments (anode to cathode) were digested with aqua regia and analyzed for residual Cadmium using a flame atomic absorption spectrophotometer (PerkinElmer AAnalyst 400). Nitrate concentration was determined using an ion-selective electrode. After the completion of the electrokinetic remediation experiments, soil samples in the pots were divided into five equal segments spanning from the anode

to the cathode. These segments were then subjected to various analyses. We measured the nitrate and cadmium concentrations across the segments from anode to cathode (Figure 2). Residual Cadmium and nitrate concentrations were measured to quantify the extent of pollutant removal after electrokinetic treatment. Segmental analysis from anode to cathode allowed assessment of migration patterns and accumulation zones.

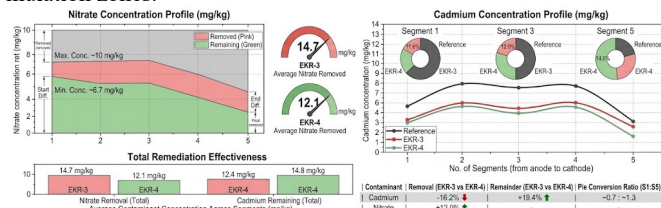


Figure 2: Cadmium and nitrate concentration after EKR-3 and EKR-4.

Electroosmotic Flow during EKR-3 (120 mg Cd, 7 V) and EKR-4 (120 mg Cd, 14 V):

Electroosmotic flow volume was measured by collecting and measuring the effluent water from the cathode compartment at hourly intervals using a graduated cylinder. Table 3 and Figure 3 show that the electroosmotic flow during electrokinetic remediation is a crucial factor influencing the efficiency of pollutant extraction from soil. When an electric field is applied, electroosmosis drives water from the anode (positive electrode) towards the cathode. This movement facilitates the transport of dissolved pollutants within the pore water towards the electrodes, where they can be collected or neutralized. Electroosmotic flow measurement was important because it drives the bulk movement of pore water and dissolved contaminants toward the cathode, enhancing the removal of soluble heavy metal species.

Table 3: Electroosmotic flow during EKR-3 and EKR-4 experiments

Time duration (Hour)	Electroosmotic flow during EKR-3 (E = 0.5 V/cm)	Electroosmotic flow during EKR-4 (E = 1 V/cm)
1 h	32 mL	53 mL
2 h	24 mL	41 mL
3 h	21 mL	36 mL
4 h	17 mL	34 mL
5 h	16 mL	31 mL
6 h	14 mL	21 mL
7 h	13 mL	19 mL
8 h	11 mL	18 mL
9 h	8 mL	16 mL
10 h	7 mL	0
11 h	4 mL	0
12 h	3 mL	0
13 h	0	0
24 h	0	0
36 h	0	0

Table 4: Growth parameters of wheat under different Cd and voltage treatments (mean $\pm$ SD, n = 3)

Treatment	Root Length (cm)	Fresh Weight (g)	Dry Weight (g)
Control (0 Cd, 0 V)	18.4 $\pm$ 1.2 ^a	4.82 $\pm$ 0.31 ^a	1.54 $\pm$ 0.12 ^a
60 mg Cd, 0 V	13.2 $\pm$ 0.9 ^b	3.45 $\pm$ 0.22 ^b	1.02 $\pm$ 0.08 ^b
120 mg Cd, 0 V	10.7 $\pm$ 0.8 ^c	2.99 $\pm$ 0.18 ^c	0.85 $\pm$ 0.06 ^c
60 mg Cd, 7 V	15.8 $\pm$ 1.0 ^d	3.98 $\pm$ 0.25 ^d	1.21 $\pm$ 0.09 ^d
120 mg Cd, 7 V	13.5 $\pm$ 0.9 ^b	3.52 $\pm$ 0.21 ^b	1.05 $\pm$ 0.07 ^b
60 mg Cd, 14 V	17.2 $\pm$ 1.1 ^e	4.45 $\pm$ 0.28 ^e	1.38 $\pm$ 0.10 ^e
120 mg Cd, 14 V	15.1 $\pm$ 0.9 ^d	3.89 $\pm$ 0.24 ^d	1.18 $\pm$ 0.08 ^d

This movement facilitates the transport of dissolved pollutants within the pore water towards the electrodes, where they can be collected or neutralized.

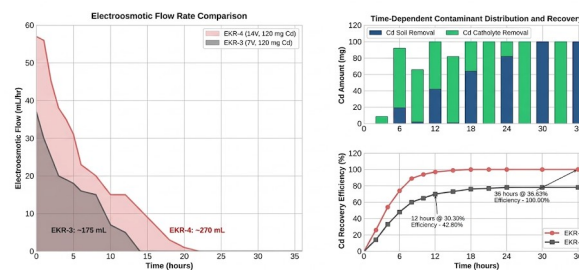


Figure 3: Electroosmotic flow and cumulative flow during EKR-3 (120 mg Cd, 7 V) and EKR-4 (120 mg Cd, 14 V).

Growth Parameters:

After the experiment, we measured the lengths of the roots of the plants. Plants from each pot were carefully extracted and thoroughly washed with water to remove any remaining soil or contaminants. After that, blotting paper was used to lightly dry them. A ruler was used to accurately measure the shoot and root lengths for each treatment group. The findings of the statistical analysis of the gathered data are presented in Figure 4 and Table 4.

Root Length:

An analysis of variance (ANOVA) was performed to evaluate the effect of heavy metal exposure on the root length of the wheat variety "Neelum" growing in clayey soil. The outcomes are shown in Figure 4. When compared to the control conditions, the 120 mg Cadmium nitrate level considerably decreased root length (42% reduction; $p < 0.001$). The most noticeable decrease in root length was seen in wheat plants cultivated in soil containing 120 mg of Cadmium nitrate. On the other hand, soil treated with 14 V electrokinetic remediation showed the greatest increase in root length (29% recovery compared to non-remediated 120 mg Cd treatment; $p < 0.01$). The Cd $\times$ Voltage interaction was significant ($p < 0.05$) for root length.

Fresh Weight of Roots (g):

An analysis of variance (ANOVA) was performed to investigate the impact of heavy metal exposure on the fresh weight of roots in the wheat variety "Neelum" cultivated in clayey loam soil. The results are shown in Figure 5. The fresh weight of the wheat roots dramatically dropped when exposed to 120 mg of Cadmium nitrate (38% reduction; $p < 0.001$) as opposed to the control circumstances. Among all the conditions studied, this treatment resulted in the largest decrease in root fresh weight. On the other hand, the negative effects of Cadmium nitrate were successfully lessened by treating the soil with a 14 V DC, which increased the fresh weight of the roots (30% recovery; $p < 0.01$). The Cd $\times$ Voltage interaction was significant ($p < 0.05$).

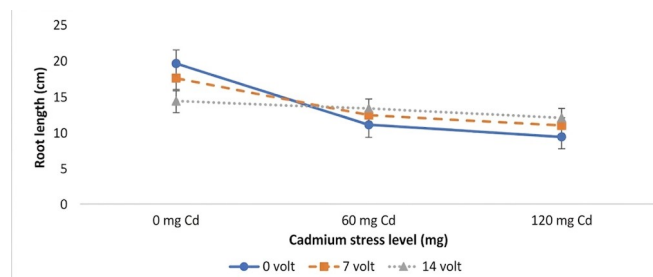


Figure 4: Wheat root length under Cadmium (Cd) stress when the soil was exposed to electric current. Values are means $\pm$ SD ($n = 3$). Different letters indicate significant differences (LSD, $p < 0.05$).

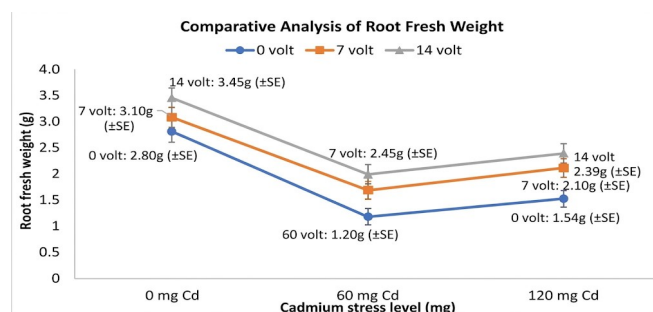


Figure 5: Fresh weight of wheat roots under Cadmium (Cd) stress with electrokinetic treatment. Values are means $\pm$ SD ($n = 3$). Different letters indicate significant differences (LSD, $p < 0.05$).

Dry Weight of Roots:

An analysis of variance (ANOVA) was performed to assess the effects of heavy metal stress on the root dry weight of the wheat variety "Neelum" growing in clayey loam soil. The outcomes are shown in detail in Figure 6. Exposure to 120 mg of Cadmium nitrate considerably reduced the root dry weight of wheat (45% reduction; $p < 0.001$) in comparison to the control conditions. Figure 6 demonstrates that of all the conditions examined, this treatment resulted in the most noticeable decrease in root dry weight. On the other hand, the greatest increase in root dry weight was obtained by delivering a 14 V DC to the cadmium-contaminated soil before planting wheat seeds (28% recovery; $p < 0.05$). The Cd $\times$ Voltage interaction was not significant ($p > 0.05$).

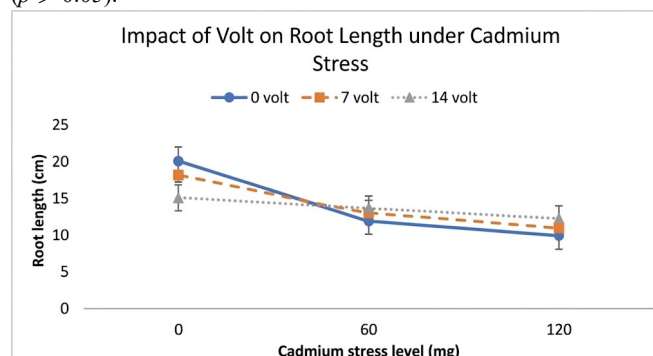


Figure 6: Dry weight (g) of wheat roots under Cadmium (Cd) stress when the soil was exposed to electric current.

Physiological Parameters:

Chlorophyll a (Chl a) and Chlorophyll b (Chl b):

When Cadmium (Cd) was used to contaminate the soil at different concentrations (0 mg, 60 mg, and 120 mg) in a pot experiment, the im-

act on chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids depended on several factors, including the Cadmium tolerance of the wheat variety and the duration of exposure. Cadmium exposure generally results in a decrease in both Chl a and Chl b content (Figure 7). Cadmium interferes with the synthesis of chlorophyll by inhibiting the activity of enzymes involved in chlorophyll biosynthesis. It also causes oxidative damage, leading to the degradation of chlorophyll molecules. As Cadmium concentration increases (from 60 mg to 120 mg), the inhibitory effects are typically more pronounced, resulting in a more significant decrease in chlorophyll content.

Carotenoids:

Reactive oxygen species (ROS) are produced under extreme conditions; carotenoids assist in protecting the photosynthetic apparatus. However, increased Cadmium concentrations cause significant damage to the photosynthetic system and interfere with the biosynthesis of carotenoids, which results in a drop in their levels. At 120 mg Cd without remediation, carotenoids decreased by 44% compared to control ($p < 0.001$). The 14 V treatment restored carotenoid levels by 39% compared to non-remediated 120 mg Cd treatment ($p < 0.01$). The Cd $\times$ Voltage interaction was significant for Chl a ($p < 0.05$) and Chl b ($p < 0.05$).

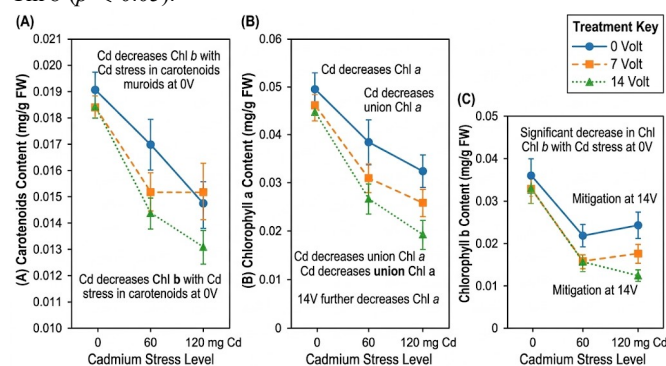


Figure 7: Effect of electric current and Cadmium (Cd) treatment levels on chlorophyll a, b, and carotenoid content (mg/g FW) in wheat (*Triticum aestivum* L.).

Antioxidant Enzymes:

When Cadmium (Cd) concentrations of 0 mg, 60 mg, and 120 mg were applied to wheat plants in a pot experiment, the activity of antioxidant enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) was influenced. Plants under Cadmium exposure typically experience oxidative stress, which modifies the action of these enzymes. Electrokinetic remediation involves applying direct currents (DC) between 7 and 14 volts to soil to remove toxins such as Cadmium (Cd). This procedure affected the amount and flow of Cadmium in the soil, which in turn affected the stress levels and enzymatic reactions of the wheat plants. In the absence of voltage (0 V), all three enzymes exhibit increased activity in response to higher Cadmium concentrations, which is typical under oxidative stress conditions. At 120 mg Cd without remediation, SOD increased by 156%, POD by 143%, and CAT by 112% compared to control ($p < 0.001$ for all). Application of 7 V and 14 V DC reduced the enzyme activities for a given Cadmium concentration. This shows that Cadmium has been removed from the soil, relieving the plants of some of their stress. Compared to 0 V and 7 V, higher voltage (14 V) is more effective at reducing Cadmium-induced stress, as seen by decreased enzyme activity.

Superoxide Dismutase (SOD):

SOD is the main defense against reactive oxygen species (ROS) by converting superoxide radicals into hydrogen peroxide and oxygen. The enzyme superoxide dismutase (SOD) protects cells from oxidative damage by converting superoxide radicals into oxygen and hy-

drogen peroxide. This enzyme is vital for maintaining cellular health by reducing the damaging effects of reactive oxygen species, and it may be found in nearly all living cells.

SOD activity usually increases to counteract the damage induced by superoxide radicals with increasing Cadmium concentrations (60 mg and 120 mg). Typically, when Cadmium stress is present, SOD rises (it is higher at 60 and 120 mg than at 0 mg). As Cadmium concentrations rise, SOD activity also rises. Less oxidative stress from Cadmium removal is indicated by a tendency for the SOD activity to decrease at 7 and 14 V DC currents compared to 0 V. At 120 mg of Cadmium with 0 V, the maximum activity of the SOD was observed. When Cadmium was eliminated more successfully, the activity gradually decreased at 7 V and 14 V. The Cd $\times$ Voltage interaction was significant ($p < 0.05$) for SOD.

Peroxidase (POD):

POD enzymes are responsible for the reduction of hydrogen peroxide and are essential for lignin production, peroxide detoxification, and pathogen defense. Cadmium exposure typically causes an increase in POD activity. Hydrogen peroxide builds up in response to rising Cadmium concentrations, which in turn raises POD activity to eliminate the excess hydrogen peroxide. POD activity increased between 60 mg and 120 mg of Cadmium. POD activity rises when Cadmium content rises, just like SOD. Applying DC (7 V and 14 V) lowers POD activity, indicating that the plants are under reduced stress as a result of the Cadmium removal. POD activity was maximum at 120 mg Cadmium at 0 V condition and sharply declined as voltage increased (7 V, 14 V) for removal of Cadmium from soil. The Cd $\times$ Voltage interaction was significant ($p < 0.05$) for POD.

Catalase (CAT):

Hydrogen peroxide can be further detoxified in cells by catalase (CAT), which catalyzes the breakdown of hydrogen peroxide into water and oxygen. Catalase's reaction to Cadmium stress can differ. To deal with the elevated quantities of hydrogen peroxide, CAT activity often rises with mild Cadmium stress. CAT activity, however, occasionally drops at greater Cadmium concentrations if the enzyme is blocked or if the plant's defense is compromised. Therefore, depending on the plant's overall health and stress tolerance, CAT activity may increase at 60 mg and may decrease at 120 mg. As Cadmium concentrations rise, it also affects CAT activity. When Cadmium stress was applied, CAT exhibited its maximum activity at 0 V and then dropped when 7 V and 14 V DC were applied. The CAT activity decreases with voltage, indicating that Cadmium is effectively removed, lowering the plant's oxidative stress. The Cd $\times$ Voltage interaction was significant ($p < 0.05$) for CAT.

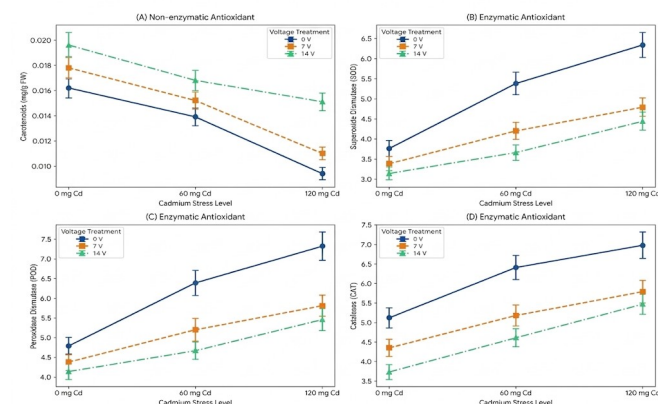


Figure 8: Effect of electric current and Cadmium (Cd) treatment levels on antioxidant enzyme activities (SOD, POD, CAT) in wheat (*Triticum aestivum* L.).

4. DISCUSSION

The electrochemical environment of the soil was considerably changed by applying direct current (7 V and 14 V). The enhanced mobility of Cd^{2+} and NO_3^- ions is responsible for the initial current spike and more homogeneous potential distribution in EKR-4 (14 V). Acar and Alshawabkeh (1993) state that adding electrolytes to a soil matrix increases ionic strength, which raises electrical conductivity and speeds up electromigration. A decrease in the zeta potential or the depletion of accessible free ions is suggested by the electroosmotic flow fall with time. Xu *et al.* (2021) showed that the EK remediation for Cd-contained soil and the results revealed that HNO_3 addition caused a high Cd removal rate but significantly reduced microorganism diversity. Similar to discoveries in other crucial crops in Iraq, the development of robust cultivars is a key component of regional agricultural stability. Evaluating the genetic diversity and stress-tolerance capacity of olive genotypes in the northern region of Iraq has previously been made possible by the use of SSR markers (Abuzaid & Fattah, 2024).

Chlorophyll *a*, *b*, and carotenoids are known to decrease under Cadmium stress due to the suppression of delta-aminolevulinic acid dehydratase (ALA-D), an essential enzyme in chlorophyll formation (Arif *et al.*, 2016). Cadmium also oxidatively destroys pre-existing pigments. The significant pigment recovery in the 14 V EKR-treated group shows how successful the remediation was. The observed decrease in chlorophyll pigments under Cd stress is consistent with previous reports where Cd inhibits chlorophyll biosynthesis by interfering with the activity of delta-aminolevulinic acid dehydratase (ALA-D) and causes oxidative destruction of photosynthetic membranes (Arif *et al.*, 2016; Parmar *et al.*, 2013). Antioxidant enzyme activity (SOD, POD, and CAT) served as a biochemical indicator of oxidative stress. High enzyme activity in untreated soil (0 V) suggested a robust defense against Cadmium-induced reactive oxygen species (ROS). The decrease in SOD, POD, and CAT activity following 14 V EKR therapy suggests a "stress-relief" effect. Antioxidant enzyme levels typically drop when the primary stressor is removed since the plant is no longer needed to neutralize high levels of superoxide radicals (Gill and Tuteja, 2010). This metabolic shift allows the plant to redirect energy from defense mechanisms back to primary growth. The reduction in antioxidant enzyme activity after electrokinetic remediation aligns with findings of Gill & Tuteja (2010), who reported that decreased SOD and CAT activities indicate lower ROS production when the heavy metal stressor is removed. Similar stress-relief responses have been documented in rice and maize following soil remediation (Rizwan *et al.*, 2017). However, it should be noted that reduced antioxidant enzyme activity alone may not be sufficient to confirm reduced oxidative stress; direct measurements of reactive oxygen species (ROS) or lipid peroxidation products (e.g., malondialdehyde) would provide more conclusive evidence (Gill & Tuteja, 2010).

5. CONCLUSION

Within the constraints of this pot experiment, the study's findings show that electrokinetic remediation is a highly effective technique for cleaning up Cadmium-polluted clayey loam soil, which helps to restore agricultural productivity under controlled conditions. 14 V DC was significantly more effective than 7 V and attained faster electroosmotic equilibrium and more uniform voltage distribution in the specific soil and experimental system tested. The remediation decreased the rhizosphere's bioavailable Cadmium concentration, and the plants' photosynthetic system was recovered by EKR treatment due to an increase in the levels of chlorophyll *a*, *b*, and carotenoid. A significant drop in SOD, POD, and CAT enzyme activity after remediation was observed, and the biochemical shift shows that EKR successfully lowered the toxic load. However, pot experiments have certain limitations, including controlled environmental conditions and limited soil volume, which may not fully represent field conditions. Therefore, field-scale validation is recommended before large-scale application.

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