

Electro-kinetic assisted phytoremediation of zinc using ASA- and APAP-based chelated complexes in paddy plants

Abhijit Kantankar¹, K. Anuradha²

¹ Assistant Professor of Chemistry, Department of Chemistry

² Department of Botany, Assistant Professor of Botany

^{1,2} New Government Degree College, Serilingampally, Hyderabad, India

Abstract—Zinc (Zn) is an essential micronutrient for plants; however, excessive Zn availability induces phytotoxicity and limits uptake efficiency. The present study evaluated zinc absorption and phytoaccumulation in paddy (*Oryza sativa* L.) plants exposed to Zn supplied as Zinc sulphate (ZnSO₄), zinc–aspirin (Zn–ASA), and zinc–paracetamol (Zn–APAP) complexes under light, dark, and electro-kinetic phytoremediation (EK-PR) conditions. Hydroponically grown plants at the fifth-leaf stage were treated with solutions containing an equivalent Zn concentration of 227.3 ppm for 72 h. Zinc content was quantified using atomic absorption spectroscopy. Results demonstrated significantly higher Zn uptake from chelated Zn–ASA and Zn–APAP complexes compared to ionic ZnSO₄, indicating reduced Zn²⁺ toxicity and improved plant tolerance. Zn–APAP complexes exhibited the highest accumulation, particularly under dark and EK-PR conditions. Electro-kinetic stimulation further enhanced Zn uptake across all treatments. Overall, the findings highlight the effectiveness of pharmaceutical chelating agents, especially APAP, in enhancing zinc bioavailability and phytoremediation efficiency. The combined use of metal–drug chelation and electro-kinetic enhancement represents a promising strategy for remediating Zn-contaminated environments and potentially other heavy metals.

Index Terms—Zinc absorption, ASA complex, APAP complex, phytoremediation, Electro kinetic remediation, chelation.

ABBREVIATIONS

- Zn – Zinc
- Zn²⁺ – Divalent zinc ion
- ZnSO₄ – Zinc sulphate
- ASA – Acetylsalicylic acid (Aspirin)

- APAP – Acetaminophen (Paracetamol)
- Zn–ASA – Zinc–acetylsalicylic acid complex
- Zn–APAP – Zinc–acetaminophen (paracetamol) complex
- EK-PR – Electro-kinetic phytoremediation
- AAS – Atomic Absorption Spectroscopy
- ROS – Reactive Oxygen Species
- ppm – Parts per million
- h – Hour(s)
- DC – Direct current

I. INTRODUCTION

Plants require sixteen essential mineral nutrients in varying concentrations to sustain normal growth and development, among which zinc (Zn) is an indispensable micronutrient. Zinc is primarily absorbed by plants in its Zn²⁺ form and functions as a structural and catalytic cofactor for numerous enzymes involved in key physiological and biochemical processes, including photosynthesis, protein synthesis, and chlorophyll biosynthesis [1],[2]. Adequate Zn availability is therefore critical for maintaining cellular metabolism and overall plant productivity.

Although Zn is essential at trace levels, its excessive accumulation poses significant toxicity risks. Anthropogenic activities such as industrial emissions, mining, agricultural inputs, and wastewater discharge have resulted in elevated Zn concentrations in soils and aquatic environments [3]. High Zn levels induce phytotoxic effects, including oxidative stress, lipid peroxidation, inhibition of root growth, and impairment of nutrient uptake mechanisms [4],[5]. Furthermore, excessive Zn accumulation can interfere

with the absorption of other essential micronutrients, notably copper and iron, in both plants and humans [6].

Heavy metal contamination of soil and water remains a persistent global environmental concern. Conventional remediation techniques are often costly, energy-intensive, and environmentally disruptive. In contrast, phytoremediation has emerged as a cost-effective, sustainable, and eco-friendly alternative that utilizes plants to remove, stabilize, or accumulate contaminants from polluted environments [7]. Although the concept of phytoremediation dates back several centuries, with early applications in wastewater treatment [8], its mechanistic understanding and technological optimization continue to evolve.

Zinc uptake by plants typically occurs from soluble sources such as $ZnSO_4$, where Zn exists predominantly as free Zn^{2+} ions. However, elevated free-ion concentrations are highly toxic and restrict Zn bioavailability and transport within plant tissues [9], [10]. Despite these limitations, certain plant species exhibit the capacity to tolerate and accumulate substantial quantities of Zn, highlighting the potential for enhanced phyto extraction strategies [7].

Chelation represents a promising approach to mitigate Zn-induced ionic stress and improve metal uptake efficiency. ASA and APAP are widely used pharmaceutical compounds that can function as chelating ligands, forming stable coordination complexes with metal ions. Chelating ligands bind to central metal atoms, reducing free-ion activity and associated toxicity while enhancing metal solubility and transport [11]. The formation of Zn-ASA and Zn-APAP complexes may therefore facilitate improved Zn absorption and biological availability in plants.

In addition, EK-PR has gained attention as a method to enhance metal mobility in soils through the application of low-intensity electric fields, thereby promoting increased metal uptake by plant roots.

The present study aims to comparatively evaluate the absorption and phyto-accumulation of zinc in paddy plants supplied as $ZnSO_4$, Zn-ASA, and Zn-APAP complexes, under conditions with and without electrokinetic enhancement. The primary objective is to quantitatively assess the capacity of paddy plants to absorb and accumulate Zn-drug complexes and to elucidate their potential application in advanced phytoremediation strategies.

II. MATERIALS AND METHODS

Plant Material and Growth Conditions

Healthy paddy (*Oryza sativa* L.) seedlings at the fifth-leaf developmental stage were selected for the experiment. The plants were cultivated under controlled hydroponic conditions to eliminate soil-mediated variability and ensure uniform exposure to zinc treatments. The nutrient medium consisted of aqueous solutions containing zinc supplied either as zinc sulphate ($ZnSO_4$), zinc-aspirin (Zn-ASA), or zinc-paracetamol (Zn-APAP) complexes. All treatments were standardized to an equivalent zinc concentration of 227.3 ppm to enable comparative evaluation of zinc uptake efficiency.

Synthesis of Zinc-Drug Complexes

Zn-ASA complexes were synthesized by refluxing stoichiometric amounts of $ZnSO_4 \cdot 7H_2O$ with acetylsalicylic acid (ASA) in an aqueous medium under controlled temperature conditions to facilitate coordination bonding between Zn^{2+} ions and the ligand. Zn-APAP complexes were prepared in a methanolic medium, allowing effective chelation between zinc ions and paracetamol (APAP) molecules. The resulting complexes were cooled, filtered, and reconstituted in aqueous solution prior to plant exposure.

Experimental Treatments

Paddy plants were exposed to the respective zinc treatments for a total incubation period of 72 hours. To assess the influence of photoperiod on zinc absorption, plants were maintained under two environmental regimes:

- Light condition: 16 h light / 8 h dark photoperiod
- Dark condition: Continuous darkness

All experiments were conducted at ambient temperature, and solutions were renewed as necessary to maintain consistent zinc availability.

Electrokinetic Phytoremediation (EK-PR)

To evaluate the effect of electrokinetic enhancement on zinc uptake, an electrokinetic phytoremediation (EK-PR) setup was employed. A direct current electric field of 2 V was applied across the hydroponic system for 8 hours per day throughout the treatment period. This approach was intended to promote ionic mobility, reduce diffusion limitations, and enhance the transport

of Zn²⁺ and Zn–drug complexes toward the plant root system, as described by Cameselle et al. (2013).

Sample Preparation and Zinc Analysis

Following the incubation period, plants were harvested and thoroughly rinsed with distilled water to remove surface-bound zinc residues. The plant samples were then oven-dried at 65 °C until constant weight was achieved. Dried tissues were finely ground and subjected to acid digestion prior to metal analysis. Zinc concentrations in plant tissues were quantified using Atomic Absorption Spectroscopy (AAS), ensuring high sensitivity and accuracy in trace metal determination.

III. RESULTS

The Quantitative estimation of zinc accumulation in paddy plants revealed a pronounced dependence on the chemical form of zinc, illumination regime, and electro-kinetic stimulation. Basal zinc content in

control plants grown under natural conditions was 43.52 ppm, representing endogenous Zn levels required for normal physiological functions

Plants exposed to ZnSO₄ solution (227.3 ppm Zn equivalent) showed moderate Zn accumulation, with higher uptake under light conditions (109.41 ppm) compared to dark conditions (93.84 ppm). In contrast, plants treated with zinc–drug complexes exhibited significantly enhanced Zn accumulation, indicating improved bioavailability and reduced ionic toxicity of chelated Zn.

Figure 1 illustrates Zn–ASA–treated plants accumulated 81.85 ppm Zn under light and 137.36 ppm under dark conditions, demonstrating a substantial increase in Zn uptake in the absence of light. A further enhancement was observed with Zn–APAP complexes, which resulted in the highest Zn accumulation among non-electrokinetic treatments: 170.33 ppm under light and 216.41 ppm under dark conditions.

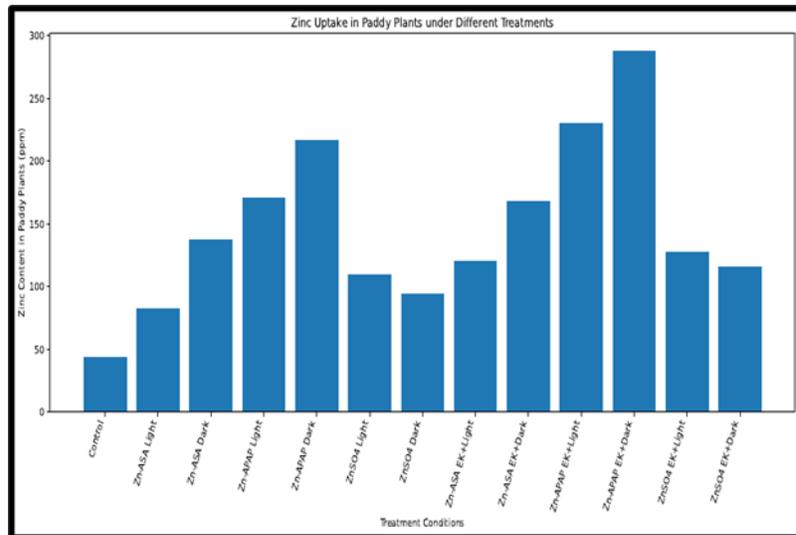


Figure 1: Zinc Accumulation in Paddy Plants under

Different Chelation and Electro-Kinetic Treatments

Table I. Zinc Content Estimated in Paddy plants.

S.No.	Culturing Complex	Concentration of Zn Equivalent in Culturing Solution (ppm)	Type of Culture / Condition	Phyto-absorption of Zn in ppm
1.	Control Plant	Natural	Normal	43.52

2.	Zn-ASA	227.3	Light	81.85
3.	Zn-ASA	227.3	Dark	137.36
4.	Zn-APAP	227.3	Light	170.33
5.	Zn-APAP	227.3	Dark	216.41
6.	ZnSO ₄ Solution	227.3	Light	109.41
7.	ZnSO ₄ Solution	227.3	Dark	93.84
8.	Zn-ASA	227.3	EK-PR+Light	120.35

9.	Zn-ASA	227.3	EK-PR+ Dark	167.57
10.	Zn-APAP	227.3	EK- PR+Ligh t	230.45
11.	Zn-APAP	227.3	EK- PR+Dark	287.89
12.	ZnSO ₄ Solution	227.3	EK- PR+Ligh t	127.35
13.	ZnSO ₄ Solution	227.3	EK- PR+Dark	115.35

Application of electro-kinetic phyto-remediation (EK-PR) markedly increased Zn uptake across all treatments. Under EK-PR conditions, Zn-ASA exposure resulted in Zn concentrations of 120.35 ppm (light) and 167.57 ppm (dark). Zn-APAP complexes showed the most pronounced response to electro-kinetic enhancement, achieving 230.45 ppm Zn under light and a maximum of 287.89 ppm under dark conditions. ZnSO₄-treated plants under EK-PR accumulated 127.35 ppm (light) and 115.35 ppm (dark), indicating that electro-kinetic stimulation partially overcomes ionic transport limitations even in non-chelated systems.

Overall, the hierarchy of zinc accumulation was: Zn-APAP > Zn-ASA > ZnSO₄, with dark conditions and EK-PR consistently favoring higher uptake.

IV. DISCUSSION

The results clearly demonstrate that chelation of Zn²⁺ with pharmaceutical ligands such as aspirin (ASA) and paracetamol (APAP) significantly enhances zinc uptake and accumulation in paddy plants compared to inorganic ZnSO₄. Free Zn²⁺ ions are known to induce oxidative stress by generating reactive oxygen species (ROS), leading to lipid peroxidation, membrane destabilization, and inhibition of root growth. Chelation reduces the effective ionic activity of Zn²⁺, thereby mitigating toxicity and facilitating safer translocation across root membranes.

Among the two ligands studied, APAP proved to be a more effective chelating agent than ASA, as reflected by consistently higher Zn accumulation under both normal and electro-kinetically enhanced conditions. This may be attributed to stronger coordination interactions, improved stability of Zn-APAP complexes, and enhanced membrane permeability,

resulting in greater bioavailability of Zn to plant tissues.

An intriguing observation was the consistently higher zinc accumulation under dark conditions compared to light. This phenomenon may be associated with altered metabolic demand, reduced photosynthetically driven Zn utilization, or changes in membrane transport dynamics and ion channel regulation during dark incubation. Reduced photochemical stress may also enhance tolerance to metal complexes, allowing greater accumulation.

Electro-kinetic phyto-remediation significantly amplified Zn uptake in all treatments by promoting directional ion migration, increasing metal mobility, and improving contact between root surfaces and Zn species. The synergistic effect of chelation and electro-kinetic stimulation was most evident in Zn-APAP treatments, indicating that EK-PR can effectively complement ligand-assisted phyto-accumulation strategies.

In conclusion, ligand-mediated chelation combined with electro-kinetic enhancement represents a promising approach for improving zinc phyto-accumulation while minimizing metal-induced toxicity. The superior performance of Zn-APAP complexes highlights their potential application in environmentally sustainable remediation of zinc-contaminated systems.

V. CONCLUSION

The present study clearly demonstrates that chelation of zinc with acetylsalicylic acid (ASA) and paracetamol (APAP) significantly enhances zinc uptake and phyto-accumulation in paddy plants when compared to conventional ionic zinc solutions. The reduced toxicity of chelated Zn²⁺ ions improve plant tolerance, allowing for higher accumulation without inducing oxidative stress. Among the two chelating agents, APAP proved to be more effective than ASA, yielding the highest zinc uptake under both normal and electro-kinetically enhanced conditions.

Electro-kinetic phyto-remediation further augmented zinc absorption by enhancing ion mobility and availability in the growth medium, highlighting the synergistic potential of combining chemical chelation with electro-kinetic stimulation. The notably higher zinc uptake observed under dark conditions suggests a

role of metabolic and physiological regulation in heavy metal accumulation.

Overall, the integration of drug-based chelation and electro-kinetic enhancement represents a promising, cost-effective, and environmentally sustainable strategy for improving phyto-remediation efficiency. This approach has potential applicability not only for zinc-contaminated environments but also for the remediation of other toxic heavy metals such as lead, mercury, and cadmium.

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