**Zn nanocomposite Reducing Cadmium Toxicity and Accumulation in Lettuce**

**Introduction**

Cadmium (Cd) is a persistent toxic heavy metal linked to severe health issues, including organ damage, osteomalacia, and carcinogenic effects (…). The primary human exposure route is through the consumption of Cd-contaminated edible plants (.). In plants, Cd disrupts growth, induces necrosis, and impairs photosynthesis by reducing carbon fixation and chlorophyll content (.). Cd exposure also triggers osmotic stress, ROS overproduction, and nutrient imbalances, particularly affecting iron (Fe) and zinc (Zn) uptake and transport mechanisms (.). Consequently, the uptake of essential elements is disrupted, which impairs the accumulation of these minerals and, ultimately, inhibits the growth of plants (.). Therefore, effective Cd removal is crucial; however, conventional methods like ion exchange and membrane separation are significant financial and technical challenges (.).

Nanomaterials represent a novel category of materials that are increasingly applied in agricultural studies (.). Over the past decades, an abundance of NPs has been manufactured using diverse procedures and employed for environmental uses such as identification of enduring pollutants, and remediation of soil/ aqueous environments (.). Recently, NPs have emerged as a novel strategy for directly ameliorate heavy metal stress and functioning as nano-fertilizers. Studies on various plant species have emphasized the significance of nanotech innovations for sustainable agriculture, particularly in enhancing resilience against environmental stressors like heavy metals (.). Among all engineered NPs, Zn NPs are notable for their affordability and accessibility, ranking as the fourth most prevalent globally across spanning industries from rubber, paints, and plastics to glass, ceramics, cement, pigments, agriculture, food additives, and batteries (..). Notably, utilizing Zn nanostructures has gained significant attention in agriculture, offering the potential to supply up to eighty percent of Zn requirements in edible leafy plants (..). Zn NPs have also demonstrated beneficial influence on plant via stimulating growth and augmenting essential element uptake (.1). Moreover, Zn NPs mitigate oxidative damage caused by salt stress, owing to their high surface area and strong adsorption capabilities, enabling the sequestration of heavy metal ions (.).

Research has shown that Zn NPs can alleviate cadmium toxicity symptoms in lettuce seedlings, enhancing growth through increased biomass production and elevated oxidative stress mitigation enzyme function (.).

In this study, we investigate, for the first time, the effects of a novel Zn nanocomposite on increasing growth and reducing Cd accumulation in lettuce. The results of the current study provide a low-cost, sustainable approach to counteract the harmful impacts of Cd exposure in hydroponic systems, and bring new clarity to the application of Zn nanocomposite for enhancing growth and mitigating Cd accumulation in leafy green vegetables.

**Plant material and treatments**

For seed germination, lettuce was sown in experimental pots containing perlite, in an environmentally controlled growth chamber. Subsequently, uniform seedlings were transferred to half-liter hydroponic pots containing an amended nutrient solution (.). After a duration of 5 days of hydroponic cultivation, the plant seedlings were subjected to the specified treatments for an additional 14 days, encompassing: 1) control (nutrient solution only); 2) nutrient solution + 20 mg/L Zn nanocomposite ; 3) nutrient solution + 40 mg/L Zn nanocomposite ; 4) 18 mg/L Cd; 5) 18 mg/L Cd + 20 mg/L Zn nanocomposite; 6) 18 mg/L Cd + 20 mg/L Zn nanocomposite . Following a treatment duration of 14 days, the plants underwent harvesting, and the separation of root and shoot biomass was conducted for length measurement.

**Determination of Cd concentration**

Plant samples for Cd concentrations was determined in oven-dried samples and digested using nitric acid and hydrogen peroxide. Cd concentrations was determined by an atomic absorption spectrometry

**Bioaccumulation and translocation factors, and tolerance index**

Metal accumulation capacity (bioaccumulation factor (BF)), transport efficiency (translocation factor (TF)), and physiological tolerance (tolerance index (TI)) were considered using the following respective ratios:

$$BF of root=\frac{The concentration of total Cd in plant root (\frac{mg}{kg})}{The concentration of total Cd in the medium (\frac{mg}{kg})}$$

$$BF of shoot=\frac{The concentration of total Cd in plant shoot (\frac{mg}{kg})}{The concentration of total Cd in the medium (\frac{mg}{kg})}$$

$$TF=\frac{The concentration of Cd in plant shoot (\frac{mg}{kg})}{The concentration of Cd in plant root (\frac{mg}{kg})}$$

$$TI=\frac{Dry weight of stressed plants \left(g\right)}{Dry weight of control plants \left(g\right)}$$

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**Zn nanocomposite** **reduce Cd concentration, BF, TF, and TI**

As shown in Figure A, compared to the control plants, Cd treatment significantly enhanced the Cd accumulation in both roots and shoots. The highest Cd accumulation was observed in the root (701.6 mg Kg -1), which was 8.6 times more than the concentration in the shoots, which could be due to the roots being directly exposed to the contaminated solution by Cd. However, applying Zn nanocomposite at a concentration of 20 mg/L led to a significant reduction in Cd concentration in the roots by 42%, compared to the Cd treatment (Figure A). Furthermore, compared to the Cd treatment, Cd concentration decreased by 53% in the presence of Zn nanocomposite at 40 mg/Lin the shoots. To claim the application of Zn nanocomposite in mitigating the Cd stress, we quantified the content of Zn in lettuce plant under different treatments. The application of 20 and 40 mg/L of Zn nanocomposite considerably enhanced the Zn concentration in the roots by 3.3- and 5.4-fold, respectively, compared to Cd-free. Moreover, a parallel increasing trend was apparent in the shoots, where the using of 20 and 40 mg/L Zn nanocomposite significantly increased the Zn concentration by 1.6- and 2.6-fold, respectively, compared to Cd-free (Figure 5D). On the other hand, exposure to 18 mg/L Cd reduced Zn concentration by 54% in roots and 32% in shoots, compared to Cd-free. However, the using of Zn nanocomposite (in ZHCs+Cd treatment) significantly enhanced the Zn concentration, compared to the Cd treatment. The using of Zn nanocomposite (40 mg/L) enhanced the Zn concentration in the roots by 8.4-fold and shoots by 1.7-fold, compared to the Cd treatment.

The BF, TF, and TI were considered in lettuce plants to evaluate the effect of Zn nanocomposite on absorbing, translocating, and tolerating Cd stress (Figure B). Applying Zn nanocomposite in the Cd-contaminated solution significantly reduced the BF in lettuce. The using of Zn nanocomposite in the Cd-contaminated solution significantly reduced the BF in both the roots and shoot. Surprisingly, this reduction was more pronounced in the shoot, where applying Zn nanocomposite at 40 mg/L decreased BF by 52% compared to the Cd-treated plant. Applying Zn nanocomposite (40 mg/L) triggered a major decrease in TF, compared to the Cd treatment. Measurements demonstrate that applying Zn nanocomposite at concentration of 40 mg/L triggered reductions in the Cd uptake and accumulation in shoot biomass of lettuce plants.