IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article Open access Int J Agric For Life Sci (2021) 5(2): 154-162

An investigation on the effect of nano-ZnO application on cadmium phytoextraction by safflower

Fatih Ahmet Yentür[1] Hatice Dağhan[2*]

[1]Republic of Turkey, Ministry of Agriculture and Forestry, Ankara, Turkey
[2]Eskişehir Osmangazi University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Eskişehir, Turkey

*Corresponding author e-mail: hdaghan@ogu.edu.tr

Abstract

Nowadays, soils contaminated with heavy metals are one of the biggest environmental pollution problems in the world. Phytoextraction method is the most effective and well-known plant remediation method that can be used to clean-up agricultural soils contaminated with heavy metals. Nanoparticle (NP) applications have recently been introduced to remove pollutants, promote plant growth and improve pollutant phyto-availability to improve the effectiveness of this method. In this study, it is aimed to use phytoextraction method and nanomaterial together for the cleaning of cadmium (Cd) contaminated growth media and to investigate the effects of nanomaterial on plant properties. For this purpose, a hydroponic culture was planned and zinc oxide nanoparticles (ZnO-NP), a nanomaterial which was determined by OECD as a priority, was used for the experiment. As safflower (*Carthamus tinctorius*) can be grown in different ecological conditions, it was selected. Safflower seeds were germinated in a mixture of peat-perlite (1:1), and after 2-3 leaves, they were transferred to the Hoagland nutrient solution. In order to see the effects of Cd × ZnO-NP applications, morphological observations of the plants were made and chlorophyll contents were measured before the harvest by applying ZnO-NP and (0-3-6 mg / L) Cd in increasing doses (0, 5, 10 mg / L) to the nutrient solution. Plants were harvested 20 days after the transplanting. Shoot and root dry weights of plants, Zn, and Cd concentrations were determined. The results showed that Cd accumulation of the plant increased due to increasing doses of ZnO-NP. In the shoot of the safflower plant, Cd has accumulated 5.2 to 8.7 times more Cd than the hyperaccumulation critical threshold value (100 µg / g). The research showed that the safflower can have a promising Cd phytoremediation potential in the environment.

Key words: Phytoextraction, cadmium, ZnO nanoparticle, safflower, hydroponic culture.

Introduction

Soil is the primary source of agricultural production. Unfortunately, soils are polluted especially because of human activities (misuse of soils, industrial activities, excessive use of chemical fertilizers and pesticides, erosion, polluted waters, nuclear accidents, urban wastes, mining, etc.). Because of this, agricultural production areas are decreasing, ecological balance is disrupted and pollutants are included in the food chain and threatening living organisms or causing their extinction (Esetlili and Anaç, 2015). The most common pollutants in agricultural soils are heavy metals, which are among the inorganic pollutants (Çağlarırmak et al., 2010; Dağhan and Öztürk, 2015; Köleli et al., 2018). Unlike organic pollutants, heavy metals remain intact even if they change form in their environment (Köleli et al., 2018). Since these pollutants persistent in the soil, they limit the growth of microorganisms and plants, inhibit the development of plant roots and leaves, and can destroy the soil ecosystem health. Heavy metals enter into the food chain through plants from contaminated soil and water; also threaten animals and human beings' life (Zhu et al., 2019).

Cite this artile as:
Yentur, F.A., Daghan, H. (2021). An investigation on the effect of nano-ZnO application on cadmium phytoextraction by safflower. Int. J. Agric. For. Life Sci., 5(2): 154-162.
ORCID and Mail:
Daghan, H.: 0000-0002-0150-5882 (hdaghan@ogu.edu.tr); Yentur, F.A.: 0000-0002-8288-7500 (fatihahmet.yentur@tarimorman.gov.tr)
Received: 25.11.2021 **Accepted:** 16.12.2021 **Published:** 16.12.2021
Year: 2021 **Volume:** 5 **Issue:** 2 (December)
Available online at: http://dergipark.gov.tr/ijafls

Heavy metals such as lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni), mercury (Hg), which the living and non-living environment are exposed in nature are defined as metals with a density higher than 5 g/cm^3 (Seven et al., 2018). Cadmium is one of the most dangerous heavy metals and it is a non-essential element for plants. This heavy metal is presented seventh of substance in the Substance Priority List (included 275 substances), which is prepared by the Agency for Toxic Substances and Disease Registry of United State (US-ATSDR) (ATSDR, 2021). In general, as little as 0.05 to 0.2 mg/kg dry weight Cd concentrations cause toxic effect in plant tissues (Kabata-Pendias, 2000). Over that, doses of leaf chlorophyll pigments damage, chlorosis and necrosis appears in plant leaves, and biomass production of shoot and roots decreases, plant length reduces in plants (Rizwan et al., 2019a). Therefore, especially the problem of Cd contaminated soil should be addressed urgently.

Phytoextraction method is used for cleaning soils contaminated with heavy metals. This method is more economical than many methods involving physical, chemical, thermal and biological processes, is environmentally friendly, does not require special equipment, and allows the reuse of the reclamation area. The plant which is used in phytoextraction should grow fast, have deep rooting and high shoot biomass production, accumulate high metal in its harvestable parts, tolerate heavy metal accumulated and be easily harvested. This method is very effective for low pollutant concentrations (Daghan et al., 2012; Köleli et al., 2018; Dağhan, 2019).

Phytoextraction is known as an environmentally friendly cleaning method. However, it takes a long time to clean-up the heavy metal contaminated areas. Therefore, the combined use of plants and nanomaterials in environmental management has been popular approach, since some nanomaterials can support plant seed germination and plant growth (Song et al., 2019). Nanomaterials could play a role in phytoremediation technology by removing pollutants, promoting plant growth, and increasing pollutant's phyto availability (Song et al., 2019).

Zinc oxide nanoparticles are the most well-known and widely used nanoparticle in all metallic nanoparticles. Due to their high metal adsorption capacity, ZnO-NPs are used to rehabilitate metal-contaminated water and soil (Hua et al., 2012; Tang et al., 2014; Hussain et al., 2021). The appropriate concentration of ZnO-NPs can significantly increase the phyto-accumulation potential of heavy metals in metal-contaminated water sources. It has been reported that ZnO-NPs can be used as potential supports for phytoremediation in heavy metal-contaminated areas (Hussain et al., 2021).

The hypothesis of this study was using ZnO-NP to stimulate plant growth while alleviating Cd toxicity on plant and on the other side enhancement Cd uptake by plants. For this aim phytoextraction method and ZnO-NPs were used together to clean-up Cd from contaminated growth media to investigate the effects of ZnO-NPs treatments on Cd phytoextraction by safflower plants.

Materials and Methods

Material

In this study, the ZnO-NPs were synthesized with the sol-gel method (Gokhale et al., 2009), which was modified by Dr. Birol Karakaya. Zinc oxide nanoparticle was chosen as a nanoparticle material in this research because it has been determined by the Organization for Economic Cooperation and Development (OECD) as a priority nanomaterial (OECD, 2013).

Dinçer variety of safflower (*Carthamus tinctorius*) was used as plant material in the experiment. Plant seeds were purchased from Eskişehir Transition Zone Agricultural Research Institute.

Method

Characterization of ZnO-NPs

Structural properties, shapes, particle sizes and elemental compositions of ZnO-NPs, which were synthesized according to the sol-gel method, were determined by using scanning electron microscopy with energy-dispersive X-Ray Spectroscopy (SEM-EDX) (Carl Zeiss, Supra 55).

In addition, the crystal structures of ZnO-NPs were determined by using X-ray diffraction analysis (XRD) RadB-DMAX II Computer Controlled X-ray Diffractometer device.

Hydroponic experiment

Safflower seeds were germinated in the peat and perlite mixture (1:1, w/w) medium. When the seedlings were had 2-3 leaves and rooted slightly, they were transferred into the Hoagland nutrient solution (Hoagland and Arnon, 1950) medium (pH 5.2). The macro and micro nutrients and their concentrations in the Hoagland nutrient solution were as follows: 1 mM KH_2PO_4, 3 mM KNO_3, 0.25 mM $MgSO_4.7H_2O$, 2 mM $Ca(NO_3)_2.4H_2O$, 2.5x10^{-2} mM KCl, 1 µM $MnSO_4.H_2O$, 0.25 µM $CuSO_4.H_2O$, 0.25 µM $(NH_4)_6Mo_7O_{24}$, 0.125 µM H_3BO_3, 0.1 mM Fe-EDTA.

The seedlings were transferred to the 4.5 L polyethylene pots full with Hoagland solution. Safflower seedlings were placed separately as 2 plants in each pot. The experiment was set-up in completely randomized design with three replications in the factorial arrangement (3x3). The nanomaterial was applied to Hoagland nutrient solution in the form of ZnO and at doses of 0, 5, 10 mg/L. Cadmium was applied to the solution in the form of $CdSO_4.8H_2O$ at doses of 0-3-6 mg/L. Cadmium doses were determined considering the toxic limit value (3 mg/L) of Cd in the plant. The nutrient solution was changed every 2-3 days. Plants were grown in nutrient solution medium under controlled conditions (16/8 hours light/dark, 25/20 ºC temperature and 60% humidity, light intensity 10 Klux) for 20 days. Before harvest, the chlorophyll content of leaf was measured with Konica-Minolta SPAD-502 chlorophyll meter as soil plant analysis development value (SPAD value) (Dağhan, 2018).

The plant samples were harvested as the shoot and root, washed with distilled water, and then dried at 65 °C until they reach a constant weight. Then the samples were ground and homogenized by means of agate mill (Retsch MM-301 Mixer Mill, Retsch, Nordrhein-Westfalen, Germany) for analysis.

Plant analysis

The ground plant materials were digested with acids (HNO_3 and H_2O_2) in the microwave oven (MarsXpress 6 CEM, Matthews, USA) (Müftüoğlu et al., 2012). In this method, 0.2 g plant sample were digested for elemental analysis with 2 mL deionized water, 2 mL 35% H_2O_2 and 5 mL 65% HNO_3 for 45 minutes in the microwave oven.

The total Cd and Zn concentrations of the digest were measured by using Atomic Absorption Spectrophotometer (AAS, NovAA 350, Analytic Jena).

Statistical analysis
The data set were subjected to ANOVA by using the SPSS-20 statistical analysis package program. The mean separation were performed by the Duncan' multiple range test at $p<0.05$ probability level.

Results and Discussions

Characterization of ZnO-NPs
Scanning electron microscope (SEM) image of the ZnO-NPs material is given in Figure 1. According to the image, ZnO-NPs have spherical shape and the size of ZnO-NPs was below 100 nm and the smallest size was 44.69 nm. In the EDX analysis results, mostly Zn and ZnO peaks were observed and most of them were in ZnO form (Figure 2).

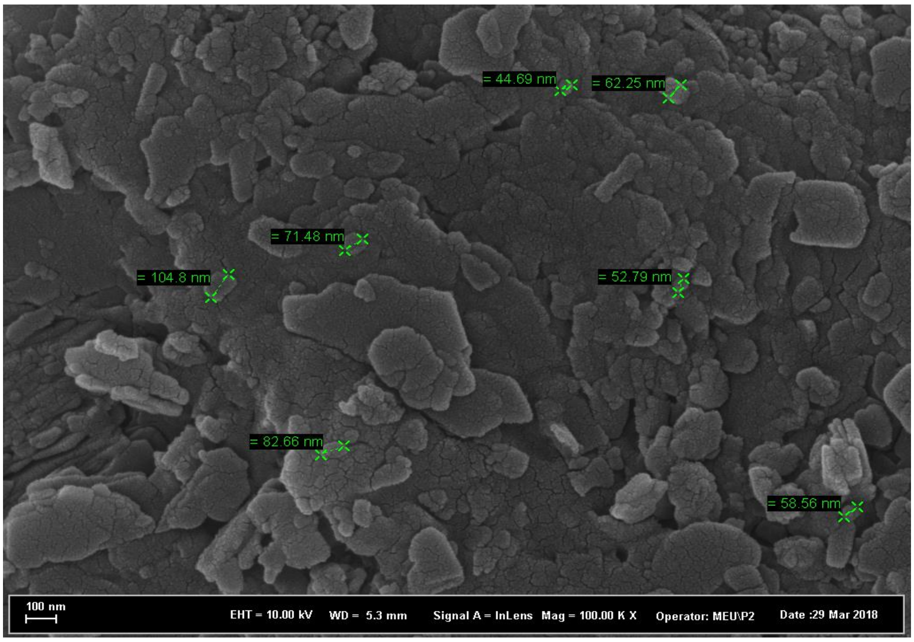

Figure 1. Scanning Electron Microscope (SEM) image of ZnO-NPs (×100K)

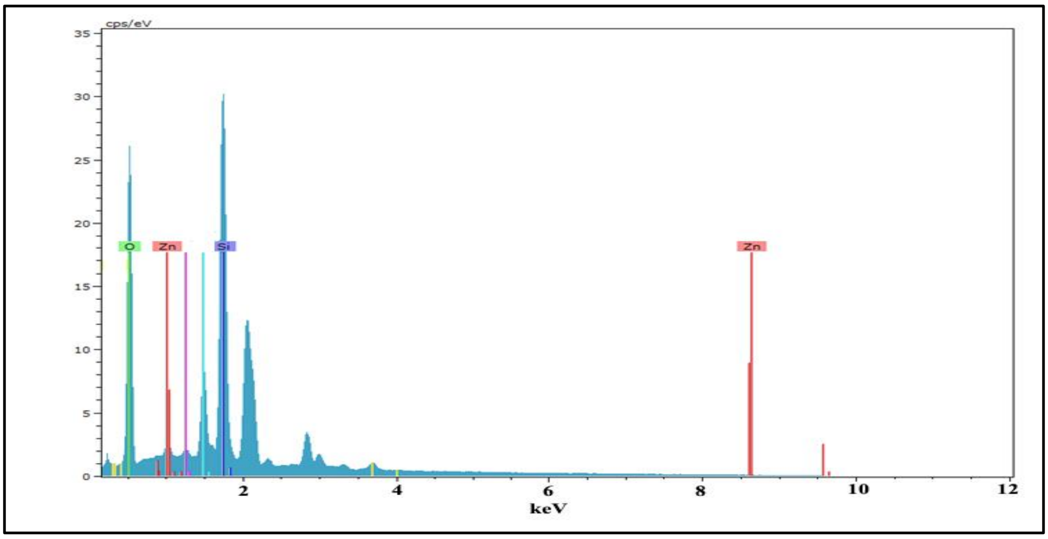

Figure 2. SEM-EDX spectrum of ZnO-NPs

The crystal structures of ZnO-NPs were analyzed by XRD (Figure 3). The results show that the corresponding sharp peaks at 100^o, 002 101^o, 102^o 200^o, 110^o and 112^o at 2θ are the peaks showing the crystal structure of ZnO-NPs (Figure 3). Debye-Scherrer's equation (Eren ve Baran, 2019):

$$D = K\lambda / (\beta \cos\theta) \text{ (Eq:1)}$$

From this equation, the size of the nanoparticle crystals was calculated as 24.89 nm, which is consistent with the zeta potential results. As expressed in inequality, D= Size of the particle (nm), K= Constant (0.90), λ = Wavelength of X-ray (1.5406 °A), β = Half the value of the highest peak as radian unit (FWHM), θ = Refraction angle.

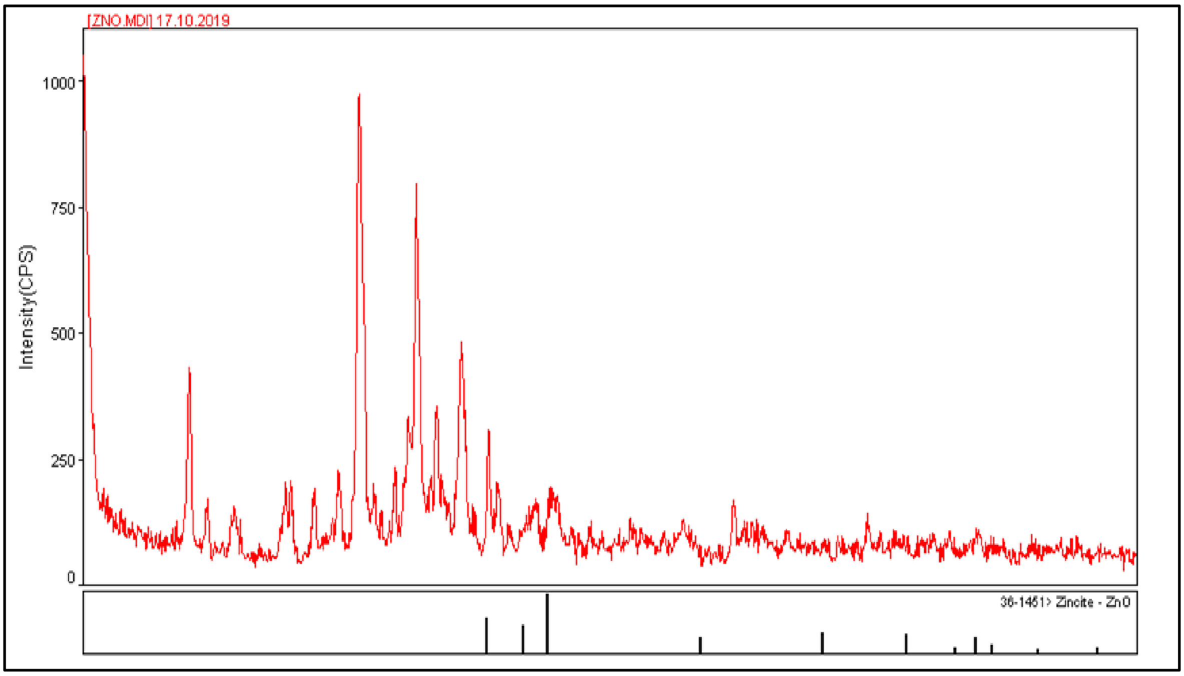

Figure 3. ZnO-NP synthesized by XRD green light spectra method

Morphological Observation

The plants treated with increasing doses of Cd without ZnO-NP were compared to the control; the severe toxic effect of Cd was observed (Fig. 4. A). While the control plant had high shoot biomass with healthy leaves, it was observed that chlorosis and necrosis appeared in Cd-treated plants (Fig. 4. A). In plants treated with 5 and 10 mg ZnO-NP/L, growth reduction was observed in plants compared to control, severe chlorosis and stunting growth at 3 mg Cd/kg doses, and severe necrosis and stunting at 6 mg Cd/kg doses were apparent (Fig. 4. B and C).

On the other hand, it was observed that the plants treated with increasing doses of ZnO-NP and without Cd had grown well and their leaves were dark green (Fig. 4. D). However, it has been determined that the plants become stunted, the leaves become smaller, and very severe chlorosis appears in the old leaves with Cd applications (Fig. 4. E and F). The applications of ZnO-NP had positively affected the plant growth while Cd had negatively affected the plants growth.

Chlorophyll Content

The effects of ZnO-NP (0, 5 and 10 mg/L) and Cd (0, 3 and 6 mg/L) application at increasing doses on the chlorophyll content, root and shoot dry weights of the safflower plant were investigated in hydroponic culture experiment conducted under the control environment conditions.

Table 1 shows that ZnO-NP and Cd applications were significant ($p<0.01$) according to the variance analysis results of leaf chlorophyll (SPAD Unit) contents of safflower plant. The highest chlorophyll content (42.9 SPAD units) was obtained from plants treated with 10 mg/L ZnO-NP without Cd treatment. The lowest chlorophyll value (6.23 SPAD units) was obtained from 6 mg/L Cd application without ZnO-NP. Exposure of ZnO-NPs has positively affected leaf chlorophyll content and morphological observations supported this result.

Dry Weights

Higher biomass production of shoot and root under metal stress is the one of the main criteria for determination of phytoremediation potential of a plant. The effect of ZnO-NP and Cd applications on the shoot dry weights of the safflower plant was found to be statistically significant (Table 1). The highest dry weight of the shoot was obtained at 5 and 10 mg/L ZnO-NP (0.505 g and 0.498 g, respectively) doses without Cd treatment. The effects of ZnO-NP and Cd applications on the dry weight of shoots were found to be statistically significant at $p<0.01$. The highest root dry weight (0.118 g/pot) was determined in control plant without Cd treatment. In addition, it was determined that low-dose ZnO-NP applications increased the shoot biomass ($ZnO\text{-}NP_0$: 0.422, $ZnO\text{-}NP_5$: 0.505 and $ZnO\text{-}NP_{10}$: 0.498) compared to control plants (Table 1).

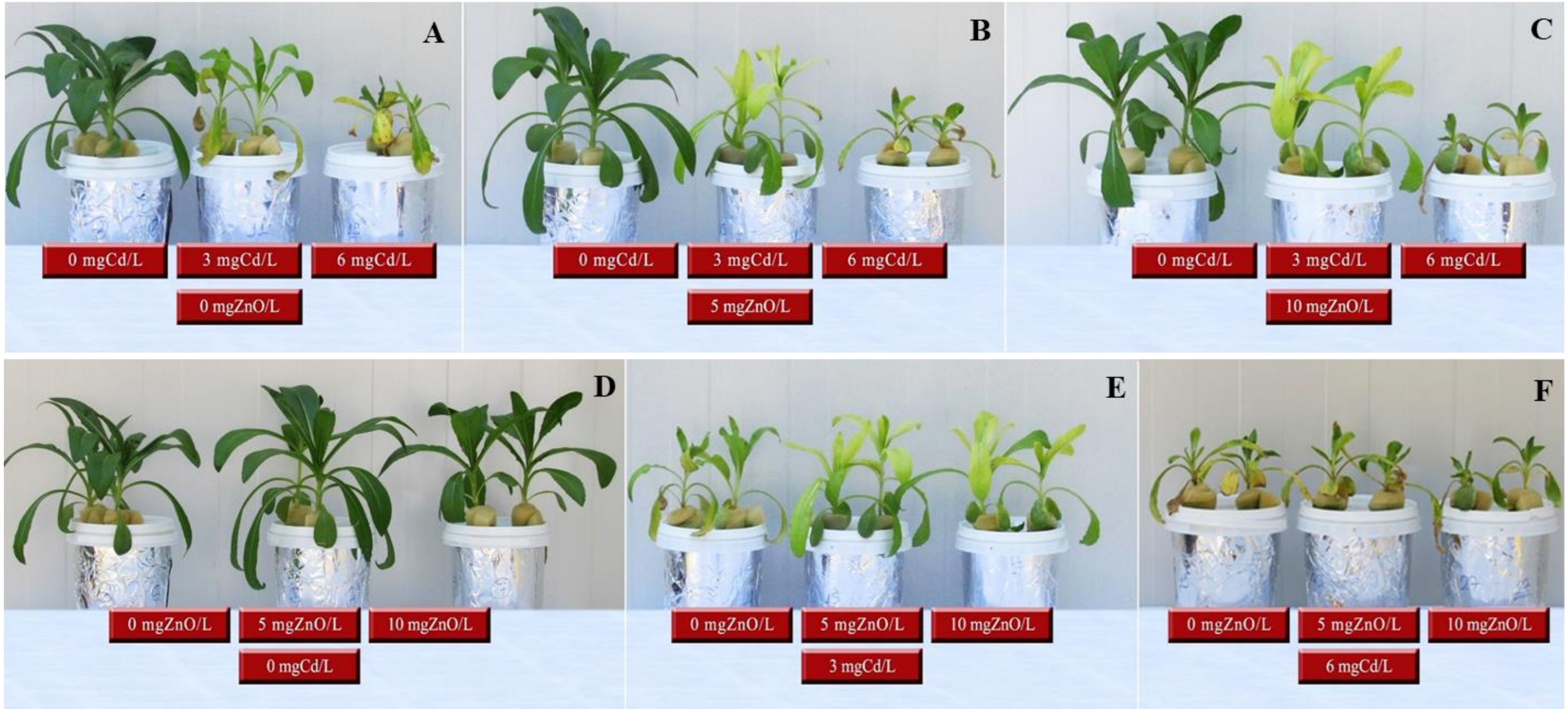

Figure 4. Morphological changes of safflower plant under ZnO-NP and Cd treatments

The dry weights of root was decreased with increasing dose of Cd. The effects of Cd applications on root dry weight of safflower were found to be statistically significant ($p<0.01$). However, ZnO-NP applications were not significantly influenced the root biomass. The enhancement of dry weight in safflower with ZnO-NPs treatment could be related to improving growth with increasing chlorophyll content. On the other hand, Cd application severely decreased shoot and root dry weights. However, plants' growth and chlorophyll content depend on the application dose of ZnO-NPs. Because overdose of ZnO-NP application may cause toxicity on plants.

Various researchers (Zhang et al., 2019; Khan et al., 2019; Venkatachalam et al., 2017) have reported the positive effect of ZnO-NP and toxic effects of Cd on plants. Zhang et al. (2019) reported that, comparing to the control, rice biomass increased by 13-22% and 25-43% at 2.5 Cd mg/kg and 5.0 mg Cd/kg treatments, respectively, with ZnO-NP applications (0, 25, 50 and 100 mg/kg). In addition, increasing rates of ZnO-NP applications increased the dry weight of the wheat plant grown in Cd contaminated soil and decreased the oxidative stress in the plant (Khan et al., 2019). Venkatachalam et al. (2017) compared the individual effects of Cd and Pb applications with and without ZnO-NPs applications and reported an increased growth tolerance index.

Table 1. The effect of increasing doses of ZnO-NP and Cd applications on the chlorophyll content, shoot and root dry weights of the safflower (n=3)

Doses (mg/L)		**Chlorophyll Concentration**	**Shoot**	**Root**
		(SPAD Unit)	**Dry Weight (g)**	
$ZnO\text{-}NP_0$	**Cd_0**	42.3ab	0.422b	0.118a
	Cd_3	21.4c	0.181c	0.050c
	Cd_6	6.23e	0.125d	0.018d
$ZnO\text{-}NP_5$	**Cd_0**	41.5b	0.505a	0.114a
	Cd_3	8.50d	0.213c	0.071b
	Cd_6	8.80d	0.115d	0.027d
$ZnO\text{-}NP_{10}$	**Cd_0**	42.9a	0.498a	0.106a
	Cd_3	8.20d	0.202c	0.066b
	Cd_6	8.90d	0.116d	0.029d
ZnO-NP		67.7**	5.62**	2.49
Cd		5.6**	563**	264.8**

**: $p<0.01$ *: $p<0.05$, n.s: not significant

Zinc and Cadmium concentrations of plant

Cadmium toxicity in plants may be alleviated, to some extent, by Zn treatments (Rizwan et al., 2019b). Because Zn is an essential plant nutrient and it has many important function in the metabolic processes of plants (Marschner, 1995). Cadmium is a divalent ion (Cd^{+2}) and behave very like Zn^{2+} and therefore depending on their concentrations in soil solution Cd may compete with Zn uptake by plant roots. Köleli et al. (2004) reported that Cd toxicity could be more hazardous under Zn deficiency.

The Zn and Cd concentrations of shoots and roots of the safflower plant were shown in Table 2. The effects of both treatments (ZnO-NP and Cd) on Zn and Cd concentrations of plant shoot were found to be statistically significant ($p<0.01$). In shoots, the lowest Zn concentration (6.20 mg/kg) was determined in 0 mg Cd/L and 0 mg ZnO-NP/L applications, while the highest Zn concentration (142.5 mg/kg) was determined in 10 mg ZnO-NP/L + 3 mg Cd/L applications. It was determined that the root Zn concentration of the safflower plant ranged from 19.6 mg/kg at 0 mg ZnO-NP + 3 mg Cd/L to 162.3 mg/kg at 10 mg ZnO-NP + 6 mg Cd/L (Table 2). With the increase in ZnO-NP and Cd doses, the Zn concentration of the roots also increased.

Table 2. The effect of increasing doses of ZnO-NP and Cd applications on the Zn and Cd concentrations of shoot and root (n=3)

Doses (mg/L)		Shoot		Root	
		Zn	Cd	Zn	Cd
		(mg/kg)			
$ZnO\text{-}NP_0$	Cd_0	6.20h	0.00e	22.1f	0.00e
	Cd_3	23.5g	534d	19.6f	1417c
	Cd_6	37.4f	654c	28.9f	2441a
$ZnO\text{-}NP_5$	Cd_0	39.1f	0.00e	64.6d	0.00e
	Cd_3	121.6b	524d	41.8e	1188d
	Cd_6	79.7d	878a	83.0c	2420a
$ZnO\text{-}NP_{10}$	Cd_0	51.4e	0.00e	99.0b	0.00e
	Cd_3	142.5a	520d	76.3c	1144d
	Cd_6	100.0c	743b	162.3a	1994b
ZnO-NP		276**	13.24**	441**	20.7**
Cd		183**	1526**	118**	1822**

**: $p<0.01$ *: $p<0.05$, n.s: not significant

The effects of ZnO-NP and Cd treatments on the shoot and root Cd concentrations of the safflower plant were significant ($p<0.01$). The highest Cd concentration (878 mg/kg) of shoot was obtained from the 5 mg ZnO-NP/L + 6 mg Cd/L doses. The root Cd concentrations were increased with Cd applications (Table 2). The highest Cd concentration in the plant root (2441 mg/kg) was obtained from 6 mg Cd/L without ZnO-NP application.

Hyperaccumulator plants can accumulate more than 100 mg/kg (0.01%) Cd in their shoots, which are also defined as Cd hyperaccumulator plants (Van der Ent et al., 2013). While normal plants can accumulate very low amounts of Cd (0.03-5.0 mg Cd/kg in many plant species) in their aboveground parts.

However, despite such a toxic amount, the safflower plant accumulated 5.2 to 8.7 times higher Cd than the hyperaccumulation threshold value in its harvestable parts (Table 2). This result shows that the Cd phytoremediation potential of the safflower plant is high. Gowayed (2017) reported that the Zn or Cd concentrations of shoots were less than the root concentration with both (ZnO-NP and Cd) applications, and this could be due to the chemical and physical similarities that cause interaction between Zn and Cd. Khan et al. (2019) found an enhancement in root translocation factor with the increasing dose of ZnO-NP (0 - 25 - 50 and 100 mg/kg) applications to the Cd contaminated soil. Keller et al. (2015) reported that the translocation of metals from roots to the aboveground part of the plant restricted when the higher accumulation of metal in roots. On the other hand, at low metal concentrations, the plant defense system may not be active and higher amounts of metals may be transferred to the aboveground part of the plant (Khan et al., 2019). On contrary to our findings, Angelova et al. (2016) found that the safflower plant, which was subjected to varieties of heavy metals (Pb, Zn and Cd), was accumulated less heavy metals in the roots than the aboveground parts. The differences in results may be because Zn and Cd may dissimilarly interact with each other under different conditions, and the Zn application may increase or equilibrate the phytotoxicity of Cd (Khurana et al., 2012).

Conclusion

In this study, the use of the phytoextraction method and ZnO-NPs together to improve Cd uptake by safflower plants were investigated. The results showed that Cd accumulation of the plant increased due to increasing doses of ZnO-NP. In the shoots of the plant Cd has accumulated 5.2 to 8.7 times more than the hyperaccumulation critical threshold value (100 μg/g). This investigation showed that the safflower plant has high phytoremediation potential to clean-up Cd contaminated soil.

The use of metal-based nanoparticles such as ZnO-NP to increase the efficiency of the phytoextraction method is an idea that has emerged with the development of nanotechnology and phytoremediation technology. However, the use of these two technologies together may bring some additional challenges in field practices. Not knowing the possible environmental risk that metal-based nanoparticles to be used may create in the soil ecosystem is worrisome. It is also important to investigate the toxic effects of the nanoparticles to be applied for animals, plants and microbial communities in the soil. Thus, more research is needed regarding environmental risks of nanoparticles.

Author Contributions

Dağhan H. devised and supervised, the main conceptual ideas, proof outline of the project and wrote the current manuscript. Yentür F. A. carried out the hydroponic experiment, analyzed plant materials.

Conflict and Interest

Authors declare no conflict and interest.

Acknowledgements

The authors wish to thank Dr. Birol Karakaya for the synthesis of ZnO-NPs. This article was written from the master's thesis,

which was carried at the University of Eskişehir Osmangazi, Institute of Science, Department of Soil Science and Plant Nutrition.

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