Avicenna J Environ Health Eng. 2020 June;7(1):20-28 http://ajehe.umsha.ac.ir

Original Article

doi 10.34172/ajehe.2020.04

Effect of Organic Nano-Materials on Heavy Metals Concentration of Wheat Plants Inoculated With *Piriformospora indica* Fungus and Irrigated With Wastewater of Plating Industry

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Published online June 29, 2020

Abstract
Removal of heavy metals from industrial effluents is one of the most important issues in environmental research. This study was done to investigate the adsorption effect of multiwall carbon nanotubes (MWNTs) and nano zinc oxide (ZnO) on the decrease of chromium (Cr) and cadmium (Cd) concentration in wheat that was inoculated with *Piriformospora indica* and irrigated with plating industry wastewater. Treatments consisted of applying MWCNs (0 and 0.5% (W/W)) and nano ZnO (0 and 1.5% (W/W)) in the soil irrigated with the plating industry wastewater under the cultivation of wheat inoculated with *P. indica*. This study was done as a factorial experiment in a completely randomized block design in three replications. After 90 days, plants were harvested and the concentration of Cd and Cr in the plant was measured using atomic absorption spectroscopy. The use of MWCNs had a significant effect on the increase of cation exchangeable capacity of the soil. The application of 0.5% (W/W) MWCNs in the soil irrigated with wastewater significantly decreased the soil Cd concentration by 11.7%, while the plant biomass was increased by 13.4%. In addition, applying nano ZnO (1.5% (W/W)) significantly decreased the negative effect of heavy metal toxicity that can be related to the role of nano ZnO in improving soil sorption properties. However, the interaction effect of Zn and Cd cannot be ignored. The results of this study showed that applying MWCNs and nano ZnO has a significant effect on the decrease of phytoremediation efficiency in soil irrigated with plating industry wastewater. However, the type and the amount of heavy metals in wastewater cannot be ignored.
Keywords: Multiwall carbon nanotubes, Wastewater, Nano ZnO, Heavy metals

Received February 4, 2020; **Revised** May 1, 2020; **Accepted** May 10, 2020

1. Introduction

Water is one of the most vital components in today's world and plays a key role in the proper functioning of the Earth's ecosystem. Unfortunately, the wastewater and toxic materials such as heavy metals enter the available freshwater with the development of new civilization and industrialization of societies, as well as agricultural and geological activities (1,2). As the societies are industrialized, the production, disposal, and recycling of industrial effluents become major concerns of these countries due to the existence of heavy metals in them. The effluents contain low to medium levels of heavy metals, which are often caused by metal plating activities, mining industries, making fertilizers, battery manufacturing factories, dye production, drug manufacturing, and so on (3,4).

The severe increase in the production and consumption of heavy metals over the past few decades has caused large amounts of these materials to enter the water in the form of cations. Due to their non-degradable property and resistance to biological changes, heavy metals can remain stable for a long time and continue their life cycle after entering the environment. Most of these ions are toxic to living organisms. Some of them have accumulation property and accumulate in tissues of living organisms. Finally, their concentration in the tissues reaches a level that causes several physiological disorders. Of these disorders, we can refer to carcinogenicity and the effect on the nervous system, skin, hematopoietic system, cardiovascular system, and renal damage (5,6). Therefore, the detection and control of their availability in water and soil are of paramount importance. The mortality and morbidity after heavy metal poisoning have been widely reported. Therefore, broad research has been conducted on the recycling and sanitation of the resulting effluents, and methods such as sedimentation, cation exchange, flocculation, flotation, membrane filtration, biological process, electrochemical process, and chemical reactions have been used to remove these metals. The application of each of the above-mentioned methods is accompanied by some advantages and limitations in addition to low efficiency. Considering that they mainly cause environmental damage and life degradation, researchers are looking for more effective and cheaper methods with higher efficiency (7-10).

The adsorption process is an appropriate technique for the removal of organic and inorganic pollutants from effluents due to its important advantages such as low cost, accessibility, profitability, and ease of operation. Additionally, it is more efficient than other methods (11,12). The retention and adsorption of these metals by multiwall carbon nanotubes (MWNTs) and nano zinc oxide (ZnO) can be very useful owing to their particular crystalline structure. Considering the advantages of these compounds compared to other natural adsorbents, they can be very effective in recycling chemical and industrial effluents due to their porous and resistant structure, lower cost, ease of processing, and easy access (13,14). Nowadays, MWCNs have gained significant attention because of their large surface area, outstanding structural properties, and surface-related characteristics (15) which introduce them as suitable components to adsorb different heavy metal ions. In recent years, nanotechnology has offered new solutions for the use of ecofriendly and efficient adsorbents for wastewater treatment (16). Furthermore, ZnO nanoparticles have importance due to their vast area of applications such as gas sensor, drug delivery, cosmetics, and solar cells (17). Due to easy fabrication, non-toxic synthesis procedure, and environmentally friendly properties, ZnO nanoparticles have also been applied as adsorbent to pick up heavy metal ions (18). Wang et al have prepared modified porous ZnO hollow microspheres with high surface activity, high specific surface area, and high stability against aggregation through modification of hydrothermal method. ZnO hollow microspheres with exposed porous nano-sheets surface indicated more adsorption capacities as compared to the commercial ZnO nano-powders (19).

Sharififard et al studied the removal of cadmium (Cd) from wastewater using nano-clay/TiO_2 composite and concluded that using these composites has a significant effect on the removal of heavy metals from wastewaters. In addition, they mentioned that the modified nano-clay has a significant effect on the reduction of heavy metals in aqueous solution. However, their results are limited to laboratory conditions (20).

It is important to note that conducting such research in the soil environment can have different results, as the adsorption conditions of heavy metals change and the findings of these studies can play an important role in the phytoremediation studies. On the other hand, the use of methods that can reduce the availability of heavy metals in plants can play an important role in growing plants in contaminated soils. Among them, root endophytic fungus *Piriformospora indica* can improve plant growth in contaminated soils. However, the increase in plant growth depends on the type and amount of heavy metal and plant physiology. In addition, the symbiotic association of plants with arbuscular mycorrhizal fungi or *P. indica* can also play an important role in the plant defense system as it can effectively restrict and alleviate heavy metals in the soil (21). Shahabivand et al reported that *P. indica* can affect the plant growth, Cd partitioning, and chlorophyll fluorescence of sunflower under Cd stress (22). However, they did not consider the simultaneous effect of heavy metals, which could play an important role in plant growth in heavy metal contaminated soils. Therefore, this study was conducted to evaluate the effect of MWCNs and nano ZnO on the decrease of chromium (Cr) and Cd concentration in wheat that was inoculated with *P. indica* and irrigated with plating industry wastewater.

2. Materials and Methods

To reuse plating industry wastewater for soil irrigation of agricultural land, a factorial experiment (23) in the layout of randomized completely block design in three replications (as a greenhouse experiment) was used. Treatments (N=48) included the application of MWCNs (0 and 0.5% (W/W)) and nano ZnO (0 and 1.5% (W/W)) in the soil irrigated with the plating industry wastewater (with the different pollution levels of Cd (0 and 20 mg L^{-1}) and Cr (0, 50 and 100 mg L^{-1}) under cultivation of wheat (Pishtaz Cv.) that was inoculated with *P. indica*. This study was performed in three replications. The MWCNs and nano ZnO used with a purity of 90 to 95% in this study were purchased from the Research Institute of Iranian Petroleum Industry. To investigate the effects of MWCNs and nano ZnO on the decrease of the heavy metals pollution entering the soil via irrigation with Cd and Cr polluted plating industry wastewater, a soil with a low organic carbon content and low salinity was selected. Selected soil physico-chemical characteristics measured in this study are shown in Table 1.

The soil was amended with MWCNs and nano ZnO at concentrations of 0.5 and 1.5% (W/W), respectively and

Table 1. Some Selected Physicio-chemical Characteristics Measured in This Study

Property	Unit	Value
Soil texture	-	Loam
pH	-	7.3
EC	dS m^{-1}	0.6
Organic carbon	%	0.4
$CaCo_3$	%	7
Pb availability	mg kg^{-1}	ND
Cd availability	mg kg^{-1}	ND
Cr availability	mg kg^{-1}	ND
Ni availability	mg kg^{-1}	ND
Cation exchange capacity	meq/100 g soil	10.2

incubated for two weeks to reach equilibrium. The fungal strain of *P. indica* used in this study was obtained from Water and Soil Research Institute. The inoculum for the experiments was prepared in the Soil Biology Laboratory of Isfahan University of Technology according to the methods used by Zamani et al (24). The kernel of the wheat plant (Pishtaz Cv.) was sterilized in 70% ethanol solution for 5 minutes followed by NaClO solution for 8 minutes (0.75% Cl), and then washed with deionized water. Afterwards, the seeds were germinated for 2 days on an agar medium in closed Petri dishes at 25°C. Then, two uniform sets of seedlings with radicles of about 1 cm length were chosen to continue the experiment. Then, half of the seeds were inoculated with *P. indica* by immersion in inoculums (= 2×10^6) under gentle shaking for 3 hours. The other half of the seedlings were dipped in sterilized distilled water containing Tween 0.02%. Thereafter, the experimental pots (5 kg) were filled with the treated soils. The inoculated or non-inoculated seedlings (5 seeds) were cultivated at a depth of 1 cm in uncontaminated top soil layer in each pot and watered with the different contaminated plating industry wastewater. The water needed for the plant was evaluated based on the moisture curve (25). Finally, the wheat plants (Rushan Cv.) were harvested at grain maturity (90 days). Soil and above-ground parts of the plant in each pot were separated and transferred to the lab. The availability of Cd and Zn in the soil was measured according to the Lindsay method (26). Plant samples under study were first digested using wet digestion method. Briefly, approximately 0.2 g of the sample was taken in a 100-mL volumetric flask and about 4 mL of HNO_3 was added. The solution was allowed to stand for few hours. Then, it was carefully heated over a water bath until red fumes coming from the flask completely ceased. The flask was allowed to cool at room temperature and then about 4 mL of perchloric acid was added. Then, the flask was heated again over a water bath to evaporate until a small portion remained, which was then filtered through filter paper No.42 and the volume was made up to 100 mL with distilled water. Cd and Cr concentration of plant was also measured using AAS (27).

Statistical analyses of the data were done using the ANOVA procedure in SAS software. The least significant difference test was used to determine the differences between the means. In addition, the significant difference between the means was determined using the probability value of 95% ($P=0.05$).

3. Results and Discussion

The addition of MWCNs and nano ZnO had a significant effect on soil Cd concentration (Table 2).The results of this study showed that using 1.5% (W/W) MWCNs and nano ZnO significantly decreased the concentration of Cd that enters the soil through irrigation with plating industry wastewater by 3.8 and 4.9%, respectively, which may be related to the effect of organic nano-materials on soil cation exchange capacity (CEC). Based on the results of our study, significant increases (9.8% and 11.3%) were observed in soil CEC (Fig. 1) when the studied soil was amended with 0.5% (W/W) MWCNs and 1.5% (W/W) nano ZnO, respectively.

In addition, soil amended with these organic amendments had additive effect. Accordingly, the simultaneous use of MWCNs and nano ZnO had also a significant effect on soil CEC that is a positive point in the removal of heavy metals from soils. The important point in this research is that the application of zinc nano-oxide played an important role in increasing the soil Zn availability (Table 3) that can help to decrease Cd availability in soil. The competitive effects of Zn and Cd are mentioned by many researchers (28,29). The greater decrease in soil Cd availability due to the application of nano-Zn oxide confirms our results clearly.

Soil pollution with Cr had also a significant effect on soil Cd concentration (Table 2). For instance, increasing Cd concentration in the wastewater added to the soil (0 to 20 mg/L) significantly decreased Cr concentration in the soil by 5.8%, which can be related to the greater solubility of Cd compared to Cr. Jahanbakhshi et al investigated the comparison effect of phytoremediation in Cd and Cr contaminated soil in *Spinacia oleracea* and concluded that increasing soil pollution with Cd caused

Table 2. The Effect of MWCNs, Nano ZnO, and the Presence of *Piriformospora indica* on Cd Availability (mg/kg) in the Soil Irrigated With the Plating Industry Wastewater

Nano ZnO (%)	Multi-Walled Carbon Nanotubes	Cd Concentration (mg/L)	+*P. indica*			-*P. indica*		
			Cr Concentration (mg/L)			Cr Concentration (mg/L)		
			0	50	100	0	50	100
0	0	0	ND**	ND	ND	ND	ND	ND
	0.5		ND	ND	ND	ND	ND	ND
	0	20	18.39a*	18.18c	18.00d	18.33a	18.11c	18.07d
	0.5		18.19c	17.79f	17.28h	18.11c	17.73f	17.24h
1.5	0	0	ND	ND	ND	ND	ND	ND
	0.5		ND	ND	ND	ND	ND	ND
	0	20	18.22b	18.11c	17.88e	18.20b	18.13c	17.81e
	0.5		18.00d	17.65g	17.11j	18.07d	17.60g	17.15j

* Different letters show the significant differences ($P=0.05$), **ND: not detectable by AAS.

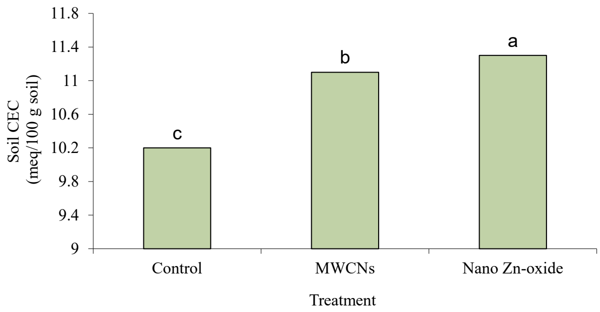

Fig. 1. Effect of Applying MWCNs and Nano ZnO on Soil CEC

a significant decrease in Cr concentration of the plant which was related to the greater solubility of Cd compared to Cr. In addition, they mentioned that plant type had also a significant effect on heavy metals uptake. However, they did not mention the role of the chemical properties of soil in soil heavy metal availability (30). Based on the results of this study, the highest Cr concentration of the soil belonged to soil without any pollution with Cd, while the lowest was measured in the soil with the highest level of Cd.

Cr concentration in the root (Table 4) was significantly affected by soil Cd concentration (Table 2). Based on the results of this study, increasing soil pollution with Cd significantly decreased the Cr concentration in the plant. However, the Cd concentration of the plant showed a significant increase, which can be related to the competitive effect of Cd and Cr on the sorption by plants. In addition, the greater solubility of Cd compared to Cr cannot be ignored. Nojabaee et al investigated the concentration of Pb and Cr in leaves of cress and parsley in soils irrigated with contaminated water and reported the competitive effect of heavy metals on the sorption by plants (31).

The addition of MWCNs and nano ZnO had a significant effect on the decrease of Cd (Table 5) and Cr concentrations in the plant root. Based on the results of this study, adding 1.5% (W/W) MWCNs and nano ZnO caused a significant decrease in Cd concentration of the root by 9.6% and 12.4%, respectively. The Cr concentration in the plant root also decreased by 7.2% and 9.4% that can be related to the effect of applying organic amendments with high specific surface area, thereby increasing the soil CEC. The decrease of heavy metal concentration in the plant due to applying soil organic amendments has been mentioned by researchers. Generally, organics increase surface charge and area and

Table 3. The Effect of MWCNs, Nano ZnO, and the Presence of *Piriformospora indica* on Zn Availability (mg/kg) in the Soil Irrigated With the Plating Industry Wastewater

Nano ZnO (%)	Multi-Walled Carbon Nanotubes	Cd Concentration (mg/L)	*+P. indica*			*-P. indica*		
			Cr Concentration (mg/L)			Cr Concentration (mg/L)		
			0	50	100	0	50	100
0	0	0	1.11j*	1.03k	0.93l	1.15j	1.07k	0.99l
	0.5		1.45g	1.31h	1.24i	1.41g	1.37h	1.29i
	0	20	1.22i	1.11j	1.03k	1.27i	1.16j	1.08k
	0.5		1.36h	1.27i	1.17j	1.32h	1.22i	1.13j
1.5	0	0	3.00b	2.73c	2.55e	3.08b	2.79c	2.58e
	0.5		3.12a	3.04b	2.72c	3.10a	3.09b	2.78c
	0	20	2.61d	2.52e	2.41f	2.65d	2.55e	2.48f
	0.5		2.78c	2.69d	2.54e	2.74c	2.63d	2.50e

* Different letters show the significant differences ($P=0.05$).

Table 4. The Effect of MWCNs, Nano ZnO, and the Presence of *Piriformospora indica* on Cr Availability (mg/kg) in the Root Grown in the Soil Irrigated With the Plating Industry Wastewater

Nano ZnO (%)	Multi-Walled Carbon Nanotubes	Cd Concentration (mg/L)	*+P. indica*			*-P. indica*		
			Cr Concentration (mg/L)			Cr Concentration (mg/L)		
			0	50	100	0	50	100
0	0	0	ND**	54.2j*	115.8a	ND	52.2k	113.9c
	0.5		ND	49.3n	114.9b	ND	48.1o	107.2g
	0	20	ND	51.3l	115.1a	ND	48.5o	111.3d
	0.5		ND	45.7q	112.9c	ND	44.2r	108.1f
1.5	0	0	ND	50.5m	115.3a	ND	48.3o	112.7c
	0.5		ND	47.1p	112.9c	ND	45.2q	106.9h
	0	20	ND	47.4p	112.4c	ND	44.3r	108.9f
	0.5		ND	44.8r	110.6e	ND	43.1s	105.9i

* Different letters show the significant differences ($P=0.05$), **ND: not detectable by AAS

number of exchange sites in the soil, thereby reducing metal mobility by surface adsorption (32) and stable complexation (33). Carbon compounds are the main constituents of organic amendments which are involved in metal precipitation and their conversion to stable forms (34). Xiong et al investigated the effect of nano-zeolite on chemical fractions of Cd in soil and its uptake by cabbage and concluded that the plant biomass was significantly increased by Nano zeolite. In addition, they mentioned that applying nano-zeolite significantly improved the sorption properties of the soil, thereby decreasing the Cd uptake by plant (35), which is similar to our results. However, they did not consider the simultaneous effect of heavy metals in soil. Based on the results of our study, the additive effect of applying MWCNs and nano ZnO significantly increased the soil CEC, thereby decreasing the heavy metal uptake by plant.

Wheat plants inoculated with *P. indica* showed significantly increased Cd and Cr concentrations in the root. Based on the results of this study, significant increases (12.8% and 14.3%, respectively) in Cr and Cd concentrations were observed in the root when the plants were inoculated with *P. indica*. However, inoculation with *P. indica* can protect against the negative effects of plant stresses. Khalvandi et al investigated the effects of symbiotic fungus *P. indica* on the quantity of essential oil and some physiological parameters of peppermint in saline conditions and concluded that plant inoculation with *P. indica* can diminish the negative effects of abiotic stress such as salinity (36). Shahabivand et al mentioned that inoculation with *P. indica* can affect growth, Cd partitioning, and chlorophyll fluorescence of sunflower under Cd toxicity. In addition, the results of their study showed that plant inoculation with *P. indica* can increase and decrease heavy metal concentration in plant root and shoot, respectively (22), which confirm our results clearly.

The highest Cr concentration in plant shoot (Table 6) belonged to the plants without any inoculation which were cultivated in the non-Cd polluted soil, while the lowest was measured in the plants that was inoculated with *P. indica* and grown in the soil highly polluted with Cd and non-polluted with Cr. Increasing soil pollution with Cr significantly increased the Cr concentration in plant shoot, which can be related to the increase of Cr availability in the soil. However, plant inoculation with *P. indica* significantly decreased the translocation of Cr from root to shoot that is a positive point in environmental studies. Dianat Maharluei et al investigated the effect of the presence of *P. indica* on corn yield in Zn-contaminated soil and concluded that plant inoculation with *P. indica*

Table 5. The Effect of MWCNs, Nano ZnO and the Presence of *Piriformospora indica* on Cd availability (mg/kg) in the Root Grown in the Soil Irrigated With the Plating Industry Wastewater

Nano ZnO (%)	Multi-Walled Carbon Nanotubes	Cd Concentration (mg/L)	+*P. indica*			-*P. indica*		
			Cr Concentration (mg/L)			Cr Concentration (mg/L)		
			0	50	100	0	50	100
0	0	0	ND**	ND	ND	ND	ND	ND**
	0.5		ND	ND	ND	ND	ND	ND
	0	20	20.4a*	19.8e	19.7f	20.2b	19.7f	19.6g
	0.5		20.1c	20.0d	19.6g	19.8e	19.7f	19.5h
1.5	0	0	ND	ND	ND	ND	ND	ND**
	0.5		ND	ND	ND	ND	ND	ND
	0	20	20.0d	19.8e	19.3j	19.8e	19.7f	19.5h
	0.5		19.8e	19.6g	19.1k	19.6g	19.4i	19.3j

* Different letters show the significant differences ($P=0.05$), **ND: not detectable by AAS

Table 6. The Effect of MWCNs, Nano ZnO, and the Presence of *Piriformospora indica* on Cr Availability (mg/kg) in the Shoot Grown in the Soil Irrigated With the Plating Industry Wastewater

Nano ZnO (%)	Multi-Walled Carbon Nanotubes	Cd Concentration (mg/L)	+*P. indica*			-*P. indica*		
			Cr Concentration (mg/L)			Cr Concentration (mg/L)		
			0	50	100	0	50	100
0	0	0	ND**	38.2i*	75.2a	ND	36.2k	72.1d
	0.5		ND	37.8j	74.2b	ND	35.4l	70.5e
	0	20	ND	37.5j	73.6c	ND	35.1l	69.3f
	0.5		ND	36.7k	72.7d	ND	35.0l	67.2g
1.5	0	0	ND	37.2j	74.3b	ND	35.5l	70.1e
	0.5		ND	36.3k	72.6d	ND	34.1m	67.3g
	0	20	ND	35.1l	72.1d	ND	33.2n	67.2g
	0.5		ND	34.7m	70.1e	ND	32.1o	65.2h

* Different letters show the significant differences ($P=0.05$), **ND: not detectable by AAS.

can increase the plant biomass and this increase occurred due to the provision of the nutritional needs of the plant and plant inoculation (37). However, storing heavy metals in fungal hyphae in the root and preventing them from being transferred to the aerial parts of the plant should not be overlooked. In addition, the results obtained by some researchers suggest that changes in pH in the rhizosphere of the plant due to inoculation of the plant with fungi can also be an effective factor in the availability of heavy metals in soil and plants (38,39).

Inoculation with *P. indica* can improve the uptake of nutrient such as P, thereby decreasing the heavy metal uptake by plants. Baghaie et al reported that soil indigenous arbuscular mycorrhizal fungi can alter chemical properties of the soil, thereby decreasing the heavy metal uptake by wheat (40). It should be noted that inoculation of the plant cultivated in soil that was contaminated with different types of heavy metals can also reduce the sorption of heavy metals by the aerial parts, but the type and amount of heavy metals uptake by plant depend on the chemical properties of the soil (40). Based on the results of our study, increasing soil pollution with Cd significantly decreased the Cr concentration of the shoot due to its greater solubility compared to Cr. However, Cd uptake showed similar conditions as Cr. Adding MWCNs and nano ZnO had a significant effect on the decrease of heavy metal concentration of the shoot. Based on the results of this study, the highest Cd (Table 7) and Cr concentration of the shoot belonged to the non-inoculated plants cultivated in the soil polluted with the greatest level of Cd and Cr (without receiving MWCNs and nano ZnO), respectively. However, the lowest Cd and Cr concentration of the shoot was observed in the inoculated plant grown in the soil with the lowest Cd and Cr concentration, respectively, which received the greatest level of MWCNs and nano ZnO. Adding 1.5% (W/W) nano ZnO and 0.5% (W/W) MWCNs significantly decreased the Cr concentration of the shoot by 11.3% and 10.2%, respectively, while for Cd (Table 7), it decreased by 13.1% and 11.8%, respectively. This can be related to the role of organic amendments in increasing sorption properties of the soil such as soil CEC, thereby decreasing heavy metal availability in the soil and consequently decreasing heavy metal uptake by the plant. However, the simultaneous effect of applying MWCNs and nano ZnO has additive effects on the decrease of heavy metal availability in the shoot.

The highest plant biomass (Table 8) belonged to the plants that were inoculated with *P. indica* and cultivated in the soil that was not polluted with heavy metals, while

Table 7. The Effect of MWCNs, Nano ZnO, and the Presence of *Piriformospora indica* on Cd Availability (mg/kg) in the Shoot Grown in the Soil Irrigated With the Plating Industry Wastewater

Nano ZnO (%)	Multi-Walled Carbon Nanotubes	Cd Concentration (mg/L)	+*P. indica* Cr Concentration (mg/L)			-*P. indica* Cr Concentration (mg/L)		
			0	50	100	0	50	100
0	0	0	ND**	ND	ND	ND	ND	ND
	0.5		ND	ND	ND	ND	ND	ND
	0	20	11.7b*	10.8g	10.3k	11.9a	11.0f	10.5i
	0.5		11.5c	10.5i	10.1m	11.7b	10.8g	10.2l
1.5	0	0	ND	ND	ND	ND	ND	ND
	0.5		ND	ND	ND	ND	ND	ND
	0	20	11.2d	10.3k	10.1m	11.5c	10.5i	10.3k
	0.5		11.1e	10.2l	9.7o	10.7h	10.4j	10.0n

* Different letters show the significant differences ($P=0.05$), **ND: not detectable by AAS.

Table 8. The Effect of MWCNs, Nano ZnO, and the Presence of *Piriformospora indica* on Dry Biomass (g) of the Plant Grown in the Soil Irrigated With the Plating Industry Wastewater

Nano ZnO (%)	Multi-Walled Carbon Nanotubes	Cd Concentration (mg/L)	+*P. indica* Cr Concentration (mg/L)			-*P. indica* Cr Concentration (mg/L)		
			0	50	100	0	50	100
0	0	0	4.90f*	4.80n	4.75q	4.82l	4.71s	4.62v
	0.5		4.90f	4.8j5	4.80n	4.85j	4.80n	4.72r
	0	20	4.80n	4.82l	4.75q	4.72r	4.70t	4.65u
	0.5		4.85j	4.83k	4.80n	4.80n	4.78o	4.75q
1.5	0	0	4.95b	4.91e	4.88g	4.91e	4.88g	4.81m
	0.5		4.98a	4.93c	4.90f	4.93c	4.91e	4.88g
	0	20	4.92d	4.87h	4.83k	4.88g	4.85j	4.80n
	0.5		4.90f	4.87h	4.83k	4.86i	4.80n	4.77p

* Different letters show the significant differences ($P=0.05$).

the lowest was recorded in the plants grown in the soil polluted with the highest level of Cd and Cr and without receiving MWCNs and nano ZnO. The addition of 1.5% (W/W) nano ZnO and 0.5 % (W/W) MWCNs in a soil polluted with the highest level of Cr significantly decreased the plant biomass by 13.3% and 12.1%, respectively, which can be related to the role of adding organic amendments in decreasing Cd concentration of the plant, thereby increasing plant biomass. However, the increase in plant biomass due to the increase of Zn concentration of the plant and consequently the decrease of Cd concentration in the plant cannot be ignored. The competitive effect of Cd and Zn has been mentioned by researchers (41,42). Furthermore, for Cd polluted soil, applying 1.5% (W/W) nano ZnO and 0.5% (W/W) MWCNs significantly increased the plant biomass by 11.1% and 9.8%, respectively. Based on this, we can conclude that the effect of applying MWCNs and nano ZnO on the increase of plant biomass are more effective in soil polluted with Cr compared to Cd, which may be attributed to the higher solubility of Cd compared to Cr.

Plant inoculation with *P. indica* had a significant effect on the increase of plant biomass (Table 8). Based on the results of our study, the highest and lowest plant biomass belonged to the inoculated and non-inoculated plant, respectively. The inoculation of the plant (with *P. indica)* which was cultivated in the soil polluted with Cd (20 mg/kg) and Cr (50 mg/kg) significantly increased the plant biomass by 14.3% and 15.7%, respectively, which can be related to the effect of *P. indica* on the prevention of the heavy metal translocation to the aerial part of the plant or reducing the availability of heavy metals. Mohd et al studied the role of *P. indica* in translocation of heavy metals to the aerial parts of the plant and concluded that *P. indica* can protect the plant against arsenic toxicity by three different mechanisms via reducing the availability of free arsenic in the plant environment, bio-transformation of toxic arsenic salts into insoluble particulate matter, and modulating the antioxidative system of the host cell (43). In addition, they mentioned that the induced heavy metal tolerance imparted by the fungus is due to selective clearance of arsenic by the extraradical hyphae of the fungus from the media and this is why root receives a large amount of arsenic, while a small fraction of it can translocate to the shoot (43), which clearly confirms our results. However, the additive effect of soil pollution with Cd and Cr had negative effects on plant growth processing.

4. Conclusion

According to the results of this study, using nano-clays can play an important role in the treatment of the plating industry wastewater. Accordingly, the application of MWCNs and nano ZnO has played a more important role in reducing the pollution adsorbed by wheat plants in the soil irrigated with industrial wastewater. However, Nano ZnO showed a greater efficiency in removing heavy metals compared to MWCNs that can be related to the interaction role of Zn and Cd in heavy metal sorption by plant. It is necessary to mention that the amount and type of metal have also played an important role in the efficiency of nanoparticles in removing heavy metals. On the other hand, inoculation of the plant with *P. indica* has also been able to improve resistance of the plant to abiotic stress such as heavy metal toxicity. Due to the fact that the effluent of industrial plants can be contaminated with several heavy metals, it is necessary to investigate the simultaneous effect of heavy metals. In addition, the economic aspect of using treated wastewater and its role in phytoremediation efficiency in these studies cannot be ignored.

Conflict of Interest Disclosures

The author declares that he has no conflict of interests.

Acknowledgements

The author of this study gratefully would like to thank Islamic Azad University, Arak Branch, for cooperating in analysis of samples.

References

1. Bhere SM, Patil GK. The climate change analysis on water resources over western Ghat by deficit calculation method. J Water Resour Eng Manag. 2020;7(1):16-35.
2. Dadhich U, Shaban A. Rainfall deficiency and water conservation mechanisms in Western Rajasthan. In: Bandyopadhyay S, Magsi H, Sen S, Ponce Dentinho T, eds. Water Management in South Asia. Cham: Springer; 2020. p. 203-32. doi: 10.1007/978-3-030-35237-0_12.
3. Bhagat SK, Tung TM, Yaseen ZM. Development of artificial intelligence for modeling wastewater heavy metal removal: state of the art, application assessment and possible future research. J Clean Prod. 2020;250:119473. doi: 10.1016/j.jclepro.2019.119473.
4. Almomani F, Bhosale R, Khraisheh M, kumar A, Almomani T. Heavy metal ions removal from industrial wastewater using magnetic nanoparticles (MNP). Appl Surf Sci. 2020;506:144924. doi: 10.1016/j.apsusc.2019.144924.
5. Adimalla N, Chen J, Qian H. Spatial characteristics of heavy metal contamination and potential human health risk assessment of urban soils: a case study from an urban region of South India. Ecotoxicol Environ Saf. 2020;194:110406. doi: 10.1016/j.ecoenv.2020.110406.
6. Mukherjee I, Singh UK, Singh RP, Anshumali, Kumari D, Jha PK, et al. Characterization of heavy metal pollution in an anthropogenically and geologically influenced semi-arid region of east India and assessment of ecological and human health risks. Sci Total Environ. 2020;705:135801. doi: 10.1016/j.scitotenv.2019.135801.
7. Awa SH, Hadibarata T. Removal of heavy metals in contaminated soil by phytoremediation mechanism: a review. Water Air Soil Pollut. 2020;231(2):47. doi: 10.1007/s11270-020-4426-0.
8. Jaskulak M, Grobelak A, Vandenbulcke F. Modelling assisted phytoremediation of soils contaminated with heavy metals–main opportunities, limitations, decision making and future prospects. Chemosphere. 2020;249:126196. doi: 10.1016/j.chemosphere.2020.126196.
9. de Souza CB, Silva GR. Phytoremediation of effluents contaminated with heavy metals by floating aquatic

macrophytes species. In: Biotechnology and Bioengineering. IntechOpen; 2019. p. 1-19. doi: 10.5772/intechopen.83645.
10. Haq S, Bhatti AA, Dar ZA, Bhat SA. Phytoremediation of heavy metals: an eco-friendly and sustainable approach. In: Hakeem K, Bhat R, Qadri H, eds. Bioremediation and Biotechnology. Cham: Springer; 2019. p. 215-31. doi: 10.1007/978-3-030-35691-0_10.
11. Abdelsalam H, Teleb NH, Yahia IS, Zahran HY, Elhaes H, Ibrahim MA. First principles study of the adsorption of hydrated heavy metals on graphene quantum dots. J Phys Chem Solids. 2019;130:32-40. doi: 10.1016/j.jpcs.2019.02.014.
12. Soliman NK, Moustafa AF. Industrial solid waste for heavy metals adsorption features and challenges; a review. J Mater Res Technol. 2020;9(5):10235-53. doi: 10.1016/j.jmrt.2020.07.045.
13. Thomas P, Rumjit NP, George PJ, Lai CW, Tyagi P, Johan MRB, et al. Remediation of heavy metal ions using nanomaterials sourced from wastewaters. In: Thangadurai D, Sangeetha J, Prasad R, eds. Nanotechnology for Food, Agriculture, and Environment. Cham: Springer; 2020. p. 255-96. doi: 10.1007/978-3-030-31938-0_12.
14. Alothman ZA, Habila MA, Moshab MS, Al-Qahtani KM, AlMasoud N, Al-Senani GM, et al. Fabrication of renewable palm-pruning leaves based nano-composite for remediation of heavy metals pollution. Arab J Chem. 2020;13(4):4936-44. doi: 10.1016/j.arabjc.2020.01.015.
15. Ihsanullah, Abbas A, Al-Amer AM, Laoui T, Al-Marri MJ, Nasser MS, et al. Heavy metal removal from aqueous solution by advanced carbon nanotubes: critical review of adsorption applications. Sep Purif Technol. 2016;157:141-61. doi: 10.1016/j.seppur.2015.11.039.
16. Khan NA, Khan SU, Ahmed S, Farooqi IH, Dhingra A, Hussain A, et al. Applications of nanotechnology in water and wastewater treatment: a review. Asian J Water Environ Pollut. 2019;16(4):81-6. doi: 10.3233/ajw190051.
17. Kumar R, Chawla J. Removal of cadmium ion from water/wastewater by nano-metal oxides: a review. Water Qual Expo Health. 2014;5(4):215-26. doi: 10.1007/s12403-013-0100-8.
18. Hua M, Zhang S, Pan B, Zhang W, Lv L, Zhang Q. Heavy metal removal from water/wastewater by nanosized metal oxides: a review. J Hazard Mater. 2012;211-212:317-31. doi: 10.1016/j.jhazmat.2011.10.016.
19. Wang X, Cai W, Liu S, Wang G, Wu Z, Zhao H. ZnO hollow microspheres with exposed porous nanosheets surface: structurally enhanced adsorption towards heavy metal ions. Colloids Surf A Physicochem Eng Asp. 2013;422:199-205. doi: 10.1016/j.colsurfa.2013.01.031.
20. Sharififard H, Ghorbanpour m, Hosseinirad S. Cadmium removal from wastewater using nano-clay/TiO2 composite: kinetics, equilibrium and thermodynamic study. Adv Environ Technol. 2018;4(4):203-9. doi: 10.22104/aet.2019.3029.1149.
21. Nanda R, Agrawal V. *Piriformospora indica*, an excellent system for heavy metal sequestration and amelioration of oxidative stress and DNA damage in *Cassia angustifolia* Vahl under copper stress. Ecotoxicol Environ Saf. 2018;156:409-19. doi: 10.1016/j.ecoenv.2018.03.016.
22. Shahabivand S, Parvaneh A, Aliloo AA. Root endophytic fungus *Piriformospora indica* affected growth, cadmium partitioning and chlorophyll fluorescence of sunflower under cadmium toxicity. Ecotoxicol Environ Saf. 2017;145:496-502. doi: 10.1016/j.ecoenv.2017.07.064.
23. Ghaedi AM, Panahimehr M, Rayegan Shirazi Nejad A, Hosseini SJ, Vafaei A, Baneshi MM. Factorial experimental design for the optimization of highly selective adsorption removal of lead and copper ions using metal organic framework MOF-2 (Cd). J Mol Liq. 2018;272:15-26. doi: 10.1016/j.molliq.2018.09.051.
24. Zamani J, Hajabbasi MA, Alaie E, Sepehri M, Leuchtmann A, Schulin R. The effect of *Piriformospora indica* on the root development of maize (*Zea mays* L.) and remediation of petroleum contaminated soil. Int J Phytoremediation. 2016;18(3):278-87. doi: 10.1080/15226514.2015.1085831.
25. Fang J, Su Y. Effects of soils and irrigation volume on maize yield, irrigation water productivity, and nitrogen uptake. Sci Rep. 2019;9(1):7740. doi: 10.1038/s41598-019-41447-z.
26. Lindsay WL, Norvell WA. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J. 1978;42(3):421-8. doi: 10.2136/sssaj1978.03615995004200030009x.
27. Akram S, Najam R, Rizwani GH, Abbas SA. Determination of heavy metal contents by atomic absorption spectroscopy (AAS) in some medicinal plants from Pakistani and Malaysian origin. Pak J Pharm Sci. 2015;28(5):1781-7.
28. Abdin Y, Usman A, Ok YS, Tsang YF, Al-Wabel M. Competitive sorption and availability of coexisting heavy metals in mining-contaminated soil: contrasting effects of mesquite and fishbone biochars. Environm Res. 2020;181:108846. doi: 10.1016/j.envres.2019.108846.
29. Qin S, Liu H, Nie Z, Rengel Z, Gao W, Li C, et al. Toxicity of cadmium and its competition with mineral nutrients for uptake by plants: a review. Pedosphere. 2020;30(2):168-80. doi: 10.1016/s1002-0160(20)60002-9.
30. Jahanbakhshi S, Rezaei MR, Sayyari-Zahan MH. Comparison effect of phytoremediation in cadmium and chromium contaminated soil in *Spinacia oleracea* and *Lepidium sativum*. J Water Soil Sci. 2015;18(70):1-12.
31. Nojabaee SA, Ghajar Sepanlou M, Bahmanyar MA. Concentration of lead and chromium in leaves of cress and parsley in soils irrigated with contaminated water. Iranian Journal of Water Research in Agriculture. 2017;31(2):181-94. [Persian].
32. Ghaedi AM, Ghaedi M, Vafaei A, Iravani N, Keshavarz M, Rad M, et al. Adsorption of copper (II) using modified activated carbon prepared from Pomegranate wood: optimization by bee algorithm and response surface methodology. J Mol Liq. 2015;206:195-206. doi: 10.1016/j.molliq.2015.02.029.
33. Hamid Y, Tang L, Hussain B, Usman M, Lin Q, Rashid MS, et al. Organic soil additives for the remediation of cadmium contaminated soils and their impact on the soil-plant system: a review. Sci Total Environ. 2020;707:136121. doi: 10.1016/j.scitotenv.2019.136121.
34. Anwar Z, Irshad M, Mahmood Q, Hafeez F, Bilal M. Nutrient uptake and growth of spinach as affected by cow manure co-composted with poplar leaf litter. Int J Recycl Org Waste Agric. 2017;6(1):79-88. doi: 10.1007/s40093-017-0154-x.
35. Xiong SJ, Xu WH, Xie WW, Chen R, Chen YQ, Chi SL, et al. [Effect of nano zeolite on chemical fractions of Cd in soil and its uptake by cabbage]. Huan Jing Ke Xue. 2015;36(12):4630-41.
36. Khalvandi M, Amerian MR, Pirdashti H, Baradaran Firoozabadi M, Gholami A. Effects of *Piriformospora indica* fungi symbiotic on the quantity of essential oil and some physiological parameters of peppermint in saline conditions. Iran J Plant Biol. 2017; 9(32):1-19. [Persian].
37. Dianat Maharluei Z, Yasrebi J, Sepehri M, Ghasemi R. Effect of rice husk biochar and *Piriformospora indica* endophytic fungus on corn yeild in Zn contaminated soil. Electronic Journal of Soil Management and Sustainable Production. 2018; 8(3):61-78. [Persian].
38. Cui G, Ai S, Chen K, Wang X. Arbuscular mycorrhiza augments cadmium tolerance in soybean by altering accumulation and

partitioning of nutrient elements, and related gene expression. Ecotoxicol Environ Saf. 2019;171:231-9. doi: 10.1016/j.ecoenv.2018.12.093.

39. Gao X, Akhter F, Tenuta M, Flaten DN, Gawalko EJ, Grant CA. Mycorrhizal colonization and grain Cd concentration of field-grown durum wheat in response to tillage, preceding crop and phosphorus fertilization. J Sci Food Agric. 2010;90(5):750-8. doi: 10.1002/jsfa.3878.
40. Baghaie AH, Aghili F, Jafarinia R. Soil-indigenous arbuscular mycorrhizal fungi and zeolite addition to soil synergistically increase grain yield and reduce cadmium uptake of bread wheat (through improved nitrogen and phosphorus nutrition and immobilization of Cd in roots). Environ Sci Pollut Res Int. 2019;26(30):30794-807. doi: 10.1007/s11356-019-06237-0.
41. Khaliq MA, James B, Chen YH, Ahmed Saqib HS, Li HH, Jayasuriya P, et al. Uptake, translocation, and accumulation of Cd and its interaction with mineral nutrients (Fe, Zn, Ni, Ca, Mg) in upland rice. Chemosphere. 2019;215:916-24. doi: 10.1016/j.chemosphere.2018.10.077.
42. Xiao R, Wang P, Mi S, Ali A, Liu X, Li Y, et al. Effects of crop straw and its derived biochar on the mobility and bioavailability in Cd and Zn in two smelter-contaminated alkaline soils. Ecotoxicol Environ Saf. 2019;181:155-63. doi: 10.1016/j.ecoenv.2019.06.005.
43. Mohd S, Shukla J, Kushwaha AS, Mandrah K, Shankar J, Arjaria N, et al. Endophytic fungi *Piriformospora indica* mediated protection of host from arsenic toxicity. Front Microbiol. 2017;8:754. doi: 10.3389/fmicb.2017.00754.