

ORIGINAL RESEARCH PAPER

Nano Fe-oxide and nano-hydroxyapatite can decrease the Cd uptake by plant in a soil treated with Cd-polluted sewage sludge

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ABSTRACT

Decreasing heavy metal availability has important key factors in environmental studies. This research was done to investigate the effect of nano-Fe oxide and nano-hydroxyapatite (NHAP) on Cd uptake by the plant cultivated in the sewage sludge amended soil. Treatments consisted of applying Cd (0, 10 and 20 mg Cd/kg)-polluted sewage sludge at the rates of 0, 15 and 30 t/ha, nano-Fe oxide (0 and 0.5 ppm) and NHAP (0, 0.5 and 1 % (W/W)). The plant in this experiment was the pinto bean (Cv. Khomein). After 90 days, the plants were harvested and the plant Zn, Cd, and Fe concentrations were measured using atomic absorption spectroscopy. In addition, soil microbial respiration was calculated. Soil application of NHAP significantly increased the Zn and Fe concentration of the plants cultivated in the soil which was amended with 15 t/ha sewage sludge by 12.8 and 14.5%, respectively. However, the Cd concentration was decreased by 17.2%. Using 15 and 30 t/ha sewage sludge significantly increased the plant Zn and Fe by 13.1 and 14.6%, respectively. Foliar application of nano-Fe oxide at the rate of 1 ppm significantly decreased the plant Cd uptake by 18.3%. Using NHAP (0.5 % (W/W)) and sewage sludge (30 t/h) significantly increased the soil microbial respiration by 14.2 and 15.3%, respectively. The results of this study showed that using organic amendments such as NHAP and sewage sludge or foliar application of nano-Fe oxide can affect in decreasing the heavy metal uptake by plants that is a positive point in environmental studies.

Keywords: Sewage sludge, Cd, NHAP, Remediation, Nano-Fe oxide

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INTRODUCTION

Soil is the habitat of soil organisms, especially plants. The life of living organisms in the soil depends on the presence of organic matter as a source of energy and nutrients [1]. Organic matter is very important in terms of food supply. In arid and semi-arid climates, which include most of Iran, the lack of adequate vegetation reduces the return of plant debris to the soil and thus causes the lack of organic matter [2]. On the other hand, the soils of these regions often have a lack of absorbable nutrients, especially trace elements

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such as manganese, copper, iron, and zinc, due to their alkaline properties and a high percentage of lime [3, 4].

After the introduction of chemical fertilizers in agriculture and its use by farmers due to the yield increasing, cheap price, and ease of preparation, the importance of organic fertilizers has been forgotten and unfortunately due to excessive use of chemical fertilizers in the last two or three decades, physical and chemical properties of soils have low quality [5, 6]. Organic matter is known as one of the main important factors of soil fertility due to its positive effects on physicochemical properties



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and soil fertility. However, more than 60% of the soils of arid and semi-arid regions have less than one percent organic matter [7, 8].

Due to the lack of organic matter in the soils of arid and semi-arid regions, the use of any compound containing organic matter, especially sewage sludge is suitable to strengthen the physical properties of the soil and improve the structure and increase the permeability of water holding capacity and stability of aggregates [9, 10]. However, sewage sludge contains a large number of heavy elements such as Pb, Cd, and other toxic metals, and when added to the soil, plants absorb these elements in addition to essential elements.

Research has shown that long-term use of sewage sludge causes the accumulation of elements such as lead, cadmium, and other metals in the soil, which may lead to more absorption of these elements by plants and as a result of these compounds entering the human food chain. Therefore, it is necessary to prevent the excessive entry of heavy metals into the food chain by considering the appropriate solutions [11, 12].

The use of the stabilization method of heavy metals as an alternative to traditional methods to cleaning soil heavy metal polluted soils is expanding and progressing [13]. In fact, soil remediation and control of heavy metals in contaminated soils are laborious and costly [14]. In situ stabilization technique is less expensive than other methods and its long-term modification causes the formation of metal minerals and thus reduces their solubility. In situ stabilization techniques do not remove contaminants from the soil, but rather give a type of contaminant to less mobile forms through the formation and deposition of chemicals and the formation of more stable minerals [13].

This technique involves the addition of chemicals to contaminated soils to reduce the solubility and bioavailability of metals through adsorption and deposition processes and prevent the transfer of contaminants to deeper soil layers and into groundwater. In addition, replanting of highly polluted sites will be possible after the stabilization of heavy metals [15].

The low cost as well as the minimal effects on soil properties make this technique extremely useful. Compared to other remediation techniques, in situ stabilization technique has a longer and more positive effect on soil remediation and forms minerals with low solubility, thus reducing the availability of heavy metals [16].

Among the methods used, the use of organic additives, phosphorus compounds such as nano-hydroxyapatite (NHAP) can be a useful method to reduce the availability of heavy metals in organic compounds and prevent the direct entry of heavy metals into the soil [17]. According to this, Ashrafi et al. studied the immobilization of Pb, Cd, and Zn in contaminated soil using eggshell and banana stem amendments and concluded that using these organic amendments had a significant effect on decreasing soil heavy metal availability. However, they did not study the interaction effect of heavy metals with nutrient elements to decreasing the heavy metal uptake by plants [18]. To overcome the remediation of heavy-metal-polluted soil, many methods have been introduced such as soil washing, electrokinetic, and phytoextraction. However, in most of them, a secondary method is required to complete the step of the remediation process since the heavy metals are still in a bio-available form in the environment [19]. On the other hand, using suitable methods to improving plant growth is or decreasing heavy metal availability is necessary. One of the most important methods for decreasing the heavy metal uptake by plants is the method based on the interaction effect between the heavy metal and nutrient elements. Accordingly, Tabarteh et al. investigated the interaction effect of Fe and Pb on decreasing the heavy metal availability in a Pb-polluted soil and concluded that soil application of Fe fertilizer had a positive effect on decreasing the heavy metal uptake by the plant [20].

In the soils of arid and semi-arid regions, the possibility of soil nutrients stabilization such as Fe can affect the efficiency of phytoremediation which has not been considered in their research. In addition, they did not mention the role of the foliar application of soil nutrients on phytoremediation efficiency. Regardless of the type of Fe sources, the stabilization efficiency of heavy metals in the soil depends on various factors such as soil physical and chemical properties and the type of plant, which should be considered separately in each study. Thus, this research was done to investigate the effect of nano-Fe oxide and NHAP on plant Cd uptake in soil treated with Cd-polluted sewage sludge.

MATERIAL AND METHODS

To investigate the effect of organic amendments on decreasing soil Cd availability, a non-Cd polluted soil with low organic carbon was selected from Pakal village in Markazi province, Iran.

Table 1. Selected physic-chemical properties of soil and sewage sludge samples

Parameter	Unit	Amount	
		Soil	Sewage sludge
EC	dS/m	1.3	6.7
pH	--	7.2	6.8
Total Pb	mg/kg	ND*	ND
Total Cd	mg/kg	ND	ND
K availability	mg/kg	101	3200
Organic matter	%	1.1	11.4
Organic carbon	%	0.6	6.37

*ND: Not detectable by atomic absorption spectroscopy (AAS)

Table 2. Effect of foliar application of Nano-Fe oxide, NHAP, and Cd- polluted sewage sludge on soil Cd concentration (mg/kg)

Sewage sludge (t/ha)	Cd concentration (mg/kg)	Nano-Fe oxide (g/lit)					
		0			0.5		
		NHAP (% (W/W))					
		0	0.5	1	0	0.5	1
0		ND*	ND	ND	ND	ND	ND
15	0	ND	ND	ND	ND	ND	ND
30		ND	ND	ND	ND	ND	ND
0		10.6±0.1o**	10.4±0.2p	10.1±0.2r	10.3±0.2q	10.0±0.2s	9.8±0.1u
15	10	10.3±0.2q	10.1±0.4r	9.6±0.4w	10.0±0.1s	9.7±0.2v	9.4±0.2y
30		9.9±0.1t	9.6±0.1w	9.3±0.1z	9.5±0.3x	9.2±0.4a'	8.4±0.4b'
0		19.4±0.3a	19.1±0.4c	18.8±0.2d	19.2±0.1b	18.7±0.2e	18.5±0.3g
15	20	19.2±0.1b	18.6±0.2f	18.4±0.1h	18.7±0.6e	18.3±0.1i	18.1±0.1k
30		18.8±0.3d	18.3±0.1i	18.0±0.3l	18.2±0.4j	17.9±0.5m	17.7±0.3n

*ND: Not detectable by AAS, ** Data with the similar letters are not significant (P<0.05)

Selected physic-chemical properties of soil studied in this research were shown in Table 1.

This research was conducted as a factorial experiment in the layout of randomized completely block design in three replicates as a pot experiment. Treatments consisted of applying Cd polluted (0, 10, and 20 mg Cd/kg) sewage sludge at the rates of 0, 15, and 30 t/ha, foliar application of nano-Fe oxide (0 and 0.5 ppm), and soil application of nano-hydroxyapatite (NHAP) at the rates of 0, 0.5 and 1% (W/W).

The sewage sludge was polluted with Cd at the rates of 0, 10, and 20 mg Cd/kg and incubated for one month to equilibrium. After that, the Cd-polluted sewage sludge was added to the soil at the mentioned rates and incubated for a month. The plant seeds of pinto bean (Cv. Khomein) were prepared from the station of the Agricultural Research Center in Markazi Province. They were soaked in water for a few minutes and then immersed in 96% alcohol for 15 seconds in laminar and then put in sodium hypochlorite solution (1:10 (v/v)) for one minute. After that, the seeds were sterilized several times with distilled water and ready for culture, and germinated in quartz sand moistened with distilled water. After 90 days of

planting, the plants were harvested and the plant Cd, Zn, and Fe was measured [21] using atomic absorption spectroscopy (Perkin-Elmer model 3030). In addition, the soil microbial respiration was measured according to Zamani et al. [22]. Accordingly, 15 g soil samples were incubated for seven days at 25°C in 250-mL glass containers closed with rubber stoppers in three replicates. A test tube containing 10 mL of a 0.5 M NaOH solution was placed into the containers to trap the evolving CO₂. The trapped CO₂ was measured by titrating the excess in alkali using 2N hydrochloric acid. Three glass containers without soil were considered as the control samples [22].

STATISTICAL ANALYSIS

Statistical analyses were calculated according to the ANOVA procedure using SAS software. The differences between means were evaluated using the least significant difference (LSD) test. The P<0.05 value was considered to determine the significant difference.

RESULTS AND DISCUSSION:

The greatest soil Cd concentration (Table 2)

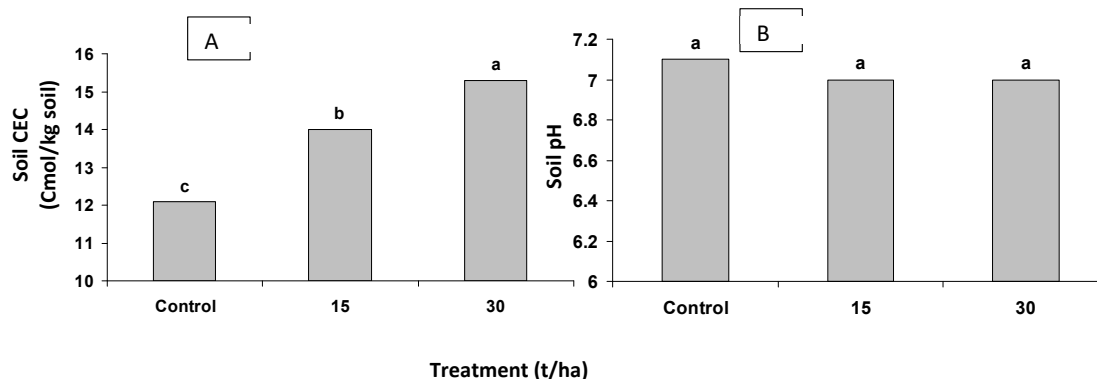


Fig. 1. The simple effect of using sewage sludge on soil CEC (A) and pH (B), the similar letters on the columns in each parameter shows no significant differences ($P < 0.05$) between treatments.

Table 3. Effect of foliar application of Nano-Fe oxide, NHAP, and Cd- polluted sewage sludge on plant Cd concentration (mg/kg)

Sewage sludge (t/ha)	Cd concentration (mg/kg)	Nano-Fe oxide (g/lit)					
		0			0.5		
		NHAP (% (W/W))					
		0	0.5	1	0	0.5	1
0		ND*	ND	ND	ND	ND	ND
15		ND	ND	ND	ND	ND	ND
30		ND	ND	ND	ND	ND	ND
0	0	8.9±m**	8.8±0.2n	8.5±0.3p	8.5±0.4p	8.3±0.2q	8.2±0.1r
15	10	8.6±0.1o	8.3±0.3q	8.0±0.1t	8.3±0.2q	8.1±0.1s	7.8±0.2u
30	10	8.3±0.1q	8.2±0.1r	7.8±0.2u	8.0±0.1t	7.7±0.4v	7.2±0.5w
0	20	15.6±a	15.3±0.4b	15.1±0.2d	15.2±0.1c	15.0±0.1e	14.7±0.1g
15	20	15.3±b	15.0±0.2e	14.8±0.1f	15.0±0.2e	14.6±0.2h	14.3±0.2j
30	20	15.2±c	14.8±0.1f	14.5±0.3i	14.7±0.3g	14.2±0.1k	13.6±0.3l

*ND: Not detectable by AAS, ** Data with the similar letters are not significant ($P < 0.05$)

has belonged to the soil without receiving any sewage sludge, while the lowest that were measured in the soil with the highest rate of sewage sludge. Increasing the application rate of sewage sludge significantly decreased the soil Cd concentration, as, our results showed that increasing the application rate of sewage sludge from 15 to 30 t/ha significantly decreased the soil Cd concentration by 11.9%.while, the soil sorption properties (soil cation exchange capacity (soil CEC)) has been increased (Fig. 1-a). Applying sewage sludge had no significant effect on soil pH (Fig. 1-b)

Using NHAP had a significant effect on decreasing the soil Cd concentration. For instance, adding NHAP to the sewage sludge at the rate of 1 % (W/W) significantly decreased the soil Cd concentration by 12.8% (Table 2) which can be related to the formation of insoluble Cd phosphate minerals. However, the amount of sewage sludge pollution with Cd directly affected the soil Cd concentration. With increasing the Cd pollution

of sewage sludge from 10 to 20 mg Cd/kg, the soil Cd availability was significantly increased by 13.1%. In addition, foliar application of nano-Fe oxide had a significant effect on decreasing soil Cd concentration that can be related to the antagonistic effects of heavy metals with nutrient elements. However. The role of applying nano-Fe-oxide in increasing soil sorption properties and thereby decreasing the soil Cd availability cannot be ignored.

The greatest plant Cd concentration (Table 3) has belonged to the plants cultivated in the soil that amended with Cd-polluted sewage sludge (20 mg Cd/kg). Increasing the application of sewage sludge from 0 to 30 t/ha significantly decreased the plant Cd concentration, as, the results of this showed that using 30 relative to 15 t/ha sewage sludge significantly decreased the plant Cd concentration by 13.2% that can be related to the role of applying sewage sludge on decreasing soil Cd concentration and thereby decreasing the plant Cd concentration.

Using NHAP had a significant effect on decreasing plant Cd concentration, as, increasing soil application of NHAP from 0 to 1% (W/W) significantly decreased the plant Cd concentration by 12.6% which can be attributed to the formation of Cd insoluble minerals and thereby decreasing the soil and plant Cd concentration. Foliar application of nano-Fe oxide had a significant effect on decreasing the plant Cd concentration. For instance, using 0.5 ppm nano-Fe oxide on the plants cultivated in the soil that amended with 30 t/ha sewage sludge significantly decreased the plant Cd concentration by 11.9% that can be related to the interaction effects of Cd and Fe. Bagheri et al. investigated the effect of iron slag enriched vermicompost on the changes in heavy metal uptake by corn and concluded that using iron slag for enriching the vermicompost had a positive effect on increasing and decreasing the plant Fe and Cd, respectively [23]. However, the soil physico-chemical properties and plant type physiology can be different from plant to plant and the different conditions should be considered separately.

Generally, heavy metals have interaction effects with nutrient elements, and using organic amendments in the form of foliar or soil application can decrease the plant-heavy metals uptake that is a positive point in heavy metal polluted soils. On the other hand, using organic additives such as sewage sludge can improve the soil nutrients that help to reduce the uptake of the heavy metal by plants that can confirm our results. In addition, using sewage sludge can increase the soil sorption properties such as soil CEC (Fig. 1) and thereby decrease the soil and plant Cd availability that is in line with our results. Hamid et al. investigated the role of organic amendments in decreasing the heavy metal uptake by plants and concluded that organic materials make soluble or insoluble complexes with heavy metals that decrease metal uptake by plants, which ultimately reduces risk to the food chain [24]. Generally, strong adsorption by natural organic matter in residuals by the formation of metal chelates reduces the solubility of heavy metals in the soil [25]. Saengwilai et al. investigated the effect of organic amendments on Cd immobilization and its effects on rice growth performance and concluded that using these organic amendments had a significant effect on decreasing soil and plant Cd availability via increasing soil Cd sorption. However, they mentioned that the concentration of accumulated heavy metals in different plant parts

is based on processes including root uptake, xylem loading, and translocation, which are determined by morphological, biochemical, and molecular characteristics [26].

With increasing the Cd pollution in sewage sludge, the soil and plant Cd pollution significantly increased. Therefore, using a suitable way for decreasing the Cd availability in sewage sludge is necessary. According to this, using inorganic amendments such as NHAP can help to decrease the soil Cd availability. The results of the study showed that using NHAP at the rate of 0.5 and 1% (W/W) significantly decreased the soil Cd concentration by 11.6 and 14.1% that can be related to the formation of insoluble compounds and thereby decreasing the soil heavy metal concentration. Generally, phosphate compounds can immobilize metals in soils by decreasing their bioavailability. However, its effectiveness depends on the soil's physico-chemical properties. Today, chemical immobilization is a method where relatively cheap materials are added to contaminated soil to decrease mobility, bioavailability, and bio-accessibility of heavy metals [27]. Zhao et al. reported that increasing phosphate can prevent the plant's Cd uptake and can promote the synthesis of amino acids in rice grains [28]. The remarkable point of this research is that the application of phosphate nanoparticles has been able to reduce the availability of heavy metals in soil and plants and thus increase the plant nutrient concentration. Accordingly, based on the results of our study, increasing decreasing soil Cd availability due to NHAP application significantly increased the plant Fe and Zn by 12.6 and 15.1%, respectively. However, the role of applying sewage sludge in increasing the plant nutrient concentration cannot be ignored.

Plant Fe concentration (Table 4) was also affected by the treatments. Accordingly, the greatest plant Fe concentration has been measured in plants with the lowest plant Cd uptake (Table 3) which can be attributed to the antagonistic effects of Fe and heavy metals. Foliar application of nano-Fe oxide had a significant effect on increasing the plant Fe concentration, as, the results of this study showed that using 0.5 ppm nano-Fe oxide significantly increased the plant Fe concentration by 15.6%. In this way, Bagheri et al. investigated the effect of enriched vermicompost with iron slag on corn Fe availability in a Cd polluted and concluded that using iron-enriched organic amendments can increase and decrease the plant Fe and Cd

Table 4. Effect of foliar application of Nano-Fe oxide, NHAP, and Cd- polluted sewage sludge on plant Fe concentration (mg/kg)

Sewage sludge (t/ha)	Cd concentration (mg/kg)	Nano-Fe oxide (g/lit)					
		0			0.5		
		NHAP (% (W/W))					
		0	0.5	1	0	0.5	1
0	0	15.3±0.7o'	16.8±0.1m'	17.6±0.3j'	31.0±0.1k	31.6±0.5j	32.1±0.5h
15		29.0±0.5r	28.4±0.3u	29.4±0.2q	45.5±0.3f	46.2±0.3f	46.4±0.3e
30		29.6±0.2p	28.9±0.6s	30.2±0.1o	48.7±0.2c	49.1±0.2b	49.5±0.2a
0	10	15.0±0.3p'	14.5±0.3r'	17.1±0.4l'	30.2±0.1o	30.6±0.5n	31.5±0.4k
15		26.0±0.2b'	25.7±0.2c'	26.5±0.2a'	29.4±0.4q	30.2±0.1o	30.7±0.1m
30		26.8±0.3z	26.0±0.1b'	27.0±0.6y	32.0±0.5i	32.6±0.5f	31.0±0.3l
0	20	14.7±0.1q'	14.2±0.4s'	16.1±0.7n'	27.0±0.2y	27.5±0.2x	28.0±0.4w
15		24.5±0.5i'	24.0±0.2j'	25.1±0.2e'	27.5±0.4x	28.0±0.2w	28.2±0.2v
30		25.4±0.2d'	25.0±0.1uf	25.7±0.1c'	28.0±0.1w	28.6±0.4t	28.9±0.5s

* Data with the similar letters are not significant (P<0.05).

Table 5. Effect of foliar application of Nano-Fe oxide, NHAP, and Cd- polluted sewage sludge on plant Zn concentration (mg/kg)

Sewage sludge (t/ha)	Cd concentration (mg/kg)	Nano-Fe oxide (g/lit)					
		0			0.5		
		NHAP (% (W/W))					
		0	0.5	1	0	0.5	1
0	0	9.0±0.14*	9.3±0.11v	9.5±0.42u	11.6±0.31h	11.8±0.31f	11.9±0.35e
15		9.9±0.25t	10.0±0.15s	10.2±0.22q	11.8±0.12f	12.0±0.14d	12.3±0.36b
30		11.2±0.51j	11.6±0.33h	11.9±0.26e	12.2±0.15c	12.3±0.22b	12.7±0.21a
0	10	8.1±0.13e'	8.2±0.33d'	8.4±0.11c'	10.4±0.18o	10.7±0.24n	11.0±0.12l
15		8.2±0.31d'	8.6±0.11a'	8.9±0.31x	11.0±0.24l	11.4±0.31i	11.6±0.13h
30		8.6±0.42a'	8.7±0.14z	9.0±0.23w	11.2±0.43k	11.6±0.42h	12.0±0.33d
0	20	7.5±0.22i'	7.8±0.15h'	8.0±0.11f'	10.1±0.33r	10.4±0.12o	10.7±0.21n
15		7.9±0.14g'	8.2±0.17d'	8.4±0.61c'	10.3±0.13p	10.7±0.15n	11.0±0.42l
30		8.2±0.98d'	8.5±0.18b'	8.8±0.22y	10.8±0.15m	11.3±0.16j	11.7±0.51g

* Data with the similar letters are not significant (P<0.05)

concentration which can be attributed to the antagonistic effects of Fe with heavy metals [23].

On the other hand, soil pollution with Cd harmed plant Fe uptake (Table 4). Increasing the Cd concentration in sewage sludge from 0 to 20 mg Cd/kg significantly decreased the plant Fe uptake by 13.1%. Using NHAP had a significant effect on increasing the plant Fe concentration. Increasing the application rate from 0 to 1 % (W/W) significantly increased the plant Fe concentration by 18.3%.

The greatest plant Zn concentration (Table 5) has belonged to the plants with the highest plant Fe uptake. Increasing soil application of sewage sludge from 0 to 30 t/ha significantly increased the plant Zn concentration. However, its Cd contamination had a negative on Zn uptake by plants. Our results showed that with increasing the Cd concentration in the sewage sludge from 0 to 10 mg Cd/kg, the plant Zn concentration was decreased by 17.3%. Using NHAP had a positive effect on increasing the plant Zn concentration. For instance, increasing

the application rate of NHAP from 0 to 1 ppm significantly increased the plant Zn concentration by 12.6%. Foliar application of nano-Fe oxide significantly increased the plant Zn concentration. Based on the results of our study, using 0.5 ppm nano-Fe oxide on the plants cultivated in the soil that amended with 30 t/ha sewage sludge significantly the plant Zn concentration by 15.2%.

The greatest soil microbial respiration (Table 6) has belonged to the soil with the greatest reviewing of soil sewage sludge that can be related to the role of applying sewage sludge as a carbon source for soil microbial activities [29]. However, increasing soil pollution to Cd had adverse effects on soil microbial activities that were shown as soil microbial respiration in this research. Farsang et al. evaluated Evaluating the effects of sewage sludge compost applications on the microbial activity, the nutrient and heavy metal content of a Chernozem soil in a field survey and concluded that using organic amendments can provide the nutrient elements for plants and micro-organisms

Table 6. Effect of foliar application of Nano-Fe oxide, NHAP, and Cd- polluted sewage sludge on soil microbial respiration (mg C-CO₂/kg soil)

Sewage sludge (t/ha)	Cd concentration (mg/kg)	Nano-Fe oxide (g/lit)					
		0			0.5		
		NHAP (% (W/W))					
		0	0.5	1	0	0.5	1
0		14.5±0.11v	14.8±0.31t	15.3±0.11r	16.4±0.32l	16.9±0.34j	17.9±0.31f
15	0	14.9±0.13t	15.6±0.35p	17.0±0.43j	17.9±0.42f	18.4±0.32r	19.2±0.53c
30		15.4±0.12q	15.6±0.21p	16.8±0.32k	18.9±0.11d	19.6±0.13b	20.0±0.32a
0		13.7±0.14z	14.1±0.34x	14.5±0.43v	15.1±0.31s	15.4±0.43q	15.9±0.43n
15	10	14.9±0.21t	14.4±0.12w	15.1±0.56s	15.8±0.43o	16.4±0.21l	17.1±0.22h
30		15.1±0.32s	15.3±0.14r	16.4±0.21l	16.1±0.11m	16.9±0.65j	17.8±0.24g
0		11.2±0.42j'	11.5±0.32i'	11.9±0.43f'	11.5±0.54i'	11.8±0.32g'	12.1±0.54e'
15	20	11.6±0.34h'	11.9±0.21f'	12.4±0.54d'	11.9±0.32f'	12.5±0.52c'	12.8±0.21b'
30		12.1±0.27e'	12.4±0.18d'	13.2±0.21a'	12.5±0.43c'	12.8±0.12b'	13.9±0.11y

* Data with the similar letters are not significant (P<0.05)

and consequently can increase the soil microbial respiration [30].

Increasing soil pollution to Cd significantly decreased the soil microbial respiration (Table 6). Based on the results of our study, increasing the Cd pollution from 0 to 20 mg Cd/kg in sewage sludge significantly decreased the soil microbial respiration by 13.9%. Foliar application of nano-Fe oxide had a positive effect on increasing the soil microbial respiration. According to our results, foliar application of nano-Fe oxide at the rate of 0.5 ppm significantly increased the soil microbial respiration by 14.3%. In addition, using NHAP had also a positive effect on increasing soil microbial respiration, as, our study showed that with increasing the application rate of NHAP from 0 to 1 % (W/W) the soil microbial respiration was significantly increased by 17.3% that may be related to the role of organic amendment on improving the plant nutrient availability and thereby decreasing the plant-heavy metal uptake that harms soil microbial respiration. On the other hand, foliar application of nano-Fe oxide has significantly decreased the soil Cd availability that can be due to the role of nano-Fe oxide on improving the plant growth (data was not shown) and consequently increasing the plant root exudate which decreases the soil Cd availability via formation of insoluble organic compounds. As a result of this research, decreasing the soil Cd availability could help to increase the soil microbial activity. In addition, using NHAP had a significant effect on increasing soil microbial respiration. In this line, the soil Cd availability was significantly decreased with soil application of NHAP indicating the positive effect of NHAP on decreasing the soil Cd availability and thereby increasing the soil microbial activity. In

addition, soil application of NHAP can increase the plant Fe and Zn (Table 4 and 5) concentration that is a suitable point for plant growth in contaminated soils with heavy metals [31].

CONCLUSION

Soil application of sewage sludge at the rate of 30 t/ha significantly increased the plant Fe and Zn concentration by 11.3 and 15.4%, respectively, while the plant's Cd concentration was decreased by 12.1%. However, increasing the Cd pollution in sewage sludge had a significant effect on decreasing the plant Zn and Fe concentration that can be related to the interaction effects of nutrient elements with heavy metals. Foliar application of nano-Fe oxide had positive effects on increasing the plant Fe and Zn concentration that is a positive point in environmental studies. However, plant physiology and soil physico-chemical properties have a direct effect on heavy metals uptake that needs to be considered in different studies. In addition, using NHAP can increase the plant nutrient concentration and consequently decrease the heavy metal uptake by plants.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts

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