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ORIGINAL RESEARCH PAPER

Effect of Carbon Nanotubes, Zeolite and AMF on Triticale Ni Concentration in a Soil Co-contaminated with Diesel Fuel and Ni

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ABSTRACT

Remediation of soils polluted with heavy metals or petroleum hydrocarbons is one of the environmental problems. This research aimed to evaluate the effect of carbon nanotubes, zeolite, and AMF on triticale Ni concentration in a soil co-contaminated with diesel fuel and Ni. Treatments consisted of applying multi-walled carbon nanotubes (MWCNs) and zeolite at the rates of 0, 1 and 2 % (W/W) in a Ni-polluted soil (0, 75 and 150 mg Ni/kg soil) which was naturally polluted with diesel fuel under cultivation of triticale plant inoculated with AMF. After 70 days, plants were harvested and soil and plant Ni concentration were measured using atomic absorption spectroscopy (AAS). Soil microbial respiration and degradation of diesel fuel were also measured. Applying 2 % (W/W) zeolite and MWCNs significantly increased the diesel fuel degradation in soil by 12.3% and 14.5 %, respectively, while the plant Ni concentration was decreased by 8.9 % and 13.1%, respectively. Increasing soil pollution with Ni from 0 to 75 mg/kg soil significantly decreased the degradation of diesel fuel in the soil under cultivation of plant inoculated with AMF by 14.4%. In addition, the soil microbial respiration was also decreased by 11.8%. The results of this study showed that the application of zeolite and MWCNs had a significant effect on increasing diesel fuel degradation in heavy metal polluted soil that is a positive point in environmental studies.

Keywords: *Nickel, Diesel fuel, Zeolite, Fungi, Biomass*

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INTRODUCTION

To develop societies and advance in industry and agriculture, humans have produced and used chemical materials such as petroleum derivatives. Extraction, transfer, and the use of these chemicals as fuel have caused irreparable damages to the environment [1].

As a result of human activities, large amounts of various pollutants enter the water, soil, and air daily. These pollutants include petroleum compounds. The major part of these compounds is entered into the soil as a result of the development of petroleum refining industries. As a part of the environment, soil provides a unique habitat for terrestrial creatures such as humans and plants. Increasing soil contamination with such compounds could endanger human health and food chain [1, 2]. Accordingly, in many cases, the simultaneous contamination of heavy metals with petroleum compounds can reduce soil quality.

In the last few decades, releasing petroleum hydrocarbons in the environment has been a usual process. The consequent risks appeared as these pollutants gradually entered into the environment. So, we should be aware of the effects of these compounds on humans as a member of the environment and the ways of eliminating these effects [3]. It should be mentioned that the soils that are polluted by petroleum compounds are not suitable for agriculture, residence, and construction of recreational places, and they cause economic, ecological, and agricultural damages.

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So, it is necessary to remove these compounds from the soil, and the societies should take proper actions for this purpose. There are various methods of removing, reducing or stabilizing the polluting compounds [4]. These methods are classified into three major categories: physical, chemical, and bioremediation methods. The last method has been paid more attention to the researchers and industries due to its less cost and more compatibility with the environment [5]. Among the bioremediation method, the use of plants for reducing pollution which is referred to as phytoremediation has been paid special attention. Recently, proper solutions have been used to achieve more success in the phytoremediation of petroleum hydrocarbon pollutants and reduce the amount of these compounds in soil. One of these solutions is the use of additives to decrease the cost and time required for remediation of the pollutants. One of the compounds that have attracted the attention of many researchers of different sciences is multi-walled carbon nanotubes (MWCNs) [6]. MWCNs are a unique product due to its tube form and large specific surface area. Due to its tube form, this particle penetrates to the root wall and increases the amount of water and minerals absorbed by the plant. So, it is effective on plant growth and absorption of pollutants [7].

Matos et al. investigated the effect of application of MWCNs on immobilizing of heavy metals in polluted soils and concluded that the application of these compounds has a significant effect on increasing and decreasing soil sorption properties and soil heavy metal availability, respectively [8]. However, they did not mention the role of soil chemical properties on the soil heavy metals availability. Generally, carbon can exist in many forms; some are natural like diamonds and graphite and others are artificial like fullerenes and carbon nanotubes. MWCNs can attract and immobilize heavy metals on its surface (adsorption), and since they have a high surface area, they can decrease soil heavy metal availability [9]. It is worth noting that the type and amount of contamination have a significant impact on reducing the availability of heavy metals in soil.

The other resource to be used in different scientific and industrial areas including petrochemical, agricultural, and animal husbandry industries, and treating the wastewater of different industries is zeolite. Large amounts of heavy metals are absorbed by zeolite minerals due to the special spatial structure of this mineral. This property results from high CEC, large special surface area, and the role of zeolite as a mono-to-poly dentate macro ligand for metal ions sorption especially for transitional metals. Generally, the coordination sphere of the metal ions such as Ni, Pb, and Cd can be shared by the zeolite and external ligands and thus decreases their availability [10].

Erdem et al. conducted the effect of natural zeolites on the removal of heavy metals from aqueous solutions and concluded that natural zeolites have great potential to remove cationic heavy metal species from industrial wastewater [11]. Overall, the zeolite structure consists of three-dimensional frameworks of AlO_4 and SiO_4 tetrahedra. The Al^{3+} ion is small enough to occupy the position in the center of the tetrahedron of four oxygen atoms, and the isomorphous replacement of Si^{4+} by Al^{3+} can produce a negative charge in the zeolite lattice. The net negative charge is balanced by the exchangeable cation. These cations are exchangeable with certain cations in solutions such as Ni, Pb, Cd, and Mn [12]. Lee et al. studied the effect of zeolite and fly ash on soil heavy metal availability and concluded that applying theses organic amendments has a significant effect on decreasing plant heavy metal concentration. However, according to their results, using zeolite has not any significant effect on the plant biomass [13].

Today, in many arid and semi-arid regions of the country, there is simultaneous contamination of heavy metals and petroleum hydrocarbons, and remediation of such compounds seems necessary. Meanwhile, increasing soil sorption properties plays an important role in reducing the availability of contaminated soils. Given that there are many reserves of zeolite in our country, using these compounds due to their high sorption capacity can help to remediate the contaminated soils [10]. On the other hand, using MWCNs can also help to modify the soil absorption capacity, especially in soils with multiple contaminants. Additionally, increasing the plant resistance against abiotic stress by different approaches such as root colonization with Arbuscular Mycorrhizal Fungi (AMF) has an additive effect on increasing the plant efficiency to remediate the contaminant from the soil [14]. Among this, the type and amount of pollutants have significant roles in phytoremediation efficiency. Thus, this research was done to evaluate the effect of MWCNs, zeolite, and AMF on plant Ni concentration in a soil co-contaminated with diesel fuel and Ni.

Table 1. Some selected Physico-chemical properties of soil used in this study

Characteristic	Unit	amount
Soil texture	-----	Sandy loam
pH	------	7.1
EC	dS/m	1.2
Available Pb	mg kg^{-1}	2
Available Cd	mg kg^{-1}	3
Available As	mg kg^{-1}	1
Available Ni	mg kg^{-1}	ND*
Organic carbon	%	0.2
CaCo3	%	8
CEC	meq/100g soil	11.2

*Before enrichment

MATERIAL AND METHODS

To investigate the effect of MWCNs, zeolite, and AMF on triticale Ni concentration in soil with diesel fuel pollution, a soil with a low percentage of soil organic matter and $CaCO_3$ were selected from the soil surface layer (0-15 cm) around Pakal village in Markazi province. This research was done as a factorial experiment in the layout of a completely randomized block design in three replicates. Selected Physico-chemical properties of studied soil are shown in Table 1.

Treatments consisted of applying zeolite (0 (Z_0), 1 (Z_1) and 2 (Z_2) %), MWCNs (0 (M_0), 1 (M_1) and 2 (M_2)%) in the presence and absence of AMF (AMF^+ and AMF^-, respectively) in the Ni (0 (Ni_0), 75 (Ni_{75}) and 150 (Ni_{150}) mg Ni/kg soil) polluted soil that was naturally polluted with diesel fuel.

To obtain a field representative AMF inoculum, we used the soil around the roots of triticale plants that were growing in the field that we sampled the soil. We applied this approach to obtain soil indigenous AMF because it has been revealed that soil-indigenous AMF species improve plant growth and nutrient uptake more than non-indigenous species due to the long-term exposure to the soil conditions [15].

To prepare the Ni polluted soil, the soil was spiked with Ni at the rates of 0, 75, and 150 mg Ni/kg soil and was incubated for two weeks to equilibrium. After that, the soil was amended with Zeolite and MWCNs at the rates of 2 % (W/W) and incubated for two weeks to equilibrium. Then the treated soil was placed in the five-kilogram plastic pots. Following that the experimental pots filled with 5 Kg of the treated soil and then half of that inoculated with AMF in the form of a fresh soil sample. To this aim, 20 g of inoculum was placed in a layer at a depth of 3 cm from the soil surface [15]. Eight seeds were sown in each pot and thinned to four plants per pot after the germination of the first leaf. After 70 days, plants were harvested and the plants' Ni concentration was measured using atomic absorption spectroscopy (AAS) according to the Intawongse et al. [16]. Accordingly, Subsamples (1 g) were digested with 10 mL of HNO_3 (65%) and 10 mL of H_2O_2 (30%). The digests were diluted to 100 mL with deionized water and filtered. The filtrate was analyzed for Ni by atomic absorption spectroscopy (AAS) (Perkin-Elmer model 3030) according to the Xu et al. [17]. The Soil Ni availability (DTPA-Ni concentration) was measured according to the Lindsay method [18].

The diesel fuel degradation in soil was determined according to Besalatpour et al. [19]. Accordingly, the diesel fuel residual in the soil samples were extracted by soxhlet using a 1:1 (v/v) dichloromethane and n-hexane (150 ml) mixture for 24 h and calculated using the weight method. The soil microbial respiration was measured as evolved CO_2 according to Besalatpour et al. [19].

Statistical analyses were calculated according to the ANOVA procedure. 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

Application of MWCNs and zeolite had an additive effect on decreasing soil Ni availability, as, the lowest soil Ni availability has belonged to the soil that received the greatest level of MWCNs and zeolite (Table 2) and polluted with 75 mg Ni/kg soil, while the greatest that has belonged to the soil without receiving any MCWN or zeolite. Soil Ni concentration in non-polluted soil was not detectable by AAS. Application of 1 and 2

Table 2. Effect of Zeolite, Ni concentration, MWCNs, and the presence of AMF on soil Ni availability (mg/kg).

MWCNs (%)	Ni concentration (mg/kg soil)	+AMF Zeolite (%) 0	+AMF Zeolite (%) 1	+AMF Zeolite (%) 2	-AMF Zeolite (%) 0	-AMF Zeolite (%) 1	-AMF Zeolite (%) 2
0	0	ND	ND	ND	ND	ND	ND**
1		ND	ND	ND	ND	ND	ND
2		ND	ND	ND	ND	ND	ND
0	75	40.1k	38.4m	36.1r	42.2g	40.6i	38.1n
1		39.2l	36.7q	35.1s	41.6h	39.1l	37.2o
2		36.5q	34.1t	33.5u	40.2j	37.1p	36.3r
0	150	44.5c	43.1e	42.7f	45.3a	44.8b	43.2d
1		42.2g	40.1k	39.1l	44.8b	42.9f	42.3g
2		41.7h	38.1n	37.2o	43.1e	42.4g	41.9h

*Date with a similar letter are not significantly different (P=0.05). **ND: not detectable by atomic absorption spectroscopy.

(W/W) MWCNs significantly decreased the soil Ni availability by 4.8% and 7.2%, respectively that may be related to the role of applying these amendments on increasing soil sorption properties. A significant increase in soil CEC by 4.9% and 6.2% due to applying zeolite and MWCNs, respectively confirm our results. In general, zeolite and MWCNs have a high capacity to stabilize heavy metals due to their high specific surface area. MWCNs is a new material of the carbon component group which, because of advantages in many various applications regarding its chemical composition, has attracted substantial attention [20].

Nuraini et al. investigated the role of carbon nanotubes on the removal of Cd from contaminated soils and concluded that the high adsorption capacity of these compounds can play an effective role in the remediation of heavy metals from contaminated soils [20]. Matos et al. also investigated the role of applying MWCNs on immobilizing of heavy metals in polluted soils and concluded that soil amended with MWCNs can increase soil sorption properties and thus decreasing soil heavy metal availability [8].

On the other hand, using zeolite in the soil also significantly decreased the soil Ni concentration which can be attributed to its role in soil adsorption characteristics and thus decreasing the heavy metal availability in soil. According to the results of this study, applying 2 % (W/W) zeolite significantly decreased the soil Ni concentration by 5.2%. Generally, zeolite, due to its structure, has specific physicochemical properties (such as ion exchange and adsorption), which can be used in a wide range of industrial and environmental applications. Vrînceanu et al. studied the effect of natural zeolite on the immobilization of heavy metals in contaminated soil and concluded that applying zeolite had a significant effect on decreasing heavy metal availability in soil [21]. However, they did not consider the role of soil chemical properties on soil heavy metal availability. Davari et al. mentioned several methods that have been suggested to remediate contaminated soils, including stabilization, vitrification, membrane, excavation, filtration, oxidation/reduction, ion exchange, phytoremediation, and bioremediation. The technical solutions for the treatment of contaminated sites are usually costly, ecologically unsafe, and in many cases not feasible in practice. Among the above-mentioned measures methods, in-situ immobilization is one of the key techniques used for remediation [22]. Nevertheless, increasing the concentration of heavy metals in the soil can reduce the adsorption capacity of zeolite and consequently reduces the immobilization of heavy metals in the soil.

The presence of AMF had significantly

Table 3. Effect of Zeolite, Ni concentration, MWCNs, and the presence of AMF on root Ni concentration. (mg/kg).

MWCNs (%)	Ni concentration (mg/kg soil)	+AMF Zeolite (%)			-AMF Zeolite (%)		
		0	1	2	0	1	2
0	0	ND	ND	ND	ND	ND	ND**
1	0	ND	ND	ND	ND	ND	ND
2	0	ND	ND	ND	ND	ND	ND
0	75	80.1h	75.4m	70.1o	83.4e	79.1i	77.4k
1	75	78.3j	73.3n	65.6p	80.1h	78.4j	75.1m
2	75	75.4m	70.20	62.4q	78.7j	76.3l	73.6n
0	150	85.5c	84.1d	81.6g	87.2a	85.6c	83.1e
1	150	84.2d	82.5f	80.3h	86.3b	84.1d	82.4f
2	150	82.4f	80.3h	78.2j	85.7c	83.6e	80.4h

*Date with a similar letter are not significantly different (P=0.05). **ND: not detectable by atomic absorption spectroscopy.

decreased soil Ni availability (Table 2). Based on the results of this study, plant inoculated with AMF significantly decreased the soil Ni concentration by 12.3% in the soil the received 2 % (W/W) MWCNs. Atakan et al. investigated the effects of AMF on heavy metal and salt stress and concluded that AMF can alter root morphology and plant physiology that can increase the nutrients and water uptake from the soil and decrease the negative effect of heavy metals in contaminated soils [23]. However, they mentioned that the number of heavy metals in their studied soil was not at a level that limits the development of AMF. Based on the results of different studies, AMF is not only a way of reducing or eliminating soil pollutants, but also a method of helping the plant to uptake nutrients by improving the structure of the soil [24]. However, plant inoculation with AMF cannot alone remediate the organic and inorganic pollutants in contaminated soils. Thus, it is necessary to use some chemical compounds besides of this biological method (AMF inoculation).

The greatest root Ni concentration was belonged to the soil without receiving any organic amendments in the soil treated with 150 Ni/kg soil (Table 3), while the lowest that was measured in the Ni-polluted soil (75 mg Ni/kg soil) amended with 2 % (W/W) Zeolite and MWCNs. The root Ni concentration in non-polluted soil was not detectable by AAS.

Plant inoculation with AMF has a significant effect on decreasing root Ni availability, as, the results of this study showed that a significant decrease by 13.4 % was observed when the plant inoculated with AMF and cultivated in the Ni-polluted soil (150 mg N/kg soil). Increasing soil Ni pollution from 0 to 75 mg Ni/kg soil significantly increased the root Ni concentration in inoculated and non-inoculated plants by 15.1 % and 12.1%, respectively. The application of MWCNs and zeolite had an additive effect on decreasing plant Ni concentration. A significant decrease by 11.8% in root Ni concentration was measured the plant cultivated in the soil amended with 1% (W/W) zeolite and MWCNs. Azogh, et al. investigated the effect of zeolite on the absorption and distribution of heavy metal concentrations in roots and shoots of wheat and concluded that applying these organic amendments has a positive effect on plant biomass that cultivated in the contaminated soil [25].

The greatest shoot Ni concentration (Table 4) has belonged to the plants cultivated in the Ni-polluted soil (150 mg Ni/kg soil) without receiving any organic amendments, while the lowest that has measured in the plants that cultivated in the Ni-polluted soil (75 mg Ni/kg soil) with the greatest receiving of MWCNs and zeolite. The shoot Ni concentration in non-polluted soil was not detectable by AAS. Increasing soil pollution to Ni significantly increased the shoot Ni concentration,

Table 4. Effect of Zeolite, Ni concentration, MWCNs, and the presence of AMF on shoot Ni concentration (mg/kg).

MWCNs (%)	Ni concentration (mg/kg soil)	+AMF			-AMF		
		Zeolite (%)			Zeolite (%)		
		0	1	2	0	1	2
0	0	ND	ND	ND	ND	ND	ND**
1		ND	ND	ND	ND	ND	ND
2		ND	ND	ND	ND	ND	ND
0	75	39.2l	37.3o	35.7r	41.5h	40.0j	39.1l
1		37.8n	35.5s	33.4t	39.2l	38.3m	37.1p
2		35.4s	32.6u	30.4v	37.8n	36.7q	35.4s
0	150	43.1d	42.5f	41.4h	44.8a	44.1b	43.6c
1		42.1g	41.4h	40.8i	43.5c	42.7e	42.2g
2		41.3h	40.7i	39.5k	43.0d	42.1g	41.5h

*Date with a similar letter are not significantly different (P=0.05). **ND: not detectable by atomic absorption spectroscopy.

as, the results of this study showed that increasing soil pollution to Ni significantly increased the shoot Ni concentration by 11.8 % in the soil treated with 2 % (W/W) zeolite and MWCNs. On the other hand, the effect of simultaneous application of MWCNs and zeolite was additive on decreasing shoot Ni concentration. For example, the simple effect of applying 2% (W/W) MWCNs or zeolite and its interaction had significantly decreased the shoot Ni concentration by 5.8%, 4.7 %, and 11.8%, respectively.

The presence of AMF had a significant effect on increasing plant biomass (Table 5), as, the results of this study showed that a significant increase by 11.4 % was observed when the triticale plant was inoculated with AMF. However, increasing soil pollution in Ni significantly decreased plant biomass. However, the results of this study showed that the application of MWCNs or zeolite significantly increased the plant biomass by decreasing the soil Ni concentration. Also applying MWCNs and zeolite had an additive effect on increasing plant biomass. Accordingly, soil amended with 2 % (W/W) MWCNs and zeolite in the soil polluted with 75 mg Ni/kg soil significantly increased the plant biomass by 8.9%. While it was increased only in soil polluted with 75 mg Ni/kg soil by 3.2%.

Increasing plant biomass can affect on Ni phytoremediation efficiency. Generally, toxic elements have an adverse effect on soil chemical properties. For instance, soils with high concentrations of heavy metals such as Cd, Pb, and Ni show a decline in microbial biomass and nitrogen fixation [26]. It is mentioned that the ability of AMF to effectively remove heavy metals depends on the plant species and fungi colonizes. Chen et al. (2007) reported that the effect of AMF was significant when a legume (*Trifolium repens*) and two native plants (*Coreopsis drummondii* and *Pteris vittata*) were planted on soil with high heavy metal concentration. On the other hand, using turf grass (*Lolium perenne*) did not produce significant results [27].

Mühlbachová et al. investigated the effects of zeolite amendment on microbial biomass and respiratory activity in heavy metal contaminated soils and concluded that using zeolite can diminish the negative effect of heavy metals and thereby increase the soil microbial community [28]. Therefore, it can be concluded that applying organic amendments with the high specific surface area has a positive effect on plant growth in heavy metal contaminated soil that can be related to the role of these components on decreasing soil heavy metal availability and thereby, increasing soil microorganism activity and its positive role on the plant growth. However, in many industrial

Table 5. Effect of Zeolite, Ni concentration, MWCNs, and the presence of AMFon plant biomass (g).

MWCNs (%)	Ni concentration (mg/kg soil)	+AMF			-AMF		
		Zeolite (%)			Zeolite (%)		
		0	1	2	0	1	2
0	0	3.84jk	3.95f	4.05c	3.77q	3.80no	3.85ij
1		3.89gh	4.00d	4.08b	3.82lm	3.85ij	3.91g
2		3.92g	4.04c	4.12a	3.86i	3.91g	3.97e
0	75	3.80no	3.84jk	3.91g	3.74s	3.78pq	3.82lm
1		3.85ij	3.91g	3.96e	3.72t	3.77q	3.81mn
2		3.88h	3.95f	4.04c	3.82lm	3.85 ij	3.91g
0	150	3.75r	3.80no	3.84jk	3.70u	3.74s	3.79op
1		3.81mn	3.85 ij	3.90g	3.77q	3.81mn	3.86i
2		3.85 ij	3.91g	3.95f	3.79op	3.83kl	3.88h

*Date with a similar letter are not significantly different (P=0.05).

Table 6. Effect of Zeolite, Ni concentration, MWCNs and the presence of AMF on APX enzyme activity (μ mol/min g FW)

MWCNs (%)	Ni concentration (mg/kg soil)	+AMF			-AMF		
		Zeolite (%)			Zeolite (%)		
		0	1	2	0	1	2
0	0	13.00s	12.74t	12.44v	13.12r	13.02s	12.73t
1		12.55u	12.32w	12.10x	13.00s	12.73t	12.45v
2		12.31w	12.00x	11.78y	12.74t	12.31w	12.11x
0	75	18.54k	18.42l	18.31m	18.89h	18.71i	18.63j
1		18.32m	18.12n	18.020	18.71i	18.64j	16.52q
2		18.13n	18.010	17.83p	18.53k	18.41l	18.32m
0	150	20.26c	20.17e	20.11f	20.33a	20.27c	20.23d
1		20.23d	20.17e	20.11f	20.30b	20.22d	20.17e
2		20.16e	20.10f	20.02g	20.26c	20.16e	20.11f

*Date with a similar letter are not significantly different (P=0.05).

areas of the country with low organic carbon, there is simultaneous contamination of soil with heavy metals and petroleum hydrocarbons that prevent plant growth.

The greatest ascorbate peroxidase (APX) enzyme activity (Table 6) has belonged to the plant that cultivated in the soil with the greatest level of soil pollution with Ni and without receiving any organic amendments such as MWCNs or zeolite, while the lowest that has belonged to the non-Ni polluted soil with the greatest level of MWCNs and zeolite (2 % (W/W)). Increasing soil pollution with Ni significantly (P=0.05) increased the APX enzyme activity, as, a significant increase by 9.4%

Table 7. Effect of Zeolite, Ni concentration, MWCNs and the presence of AMF on soil microbial respiration (mg C-Co_2/kg soil)

MWCNs (%)	Ni concentration (mg/kg soil)	+AMF Zeolite (%) 0	+AMF Zeolite (%) 1	+AMF Zeolite (%) 2	-AMF Zeolite (%) 0	-AMF Zeolite (%) 1	-AMF Zeolite (%) 2
0	0	13.1mn	13.4jk	13.6i	12.2t	13.0no	13.2m
1		13.4jk	14.1ef	14.3cd	13.1mn	13.6i	14.1ef
2		13.8h	14.4bc	14.9a	13.5ij	14.2de	14.5b
0	75	12.5qr	12.7p	13.0no	11.7v	12.0u	12.3st
1		12.90	13.5ij	13.8h	12.6pq	13.0no	13.3kl
2		13.1mn	14.0fg	14.4bc	12.90	13.5ij	13.9gh
0	150	12.0u	12.4rs	12.7p	11.0x	11.4w	11.9u
1		12.6pq	13.0no	13.4jk	12.0u	12.4rs	12.90
2		12.90	13.4jk	13.9gh	12.4rs	13.0no	13.3kl

*Date with a similar letter are not significantly different (P=0.05).

in APX enzyme activity was observed, when the Ni-polluted soil was increased from 75 to 150 mg Ni/kg soil. However, applying MWCNs or zeolite had an adverse effect on the APX enzyme activity. Based on the results of this study, applying 2 % (W/W) zeolite and MWCNs significantly decreased the APX enzyme activity by 1.8 % and 3.2%, respectively.

In addition, plant inoculation with AMF significantly decreased the APX enzyme activity. Accordingly, a significant decrease by 4.3 % in the APX enzyme activity was observed when the plant that cultivated in the Ni-polluted soil (150 mg Ni/kg soil) inoculated with AMF. Generally, plant resistance systems such as antioxidant enzymes secretion can partly increase the plant resistance against heavy metal toxicity. However, high concentrations of heavy metal can disrupt these defense systems. Among this, the interaction effects of zeolite, MWCNs, and plant inoculation with AMF can help to increase the plant resistance to heavy metals in the soil. Decreasing in the antioxidant enzyme activity with increasing the plant biomass via the application of these compounds can confirm our results. Accordingly, the results of this study showed that the interaction effects of application of 2% (W/W) zeolite, MWCNs and plant inoculation with AMF can increase and decrease the plant biomass and APX enzyme activity by 15.6% and 11.2%, respectively.

Łukowski et al. studied the effect of soil pollution on the soil pollution on plant enzyme activity and concluded that increasing soil pollution to heavy metals can significantly increase the plant enzyme activity. However, they mentioned that the pollution type has a different effect on the amount of plant enzyme activity, as, the results of their studies showed that the heavy metals affected dehydrogenase activity the most.in addition, they mentioned that soil heavy metal fractions such as exchangeable or residual fraction showed a different effect on the plant enzyme activity [29].

The greatest soil microbial respiration has belonged to the soil with the greatest level of Zeolite and MWCNs (Table 7). Based on the results of this study, applying 2 % (W/W) MWCNs and zeolite in the Ni-polluted soil (75 mg Ni/kg soil) significantly decreased the soil microbial respiration by 13.4 % and 11.2 %, respectively. The lowest soil microbial respiration was belonged to the soil with the greatest soil pollution with Ni in the soil under plant cultivation in the absence of AMF. Plant inoculation with AMF had a positive effect on increasing soil microbial respiration, as, the results of this study showed that plant inoculation with AMF significantly increased the soil microbial respiration in the Ni-polluted soil (150 mg Ni/kg soil) that received 2 % (W/W) Zeolite by 13.6

Table 8. Effect of Zeolite, Ni concentration, MWCNs, and the presence of AMF on the degradation of diesel fuel (%) in soil.

MWCNs (%)	Ni concentration (mg/kg soil)	+AMF			-AMF		
		Zeolite (%)			Zeolite (%)		
		0	1	2	0	1	2
0	0	72.4k	75.6g	76.2f	70.5m	71.8l	72.4k
1		76.2f	78.3d	80.1b	71.9l	72.3k	73.8i
2		77.8e	79.2c	83.3a	72.5k	74.1h	75.5g
0	75	70.5m	72.3k	75.5g	66.4p	68.3n	70.5m
1		73.7i	75.6g	77.8e	68.4n	70.5m	72.4k
2		76.2f	77.7e	80.1b	71.8l	72.3k	74.1h
0	150	68.3n	70.5m	73.7i	64.3p	64.1q	67.1o
1		70.4m	73.8i	75.5g	66.4p	67.2o	68.4n
2		75.6g	76.2f	77.8e	68.2n	70.6m	73.1j

*Date with a similar letter are not significantly different (P=0.05).

%. Increasing soil pollution with Ni significantly decreased the soil microbial respiration. For instance, with increasing soil pollution with Ni from 0 to 150 mg Ni/kg soil, the soil microbial respiration was increased by 8.9%.

Plant inoculation with AMF had significantly increased the degradation of diesel fuel in soil (Table 8), as, the greatest diesel fuel degradation in soil has belonged to the non-Ni polluted soil under cultivation of plant inoculated with AMF, while the lowest that was observed in the soil under the cultivation of the triticale in the absence of AMF. The presence of AMF significantly increased the diesel fuel degradation in the Ni-polluted soil (75 mg Ni/kg soil) by 7.1%. However, increasing soil pollution in Ni had an adverse effect on the degradation of diesel fuel in the soil. Soil amended with MWCNs had a positive effect on increasing the degradation of diesel fuel in the soil. Based on the results of this study, the application of 2 % (W/W) MWCNs and zeolite significantly increased the diesel fuel degradation in soil by 5.3% and 4.4%, respectively.

It should be mentioned that applying MWCNs or Zeolite had a significant effect on decreasing soil Ni concentration and thereby increasing diesel fuel degradation in soil. It can be concluded that applying MWCNs or zeolite can decrease the toxicity effect of heavy metals and thereby increasing the soil microbial activity (data was not shown) that has a positive effect on diesel fuel degradation in soil. The negative effect of heavy metals on soil microbial activity is mentioned by researchers [30, 31]. Generally, high metal concentrations can significantly reduce rates of decomposition because the microbial activity is adversely affected by heavy metal toxicity [32]. Nwachukwu et al. conducted the microbial respiration as an indication of metal toxicity in contaminated organic materials and soil and concluded that increasing soil pollution to heavy metals significantly decreased the soil microbial activity. However, they did not consider the role of soil physicochemical properties on soil microbial activity [32].

Based on the results of this study, plant inoculation with AMF can significantly affect the diesel fuel degradation in soil, as, the results of this study showed that the greatest degradation of diesel fuel in the soil was related to the soil under cultivation of plant the inoculated with AMF.

Plant inoculation with AMF can significantly decrease the plant-heavy metal concentration and thereby increase the plant biomass and plant resistance to abiotic stresses [33]. Increasing plant biomass can increase the plant root exudate that is an important source carbon for the activity of microorganisms [34]. Accordingly, increasing the activity of microorganisms can increase the degradation of diesel fuel in the soil. Baumert et al. investigated the effect of root exudate on increasing soil microbial activity and concluded that plant root exudate can act as a carbon source for soil microbial activity [35]. Banks et al. was also conducted the effect of plant cultivation on the degradation of petroleum hydrocarbons in soil and concluded that plant root exudate as a carbon source can increase the soil microbial activity and thus increase the degradation of petroleum hydrocarbons in soil [36]. In addition, they mentioned that increasing soil microbial activity due to increasing plant root exudate can affect the immobilization of soil contaminants such as heavy metals and consequently can help to increase plant biomass and phytoremediation efficiency.

CONCLUSION

Increasing soil pollution with Ni had significantly decreased the diesel fuel degradation in soil that may be related to the role of heavy metal toxicity on decreasing soil microbial activity. On the other hand, the co-contamination of heavy metal and diesel fuel had an adverse effect on plant biomass and consequently decreasing the diesel fuel degradation in the soil. Applying 2 % (W/W) of MWCNs and zeolite had an additive effect on increasing diesel fuel degradation in soil and Ni phytoremediation efficiency. However, increasing soil pollution with heavy metal had an adverse effect on diesel fuel degradation in soil. It should be mentioned that soil physic-chemical properties can affect the degradation of petroleum hydrocarbons which is necessary to be considered in future research. However, the type and amount of heavy metals and petroleum hydrocarbons cannot be ignored.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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