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Effect of Drought Stress, Soil Salinity, *P. indica*, and MWCNs on Biodegradation of Diesel Oil in the Pb- and Cd-Polluted Soil Under Cultivation of Triticale Plant

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ABSTRACT

Background: Today, soil pollution with heavy metals and petroleum compounds is one of the main environmental problems. This research was done to investigate the effect of drought stress, soil salinity, *Piriformospora indica (P. indica)*, and MWCNs on biodegradation of diesel oil in the soil that contaminated with Pb and Cd under cultivation of triticale plant.

Methods: Treatments consisted of applying MWCNs (2% [W/W]) in the Pb- and Cd-polluted soil that was simultaneously polluted with diesel oil (0, 5, and 10% [W/W]), and the plants used in this study were inoculated with *P. indica*. After 70 days, plants were harvested, and plant and soil Cd were measured using AAS (atomic absorption spectrometry). In addition, the degradation percentage of diesel oil in soil was determined.

Results: Soil application of MWCNs at the rate of 2% (W/W) significantly increased the biodegradation of diesel oil in the soil in the drought and salinity (6 dS/m) stress by 11.3% and 15.6%, respectively. In addition, plant inoculation with *P. indica* significantly increased the biodegradation of diesel oil in the soil by 12.3%. For salinity (6 dS/m) and drought stress, it was increased by 8.3% and 9.4%, respectively. In addition, the Pb and Cd concentrations decreased by 14.3% and 12.8%, respectively, when the MWCNs were added to the soil (2% [W/W]).

Conclusion: Soil application of MWCNs and plant inoculation with *P. indica* not only increased the biodegradation of diesel oil but also decreased the plant Pb and Cd concentrations.

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1. Introduction

Heavy metals are the most harmful pollutants to human societies and the environment [1, 2]. When the concentration of these elements is high, they become toxic to microorganisms. Heavy metals are chemical elements with more than 5 g/cm^3 density. Lead (Pb), Cadmium (Cd), Nickel (Ni), and Arsenic (As) are some of the most dangerous heavy metals in the environment [3]. With the industrial revolution, the metals in industrial effluents have directly and indirectly entered the environment more and more. Industrial and agricultural activities lead to the release of heavy metals into the soil, groundwater, sediments, and surface water [4, 5].

Unlike other pollutants, heavy metals are very difficult to remediate from the soil and water since they are non-degradable. However, they can be oxidized, reduced, or complexed by organic matter. Therefore, appropriate methods are available to remediate metal-contaminated sites. Among different heavy metals, Pb and Cd are more dangerous in the environment. High concentrations of Pb and Cd in the human body cause osteoporosis, lung dysfunction, liver damage, and high stress. Therefore, a suitable solution should be sought to reduce the availability of these heavy metals in the soil and consequently in the food chain [6, 7].

Soil contaminated with heavy metals can be remediated via various physical, chemical, and biological methods. The high costs, as well as the relatively limited efficiency of conventional physical and chemical methods for remediation of contaminated soils, have led researchers to find new methods [8, 9]. Among these methods, phytoremediation is a simple, cheap, and environmentally friendly method in which plants are used to remove pollutants from the soil. In this method, the resistant plants eliminate or reduce the concentration of organic and inorganic pollutants and hazardous compounds from the soil [10]. Generally, this method is suitable for purifying high levels of pollution and various pollutants in shallow places, contaminated soils, and contaminated surface and underground water. However, physicochemical soil properties and climatic characteristics can affect phytoremediation efficiency. Meanwhile, limiting factors such as salinity and drought reduce phytoremediation efficiency by restricting plant growth [11]. Salinity negatively affects the phytoremediation process via increasing the solubility of heavy metals, which can reduce plant growth [12, 13].

On the other hand, salinity and drought stress reduce the decomposition of petroleum compounds by reducing the activity of soil microorganisms. Accordingly, phytoremediation in these areas cannot remediate the soils contaminated with heavy metals or petroleum compounds [14, 15]. Therefore, it is necessary to use suitable ways to reduce the adverse effects of salinity and drought stress and cultivate salinity-resistant plants in arid and semi-arid areas that are simultaneously contaminated with heavy metals and petroleum compounds.

According to the researchers' results, plant inoculation with *Piriformospora indica* (*P. indica*) may help enhance the plant resistance against abiotic stress, such as salinity or drought. However, plant physiology and soil physicochemical properties should also be considered [16, 17]. In this regard, Abdelaziz et al. investigated the role of plant inoculation with *P. indica* on altering the adverse effects of soil salinity on plant growth. They concluded that inoculation of plants with *P. indica* could increase the biomass and quality of plants grown in desert agriculture, especially in arid and semi-arid regions. However, they did not mention the role of *P. indica* in increasing the growth and quality of plants in soils contaminated with heavy metals or petroleum compounds [18]. It has also been reported that using organic amendments, such as cow manure or sewage sludge in heavy metals-polluted soils, may increase the soil sorption properties and thereby decrease the heavy metal availability [19, 20]. However, the long-term decomposition of these compounds can redistribute heavy metals in the soil, which can be a negative aspect [21, 22]. Accordingly, using organic amendments, such as Multi-Walled Carbon Nanotubes (MWCNs), can increase the soil sorption properties without changing soil components. So, Shah et al. investigated the effect of MWCNs on Cd removal from heavy metals-polluted soil. They concluded that using organic amendments, such as MWCNs, significantly decreases the soil Cd content [23]. However, they did not consider the role of MWCNs in the remediation of soils that are simultaneously polluted with different heavy metals. In addition, they did not mention the role of soil salinity on heavy metals availability because the results of other studies have shown that soil salinity has a significant effect on increasing the availability of heavy metals in the soils [24-26].

On the other hand, remediation of petroleum compounds in the soils simultaneously contaminated with heavy metals is one of the main environmental challenges, especially in industrial areas where the phytoremediation efficiency is low due to low plant biomass [27, 28]. Thus, using MWCNs materials can help increase the soil sorption properties and thereby decrease the soil heavy metal availability, promoting soil microbial activity. Improving the soil microbial activity can increase the biodegradation of petroleum hydrocarbons in the soil. However, this process depends on the soil's physicochemical properties, such as the type of pollutant, salinity and drought stress, and the physiological characteristics of the plant. In addition, plant inoculation with *P. indica* may enhance the plant biomass and consequently increase the phytoremediation efficiency. On the other hand, the use of plants that are resistant to salinity and drought stress may also have a synergistic effect on the decomposition of diesel oil in the arid and semi-arid regions, which should be studied.

The grains of the triticale plant has about 13.5% more protein than wheat, corn, rice, rye, and oats. It also contains a considerable amount of essential amino acids, different minerals, and vitamins. Triticale is more resistant than other crops in stressful conditions, such as salinity, drought, erosive rainfall, low soil acidity, lack of phosphorus, elemental toxicity, and shallow soils. In stressful and poor environments to provide forage, triticale can be used for direct grazing and grain and straw production, such as traditional wheat and barley. Thus, this research was conducted to evaluate the effect of drought stress, soil salinity, *P. indica*, and MWCNs on biodegradation of diesel oil in the Pb- and Cd-polluted soil under cultivation of triticale plant.

2. Materials and Methods

To investigate the effect of drought stress, soil salinity, *P. indica*, and MWCNs on biodegradation of diesel oil in the Pb- and Cd-polluted soil under cultivation of triticale plant, we designed a factorial experiment in the layout of completely randomized block design in three replicates as a pot experiment. To do this, a non-saline soil (EC=2 dS/m) with low organic carbon (<0.2%) that was naturally polluted with Pb and Cd was selected. Treatments consisted of application of MWCNs at the rate of 0 and 2% (W/W) in a diesel oil-polluted soil (0, 5, and 10 [W/W]) [29] that was polluted with Pb and Cd. Moreover, the plant used in this experiment was triticale inoculated with *P. indica* and cultivated in the drought and salinity (EC=2, 4, and 6 dS/m) stress condition.

The inoculum of *P. indica* was obtained from the soil laboratory of Soil and Water Research Institute of Karaj, Karaj City, Iran, and then cultured on a Hill and Käfer medium in Petri dishes [30]. The fungal samples were placed for 14 days in a temperature-controlled growth chamber at 29 °C±1 °C in the dark.

The surface of triticale seeds (Cv. Omidbakhsh) was sterilized with H_2O_2 15% for 15 min, rinsed with distilled water, and germinated on moistened filter papers for two days. Then, the strongest seedlings were selected for the experiment, and half of them were inoculated with *P. indica* by immersion in the inoculum (adjusted to 2×106) for 3 h under gentle shaking. The non-inoculated seedlings were dipped in sterilized distilled water containing Tween 20 (0.02%) [31]. The selected soil for the experiment was contaminated with diesel oil at the rate of 0, 5, and 10% (W/W) and incubated for two weeks. In addition, the treated soils were irrigated with saline water several times to reach the salinity of the desired treatments. After that, four seeds of either inoculated or non-inoculated plants were transferred to the 5 kg plastic pots, which were filled with the treated soil. Plants were irrigated under normal (full irrigation (D0)) and intensive (70% water depletion of field capacity) drought stress (D1). After 70 days, the plants were harvested, and their Pb and Cd concentrations were determined according to the method described by Maqbool et al. [32]. In addition, the soil Cd and Pb concentrations were measured according to the Lindsay method [33]. In this method, the available metal contents in the soil samples were determined by extraction with 0.005 M DTPA (diethylenetriaminepentaacetic acid) (pH=7.3). About 20 mL of DTPA solution was added to the 10 g of soil sample placed in polypropylene bottles. The bottles were shaken on a rotating shaker for 2 h and then centrifuged. Then the soil Pb and Cd concentrations were measured using atomic absorption spectroscopy. The soil microbial respiration was determined according to Besalatpour et al. [27]. The basal soil microbial respiration was measured as evolved CO_2. For this purpose, 3 replicate soil samples of each treatment were incubated for three days at 26 °C in 250-mL glass containers closed with rubber stoppers. The evolving CO_2 was trapped in NaOH solution, and the excess in alkali was then titrated with HCl.

Statistical analyses were done according to the ANOVA procedure using SAS software V.9.1. The differences between means were evaluated using the Least Significant Difference (LSD) test. P-values less than 0.05 were considered significant.

3. Results and Discussion

The greatest biodegradation of diesel oil in the soil (Table 1) belonged to the soil with the highest amount of MWCNs. This effect can be attributed to the role of MWCNs on increasing the soil sorption properties and thereby decreasing the soil Pb (Table 2) and Cd availability (Table 3). It also has a significant effect on soil microbial activ-

Table 1. The effect of treatments on biodegradation of diesel oil in the soil % (W/W)

Drought Stress	Multi-walled Carbon Nanotubes (%)	Diesel Oil % (W/W)	*+P. indica*			*-P. indica*		
			Salinity (dS/m)					
			2	4	6	2	4	6
D_0	0	0	NC*	NC	NC	NC	NC	NC
	2		NC	NC	NC	NC	NC	NC
	0	5	63.1f**	60.2i	58.4j	60.3i	57.3k	54.2m
	2		65.2d	63.7f	61.7h	63.1f	61.6h	57.8k
	0	10	68.4c	65.1d	63.8f	65.9d	64.2e	62.8g
	2		71.3a	70.4b	68.4c	70.2b	68.2c	65.9d
D_1	0	0	NC	NC	NC	NC	NC	NC
	2		NC	NC	NC	NC	NC	NC
	0	5	47.5t	44.2w	41.9z	44.3w	41.6z	40.5a´
	2		52.6o	50.4q	46.7u	48.3s	45.4v	42.1y
	0	10	52.1o	50.8q	47.4t	50.5q	46.7u	43.4x
	2		55.1l	53.8n	50.2q	53.1n	51.8p	49.4r

*NC: No Measurement; **D0: Full Irrigation; D1: Intensive Drought Stress.

Data with similar letters are not significantly different (P<0.05, LSD test).

Table 2. The effect of treatments on soil Pb concentration (mg/kg Soil)

Drought Stress	Multi-walled Carbon Nanotubes (%)	Diesel Oil % (W/W)	*+P. indica*			*-P. indica*		
			Salinity (dS/m)					
			2	4	6	2	4	6
D_0	0	0	105.1d*	107.2b	111.4y	108.2a	110.8z	115.4t
	2		100.2h	102.7g	104.7e	103.7f	106.2c	110.4z
	0	5	113.1w	116.4s	118.2q	116.1s	118.4q	120.6o
	2		110.1z	112.1x	115.3t	113.1w	116.3s	119.4p
	0	10	121.2n	124.7l	129.1f	125.6k	128.3g	131.5e
	2		117.1r	119.1p	126.7i	121.2n	125.7k	129.4f
D_1	0	0	110.5	113.9v	118.1q	113.5w	118.1q	124.5l
	2		107.5b	110.8	114.1u	110.3	115.6t	119.1p
	0	5	115.9t	118.1q	123.1m	119.2p	124.5l	127.9h
	2		112.3x	115.1t	118.3q	116.1s	120.1o	124.2l
	0	10	129.3f	131.7e	133.8c	132.4d	134.9b	137.8a
	2		125.8k	127.3h	131.3e	128.4g	131.9e	134.6b

D0: Full irrigation; D1: Intensive drought stress.

Data with similar letters are not significantly different (P<0.05, LSD test).

ity (Table 4) and thus diesel oil degradation in the soil. However, soil salinity and drought stress significantly decreased the biodegradation of diesel oil in the soil.

Researchers have mentioned enhancing the soil sorption properties due to increasing the organic amendments [34, 35]. However, the soil physicochemical properties have different effects on the phytoremediation efficiency of heavy metals or petroleum hydrocarbons that should be considered in the studies. Accordingly, Nawab reported that organic amendments could decrease the soil heavy metals availability via increasing soil sorption properties. These results are in line with our results. However, they did not mention the role of organic amendments on the changes of heavy metals availability in the soils that are simultaneously polluted with different heavy metals [36]. In addition, Matos et al. investigated the role of MWCNs on the changes in heavy metals availability in contaminated soils. They concluded that using such organic amendments can alter the availability of heavy metals via changes in soil sorption properties. Their findings are similar to our results. However, they mentioned that heavy metals immobilization in the soils depends on heavy metals. But they did not consider the role of MWCNs on the availability of the heavy metals in the soils contaminated with more than one heavy metal, which is the subject of our research. On the other hand, simultaneous soil contamination with heavy metals and petroleum compounds can also affect their solubility, which had not been investigated in their study [37].

According to our results, using MWCNs in the soil can reduce the heavy metals availability, increasing the soil microbial activity and thus helping degrade petroleum compounds. Increased soil microbial activity resulting from MWCNs can confirm our result. However, this increase depends on environmental conditions. The results of our study showed that MWCNs' effect on biodegradation of diesel oil in the soil is lower under cultivation of plants in salinity and drought stress relative to normal condition. Accordingly, using 2% (W/W) MWCNs in the Pb- and Cd-polluted soil under cultivation of plants in salinity (6 dS/m) and drought stress significantly increased the biodegradation of diesel oil by 11.8% and 14.5%, respectively. For plants under normal conditions, these figures were increased by 13.5% and 17.2%, respectively. Zahed et al. reported that using biochar as a sustainable product can increase the remediation of petroleum hydrocarbon-contaminated soil [38]. This finding is in line with the results of our study. However, they did not consider the interaction effects of heavy metals and petroleum hydrocarbons. Our results showed that inoculation of plants with *P. indica* significantly increased the biodegradation of diesel oil in the soil, which can be related to the role of *P. indica* on plant growth and thereby increasing the biodegradation of diesel oil in the soil. Also, the results of our study showed that inoculation of plants with *P. indica* significantly increased the biodegradation of diesel oil in the soil under drought and salinity (6 dS/m) stress by 12.8% and 15.3%, respectively. Zamani et al. reported that inoculation of plants with *P. indica* could increase the bioremediation of petroleum hydrocarbons in the soil [39]. This finding is in line with our results. Plant Cd (Table 5) and Pb (Table 6) concentrations were also affected by the treatments. According to our study results, the lowest plant Pb and Cd concentrations were detected in the plants cultivated in the soils with the highest amount of MWCNs (2% [W/W]). This finding can be attributed to the role of MWCNs in increasing the soil sorption properties and thereby decreasing the soil and plant Pb and Cd concentrations.

At this time, the biodegradation of diesel oil in the soil increased thanks to the role of MWCNs on increasing plant biomass (data were not shown), which may help improve the plant root exudate and thereby decrease the biodegradation of diesel oil in the soil [40]. Our results showed that plant inoculation with *P. indica* significantly reduced the soil and plant Pb and Cd concentration that can help enhance the plant growth and, as a result, increased the biodegradation of diesel oil in the soil. Also, Rahman et al. investigated the ameliorative effects of plants inoculation with *P. indica* on morphological and physiological parameters of Artemisia annua L. under heavy metals stress. They concluded that plant inoculation with *P. indica* can enhance plant growth via decreasing the plant heavy metals uptake that is similar to the results of our study. In addition, they mentioned that flavonoids biosynthetic pathway genes and signal molecules were prominently improved in inoculated stressed plants than un-inoculated ones [41]. The results of Mohd et al. have shown that plant inoculation with *P. indica* can decrease the plants' damage from abiotic stress, such as heavy metal toxicity that can help increase the plant growth and thereby the biodegradation of petroleum hydrocarbons in the soils. This finding is similar to our results [42].

Regardless of plants growing in salinity or drought stress, our results have shown that the interaction effects of using MWCNs and inoculation of plants with *P. indica* on decreasing the plant Pb and Cd concentration were positive and enhanced the plant growth in heavy metals-polluted soils [43] and consequently can improve the biodegradation of petroleum hydrocarbon in the soil [44]. However, abiotic stress, such as salinity or drought stress, negatively affects plant growth and physiology.

Table 3. The effect of treatments on soil Cd concentration (mg Cd/kg Soil)

Drought Stress	Multi-walled Carbon Nanotubes (%)	Diesel Oil % (W/W)	+*P. indica*			-*P. indica*		
			Salinity (dS/m)					
			2	4	6	2	4	6
D_0	0	0	5.4e´*	5.6c´	5.7b´	5.6c´	5.8a´	6.2
	2		5.3f´	5.5d´	5.6c´	5.4e´	5.7b´	6.1x
	0	5	5.8a´	6.0	6.4u	6.1x	6.3v	6.4u
	2		5.5d´	5.6c´	5.9	5.7b´	5.8a´	6.3v
	0	10	7.8m	7.9l	8.2i	8.0k	8.1j	8.3h
	2		7.5p	7.6o	7.9l	7.7n	7.9l	8.1j
D_1	0	0	5.5d´	5.7b´	5.8a´	5.8a´	5.9	6.3v
	2		5.1h´	5.2g´	5.4e´	5.3f´	5.5d´	5.8a´
	0	5	6.4u	6.5t	6.7r	6.5t	6.6s	6.8q
	2		6.1x	6.2w	6.6s	6.3v	6.5t	6.6s
	0	10	8.3h	8.5f	8.6e	8.5f	8.8c	9.3a
	2		8.1j	8.4g	8.5f	8.3h	8.7d	9.0b

D0: Full irrigation; D1: Intensive drought stress.

Data with similar letters are not significantly different (P<0.05, LSD test).

Table 4. The effect of treatments on soil microbial respiration (mg C-Co$_2$/kg Soil)

Drought Stress	Multi-walled Carbon Nanotubes (%)	Diesel oil % (W/W)	+*P. indica*			-*P. indica*		
			Salinity (dS/m)					
			2	4	6	2	4	6
D_0	0	0	6.1z´*	6.3y´	6.6v´	6.4x´	6.6v´	6.9t´
	2		6.0a´´	6.1z´	6.5w´	6.3y´	6.4x´	6.8u´
	0	5	9.5p´	9.8n´	10.3m´	9.8n´	10.3m´	10.8k´
	2		9.0s´	9.2r´	9.6o´	9.3q´	9.5p´	10.3m´
	0	10	11.0i´	11.3f´	11.6d´	11.2g´	11.6d´	12.1c´
	2		10.5l´	10.8k´	10.9j´	10.8k´	11.1h´	11.5e´
D_1	0	0	13.0y	13.3w	13.7u	13.3w	13.6v	14.0t
	2		12.1c´	12.3b´	12.7z	12.5a´	12.7z	13.1x
	0	5	14.9o	15.2l	15.5i	15.2l	15.5i	15.9g
	2		14.0t	14.2s	14.5q	14.3r	14.7p	15.0n
	0	10	16.4e	16.7d	17.0b	16.9c	17.0b	17.3a
	2		15.1m	15.3k	15.6h	15.3k	15.4j	16.0f

D0: Full irrigation; D1: Intensive drought stress.

Data with similar letters are not significantly different (P<0.05, LSD test).

Table 5. The effect of treatments on plant Cd concentration (mg Cd/kg Soil)

Drought Stress	Multi-walled Carbon Nanotubes (%)	Diesel Oil % (W/W)	*+P. indica*			*-P. indica*		
			Salinity (dS/m)					
			2	**4**	**6**	**2**	**4**	**6**
D$_0$	0	0	4.1d´*	4.3b´	4.4a´	4.2c´	4.5z	4.9v
	2		4.0e´	4.2c´	4.3b´	4.1d´	4.4a´	4.7x
	0	5	4.3b´	4.4a´	4.6y	4.4a´	4.6y	5.1t
	2		4.1d´	4.3b´	4.5z	4.3b´	4.4a´	4.8w
	0	10	4.9v	5.3r	5.5q	5.0u	5.5q	5.8n
	2		4.6y	4.8w	5.2s	4.8w	5.1t	5.6p
D$_1$	0	0	5.0u	5.2s	5.5q	5.1t	5.3r	5.8n
	2		4.7x	4.8w	5.0u	5.0u	5.1t	5.5q
	0	5	5.9m	6.2k	6.4i	6.0l	6.4i	6.5h
	2		5.6p	5.8n	6.0l	5.7o	6.0l	6.3j
	0	10	7.3f	7.4e	7.6c	7.5d	7.7b	7.8a
	2		7.1g	7.3f	7.5d	7.3f	7.6c	7.7b

D0: Full irrigation; D1: Intensive drought stress.

Data with similar letters are not significantly different (P<0.05, LSD test).

Table 6. The effect of treatments on plant Pb concentration (mg Cd/kg Soil)

Drought Stress	Multi-walled Carbon Nanotubes (%)	Diesel Oil % (W/W)	*+P. indica*			*-P. indica*		
			Salinity (dS/m)					
			2	**4**	**6**	**2**	**4**	**6**
D$_0$	0	0	40.1u*	43.1r	45.9p	42.5s	44.1q	47.8n
	2		38.1v	41.1t	43.2r	40.1u	42.4s	44.7q
	0	5	44.1q	46.1o	47.1n	45.7p	48.9m	51.3j
	2		42.3s	44.1q	45.9p	43.1r	45.8p	49.1l
	0	10	48.1m	50.1k	53.2h	50.1k	54.2g	56.2e
	2		45.1p	48.1m	50.1k	52.4i	53.1h	55.2f
D$_1$	0	0	45.1p	47.2n	49.3l	47.1n	50.1k	53.4h
	2		42.6s	44.1q	47.1n	44.1q	46.1o	50.1k
	0	5	50.1k	52.7i	53.4h	52.4i	54.4g	57.9d
	2		48.1m	50.2k	51.3j	50.2k	51.9j	53.4h
	0	10	54.6g	55.9f	61.3b	55.3f	57.8d	64.2a
	2		53.2h	54.1g	59.2c	54.1g	56.1e	61.4b

D0: Full irrigation; D1: Intensive drought stress.

Data with similar letters are not significantly different (P<0.05, LSD test).

4. Conclusion

Based on the study results, the application of MWCNs at the rate of 2% (W/W) significantly increased the biodegradation of diesel oil in the soil. However, the salinity and drought condition had a significant adverse effect on the biodegradation of diesel oil. In this condition, plant inoculation with *P. indica* significantly increased the biodegradation of diesel oil in the soil, while the plant Pb and Cd concentrations decreased. Also, plant inoculation with *P. indica* significantly increased the soil microbial respiration in the Pb- and Cd-polluted soil simultaneously contaminated with diesel oil. Moreover, the interaction effects of MWCNs application (2% [W/W]) and *P. indica* had an additive influence on the biodegradation of diesel oil in the soil. However, the biodegradation percentage of diesel oil depends on soil pollutant and physicochemical properties that should be considered in different studies. Besides, plant physiology and the climate condition are important factors in the biodegradation of petroleum hydrocarbon in the soil that should be investigated in future research.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Conflict of interest

The author declared no conflict of interest.

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