Original Article

Effect of Iron Slag, Zeolite, and *Piriformospora indica* Fungus on Mazut Biodegradation in a Heavy Metal-Polluted Soil that was amended with Cow Manure under Canola Cultivation

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Abstract

Aims: Petroleum hydrocarbon pollution in the soil is one of the important problems in environmental studies. Thus, this research was conducted to evaluate the effect of iron slag, zeolite, and *Piriformospora indica* fungus on mazut biodegradation in a heavy metal-polluted soil that was amended with cow manure under canola cultivation. **Materials and Methods:** Treatments included application of zeolite (0% and 2% [W/W]) and iron slag enriched cow manure (0, 15, and 30 t/ha) in a mazut-polluted soil (0%, 4%, and 8% [W/W]) under canola cultivation in the presence of *P. indica*. After 70 days, plants were harvested and the mazut biodegradation in the soil was measured. In addition, the soil and plant Pb and Cd concentration was measured using atomic absorption spectroscopy. **Findings:** Soil application of zeolite (2% [W/W]) in the mazut-polluted soil (4% [W/W]) significantly increased the mazut biodegradation in the soil by 13.1%. In addition, plant inoculation with *P. indica* significantly increased the mazut biodegradation in the soil and decreased the plant Pb and Cd concentration. Soil application of cow manure at the rate of 15 and 30 t/ha has also increased the mazut biodegradation in the soil by 14.1% and 17.4%, respectively. **Conclusion:** Using iron slag enriched cow manure and zeolite had additive effects of increasing the mazut biodegradation in the soil and decreasing the soil and plant heavy metal concentration. However, these changes depend on the plant physiology and soil physic-chemical properties that should be considered in different studies.

Keywords: Cd, cow manure, iron slag, mazut, Pb

Introduction

Soil pollution with petroleum hydrocarbons is one of the most common environmental problems.[1-3] Humans may be exposed to petroleum hydrocarbon in a variety of ways, including breathing polluted air, pesticides, working in extraction sites, or using contaminated plants or animals feeding from contaminated plants.[4] Different compounds of hydrocarbons can affect the human nervous system. The dangers of the pollutants to humans include skin and lung diseases, gastrointestinal poisoning, blood diseases, and effects on vision. Relatively high hydrophobicity of petroleum hydrocarbons significantly increases the accumulation of these pollutants in soils and sediments.[5,6]

Appropriate strategies should be adopted to reduce soil contaminants with petroleum hydrocarbons or heavy metals.[7,8] Although, the efficiency of each method depends on the physicochemical properties of the soil in each area, the physiological characteristics of each plant, and the type of contaminant.[9,10] Accordingly, Alam *et al*. reported that using vermicompost had significant effects on decreasing the heavy metal concentration in the soil.[11] Due to the interaction of heavy metals with nutrients in the soil,[12,13] the use of organic fertilizers enriched with nutrients may help reduce

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the availability of heavy metals in soil and plants. In this way, Abbaspour *et al*. investigated the application of iron slag as an iron fertilizer on increasing the soil Fe availability in calcareous soil and concluded that using this organic amendment can improve the soil Fe availability.[14]

It is noteworthy that in the soils of arid and semi-arid regions due to low soil organic matter, the plant growth is difficult. Therefore, using organic additives such as cow manure or enrichment of organic fertilizers with nutrient elements such as Fe can probably help to improve the nutritional status of the plant and thus increase plant growth. However, the role of soil pollution such as heavy metals or petroleum hydrocarbons on the plant growth cannot be ignored.[15] According to this, using soil organic amendment such as zeolite[16,17] or inoculation of plant that is cultivated in the petroleum hydrocarbons or heavy metals polluted soil with *Piriformospora indica* fungus[18,19] may help to increase the plant growth and consequently increase the biodegradation of petroleum hydrocarbons. On the other hand, the results of some studies[20,21] showed that soil application of iron slag as a by-product of the steel industry has to some extent helped to increase the nutritional status of iron in the soil, which can also affect the availability of heavy metals in the soil. Thus, this research was conducted to evaluate the effect of iron slag, zeolite, and *P. indica* fungus on mazut biodegradation in a heavy metal-polluted soil that was amended with cow manure under canola cultivation.

Materials and Methods

This study was done as a factorial experiment in the layout of randomized complete block was designed. Treatments (72 treatments) consisted of zeolite soil application (0 and 2 % (W/W)), using enriched cow manure (0, 15 and 30 t/ha) with iron slag at the rate of 0 and 2 % (W/W) in the mazut (0, 4 and 8 % (W/W)) polluted soil that was naturally contaminated with Pb (251.4 mg Pb/kg soil) and Cd (108.7 mg Cd/kg soil) under cultivation of canola (Brassica napus L. cv. Okapi). The canola seedling was first surface sterilized in 15% H_2O_2 thoroughly washed in distilled water, and pregerminated on moistened filter paper. The 5 kg plastic pots were filled with a nonsaline soil (electrical conductivity = 1.2) with the low organic carbon (0.1%) and the pH nearly 7.1.

After plant germination, the most vigorously growing seedlings were selected for the experiment. After that, half of the seeds were inoculated with *P. indica* by immersion in inoculums (adjusted nearly to 2×10^6) under gentle shaking for 3 h. The noninoculated seedlings were also dipped in sterilized distilled water containing Tween 0.02%. Thereafter, both types of inoculated and noninoculated seedlings were planted in the uncontaminated top soil layer in the center of each pot at a depth of 1 cm. After 70 days, the plants were harvested and the soil and plant Pb and Cd concentration was measured using atomic absorption spectroscopy. In addition, the mazut biodegradation in the soil was measured according to Hatami *et al*.[22] Statistical analyses were done based on the analysis of variance procedure via SAS software (ver. 9.1, SAS Institute Inc., Cary, NC, USA) via Duncan's test.

Results

The highest mazut biodegradation percentage in the soil [Table 1] has belonged to the soil amended with cow manure enriched with iron slag. With the increasing the application rate of zeolite from 0% to 2% (W/W), the mazut biodegradation in the soil had significantly increased by 11.7%. Furthermore, our results have shown that with increasing the soil pollution with mazut from 0% to 4% and 8% (W/W), the mazut biodegradation in the soil has been increased by 13.6% and 17.8%, respectively. Inoculation of plants with *P. indica* which was cultivated in the soil polluted with mazut (8% [W/W]) has

Table 1: Effect of treatments on the mazut biodegradation (percentage) in the soil

P. indica inoculation	Cow manure (t/ha)	Iron slag (percentage (W/W))	Zeolite (percentage (W/W))					
			0			2		
			Mazut (percentage (W/W))					
			0	4	8	0	4	8
With *P. indica*	0	0	37.3^{d^s}*	40.1^{a^s}	42.7^{y}	38.2^{c^s}	41.9^{z}	44.8^{w}
		2	40.1^{a^s}	41.9^{z}	43.2^{x}	42.4^{y}	44.2^{w}	47.2^{v}
	15	0	51.2^{t}	55.6^{p}	57.9^{n}	53.8^{r}	58.2^{m}	60.1^{k}
		2	53.7^{r}	58.1^{m}	59.3^{l}	56.2^{o}	57.9^{n}	62.1^{j}
	30	0	60.2^{k}	65.6^{h}	67.3^{f}	63.1^{i}	68.6^{e}	73.8^{b}
		2	65.2^{h}	67.8^{f}	70.1^{c}	68.3^{e}	69.6^{d}	75.5^{a}
Without *P. indica*	0	0	34.2^{f^s}	36.1^{e^s}	39.1^{b^s}	36.1^{e^s}	38.2^{c^s}	41.3^{z}
		2	37.8^{d^s}	39.4^{b^s}	41.2^{z}	41.2^{z}	42.8^{y}	44.1^{w}
	15	0	50.2^{u}	53.5^{r}	54.7^{q}	52.7^{s}	54.8^{q}	58.3^{m}
		2	52.1^{s}	56.4^{o}	58.1^{m}	53.8^{r}	55.9^{p}	59.4^{l}
	30	0	58.9^{m}	63.4^{i}	65.8^{h}	60.7^{k}	65.2^{h}	67.4^{f}
		2	63.1^{i}	66.2^{g}	68.9^{e}	65.7^{h}	68.2^{e}	70.1^{c}

*Similarity letters show not significant differences. *P. indica*: *Piriformospora indica*

also increased the mazut biodegradation in the soil by 15.8%. In addition, soil application of cow manure at the rates of 15 and 30 t/ha has significantly increased the mazut biodegradation in the soil by 17.7% and 22.3%, respectively.

Soil use of cow manure had significant effects on decreasing the soil Cd [Table 2] and Pb [Table 3] concentration. According to our results, adding 15 and 30 t/ha cow manure to the soil significantly decreased the soil Cd concentration by 12.7% and 16.7%, respectively. In addition, our results have shown that cow manure enrichment with iron slag (2% [W/W]) significantly decreased the soil Pb and Cd concentration by 17.2% and 19.6%, respectively. Using zeolite at the rate of 2% (W/W) showed the similar results. On the other hand, increasing the soil pollution with mazut had increased the soil Pb and Cd availability. Plant inoculation with *P. indica* had a negative effect on soil Pb and Cd concentration.

The greatest plant Pb and Cd concentration [Table 4] has belonged to the plants cultivated in the mazut-polluted soil without receiving any amendments. Adding 2% (W/W) zeolite to the soil significantly decreased the plant Cd and Pb concentration by 14.2% and 17.3%, respectively. For soil polluted with mazut, adding 2% (W/W) zeolite significantly decreased the plant Cd and Pb concentration by 11.1% and 13.8%, respectively. Plant inoculation with *P. indica* had a significant effect on decreasing the plant Pb and concentration. Based on the results of this study, inoculation of plants with *P. indica* cultivated in the soil polluted with mazut (4% [W/W]) significantly decreased the plant Pb and Cd concentration by 14.1% and 16.3%, respectively.

Table 2: Effect of treatments on soil Cd concentration (mg/kg soil) in the soil

P. indica inoculation	Cow manure (t/ha)	Iron slag (percentage (W/W))	Zeolite (percentage (W/W))					
			0			2		
			Mazut (percentage (W/W))					
			0	4	8	0	4	8
With *P. indica*	0	0	14.1^{i*}	14.4^{f}	14.8^{b}	14.0^{j}	14.2^{h}	14.5^{e}
		2	13.7^{m}	13.9^{k}	14.3^{g}	13.5^{o}	13.6^{n}	14.1^{i}
	15	0	13.7^{m}	13.9^{k}	14.4^{f}	13.5	13.7^{m}	14.3^{g}
		2	13.2^{r}	13.5^{o}	13.8^{l}	13.0^{t}	13.2^{r}	13.6^{n}
	30	0	13.0^{t}	13.3^{q}	13.4^{p}	12.3^{y}	12.5^{x}	12.9^{u}
		2	12.2^{z}	12.5^{x}	12.7^{v}	12.0$^{a'}$	12.2^{z}	12.5^{x}
Without *P. indica*	0	0	14.4^{f}	14.6^{d}	14.9^{a}	14.2^{h}	14.5^{e}	14.8^{b}
		2	13.9^{k}	14.3^{g}	14.4^{f}	13.8^{l}	14.0^{j}	14.3^{g}
	15	0	13.8^{l}	14.2^{h}	14.7^{c}	13.7^{m}	14.1^{i}	14.5^{e}
		2	13.5^{o}	13.8^{l}	14.1^{i}	13.1^{s}	13.5^{o}	14.0^{j}
	30	0	13.3^{q}	13.7^{m}	14.0^{j}	13.0^{t}	13.3^{q}	13.8^{l}
		2	12.7^{v}	13.1^{s}	13.5^{o}	12.3^{y}	12.6^{w}	13.1^{s}

*Similarity letters show not significant differences. *P. indica: Piriformospora indica*

Table 3: Effect of treatments on soil Pb concentration (mg/kg soil) in the soil

P. indica inoculation	Cow manure (t/ha)	Iron slag (percentage (W/W))	Zeolite (percentage (W/W))					
			0			2		
			Mazut (percentage (W/W))					
			0	4	8	0	4	8
With *P. indica*	0	0	151.2^{i*}	153.7^{g}	157.2^{c}	149.3^{k}	150.7^{j}	153.6^{g}
		2	147.8^{m}	149.2^{k}	154.5^{f}	145.2^{o}	146.9^{n}	150.1^{j}
	15	0	148.2^{l}	150.1^{j}	142.9^{r}	147.1^{m}	149.1^{k}	152.7^{h}
		2	145.1^{o}	147.8^{m}	148.2^{l}	143.7^{q}	144.6^{p}	150.2^{j}
	30	0	134.3^{z}	138.2^{v}	141.2^{s}	132.8$^{a'}$	136.5^{x}	138.4^{v}
		2	122.4$^{g'}$	125.6$^{e'}$	127.8$^{c'}$	119.7$^{i'}$	121.2$^{h'}$	124.1$^{f'}$
Without *P. indica*	0	0	153.6^{g}	155.5^{e}	161.2^{a}	152.4^{h}	153.6^{g}	158.1^{b}
		2	148.5^{l}	151.2^{i}	155.2^{e}	147.2^{m}	148.6^{l}	152.7^{h}
	15	0	152.7^{h}	155.3^{e}	156.7^{d}	150.3^{j}	152.4^{h}	154.5^{f}
		2	147.3^{m}	149.3^{k}	151.2^{i}	148.2^{l}	150.2^{j}	153.5
	30	0	137.1^{w}	140.1^{t}	143.5^{q}	139.2^{u}	143.2^{q}	146.7^{n}
		2	124.3$^{f'}$	126.3$^{d'}$	129.1$^{b'}$	134.2^{z}	135.1^{y}	137.8^{w}

*Similarity letters show not significant differences. *P. indica: Piriformospora indica*

According to our results, with increasing the soil pollution with mazut from 0% to 4% and 8% (W/W), the ascorbate peroxidase (APX) enzyme activity [Table 5] has significantly increased by 14.2% and 16.3%, respectively. In contract,

Table 4: Effect of treatments on plant Cd and Pb concentration (mg/kg) in the soil

P. indica inoculation	Cow manure (t/ha)	Iron slag (percentage (W/W))	Zeolite (percentage (W/W))					
			0			2		
			Mazut (percentage (W/W))					
			0	4	8	0	4	8
Plant Cd concentration (mg/kg)								
With *P. indica*	0	0	11.1^{f*}	11.2^{e}	11.4^{c}	10.9^{h}	11.0^{g}	11.3^{d}
		2	10.7^{j}	10.9^{h}	11.1^{f}	10.5^{k}	10.8^{i}	11.0^{g}
	15	0	10.2^{n}	10.4	10.7^{j}	10.1^{o}	10.2^{n}	10.5^{k}
		2	9.3^{s}	9.5^{q}	9.8^{p}	9.1^{t}	9.4^{r}	9.5^{q}
	30	0	8.1^{z}	8.2^{y}	8.5^{w}	7.8$^{c'}$	7.9$^{b'}$	8.1^{z}
		2	7.5$^{f'}$	7.7$^{d'}$	8.1^{z}	7.1$^{h'}$	7.3$^{g'}$	7.8$^{c'}$
Without *P. indica*	0	0	11.2^{e}	11.4^{c}	11.7^{a}	11.0^{g}	11.2^{e}	11.5
		2	11.0^{g}	11.1^{f}	11.5^{b}	10.7^{j}	10.8^{i}	11.4^{c}
	15	0	10.3^{m}	10.5^{k}	10.8^{i}	10.2^{n}	10.4^{l}	10.7^{j}
		2	9.5^{q}	9.8^{p}	10.1^{o}	9.3^{s}	9.5^{q}	9.8^{p}
	30	0	8.3^{x}	8.6^{v}	8.9^{u}	8.0$^{a'}$	8.2^{y}	8.5^{w}
		2	7.7$^{d'}$	7.8$^{c'}$	8.3^{x}	7.5$^{f'}$	7.6$^{e'}$	8.1^{z}
Plant Pb concentration (mg/kg)								
With *P. indica*	0	0	81.6^{g}	82.8^{f}	84.1^{d}	76.4^{l}	78.2^{j}	81.3^{g}
		2	78.4^{j}	81.2^{g}	82.9^{f}	75.4^{m}	77.1^{k}	79.8^{i}
	15	0	71.3^{q}	72.9^{p}	74.3^{n}	70.5^{r}	71.3^{q}	73.1^{o}
		2	65.1^{v}	68.3^{s}	70.1^{r}	63.8^{x}	64.7^{w}	66.4^{u}
	30	0	61.2^{z}	64.2^{w}	65.3^{v}	60.5$^{a'}$	62.7^{y}	63.9^{x}
		2	55.5$^{e'}$	56.7$^{d'}$	58.5$^{b'}$	53.5$^{g'}$	54.1$^{f'}$	57.1$^{c'}$
Without *P. indica*	0	0	83.4^{e}	87.3^{b}	89.4^{a}	80.2^{h}	81.3^{g}	83.5^{e}
		2	80.1^{h}	83.5^{e}	85.4^{c}	78.2^{j}	80.1^{h}	82.1^{f}
	15	0	74.2^{n}	76.4^{l}	78.8^{j}	72.3^{p}	75.7^{m}	77.1^{k}
		2	68.2^{s}	70.1^{r}	73.4^{o}	66.4^{u}	68.4^{s}	71.9^{q}
	30	0	63.7^{x}	66.4^{u}	68.9^{s}	62.7^{y}	64.8^{w}	67.5^{t}
		2	58.1$^{b'}$	61.2^{z}	64.2^{w}	56.2$^{d'}$	57.3$^{c'}$	60.4$^{a'}$

*Similarity letters in each parameter show not significant differences. *P. indica: Piriformospora indica*

Table 5: Effect of treatments on ascorbate peroxidase enzyme activity (U/mg protein)

P. indica inoculation	Cow manure (t/ha)	Iron slag (percentage (W/W))	Zeolite (percentage (W/W))					
			0			2		
			Mazut (percentage (W/W))					
			0	4	8	0	4	8
With *P. indica*	0	0	15.2^{m*}	15.8^{g}	16.7^{b}	15.1^{n}	15.3^{l}	15.7^{h}
		2	15.0^{o}	15.3^{l}	15.6^{i}	14.7^{r}	15.0^{o}	15.3^{l}
	15	0	15.0^{o}	15.4^{k}	15.8^{g}	14.7^{r}	14.8^{q}	15.3^{l}
		2	14.1^{z}	14.3^{y}	14.6^{r}	13.8$^{a'}$	14.2^{w}	14.5^{t}
	30	0	13.7$^{b'}$	13.8$^{a'}$	14.1^{z}	13.9^{z}	14.1^{z}	14.3^{y}
		2	12.7$^{j'}$	12.9$^{i'}$	13.2$^{f'}$	12.5$^{k'}$	12.7$^{j'}$	13.0$^{h'}$
Without *P. indica*	0	0	15.8^{g}	16.2^{d}	17.1^{a}	15.5^{j}	16.0^{e}	16.7^{b}
		2	15.3^{l}	15.6^{i}	15.9^{f}	15.1^{n}	15.5^{j}	15.9^{f}
	15	0	15.2^{m}	15.5^{j}	16.3^{c}	14.9^{p}	15.3^{l}	15.7^{h}
		2	14.4^{u}	14.7^{r}	15.1^{n}	14.0^{y}	14.6^{r}	14.9^{p}
	30	0	13.9^{z}	14.2^{w}	14.6^{r}	13.1$^{g'}$	13.3$^{e'}$	13.6$^{c'}$
		2	13.2$^{f'}$	13.5$^{d'}$	13.9^{z}	13.0$^{h'}$	13.2$^{f'}$	13.7$^{b'}$

*Similarity letters show not significant differences. *P. indica: Piriformospora indica*

inoculation of plants with *P. indica* significantly decreased the APX enzyme activity. Accordingly, plants inoculated with *P. indica* which cultivated in the soil polluted with mazut at the rates of 4% and 8% (W/W) have reduced the APX enzyme activity by 17.2% and 20.6%, respectively. Adding cow manure to the soil significantly decreased the APX enzyme activity. A significant decrease in APX enzyme activity by 11.3% and 17.4% was observed, when the soil was amended with 15 and 30 t/ha, respectively. In addition, enrichment of cow manure with iron slag significantly decreased the APX enzyme activity.

Discussion

Adding cow manure at the rates of 15 or 30 t/ha to the soil significantly increased the mazut biodegradation in the soil which can be related to the role of cow manure on increasing the soil sorption properties[23] and thereby decreasing the heavy metal availability that can help to increase the mazut biodegradation in the soil. In this regard, Wang *et al*. reported that adding cow manure to the soil can immobilize the heavy metal availability in the soil[24] that is similar to our results. On the other hand, one of the basic problems of the industrial areas is the low availability of nutrients in the arid and semi-arid soils,[25] which can hinder plant growth in areas contaminated with heavy metals. Therefore, the use of organic additives such as cow manure can help plant growth by increasing the availability of nutrients in the contaminated soil[26] and thereby increase the plant resistance to abiotic stresses such as heavy metal toxicity. According to this, Zhao *et al*. reported that using cow manure not only can improve the plant nutritional status and soil quality but also can enhance the plant growth via stabilization of heavy metals in the soils[27] that is in line with our results.

Despite the positive role of organic additives in improving plant nutritional status,[28] decomposition of such compounds in soils contaminated with heavy metals may lead to redistribution of heavy metals in the soils, which should be considered. Therefore, the use of nondegradable compounds such as zeolites or carbon nanotubes can stabilize heavy metals in the long term. According to this, Azogh *et al*. investigated the effect of zeolite on heavy metal uptake by wheat and concluded that using zeolite can decrease the soil and plant heavy metal availability[29] that is according to our research. In addition, the simultaneous application of zeolite and cow manure had additive effects on immobilization of soil heavy metal availability and thereby decreased the plant heavy metal uptake.

Iron slag enriched cow manure had additive effects on decreasing the heavy metal uptake by plants. However, it also depends on the type of metal and soil properties.[21] According to this, adding 15 and 30 t/ha cow manure enriched with 2% (W/W) iron slag to the soil significantly decreased the plant Pb and Cd concentration by 13.1% and 16.3%, respectively, which can be related to the interaction effects of Fe and heavy metals. According to this, Kazemi *et al*. reported that using iron organic amendments had a positive role on increasing the plant Fe uptake.[30] In addition, Tabarteh *et al*. had also mentioned that using cow manure enriched with iron organic compounds had significant effects on decreasing corn Pb uptake that is similar to our results.[31] Furthermore, the results of our study have shown that plant inoculation with *P. indica* can help to decrease the heavy metal uptake by plants that is in line with the research of Shahabivand *et al*.[32]

The remarkable point of this research is that with decreasing the soil and heavy metal availability, the mazut biodegradation in the soil has been increased that can be related to the role of organic amendments on decreasing the heavy metal toxicity, increasing the soil microbial activity (data was not shown) and thereby increasing the mazut biodegradation in the soil. On the other hand, inoculation of plant with *P. indica* significantly increased the mazut biodegradation in the soil, while the Pb and Cd concentration of soil under cultivation of plants inoculated with *P. indica* has been decreased. In addition, adding cow manure enriched with iron slag caused a significant decrease in soil and plant heavy metal concentration and thereby increased the mazut biodegradation in the soil. In this regard, Gafari *et al*. investigated the simultaneous effect of municipal solid waste compost and some fertilizers on biodegradation of oil-contaminated soils and concluded that using organic amendments had a significant effect on increasing the biodegradation of petroleum hydrocarbons in the soil that is in line with our results.[33]

Based on the results of this research, with increasing the plant heavy metal uptake, the APX enzyme activity has been increased to reduce the negative effects of heavy metal toxicity.[34] Accordingly, the greatest APX enzyme activity has belonged to the plants cultivated in the soil without receiving any organic amendments; In addition, with adding organic amendments to the polluted soil, the APX enzyme activity has decreased. The opposite relationship between mazut biodegradation in the soil and APX enzyme activity confirms our results clearly.

Conclusion

Using zeolite at the rate of 2% (W/W) significantly enhanced the mazut biodegradation in the soil. In addition, enrichment of cow manure with iron slag significantly increased the soil and plant heave metal concentration and as a result increased the mazut biodegradation in the soil. On the other hand, plant inoculation with *P. indica* had a significant effect on increasing the mazut biodegradation in the soil and decreasing the soil and plant heavy metal availability. However, this can depend on the plant physiology, soil physico-chemical properties, and type of the pollutant that should be considered in different researches. In addition, the role of iron slag as a by-product of steel company on improving the plant nutrient status cultivated in the heavy metal and petroleum hydrocarbon-contaminated soil should be investigated in the future studies. Furthermore, the interaction effects of heavy metals and petroleum hydrocarbons should be investigated in the field studies.

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Conflicts of interest
There are no conflicts of interest.

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