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Investigating the Impact of Industrial Wastewater from Khuzestan Steel Industries on Heavy Metal Accumulation and Morphological Characteristics of the Medicinal Plant Dracocephalum moldavica L.



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ABSTRACT

Introduction: This study examines the effects of different concentrations of industrial wastewater on the growth, yield, and heavy metal accumulation (lead, nickel, and cadmium) in Dracocephalum moldavica L. (badrashb). It aims to evaluate how wastewater irrigation affects the plant's morphological traits and the potential environmental risks of toxic metal uptake. The findings provide insights into the safe use of industrial effluent for growing medicinal plants in arid regions.

Methods: A greenhouse experiment was conducted using a completely randomized design with five treatments (0%, 25%, 50%, 75%, and 100% wastewater) and three replications. At the end of the cultivation period, heavy metal accumulation was determined after sample digestion using an AA-670G Shimadzu apparatus. Morphological characteristics were analyzed using SPSS software version 22.

Results: The results indicated that the highest increase in morphological parameters was observed in the 75% wastewater treatment. Accumulation of lead and cadmium in the roots and aerial parts showed significant differences between the various treatments (P<0.05). The highest concentrations of lead and cadmium in the shoots were 4.16 and 1.6 mg/kg, respectively, in the 100% wastewater treatment, while the lowest concentrations were found in the control treatment. Additionally, the transfer factor of cadmium from the roots to the shoots was higher than that of lead.

Conclusion: Irrigation with industrial effluent resulted in the accumulation of lead and cadmium in Dracocephalum moldavica, with cadmium levels surpassing the WHO standard for medicinal plants. This highlights the environmental risks associated with wastewater irrigation. If wastewater is to be used, it must be properly treated to meet irrigation standards in order to reduce potential health hazards.

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Introduction

The water scarcity crisis is one of the challenges currently facing the world. The limitation of water resources has drawn researchers' attention to the prudent use of unconventional water sources, such as saline water, municipal wastewater, and industrial effluents (Jalali et al., 2010). Due to urban and industrial development and increased water consumption, a significant volume of raw sewage and treated wastewater is produced daily (Jalali et al., 2010). The utilization of treated wastewater varies depending on community needs and the climatic conditions of each region. The Khuzestan Steel Company, located 12 kilometers southeast of Ahvaz, covers an area of 380 hectares. As the second-largest producer of crude steel in Iran, it is the country's first direct reduction iron and electric arc furnace production complex (Mollahoseini, 2013). The wastewater produced at the plant comes from water various consumption in units, including stockpiling and lifting, pelletizing, smelting and refining, and casting (Mollahoseini, 2013). Wastewater can be beneficial in agriculture, industry, and increasing the water supply to aquifers and rivers, as the proper use of municipal and industrial effluents in agriculture not only expands cultivated areas and increases crop production but also helps prevent environmental pollution (Jian et al., 2010). Furthermore, the low cost of using treated wastewater for irrigation reduces surface and groundwater pollution and decreases the need for chemical fertilizers (Ali khasi and Koochakzadeh, 2011).

The application of treated municipal and some industrial wastewater can enhance crop yield due to the presence of organic matter and essential nutrients for plants. However, it may also pose health risks due to a wide range of pathogens, including coliform bacteria and heavy metals (Alinejadian et al., 2014). Therefore, since wastewater is considered an unconventional water resource, its application in agriculture requires specific management to maximize its benefits while mitigating environmental and health risks to soil, plants, and surface and groundwater resources (Alinejadian et al., 2014). Thus, while the use of wastewater may partially compensate for water shortages, the potential negative impacts of these waters on

environmental pollution must be considered. The presence of heavy metals in wastewater and their accumulation in the soil is a significant environmental concern (Devkota and Schmidt, 2000). Although the concentration of heavy metals in wastewater may be low, their accumulation in the soil can lead to increased concentrations in plants cultivated in these soils (Beigi Harchegani, 2011). On the other hand, the accumulation of heavy metals in plants can introduce them into the food chain, potentially having adverse effects on consumer health. The absorption rate of each heavy metal from the soil by the plant depends on the soil type and its characteristics, the type of heavy metal and its forms, and the type of plant (Rahimi et al., 2016). The accumulation of heavy metals in soil, particularly in agricultural lands, is a slow process, and the concentration of heavy metals can gradually reach levels harmful to humans and livestoc (Chui et al., 2004).

Dracocephalum moldavica L., commonly known as badrashb or badrashbooye, is an herbaceous annual plant belonging to the Lamiaceae family. This genus comprises 45 species worldwide, with eight species found in various regions of Iran, some of which are endemic to the country (Fattahi et al., 2021). The extract of badrashb is used to alleviate headaches and colds, general body weakness, as a pain reliever for neuralgic pains, and for gastrointestinal and kidney spasms. It can also be applied as a poultice for rheumatic pains (Badalzadeh et al., 2016). Alinajad Jahromi et al. (2012) studied the effect of using municipal wastewater from Shahrekord on the growth, yield, and accumulation of lead and cadmium in the medicinal plant M. officinalis and found that irrigation with municipal wastewater not only provided water and nutrients required by M. officinalis but also increased its essential oil yield directly or indirectly by enhancing biomass. Notably, the concentrations of lead and cadmium accumulated in this plant were much lower than the permissible limits (Alinejad Jahromi et al., 2012). Rahimi et al. (2015) investigated the impact of irrigation with industrial wastewater on the changes in some heavy metals in the soil and radish plants, showing that the total and available concentrations of heavy metals in soil irrigated with treated wastewater were higher than those in

soil irrigated with normal water; however, their

amounts were below the critical limits for heavy metals in soil. Additionally, irrigation with wastewater did not significantly affect the concentration of heavy metals in the tubers and aerial parts of the radish plant (Rahimi et al., 2016). Klay et al. (2010) found that the concentration of heavy metals in the soil, particularly for lead and cadmium, increased with duration of irrigation using treated the wastewater (Klay et al., 2010). Khurana and Aulakh (2010) studied the effect of industrial wastewater irrigation on the concentration of heavy metals in corn and rapeseed plants, noting that the accumulated cadmium in the corn and rapeseed irrigated with wastewater was 2.5 to 3 times higher than in the control plants (Khurana and Aulakh, 2010). Therefore, irrigation with wastewater can have beneficial or detrimental effects on agricultural production depending on its composition and the sensitivity of the plant species involved. It is crucial to pay more attention to the reuse of wastewater concerning the increased concentrations of heavy metals, including lead, nickel, and cadmium, in soils and plants. Given the extensive use of the medicinal plant badrashb, ensuring the safety of this product is of utmost importance. Therefore, this study evaluates the growth, yield, and accumulation of lead, nickel, and cadmium in the roots and aerial parts of badrashb plants under irrigation with varying ratios of industrial wastewater.

Materials and Methods

The wastewater used in this study was derived from the treated industrial effluent of the Khuzestan Steel Company, located 12 kilometers southeast of Ahvaz. Drinking water was also utilized as the control treatment. The experiment was conducted in a completely randomized design with five treatments, including 0%, 25%, 50%, 75%, and 100% wastewater mixed with irrigation water. Each treatment was replicated three times in growth chambers at Khatam Al-Anbia Industrial University in Behbahan using pots.

The seeds of M. officinalis were placed in sterile Petri dishes containing filter paper and distilled water for cultivation. After sowing the seeds, the Petri dishes were placed in a germinator under a photoperiod of 16 hours of light and 8 hours of darkness at a temperature of 22 degrees Celsius

until germination and the emergence of the first leaves. Subsequently, the uniformly grown seedlings were transferred to plastic pots filled with a mixture of farm soil, sand, and perlite in a ratio of 1:1:2. Each treatment consisted of three pots, with three seedlings planted in each pot, and was conducted with different irrigation percentages of treated wastewater throughout the growth period (each pot received 100 milliliters of water, with 36 treatments applied during the experiment). The cultivation lasted for two months, after which the plants were harvested for the assessment of morphological traits and the measurement of heavy metal accumulation in the roots and aerial parts.

The chemical composition of the control water and wastewater was measured throughout the growing season. The pH was measured using a portable pH meter, and electrical conductivity was assessed with а conductometer. The concentrations of sodium, potassium, and calcium were measured using a flame photometer. Phosphate and nitrate levels were determined using a Hach DR-1900 spectrophotometer made in the USA. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were measured with an OxiDirect BOD meter and a Hach DRB 200 Digestion COD meter, respectively. The concentrations of heavy metals in water samples were analyzed using a Shimadzu AA-670G flame atomic absorption spectrometer.

At the end of the growth period, plant samples were taken to the laboratory, where they were divided into two parts: roots and aerial organs. These samples were washed with distilled water and dried on filter paper. Morphological traits, including plant height, leaf number, lateral stem count, and stem diameter at a point one centimeter above the root collar, were measured. The fresh weight of the organs was measured immediately after harvest using a balance with an accuracy of 0.001 milligrams. The samples were then dried in an oven at 70 degrees Celsius for 48 hours and reweighed (Alinejad Jahromi et al., 2012). The diameter of the main stems was measured one centimeter above the root collar with a caliper. The number of first-order lateral branches on the main stem and the number of leaves in each treatment were counted on the last day of the experiment. Plant height was also measured with a meter.

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To assess the concentration of heavy metals in the roots and aerial parts of the plants, the dried samples were ground. One gram of the ground material was weighed, and 5 milliliters of hydrofluoric acid, 10 milliliters of nitric acid, and 5 milliliters of hydrochloric acid were added. The mixture was heated to 100 degrees Celsius until a clear solution was obtained. The sample was then brought to volume with 4% nitric acid. The concentrations of lead and cadmium in the plant extracts were read using the Shimadzu AA-670G flame atomic absorption spectrometer. After measuring the concentrations of heavy metals in the roots and aerial parts, the transfer factor (TF) was calculated by dividing the concentrations of elements in the aerial parts by those in the roots. The obtained data were statistically analyzed using SPSS version 22, and means were compared using Duncan's multiple range test.

Results

Wastewater Quality

To assess the quality of the treated industrial wastewater for irrigation use, several chemical characteristics were measured, including salinity and alkalinity, key macronutrients, micronutrients, and heavy metals throughout the growth period of M. officinalis. The average results are presented in Table 1.

Morphological Traits

The analysis of variance for the results related to the effect of irrigation with treated wastewater on the measured traits is provided in Table 2. As shown in this table, all growth indices and performance of the medicinal plant M. officinalis, except for root dry weight, were significantly affected by irrigation with various ratios of wastewater (P < 0.05). The results of the mean comparisons regarding the impact of different irrigation treatments on morphological characteristics (plant height, main stem diameter, number of lateral stems, and number of leaves) were determined using Duncan's multiple range test and are presented in Table 3. Based on these results, the application of different percentages of wastewater led to an increase in morphological traits up to treatment T4, after which a decline was observed. Figures 2 and 3 illustrate the effects of various wastewater ratios in the irrigation water on the fresh and dry weights of the aerial parts and roots of M. officinalis, respectively. These figures indicate that with the application of different percentages of wastewater, the fresh and dry weights of the plant increased up to the 75% wastewater and 25% control water treatment, followed by a decrease.

Parameters	Water Source	Effluent	Standard Threshold for Irrigation
рН	Iran (9)	WHO (41)	Standard Threshold for Infigation
	7.3	7.02	5.6 - 8.5
Electrical Conductivity (EC, ds/m)	1.41	4.57	-
Biochemical Oxygen Demand (BOD)	0	21.62	100
Chemical Oxygen Demand (COD)	0	34.57	200
Sodium	80	670	-
Calcium	190	132	-
Magnesium	17	108	100
Potassium	3.2	16	-
Sulfate	475	600	500
Phosphate	0	5	-
Nitrate	4.5	1.8	5
Lead	ND	0.2	1
Cadmium	ND	0.175	0.05
Nickel	ND	ND	0.2
ND: indicates not detectable.			

Table 1. Mean values of some chemical characteristics of water and wastewater under study (mg/l)

Note:

Source of Variation	Degrees of Freedom	Height (cm)	Main Stem Diameter (mm)	Number of Side Stems	Number of Leaves	Fresh Weight of Aerial Parts (g)	Dry Weight of Aerial Parts (g)	Fresh Weight of Roots (g)	Dry Weight of Roots (g)
Mean Squares of Treatment	4	*728.8	0.241*	8.133*	63.533*	1.533*	0.026*	0.085*	0.001 ns
Mean Squares of Error	25	1.336	0.030	1.173	11.253	0.389	0.005	0.021	0.001
Coefficient of Variation (%)	-	13.46	13.73	25.49	13.20	14.24	14.55	14.30	15.92

Table 2: Analysis of variance for morphological traits of the basil plant under different wastewater treatments

*: Significant difference at the 5% statistical level; ns: No significant difference.

Table 3: Comparison of means for the traits examined in the basil plant under different wastewater treatments

Treatment	Height (cm)	Main Stem Diameter (mm)	Number of Side Stems	Number of Side Stems
T1	10.15 ± 1.14 a	1.555 ± 0.16 a	5 ± 1.09 a	29.33 ± 3.50 a
Τ2	10.733 ± 1.19 a	1.642 ± 0.18 a	5 ± 1.09 a	31 ± 3.74 a
Т3	$12.333 \pm 0.98 \text{ b}$	$1.918\pm0.14\ b$	$7 \pm 1.09 \text{ b}$	$35.33 \pm 3.01 \text{ b}$
Τ4	$13.00\pm1.51~b$	$2.033\pm0.22~b$	$7 \pm 1.09 \text{ b}$	$36.67\pm3.93~b$
Т5	10.783 ± 0.81 a	1.688 ± 0.12 a	5 ± 1.09 a	30.33 ± 2.33 a

In each column, different letters indicate significant differences at the 5% level based on the Duncan test



Effluent Rations

Figure 1: Effect of different ratios of wastewater on fresh and dry weight of aerial parts of the basil plant



Effluent Rations

Figure 2: Effect of different ratios of wastewater on fresh and dry weight of roots in the basil plant

Concentration of Lead, Nickel, and Cadmium in Basil Plants

The device used to measure nickel concentration did not detect any nickel in any of the samples, indicating either a low concentration or the absence of this element in the tested plants. Therefore, variance analysis and mean comparison for this element are not applicable. The main reasons for the absence of nickel in the plant samples may be attributed to the negligible concentration of this element in both wastewater and regular water, as well as the short duration of growth, which may not have been sufficient for absorption and accumulation of this element.

The statistical analysis results presented in Table 4 indicate that the concentration of heavy metals, lead and cadmium, in the aerial parts and roots of

the basil plant was affected by irrigation treatment with wastewater. The concentration of heavy metals in the plant's organs increased with the higher percentage of wastewater (Table 5).

Translocation Factor from Roots to Aerial Parts

Table 5 presents the translocation factors of heavy metals, lead and cadmium, from the roots to the aerial parts of the basil plant. As shown in this table, the translocation factor for cadmium and lead in the control treatment is equal and amounts to 0.26. However, in other treatments, the translocation factor for cadmium is greater than that for lead.

 Table 4: Statistical analysis of heavy metal concentrations (pb and cd) in the aerial parts and roots of basil plants under different wastewater treatments

Source of Variation	Degrees of Freedom	Cd in Roots (Mean Squares)	Cd in Aerial Parts (Mean Squares)	Pb in Roots (Mean Squares)	Pb in Aerial Parts (Mean Squares)
Mean Squares of Treatment	4	0.938*	0.349*	10.996*	1.752*
Mean Squares of Error	10	0.068	0.011	0.707	0.105
Coefficient of Variation (%)	-	34.52	50.79	17.30	26.33

*: Significant difference at the 5% statistical level.

Table 5: Mean concentrations of heavy metals (pb and cd) in the dry matter of basil plants under different wastewater treatments (mg/kg)

Treatment	Cd in Roots (mg/kg)	Cd in Aerial Parts (mg/kg)	Transfer Factor (Cd)	Pb in Roots (mg/kg)	Pb in Aerial Parts (mg/kg)	Transfer Factor (Pb)
	Mean \pm SD	$Mean \pm SD$		Mean \pm SD	Mean \pm SD	
T1	$0.93 \pm 0.05 \text{ a}$	$0.25 \pm 0.01 \text{ a}$	0.26	8.78 ± 1.10 a	2.30 ± 0.34 a	0.26
T2	$1.43\pm0.07~ab$	$0.40\pm0.12\;b$	0.27	$9.61 \pm 0.75 \text{ ab}$	2.30 ± 0.34 a	0.23
Т3	$1.43\pm0.08\ b$	$0.58\pm0.12~b$	0.40	$10.83\pm0.74~b$	2.81 ± 0.36 a	0.25
T4	$1.95\pm0.26\ c$	$0.91\pm0.08~c$	0.46	$13.05 \pm 0.15 \text{ c}$	2.81 ± 0.36 a	0.21
Т5	$2.39\pm0.50\ c$	$1.06 \pm 0.11 \text{ c}$	0.443	$12.90\pm1.08~\mathrm{c}$	$4.16\pm0.14\ b$	0.32

In each column, non-identical letters indicate a significant difference at the 5% level based on Duncan's test.

Discussion

By comparing the mean chemical composition of the wastewater with the effluent standards (Neai and Moreno, 2000) and the recommended limits set by the World Health Organization (2023), it was determined that the acidity of the wastewater falls within permissible levels, posing no restrictions on its utilization. However, the electrical conductivity of the wastewater exceeds the maximum allowable threshold for irrigation as per WHO recommendations (1989), indicating a severe limitation for irrigation use. Therefore, the wastewater exhibits a high concentration of total dissolved solids (TDS), necessitating caution regarding potential salt accumulation in the soil.

The parameters of chemical oxygen demand (COD) and biological oxygen demand (BOD) in the wastewater are within acceptable limits according to the effluent standards (Neai and Moreno, 2000), thus not imposing restrictions on its use. Nonetheless, the concentrations of sodium, magnesium, and nitrate in the wastewater are above the recommended limits the World Health Organization (2023). hv Additionally, the concentrations of sulfate and magnesium exceed the standards set by the Neai and Moreno (2000). The concentration of lead in the wastewater is at an acceptable level according to the standards of both the Neai and Moreno (2000) and the World Health Organization (2023), indicating no agricultural use. Conversely, issues for the concentration of cadmium poses a significant restriction.

The results indicate that with varying percentages of wastewater application, morphological parameters increased up to treatment T4 and subsequently decreased. This suggests that the wastewater derived from treatment, primarily due to its rich nutrient content beneficial for the M. officinalis plant, enhanced growth and vield in treatments T2, T3, and T4 compared to the control treatment. Supporting this, studies conducted by Rahimi et al. (2016) corroborate these findings. Golchin et al. (2013) reported that applying wastewater from the IranMayeh factory at concentrations exceeding 45% reduced the biological performance of alfalfa, while lower concentrations improved photosynthesis and biological yield due to positive effects of nutrients like magnesium, iron, phosphorus, and potassium (Golchin et al., 2013). The increase in the number of leaves and lateral stems with higher wastewater percentages can be attributed to enhanced plant growth and height, leading to more internodes and potential sites for lateral bud production. Sufficient nutrient availability would promote the growth of these buds, resulting in additional lateral stems.

The results also suggest that stem diameter is influenced by environmental factors, with the provision of essential nutrients, particularly nitrogen and potassium from the wastewater, facilitating better growth and increased plant resistance, thereby enhancing stem diameter. It appears that the nutrients present in the wastewater (nitrogen, phosphorus, potassium, sulfur, etc.) played a crucial role in plant growth. The reduction in growth and yield in treatment T5 is likely due to the gradual increase in soil salinity resulting from high wastewater concentrations, negatively impacting growth. As soil salinity rises, plants expend more vital energy to absorb a given amount of water, which is energy otherwise utilized for metabolic activities and cell development. Consequently, limited energy resources for growth would ultimately decrease yield (Homae, 2007).

Additionally, Cicek and Cakirlar, 2002 noted that increased osmotic potential due to salts in the root environment hampers root cells' ability to absorb necessary water, thereby limiting the uptake of dissolved essential nutrients in plants (Cicek and Cakirlar, 2002). Thus, plant growth and development may be restricted due to metabolic deficiencies. Heavy metals in the soil are typically immobile, often accumulating in the surface layer and at root penetration depths. Consequently, the increased uptake of cadmium and lead by M. officinalis in contaminated soils is not unexpected. Numerous reports indicate that plants can absorb lead from the environment and accumulate it in their roots (Jarvis and Leung, 2002).

According to Table 5, the concentrations of lead and cadmium in the roots of M. officinalis were higher than in aerial parts. Both cadmium and lead are absorbed by the roots and subsequently transferred to aerial organs. Plants exhibit a substantial capacity to absorb metals through roots and transport and store them in aerial parts (Ozturk et al., 2003). Lead primarily remains in the roots and exhibits limited transfer to aerial organs (Hooda, 2010). Singh and 2004 Kalamdhad attributed the greater accumulation of heavy metals in roots to their complexation with sulfhydryl groups, which impedes their transfer to aerial parts (Singh and Kalamdhad, 2004). Brunetti et al. (2011) reported that higher accumulations of chromium, copper, lead, and zinc in roots compared to aerial parts demonstrate the mechanism by which plants tolerate high metal concentrations in soil (Brunetti et al., 2011).

The accumulation of lead in the roots could be viewed positively, as it may prevent more lead from transferring to seeds and the food chain. However, it is essential to note that the non-harvesting of roots at the time of plant collection leaves these roots, containing certain concentrations of these elements, in the soil. Following the decomposition of the roots, this could lead to the accumulation of these elements in the soil and subsequent uptake by plants cultivated later (Saadat et al., 2023). In a study by Khurana and Aulakh, 2010, results indicated that cadmium levels in canola and corn irrigated with wastewater increased by 3.5% and nickel by 16% compared to the control, with uneven distribution of elements in plant organs, often showing higher accumulation in roots than in aerial parts (Khurana and Aulakh, 2010). Mollahoseini et al. (2013) associated the elevated levels of metals in soils irrigated with wastewater with soil pH (Mollahoseini, 2013). Beyond pH, soil salinity can also impact the solubility and absorption of metals by plants. Therefore, it can be concluded that high salinity in the wastewater contributed to increased heavy metal absorption in M. officinalis, which in turn increased soil salinity.

In this regard, Amini et al. (2011) associated the increase in nickel concentrations with soil salinity and its absorption capabilities (Amini et al., 2011). The lead concentration in aerial parts was within the permissible limits recommended by the World Health Organization for medicinal plants (mg/kg 10), whereas cadmium levels exceeded the permissible limit (mg/kg 0.3) in all treatments except the control. Similarly, a study in Kuwait by Alenzi et al. (2005) revealed that irrigating agricultural lands with industrial wastewater led to increased cadmium accumulation in wheat and barley, reaching toxicity levels for humans and animals, although the cadmium levels in plants remained nontoxic, aligning with the findings of this research (Al Enezi et al., 2004). Research by Ahmad et al. (2011) demonstrated that irrigation with varying amounts of municipal wastewater resulted in cadmium accumulation in canola's aerial parts reaching toxicity levels (Ahmad et al., 2011), which corroborates the results of the current study. Notably, the concentration of heavy metals in the control treatment was also relatively high, albeit not reaching toxicity levels. The elevated absorption of heavy metals in plants from the control treatment suggests that factors other than wastewater also play a significant role in heavy metal absorption by plants. It is also crucial to recognize that the dangerous concentration of cadmium for humans is considerably lower than the toxic concentration for plants. Consequently, plants growing in contaminated areas pose significant risks for consumer pollution (Saadat et al., 2023).

To investigate the capability of plants to absorb and transfer heavy metals from soil to roots and from roots to other organs, researchers proposed a factor known as the transfer factor (TF). According to Table 6, the transfer factor for cadmium from roots to aerial organs in M. officinalis was greater than that for lead across all treatments, excluding the control. This process can be attributed to the higher bioavailability of cadmium compared to lead.

Conclusion

The study concludes that using effluent for irrigation led to the accumulation of heavy metals, specifically lead and cadmium, in Moldavian Balm. Cadmium levels exceeded WHO standards for medicinal plants, highlighting serious environmental risks associated with wastewater irrigation. These findings emphasize the need for careful management and monitoring of wastewater used in agriculture to prevent potential contamination in medicinal and edible plants. If wastewater must be used, it should be properly treated to meet irrigation safety standards, ensuring both environmental and public health are protected.

Declarations

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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Consent to participate

Informed consent was obtained from all individual participants included in the study.

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Author contributions

Mosib Tayebi: Conceptualization, original draft writing, investigation, writing (including reviewing and editing), investigation, and formal analysis.

Hamidreza Pourkhabbaz: Conceptualization, supervision, and project administration.

Damon Razmjooei: Conceptualization, original draft writing, investigation, writing (including reviewing and editing).

Alireza Pourkhabbaz: Investigation, writing Saideh Javanmardi: Original draft writing.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki.

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