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Evaluation of acute phytotoxicity of raw leachate and landfill leachate using *Sorghum bicolor* seeds

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Abstract

Background: Leachate, a highly contaminated liquid, is produced by separating wastes and introducing moisture into the waste layers. Biological toxicity evaluation is a method that may be used to analyze the toxicity of leachate to organisms and plants. *Sorghum bicolor* was employed in this study to evaluate the acute toxicity of raw leachate and landfill leachate.

Methods: Leachate was collected from different locations within the refuse that was collected and mixed in a sampling container. The physiochemical characteristics of the leachate were analyzed in both raw leachate and landfill leachate. Germination rate, root weight, and root length were measured 24, 48, and 72 hours after planting for leachate dilutions of 25%, 50%, 75%, and 100%, respectively.

Results: Raw leachate had greater concentrations of metals (Fe, Zn, Cu, Mn, Cr, Cd, and Pb), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), nitrate, ammonia, and phosphate, as well as a more acidic pH (<6), as compared to landfill leachate. The results showed that landfill leachate had a lower potential for toxicity than raw leachate, with seed-germination rates of 0.1 and 0 in the presence of 75% landfill leachate after 48 and 72 hours, respectively, as opposed to 0.3 and 0.1 in the presence of raw leachate.

Conclusion: The findings show that raw leachate can include higher concentrations of metals and organic compounds, which can be one of the causes of *Sorghum* seed phytotoxicity. Waste leachate management is one of the most important pillars of environmental protection, and it should be taken into consideration by the right authorities.

Keywords: *Sorghum*, Germination, Leachate, Landfill, Phytotoxicity

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Introduction

One of the most difficult environmental problems in the world is still how to handle municipal solid waste (MSW). Population growth, industrial expansion, and urbanization all contribute to an increased daily per capita generation of municipal waste. In poor nations, open dumping and landfilling are the two most common waste management techniques (1,2). The collecting, processing, and disposal phases of this waste generate leachate. When improperly managed, it can result in the major environmental problems like air, water, and soil contamination as well as a variety of illnesses. Most often, landfilling in urban landfills is the most prevalent technique of final disposal (3-5).

The organic fraction of MSW is being treated using other practical methods, such as composting, the gasification process, burning, etc., due to the substantial amounts of degradable components in MSW and the environmental problems caused by landfills and disposal yards. This strategy reduces the strain on already-existing trash disposal infrastructure, the expense of waste disposal, and the environmental effects of landfilling organic matter (6-8). Following crushing and volume reduction to a minimum, the trash is dispersed in thin layers and covered with a layer of soil. After a landfill has been used, several changes, including physicochemical and biological reactions take place (9,10). Composting has the distinct benefit of being among the least expensive, easiest to use, and requiring the least amount of technical knowledge of all treatment methods. Additionally, as a byproduct, compost, an organic fertilizer, is produced. Leachate from such facilities, however, continues to be an issue that must be carefully managed before being released into the environment (1,11,12).

Leachate, a highly polluted liquid, is created when rainfall that enters the waste layers reacts with and decomposes the organic component. Waste leachate has extraordinarily high amounts of organic material, minerals, and microbes, all of which have the potential to produce a variety of contaminants in the environment, even if the volume and quality of waste leachate rely on some different factors (9,13,14).

The features of landfill leachate are frequently described using fundamental metrics such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), BOD/COD, suspended solids (SS), ammonia nitrogen, total nitrous oxide, and trace metals. Leachate has a variety of characteristics depending on the area. Leachate properties and landfill age have a significant correlation, which will help in choosing a treatment strategy (15,16). Waste leachate includes minor mineral nutrients that plants may utilize, such as iron, zinc, copper, manganese, and molybdenum, as well as the key mineral nutrients nitrogen, phosphorous, and potassium. Additionally, it has a significant quantity of organic matter, which might enhance the soil's permeability and structure. Furthermore, trace metals in high-salinity waste leachate should be studied for their impacts on soil, plants, and other species (13,17).

One of the most important components of managing MSW is determining the toxicity of waste leachate. The two most popular kinds of toxicity testing are in vitro and in vivo toxicology investigations. The in vitro method is used by researchers to focus on individual cells and various biological processes without being distracted or concerned by the presence of potentially disruptive factors in all species. To study the effects of a chemical or the evolution of a disease, in vivo research employs a range of experiments. Therefore, biological analysis of leachate toxicity can reflect the synergistic effects of all components and show toxicity if the compounds in the leachate are unknown (18,19).

Acute and chronic toxicities can both be evaluated physiologically. An acute assessment is made for 24 to 96 hours, and a chronic assessment is made for 7 to 30 days, to determine the impact of various leachate concentrations on biology, physiological function, and organism growth. Biomarkers in this experiment can include bacteria, microalgae, fish, and plants. Biological toxicity assessments (BTA) can be carried out on a laboratory scale, in a culture medium, or in the natural environment, i.e., in the field, to determine the cytotoxic, genotoxic, and estrogenic properties of substances (20). As a result, the BTA can effectively assess the toxicity of complex substances that cause toxicity in organisms, such as leachate (19).

As a low-cost and ecologically responsible method for the on-site treatment of leachate, phytoremediation has gained popularity recently. It is a flexible technology that may be applied in a variety of ways, such as irrigating vegetated land to grow biomass, horticultural, and energy crops, or using the nutrient film method. In the best-case scenario, phytoremediation of leachate (raw or partially treated) offers the chance to close the nutrient cycle loop and reduces the risk of environmental damages (21,22). However, in many situations, failure can be attributed to inappropriate leachate application and insufficient oversight due to an essential absence of knowledge of the plant soil ecosystem. It is crucial to comprehend how plant systems react to landfill leachate stress, specifically their tolerance mechanisms. Leguminous crops are particularly susceptible to leachate concerning their development and germination of seeds, according to several research (1,23). To investigate the toxicity of leachate, the plant *Vigna unguiculata* was selected for the present study.

It is crucial to comprehend how plant systems react to landfill leachate stress, specifically their tolerance mechanisms. Leguminous crops are particularly susceptible to leachate concerning their development and germination of seeds, according to several research (24). To investigate the toxicity of leachate, the plant *Sorghum bicolor* was chosen for the present study. The toxicity of landfill leachate has been the subject of several investigations, including those by Arunbabu et al (1), Kalčíková et al (25), and Jones et al (26). The fifth-most significant cereal in the world is *sorghum* (*Sorghum bicolor* (L.) Moench), a C4 plant. The ability of this plant to withstand abiotic challenges like salt and dehydration makes it ideally suited to semi-arid and arid environments (27). The emergence and germination phases of sorghum development have reportedly been shown to be the most instructive phases of the plant's life cycle for assessing the impact of toxicity (28).

In this study, the physicochemical properties of raw leachate and landfill leachate, including total suspended solids (TSS), total dissolved solids (TDS), COD, pH, ammonia, phosphate (PO_4^{3-}), nitrate (NO_3-N), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), chromium (Cr), cadmium (Cd), and lead (Pb) were analyzed. Additionally, the *S. bicolor* seed germination index was used to assess the acute toxicity of landfill leachate and raw leachate at the Shiraz municipal dump (Iran).

Materials and Methods

Study of landfill site

The leachates were collected from the landfill in Shiraz, Iran. The annual average temperature in Shiraz is 18°C. Currently, Shiraz city produces about 800 tons of waste every day. Organic, perishable, and organic materials constitute more than 70% of the waste in Shiraz. This garbage has a density of about 800 kg/m^3. To achieve a burial depth of 5 m and a covering layer thickness of 1 m, a minimum volume of the soil of 20 m^3/day is needed. The overall quantity of leachate generated in a year is 68%, the amount of compostable material is 75%, and 420 mm is calculated based on the data obtained from the rain gauge stations.

Leachate samples collection and characterization

In this study, the acute toxicity of raw leachate and landfill leachate was assessed using *S. bicolor* seedlings in the summer and winter of 2021. The study period was once every seven days. The samples were stored at 4°C in the refrigerator before use. The features of leachates were evaluated using the standard methods for the examination of water and wastewater (29). The leachate was collected from various areas throughout the collected waste and combined in a sampling container before being sent to the laboratory for further investigation. Then, physiochemical parameters of leachate, including TSS, total dissolved solids (TDS), COD, pH, ammoniac, PO_4^{3-}, NO_3-N, Fe, Zn, Cu, Mn, Cr, Cd, and Pb in raw leachate and landfill leachate were investigated. Landfill leachate is produced at the landfill site and, due to its lengthy retention period, undergoes purification by the bacteria that exist in the landfill, whereas raw leachate is produced directly from wastes without processing (30). COD was determined using the potassium dichromate oxidation technique (5220. B), and ammonia concentration was determined using a selective ammonia electrode (4500-NH_3. D) (24). Seven trace metals were measured in the leachate using an acid digestion technique (HNO_3) and atomic absorption spectrophotometry (3010. B) to assess the toxicity of the leachate. In this approach, 3 g of the sample was heated with three acids on a hot plate at 100 °C, then, the digested samples were put into a 25 mL flask, diluted twice with distilled water, and filtered before being examined with an atomic absorption spectrometer to quantify trace metals (31). Other leachate parameters were measured using the oxidation-reduction potential (2580. B), TDS (2540. C), EC (2520. B), TSS (2540. D), pH (pH meter, Wegtech Mi 151 22, UK), nitrate (4500-NO_3^-. D), and phosphate (4500-P. C) techniques (29).

Plant seeds preparation

The seeds for the *S. bicolor* plant were provided by Pakan Bazr Isfahan Company, Iran. To assess the acute toxicity of leachates, 10 seeds of *S. bicolor* (30 seeds three times) were sown in a petri dish with a diameter of 10 cm and a temperature of 27 °C in the incubator.

Germination and Growth

The Ecological Effects Test Guidelines OPPTS 850.4200-Seed Germination/Root Elongation Toxicity Test, which outlines the procedures and test conditions for determining the phytotoxicity of chemicals using seeds of land plants, was followed in the toxicity studies conducted on the raw leachate and landfill leachate (32). For each plate, 10 mL of leachate sample was filtered through a 0.45 µm diameter filter paper. Germination rate, root weight, and resultant root length were measured at 24, 48, and 72 hours after planting. Dilutions of the leachate of 25%, 50%, 75%, and 100% were assessed. The experiment treatments comprised a control sample of 10 mL of distilled water (33). The percentage ratios of relative root germination (Eq. 1), relative seed germination (Eq. 2), and germination index (Eq. 3) were determined using the following equations:

Relative root germination (RRG) = (R_E/R_K) *100 (1)

where R_E denotes the length of the sprouting root on the test sample, and R_K denotes the length of the sprouting root in distilled water.

Relative seed germination (RSG) = (S_E/S_K) *100 (2)

where S_E is the number of seeds germinating on the tested sample, and S_K denotes the number of seeds germinating in distilled water.

Germination index (GI) = (3)

Three replications of 10 seeds of this plant were tested (34).

Results

Characteristics of leachate samples

Table 1 demonstrates the physiochemical characteristics of landfill leachate and raw leachate. Seven significant

Table 1. Characteristics of landfill and raw leachate

Parameter	Value	
	Landfill Leachate	Raw Leachate
Nitrate (NO_3-N)	942 mg/L	1084 mg/L
COD	38000 mg/L	46570 mg/L
Ammonia	280 mg/L	510 mg/L
TDS	5200 mg/L	8730 mg/L
TSS	983 mg/L	995 mg/L
Phosphate (PO_4^{3-})	3.8 mg/L	5.6 mg/L
Cadmium (Cd)	1.15 mg/L	1.23 mg/L
Lead (Pb)	4.1 mg/L	5.2 mg/L
Iron (Fe)	<1 mg/L	<1 mg/L
Zinc (Zn)	<1 mg/L	<1 mg/L
Copper (Cu)	<1 mg/L	<1 mg/L
Manganese (Mn)	<1 mg/L	<1 mg/L
Chromium (Cr)	<1 mg/L	<1 mg/L
pH	8.3	5.9

elements, Fe, Zn, Cu, Mn, Cr, Cd, and Pb, were also measured in leachate to assess the toxicity of trace metals. In comparison with other chemical parameters, trace elements did not significantly decline. The chemical factors in raw leachate are greater than those in landfill leachate. However, acidity, nitrogen content, and electrical conductivity are typically greater in winter than in summer because of decreased COD and dissolved organic carbon (DOC) levels caused by increased summer temperatures. On the other hand, there are no discernible seasonal variations in the redox potential, metal levels, total suspended solids, or volatile suspended solids levels. Leachate from landfills had a pH of 8.3, indicating that the effluent was highly alkaline, whereas raw leachate had a pH of 5.9, indicating that it was relatively acidic. The outcome reveals that most of the compounds in the leachate are acidic and may have been produced by chemical reactions or as a consequence of the rubbish.

Germination and growth

The toxicity of raw leachate and landfill leachate on *Sorghum* seedlings is shown in Table 2. According to the findings, S*orghum* germination did not occur in any of the dilutions using raw leachate after 24 and 48 hours, 6 out of 10 seeds germinated in the dilution of leaching 25%, 4 out of 10 seeds germinated in the dilution of leachate 50%, and one out of 10 seeds germinated in the dilution of leachate 75%. After 48 hours, no germination was seen in the 100% leachate dilution. After 72 hours of incubation, 4 out of 10 seeds germinated in a 25% leachate dilution, and 2 out of 10 seeds germinated in a 50% leachate dilution. After 72 hours, there was no germination in the 75% dilution. After 72 hours, there was no germination in the 100% leachate dilution. The average length of buds exposed to raw leachate in 48-hour dilutions was 0.4, 1.1, and 1.4 cm, respectively, according to the measurements of the mean height of buds exposed to raw leachate. Bud lengths in 25% and 50% leachates were 0.9 and 1.7 cm in 72-hour dilutions, respectively. After 48 hours, the average weight of sprouts exposed to leachate was 25%, 50%, and 75%, with mean weights of 0.35, 1.4, and 0.93 g, respectively. This value was 0.84 and 2.1 g in 72-hour dilutions for buds exposed to 25% and 50% leachate, respectively. Germination was not found in any leachate percentages after 24 hours in landfill leachate dilutions. 0.7, 0.6, and 0.3 seeds germinated in 25%, 50%, and 75% dilutions after 48 hours. After 72 hours, 25%, 50%, and 75% of the seeds germinated at 0.8%, 0.5%, and 0.1% dilutions. At 100% leachate dilution, no germination was observed. After 48 hours, the average buds in dilutions 25%, 50%, and 75% were 0.7, 1.3, and 1.5 cm, respectively, and after 72 hours, were 1.1, 2.4, and 0.9 cm. After 48 hours, the mean bud weight in 25%, 50%, and 75% dilutions was 0.56, 1.9, and 2.1 g, respectively. After 72 hours, this parameter changed to 1.7, 2.2, and 1.6 g. After 24 and 48 hours, there was no germination in the blank sample, but after 72 hours, 0.9 seeds germinated, with an average length and weight of buds of 1.2 ± 0.26 cm and 1.7 ± 41 g, respectively.

Discussion

As shown in Table 1, the raw leachate has higher values for the chemical parameters than landfill leachate that may result from the leachate's bacteria, which consume organic materials for growth and contribute to leachate purification (35). Trace metals did not decrease considerably compared to other chemical parameters, which may be related to the environment's toxicity and bioaccumulation of water. On the other hand, by lowering the pH of the leachate, trace metals become more soluble, which may prevent bacteria from accessing them and eventually diminish removal effectiveness (36). Due to fluctuations in humidity level and waste mixture, and also, seasonal variables including temperature and rainfall, landfill leachate properties show significant variability. The content and characteristics of the landfill leachate can be influenced by some important factors, including landfill age (37,38). Depending on how old the landfill is, landfill leachate may be separated into three groups. Younger landfill leachate has a higher biodegradability index (BOD/COD) and a low pH level. It is typically formed of low-molecular-weight hydrophilic organic compounds. Old landfill leachate, in contrast, mostly consists of humic acid and fulvic acid with high-molecular-weights, consequently in a high pH and lower biodegradability index. Because the higher pH value affects the solubility of heavy metals, the concentrations of heavy metals often decrease with age (39,40). The pH of landfill leachate was 7.36 to 8.8, which is consistent with others landfill disposal in Brazil,

Table 2. Evaluation of acute toxicity of raw leachate and landfill leachate on *Sorghum* seeds

Leachate	Time	Germination Index				Buds' length (cm)				Buds' Weight (g)			
		25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%
	24 h	-	-	-	-	-	-	-	-	-	-	-	-
Raw	48 h	0.6	0.4	0.1	-	0.4	1.1	1.7	-	0.35	1.4	0.93	-
	72 h	0.4	0.2	-	-	0.9	1.7	-	-	0.84	2.1	-	-
	24 h	-	-	-	-	-	-	-	-	-	-	-	-
Landfill	48 h	0.7	0.6	0.3	-	0.7	1.3	1.5	-	0.56	1.9	2.1	-
	72 h	0.8	0.5	0.1	-	1.1	2.4	0.9	-	1.7	2.2	1.6	-

Ampar Tenang (Malaysia), Italy, and Malaysia (37,41-43). The amounts of several physiochemical parameters in leachate were comparable to those found in previous and present research. Nonetheless, the concentrations of heavy metals varied according to the type of buried waste, the communities' cultures, the materials and consumables utilized in the goods that the communities needed, and the waste processing technique (44,45).

Landfill leachate comprises a variety of inorganic and xenobiotic organic components, such as hydrophobic, volatile, aromatic, and aliphatic organic molecules, the combination of which has an impact on plant germination (46,47). The influence of landfill leachate was assessed, however, a toxicological examination of landfill leachate is required on the natural environment (19). The USEPA has recommended toxicity testing based on seed germination and root growth to evaluate the potential for contamination of waste and effluents released into the environment (32). This approach is usually a part of a broader effluent toxicity management program (48). According to several studies, leachate can cause both positive and negative reactions in plants (49). The harmful effect of leachate on seed germination is influenced by several factors, including the plant species and interspecific changes in seed structure, notably the seed coat, which comes in various anatomical shapes (50,51).

After 24 hours, none of the seeds germinated, which might be attributed to the plant's physiology and the lack of regular *Sorghum* germination. *Sorghum* seedlings exposed to raw leachate and landfill sprouted after 48 hours. However, the blank sample did not germinate after 48 hours. The germination rate is low in blank samples owing to the absence of nutrients that the plant needs, and based on the duration of germination time taken into account in this investigation, it was not possible to see the germination of this plant's seeds in the blank sample. Germination stimulants in raw leachate and landfill leachate might cause quicker germination in leachate exposure dilutions than in a blank sample (52). The germination rate in raw leachate was lower than that in landfill leachate, which is presumably owing to raw leachate's more significant toxicity (18,53). At 50% dilution, the maximum length and weight of buds were attained, providing the best circumstances for germination. Due to the toxicity of the leachates, the exposure to 100% leachate did not result in germination. Even 75% leachate resulted in a reduction in germination as well as the length and weight of the buds. The acute toxicity of incinerator ash on *shrimp* crop seedlings was researched by Ebrahimi et al. They found incinerator ash acutely hazardous to crop seeds (9).

Several study plants including *Hordeum vulgare* L., *Vicia faba* L., *Zea mays* L., *Lactuca sativa* L., and *Lepidium sativum* L. have been employed to examine the phytotoxicity of leachate (53-56). Olivero-Verbel et al and Pablos et al have also investigated the close associations between leachate physicochemical characteristics and toxicity. Due to its large biomass and deep roots, hemp (*C. Sativa* L.) has been shown in several studies to collect a significant quantity of trace metals from polluted soils, making it an excellent candidate for remediation (57-60). In a study by Palm et al, the physiological changes used to determine the tolerances of two herbaceous plants, *Sinapis alba* L. (mustard) and *Triticum aestivum* L. (wheat) to rising fractions of leachate generated from an MSW facility in the Czech Republic. They found that when growth liquids with > 50% leachate were used, many physiological changes were reduced at increasing leachate contents, particularly the lengthening of seedling roots. Photosystem II performance and chlorophyll pigments quantities were so much more responsive in *T. aestivum*, demonstrating species-dependent variations, whereas *S. alba* was more susceptible to greater levels of leachate according to the growth characteristics of the stem tissues (61). Anand and Palani carried out an analysis with an emphasis on the production, characterization, and toxicity evaluation including both inactive landfill leachate and ongoing landfill leachate to assess and determine their polluting potential. The findings showed that inactive landfill leachate production is greater than ongoing landfill leachate production (188.59 m^3/d vs. 49.53 m^3/d). In comparison to ongoing landfill leachate, the quantities of the primary characteristics including physiochemical and biological components were greater in inactive landfill leachate. Additionally, the germination indexes of ongoing landfill leachate (79.14%) and inactive landfill leachate (57.48%) confirmed that the former had a larger potential for phytotoxicity (62).

As shown in Table 3, comparisons between the germination index of several plant seed species and various types of leachates have been conducted in previous research. The acquired results showed that the germination index was extremely low in leachates with high concentrations of heavy metals and superfluous minerals, such as sodium chloride, which is compatible with the findings of this study. On the other hand, the amount of necessary minerals varied depending on the circumstances of the leachate that was created, which encouraged the growth of various plant species and served as an effective plant fertilizer for the seeds.

Conclusion

The toxicity of the leachate to several species, including plants, was examined in this study using the BTA. The acute toxicity of raw and landfill leachate was evaluated in this study using *S. bicolor* seeds. Metals Fe, Zn, Cu, Mn, Cr, Cd, Pb, COD, TDS, TSS, nitrate, ammonia, and phosphate were among the parameters in landfill leachate and raw leachate that were analyzed. It was found that raw leachate, on the other hand, has higher concentrations of the metals Cd, Pb, COD, TDS, TSS, nitrate, ammonia, and

Table 3. Comparative evaluation of germination index in different studies

Leachate type	Exposure Time (h)	Dilution (%)	Plant seed species	Germination index	Ref.
Raw leachate	96	10, 50, 100	*Lolium perenne* *Hordeum vulgare* L.	1.28, 1.21, 1.21 1.07, 1.28, 1.50	(63)
Digested leachate	96	10, 50, 100	*Lolium perenne* *Hordeum vulgare* L.	1.15, 1.19, 1.30 1.05, 1.06, 1.16	
Raw leachate	72	100	*Raphanus sativus* *Lycopersicum esculentum* *Lepidium sativum*	0.06 0.08 < 0.01	(64)
Treated leachate	72	100	*Raphanus sativus* *Lycopersicum esculentum* *Lepidium sativum*	0.4 0.3 0.2	
Contaminated water with landfill leachate	72	25, 50, 100	*Raphanus sativus* *Solanum lycopersicum*	0.19, 0.13, 0.05 0.19, 0.06, ND*	(65)
Landfill leachate	168	100	*Zea may* *Phaseolus vulgaris*	0.97 0.63	(66)
Crude leachate	72	100	*L. Sativa* *Cucumis sativus*	0.14 0.26	(67)
Treated leachate by rotating biological reactor	72	100	*L. Sativa* *Cucumis sativus*	1.32 1.13	
Raw leachate	72	25, 50, 75, 100	*Sorghum Bicolor*	0.4, 0.2, ND, ND	The present study
Landfill leachate	72	25, 50, 75, 100	*Sorghum Bicolor*	0.8, 0.5, 0.1, ND	

ND: not detectable

phosphate, as well as an acidic pH. Due to the increased concentration of raw leachate compounds compared to landfill leachate, the rate of root and stem development was much slower in the seeds exposed to raw leachate than that in the seeds subjected to landfill leachate. In addition, the findings showed that landfill leachate has a lower potential for toxicity than raw leachate, as evidenced by seed germination rates of 0.1 and 0 after 48 and 72 hours, respectively, in the presence of 75% landfill leachate, but 0.3 and 0.1 in the presence of 75% raw leachate. This study suggests that organic compounds and heavy metals may be found in raw leachate and landfill leachate, and they may be one of the factors contributing to sorghum seed poisoning.

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Authors' contribution

Conceptualization: Hassan Hashemi, Nasim Salehi, Saeed Rajabi.
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Funding acquisition: Hassan Hashemi.
Investigation: Nasim Salehi, Saeed Rajabi.
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Project administration: Hassan Hashemi, Nasim Salehi, Saeed Rajabi.
Resources: Hassan Hashemi.
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Supervision: Hassan Hashemi, Saeed Rajabi.
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Writing–review & editing: Hassan Hashemi, Nasim Salehi, Saeed Rajabi, Kaan Isinkaralar.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical issues

This research with the ethical code of IR.SUMS.REC.1398.492 was conducted in the Department of Environmental Health Engineering, Shiraz University of Medical Sciences, Shiraz, Iran.

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