Research Paper

Investigating the Effect of Vermifiltration With Different Bulking Agents on Some Important Properties of Municipal Sewage Influent

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ABSTRACT

Background: Farmers have been persuaded to use wastewater in agriculture due to drought and water deficiency. However, an important challenge is the environmental constraints

Methods: This study investigated the effect of vermifiltration on the properties of sewage influent. The sewage influent was obtained from the aerated lagoons of the Qahdarijan wastewater treatment plant, Isfahan Province, Iran. The sewage was mixed with Rice Husk (RH) and Wheat Straw (WS) as the bulking agents at three proportions (0%, 5%, and 10%v/v). Then, EarthWorm (EW) (*Eisenia fetida*) were added to the sewage sludge in two proportions (0 and 50 adult earthworms/6 kg sewage sludge).

Results: The results showed a significant decrease in electrical conductivity (about 100% decrease compared to the control), total dissolved solids (up to 3 times in RH0+ EW treatment less than the control), total suspended solids (up to 90 times in WS10+EW and RH+EW treatments decrease compared to the control), biochemical oxygen demand (significant decrease in RH levels+EW compared to other treatments), and chemical oxygen demand (a reverse status was observed for WS and RH treatments, but the role of vermifiltration was considerable). The concentration of Pb was significantly decreased in WS when it was enriched with earthworms (about an 8% decrease compared to control), but a reverse status was observed for RS treatment.

Conclusion: Vermifiltration may be an efficient tool for improving the properties of wastewater to use in agriculture, but more studies are suggested to evaluate different aspects of this technique.

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1. Introduction

Based on the 2020 UN World Water Development report, water use has increased six-fold over the past century and is rising by about 1% annually [1]. However, different factors, including climate change and increasing frequency and intensity of extreme events, will deteriorate the situation in developing countries undergoing 'water stress' and may generate similar challenges in regions that have not been harshly affected [1].

Composts, sewage sludge, and wastewaters are used in agriculture because of their high contents of nutrients and organic compounds to improve the nutritional status and growth of plants in large areas [2-4]. Farmers have been persuaded to use wastewater in agriculture because of drought and water deficiency [5]. The position and importance of wastewater in agricultural activities are widely known. Different investigations have reported that wastewater has a significant amount of essential nutrients and organic matter [6, 7], and its soil application as the irrigation water may change the physical, chemical, and biological properties of the soil [8].

However, municipal wastewater is a main source of pollution in aquatic environments [9]. The final quality of sewage sludge, which is the main by-product of the wastewater treatment process, is determined by the chemical composition of the influent wastewater and its treatment processes [10]. In most cases, wastewater treatment processes do not warranty the observational removal of many contaminants and thus could lead to another round of environmental pollution after discharge [11]. The most usual chemical pollutants in wastewater are potentially toxic metal ions, hydrocarbons, pesticides, nitrogenous compounds, pharmaceutical residues, detergents and phosphorus, different kinds of protozoa, viruses, and bacteria [12].

Therefore, if wastewater is treated correctly, it can be used as a potential nourishment resource and irrigation water in agriculture. The treatment of wastewater could be biological or chemical [13]. The fundamental basis for the biological treatment of wastewater is oxidation and biodegradation. Among all the existing options, green technology such as vermifiltration is the natural and sustainable choice. Vermifiltration is an extension of the vermicomposting process. It is a biofilter with earthworms, where the earthworms digest the suspended particles screened on the filter bed and degrade organic matter through enzymatic activity. In the process of ingestion, they passively aerate the system by burrowing and removing pathogens.

Contrary to conventional composting, vermicomposting creates a homogeneous yield with greater quality in terms of reduced contaminants and more soluble plant nutrients [14]. Despite the increased concentration of heavy metals in the sewage sludge during the composting process, the amount of bioavailable forms decreases. The reduction in the availability of heavy metals during the composting process is due to the formation of a complex with humus [15-18]. Based on the findings reported by Stover et al. and Karthick et al., less than 17% of zinc, lead, and cadmium and about 22% of nickel in the sewage sludge are in a form that plants can easily use [19, 20]. The use of earthworms in the composting process is a valuable technology for sludge and wastewater management. The activity of earthworms maintains aerobic conditions and increases the rate of microbial decomposition. In this process, the weight of worms increases, and the wastes of earthworms, which are very rich in nutrients, are replaced [20].

Adding bulking agents to the sewage sludge increases the activity of the worms and improves the quality of the product. In a study by Gondek and Filipek-Mazur, sawdust, cardboard, and wheat straw were used as bulking agents with a mixing ratio of 15% to prepare vermicompost from sewage sludge [21]. They observed that a higher abundance of organic matter, nitrogen, calcium, and sodium for both untreated tannery sludge and sludge composted by *Eisenia fetida* and contamination with heavy metals, apart from chromium, remained within the acceptable limits. In India, Arunugam et al. [22] used mixtures of sewage sludge, rice straw, and animal manure to prepare vermicomposting. Vermicomposting is being utilized within commercially available on-site waste treatment systems. However, few studies have examined this medium for the purpose of wastewater treatment [23]. Therefore, this research investigated the effect of vermicomposting and using different levels of rice husk and wheat straw as bulking agents on some important chemical properties of wastewater.

2. Materials and Methods

Geographical location of the research site

This study was conducted in the Ghahderijan wastewater treatment plant (Latitude: 32° 30' and Longitude: 51° 30' N), Isfahan Province, Iran, from November 1, 2019, to February 12, 2020. Ghahderijan has a semi-arid climate, with warm summers and semi-cold winters. Ac-

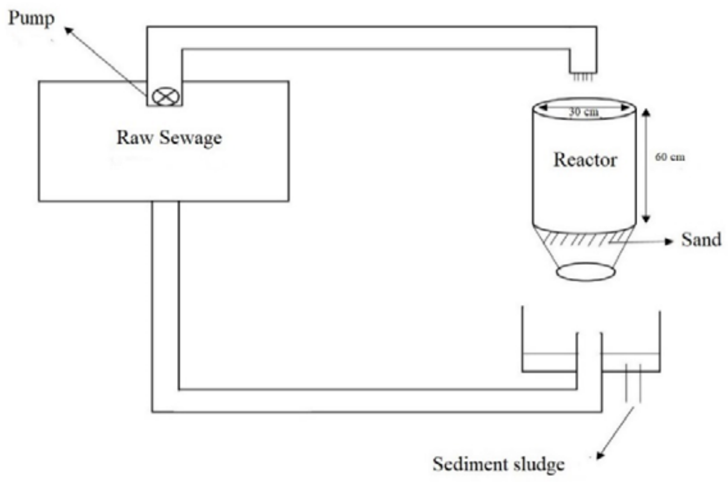

Figure 1. Reactor design in this study

cording to the World Reference Base (2015), the soil of this region is classified as Gleysol, whereas it is categorized into the Inceptisols order in Soil Taxonomy.

Study materials

The studied treatments were two organic bulking materials (Rice Husk; RH and Wheat Straw: WS) at three proportions (0%, 5%, and 10% v/v) and earthworms at two proportions (0 and 50 adult earthworms /6 kg sewage sludge) in three replications. Also, 36 reactors were used for the treatments:

36 reactors=2 types of bulking agents×2 proportions of earthworm×3 proportions of the bulking agents×3 replications

The reactors were cylinders with a height of 30 cm and a diameter of 20 cm (Figure 1). For better drainage, some washed sand was placed at the bottom of the reactors in a 10-cm layer. For the vermifiltration of the wastewater, we used *Eisenia fetida*, belonging to the Lumbricidae family and Lumbricus genus.

Experiment design

The used sewage influent in this experiment was prepared from the aerated lagoons of the Ghahderijan wastewater treatment plant. The sewage was collected in large size pre-cleaned circular plastic containers of 20 L capacity and was brought immediately to the laboratory and collected in a large-size reservoir. Wastewater was collected just before the experimentation to avoid alternation in the wastewater characteristics, mainly due to open storage of the sample. Before putting sewage influent in the experimentation cycle, a sample of sewage influent (about 1 L) was separated from stock and analyzed for its physicochemical characteristics using standard methods (Table 1). For the experiment, the sewage was mixed with Rice Husk (RH) and Wheat Straw (WS) as the bulking agents. These materials were used in this study because of their abundance in the region as an agricultural waste, their high C/N ratio, which supplies energy for microorganisms, and their high specific surface area. The size of the bulking agents (rice husk and wheat straw) was 10-15 mm and was mixed with the raw sludge in 0%, 5%, and 10% (v/v) amounts. Then, 50 adult earthworms (*Eisenia fetida*) were added to each of the 18 reactors, and 18 reactors were without vermicomposting [14]. To avoid the escaping of earthworms, the nonwoven fabric was used to cover the reactors, and the reactors were set in a dark room at 25°C, and the moisture content of the mixture was preserved at 75% field capacity because the earthworm's activity is optimum under this moisture condition [24]. The required moisture was supplied from the returned wastewater from the reactors (Figure 1) for 30 days. At the end of the 30th day, a sample of wastewater was collected in a pre-cleaned and sterilized polythene bottle of 1 L capacity from the outlet of the reactor and stored at 4°C for further investigations on changes in physicochemical characteristics of wastewater during the experiment and sent to the laboratory. The properties of the samples were determined in accordance with the standard methods for the examination of water and wastewater [25]. Before determining the nitrogen concentration, the samples were filtered through a membrane filter (polypropylene, 0.45 µm pore size; Membrane Solutions Co. Ltd., Minato-ku, Japan). The indophenol method was used to determine NH_4^+-N concentration, and the N-(1-naphthyl) ethylenediamine and UV adsorption (275 nm) methods were used to determine NO_3^--N concentrations. Total Dissolved Solids

Table 1. Some important properties of the municipal sewage influent

Trait	Values
Total Dissolved Solids (TDS)	8560 mg/L
Total Suspended Solids (TSS)	0.95 mg/L
Turbidity	60 NTU
pH	7.9
Electrical Conductivity (EC)	10.93 dS/m
Chemical Oxygen Demand (COD)	325.7 mg/L
Biochemical Oxygen Demand (BOD)	175 mg/L
Total Nitrogen	30.4 mg/L
Phosphorous	13.21 mg/L
Chromium (Cr)	0.09 mg/L
Lead (Pb)	0.65 mg/L
Cadmium (Cd)	0.065 mg/L
Nickel (Ni)	0.55 mg/L

(TDS) and Total Suspended Solids (TSS) in the wastewater samples were quantified by the gravimetric method. The Electrical Conductivity (EC) of the samples was measured with a salinometer. The pH was measured by the Hanna Instrument, which was allowed to settle for 10 minutes before measurement. The Biochemical Oxygen Demand (BOD) was determined by the standard oxidation procedure after 5 days at 20°C, while the Chemical Oxygen Demand (COD) and turbidity were determined by a UV-Vis spectrophotometer according to procedures clearly explained in detail by Sinha et al. [26]. Phosphate was determined using a UV spectrometric method [27]. To measure the concentration of heavy metals, the samples were filtered and analyzed for Pb, Ni, and Cr using (ICP-OES) type Perkin Elmer 3300 DV ICP (USA) according to other methods [25].

Statistical analysis

Statistical analysis of the data was done by SPSS software v. 16 (SPSS Inc., Chicago, Ill.), and the means were compared according to Duncan's Multiple Range Test (DMRT) ($P<0.05$).

3. Results and Discussion

The results of variance analysis are shown in Table 2.

pH and EC

The earthworms increased the pH values in the bulking agents, as the highest increase in pH happened in 10% v/v wheat straw + earthworm. Furthermore, it was observed that an increase in pH of the treatments was correlated to the rate of application of the bulking agent (Figure 2). Most studies have shown that the adsorption of metal ions is affected by the concentration of H^+ ions in the medium, and acidic pH values between 3 and 5 are suitable for the adsorption of metal ions. Since functional groups binding to metal ions, such as carboxyl groups, have an acidic nature, their availability depends on the pH of the environment [28]. In general, our results showed that pH in the absence of earthworm is lower than in the treatments with earthworm because there are more various and further cations and anions in the samples with vermicompost. This result agrees with the observation made by Zularisam et al. [29]. Furthermore, it was reported that one of the ways to precipitate heavy metals is to increase the pH solution [30]. Earthworms can increase the pH of their culture medium. Secondly, the easiest and cheapest way to remove toxic metals from the environment is to increase the pH of the environment, converting heavy metal ions to insoluble forms such as metal hydroxides [31]. Various factors affect the decrease of pH with increasing retention time, such as

Table 2. Variance analysis of the effect of the treatments on the properties of wastewater

Source of Variance	df	Mean Squares						
		P	N	TDS	TSS	EC	pH	Pb
Earthworm (W)	1	15104.4 **	1144.6 **	4851006**	0.05**	5.21**	1.152**	0.0003 ns
Bulking agent (H)	1	23.04**	94.74**	8127251**	0.45**	17.50**	0.107 ns	0.0003 ns
Rate of application (P)	2	6.11 **	2674.3 **	38468344**	0.54**	32.30 **	0.354 **	0.0002 ns
W×H	1	113.07 **	235.11 **	1013713**	0.10**	1.40 **	0.130ns	0.0025 **
W×P	2	25.56 **	613.29 **	29232533**	0.06**	28.18**	0.048ns	0.0005 ns
H×P	2	18.39 **	121.81 **	423770**	0.41**	2.00 **	0.003 ns	0.0005 ns
W×H×P	2	8.27 **	111.76 **	2128415**	0.04**	0.45 **	0.036 ns	0.0002 ns
Error	24	0.10	3.21	2157	0.0002	0.07	0.06	0.0002

Source of Variance	df	Mean Squares					
		Cr	Ni	COD	BOD	$N-NO_3$	$N-NH_3$
Earthworm (W)	1	0.00004 ns	0.004 ns	314778564 **	92323272**	42.1**	1595.2**
Bulking agent (H)	1	0.00010 ns	0.054 ns	4010013408 **	590417102 **	71.8**	63.9**
Rate of application (P)	2	0.00013 ns	0.077 ns	759869117 **	42920831 **	37.0**	14.4**
W×H	1	0.00004 ns	0.028 ns	751198464 **	121922083 **	104.2**	1336.6**
W×P	2	0.00001 ns	0.010 ns	182556872 **	9628746**	15.4**	19.5**
H×P	2	0.00013 ns	0.017 ns	676419800 **	30000272**	15.1**	65.5**
W×H×P	2	0.00001 ns	0.020 ns	173625022 **	7977518**	26.8**	52.7**
Error	24	0.00007	0.03	62157	12844	0.008	0.08

P: Phosphorous; N: Nitrogen; TDS: Total Dissolved Solids; TSS: Total Suspended Solids; EC: Electrical Conductivity; Pb: Lead; Cr: Chromium; Ni: Nickel; COD: Chemical Oxygen Demand; BOD: Biochemical Oxygen Demand.
*and ** respectively significant at statistical levels of 5% and 1%. ns: non-significant based on DMRT (P<0.05).

the decomposition of organic compounds associated with the production of carbonic acid [32]. Furthermore, increasing the intensity of the nitrification process decreases the pH [33]. Since this process is a biochemical source for the production of hydrogen ions, the denitrification process acts reversely [34]. The Environment Organization of Iran reported that the pH for wastewater used in agriculture and irrigation should be between 6 to 8.5, United States Environmental Protection Agency (USEPA) reported that for commercially processed food plants, pH should be between 6 and 9, and Environmental Protection Agency (EPA) permitted the pH from 6.5 to 8.4. Accordingly, the produced wastewater in this study has a suitable pH.

The studied treatments (both earthworm and the bulking agents) significantly decreased the EC up to 100% compared to the control treatment. The measured EC in rice husk treatments, both in the presence and absence of earthworms, was lower than in the wheat straw treatments (Figure 3). Also, in the bulking agents, vermicomposting increased EC of the wastewater, which on the one hand, is due to an increase in the temperature and the produced gases from transpiration, such as CO_2, and on the other hand, an increase in the concentration and mobility of ions in the treatments. These results are in line with those obtained by Wang et al. [35]. Also, EC is one of the most important parameters in evaluating the quality of wastewater used in agriculture [36]. According to the standard of the World Food and Agriculture Organization (FAO),

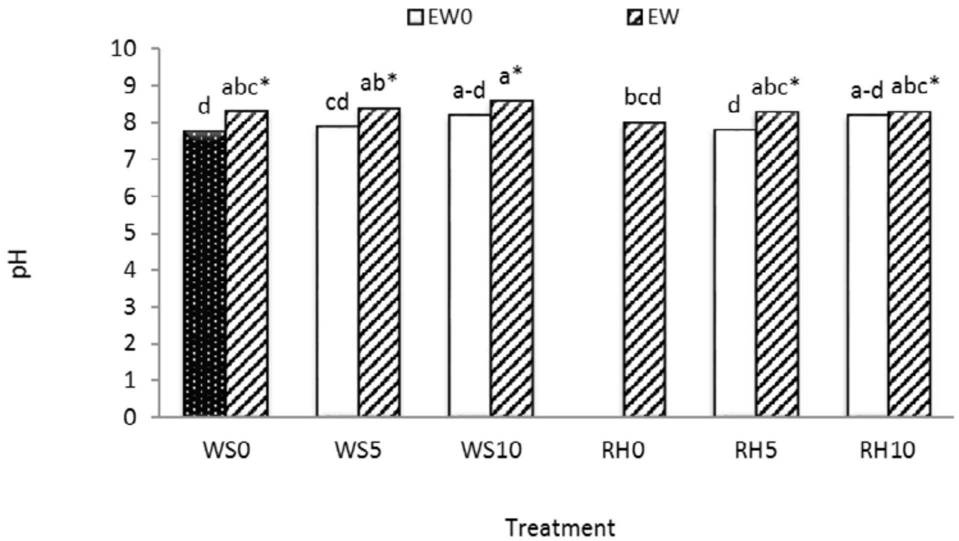

Figure 2. Effects of the treatments on the wastewater pH

EW0: without vermicomposting; EW: with vermicomposting; WS0: without wheat straw; WS5: 5% v/v wheat straw; WS10 10% v/v wheat straw; RH0 without rice husk; RH5: 5% v/v rice husk; RH10: 10% v/v rice husk.

Means in the same column followed by the same letter are not significantly different according to DMRT at (P≤0.05).

the maximum allowable amount of EC in the treated wastewater that causes a low to medium limitation for irrigation is equal to 3 dS/m, and the values that are less than 0.7 dS/m does not impose any limitations in this regard [4]. None of the studied wastewaters have reached the allowable level of 0.7 and cannot be used for irrigation in agriculture. In a similar study, Ghasemi et al. [37] stated that the use of wastewater with a salinity content between 1 and 3 dS/m could have low risks to soil and plants, and salinity management methods in the field should be considered. Therefore, RH5+EW0 and RH10+EW0, whose ECs were about 3.03 and 3 dS/m, can be used, with low to medium limitations for the soil and plant.

Total suspended solids and total dissolved solids

The studied treatments significantly decreased TSS compared to the control treatment. In the wheat straw treatments, which were enriched with earthworm, the amount of decrease of TSS in the treatments was more than in the others. In other words, vermicomposting with wheat straw as the bulking agent was the superior treatment in decreasing TSS (Table 3). The wheat straw

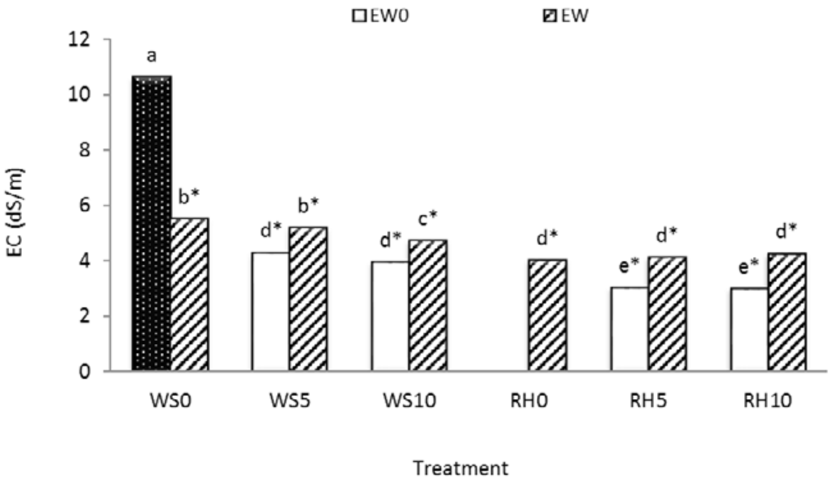

Figure 3. Effects of the treatments on wastewater EC

EW0: without vermicomposting; EW: with vermicomposting; WS0: without wheat straw; WS5: 5% v/v wheat straw; WS10: 10% v/v wheat straw; RH0: without rice husk; RH5: 5% v/v rice husk; RH10: 10% v/v rice husk.

Means in the same column followed by the same letter are not significantly different according to DMRT at (P≤0.05).

Table 3. Influence of different treatments on the status of Nitrogen (N), Phosphorous (P), Total Dissolved Solids (TDS), and Total Suspended Solids (TSS)

Treatment	N	P	TDS	TSS
	mg/L			
Control	30.40[e]	12.45[e]	8320[a]	0.97[a]
WS0EW	43[d]	45.27[d]	5245[b]	0.5[b]
WS5EW0	40.33[d]	6.04[g]	3200[f]	0.08[cd]
WS5EW	80[a]	45.33[d]	4478[c]	0.06[de]
WS10EW0	60.77[c]	5.18[h]	2900[g]	0.05[ef]
WS10EW	60.60[c]	45.37[d]	3501[d]	0.01[g]
RH0EW	31.17[e]	49.18[c]	2800[h]	0.09[c]
RH5EW0	43.33[d]	7.07[f]	2175[i]	0.03[fg]
RH5EW	60.60[c]	50[b]	3200[f]	0.07[cde]
RH10EW0	65.50[b]	4.77[h]	2001[j]	0.01[g]
RH10EW	62.77[bc]	52.25[a]	3367[e]	0.05[ef]

Control (without any bulking agents and earthworm); WS0EW and RH0EW: with earthworms and without any bulking agent; WS5EW0 and RH5EW0: respectively 5% v/v wheat straw and 5% v/v rice husk without earthworms; WS5EW and RH5EW: respectively 5% v/v wheat straw and 5% v/v rice husk with earthworms; WS10EW0 and RH10EW0: respectively 10% v/v wheat straw and 10% v/v rice husk without earthworms; WS10EW and RH10EW: respectively 10% v/v wheat straw and 10% v/v rice husk with earthworms.

Means in the same column followed by the same letter are not significantly different according to DMRT at (P≤0.05).

treatments significantly decreased TDS compared to the control treatments, but a reverse status was observed for the rice husk treatments. However, treating the bulking agents with earthworm increased TDS (Table 3). In general, vermicomposting with rice husk as the bulking agent was relatively the superior treatment in reducing TDS. Earthworms' bodies act as a 'biofilter' and remove the TDS and TSS from wastewater 'ingestion' and biodegradation of organic wastes, heavy metals, and solids from wastewater by their 'absorption' through body walls [26]. However, the measured TDS was greater than the standard value reported by WHO (450 mg/L) [36].

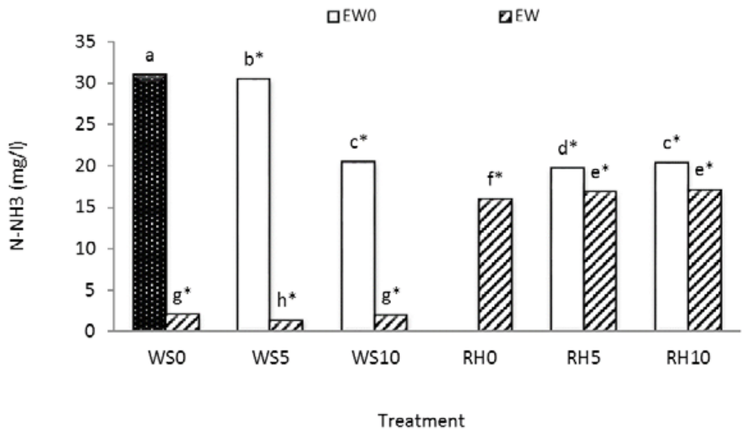

Figure 4. Effects of the treatments on the concentration of N-NH_3 in the wastewater

EW0: without vermicomposting; EW: with vermicomposting; WS0: without wheat straw; WS5: 5% v/v wheat straw; WS10: 10% v/v wheat straw; RH0: without rice husk; RH5: 5% v/v rice husk; RH10: 10% v/v rice husk.

Means in the same column followed by the same letter are not significantly different according to DMRT at (P≤0.05).

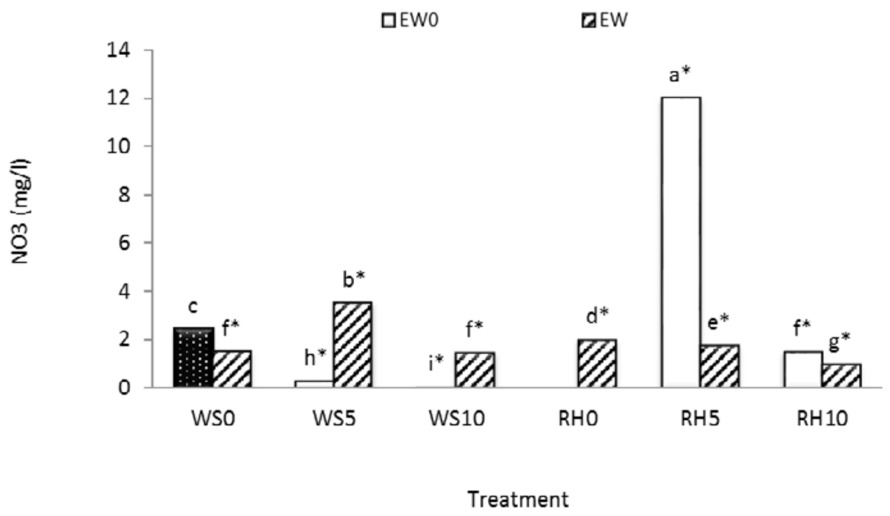

Figure 5. Effects of the treatments on the Concentration of NO_3 in the Wastewater

EW0: without vermicomposting; EW: with vermicomposting; WS0: without wheat straw; WS5: 5% v/v wheat straw; WS10: 10% v/v wheat straw; RH0: without rice husk; RH5: 5% v/v rice husk; RH10: 10% v/v rice husk.

Means in the same column followed by the same letter are not significantly different according to DMRT at ($P \leq 0.05$).

Total Nitrogen (N) and Phosphorous (P)

Investigation of the total nitrogen (N) and phosphorous (P) of the wastewater in the studied treatments showed that the studied treatments significantly affected the N and P contents of wastewater. The highest content of N was measured in 5% v/v wheat straw treatment and in the absence of earthworm, which had a significant difference from other treatments and the lowest content belonged to the control treatment (Table 3). Also, the results showed that in most treatments, vermicomposting had a positive effect on increasing N content in the wastewater. This result is in line with that obtained by Boruszko [38]

Vermicomposting also significantly increased the P content of wastewater, and the highest concentration of P was measured in the bulking agents which had received earthworm. Based on the reports of Zularisam et al. [29], vermicomposting can be an efficient technology for transforming unavailable forms of phosphorus into readily available forms for plants. It is hypothesized that vermicomposting process releases total phosphorous content from organic waste due to the activity of earthworm's phosphatases. In addition, further release of total phosphorous is attributed to the phosphorus solubilizing microorganisms present in the worm casts [29]. The results also showed that the highest concentration of P was seen in 10% v/v rice husk when treated by vermicomposting (RH10+EW) (Table 3). These results are consistent with the findings of Liu et al. [39]. They observed that vermicomposting significantly changed physicochemical parameters of wastewater in nutrients, such as the percentages of total nitrogen, phosphorous, and potassium, which were found to increase during vermicomposting.

N-NH_3 and N-NO_3

The studied treatments significantly affected the N-NH_3 and N-NO_3 content of wastewater ($P<0.01$). The highest content of N-NH_3 was measured in the control treatment, and vermicomposting with wheat straw as the bulking agent was superior to rice husk levels in reducing N-NH_3 content in wastewater (Figure 4).

Among the treatments, the amount of increase in nitrate was higher in WS+EW treatments than RH+EW treatments. In the rice husk treatments, vermicomposting decreased the amounts of nitrate nitrogen more than the non-vermicomposting treatment. The highest content of N-NO_3 was measured in 5% v/v rice husk and the absence of earthworm (i.e., RH5+ EW0), while the lowest content was recorded in WS10+EW0, which had a significant difference from other treatments (Figure 5).

As an organic fertilizer, the bioavailability of N is an important and limiting factor for the agricultural application of vermicompost because N is an essential macronutrient for plants and improvement of soil health and quality [40]. One of the mechanisms for maintaining the balance of N is nitrification which biologically adjusts the transformation of N-NH_4^+ into N-NO_3 [33]. The previous studies reported that the amount of nitrate

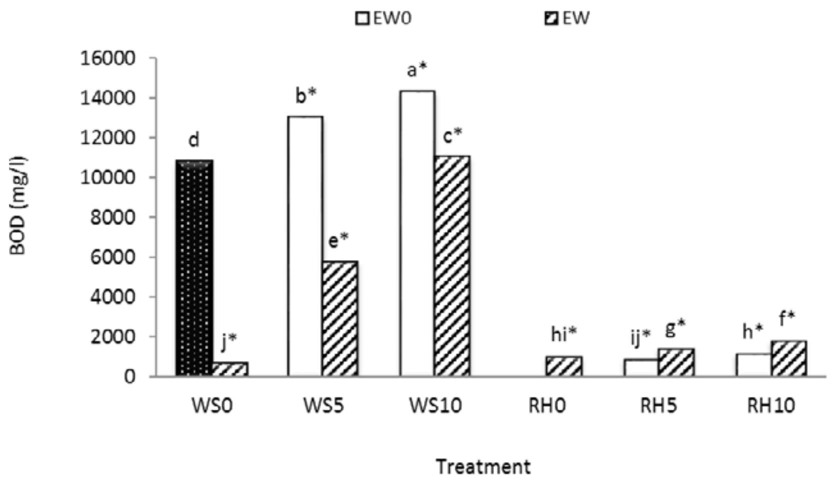

Figure 6. Effects of the treatments on BOD of the wastewater

Note: EW0, without vermicomposting; EW, with vermicomposting; WS0, without wheat straw; WS5, 5% v/v wheat straw; WS10, 10% v/v wheat straw; RH0, without rice husk; RH5, 5% v/v rice husk; RH10, 10% v/v rice husk.

Means in the same column followed by the same letter are not significantly different according to DMRT at (P≤0.05).

increased after vermicomposting [41, 42]. In general, the measurement of the present work showed that in the wheat straw treatments, vermicomposting increased the content of N-NO_3 but decreased the content of N-NH_4^+, which this result is in line with those obtained by Huang et al. [43]. They reported that earthworms facilitate the ammonia oxidization process by promoting both numbers and diversity of ammonia-oxidizing bacteria and archaea during vermicomposting.

Biochemical oxygen demand and chemical oxygen demand

The studied treatments significantly affected the BOD and COD of the wastewater (P<0.01). The highest concentrations of BOD and COD belonged to wheat straw treatments, and vermicomposting significantly decreased their concentrations. The highest BOD and COD were measured in 10% v/v WS. However, the recorded BOD and COD in the presence of earthworm in rice husk treatments were greater compared to the absence of earthworm (Figures 6 and 7). Based on the reports of Sinha et al. [26], also Sundar [44], and Natarajan and

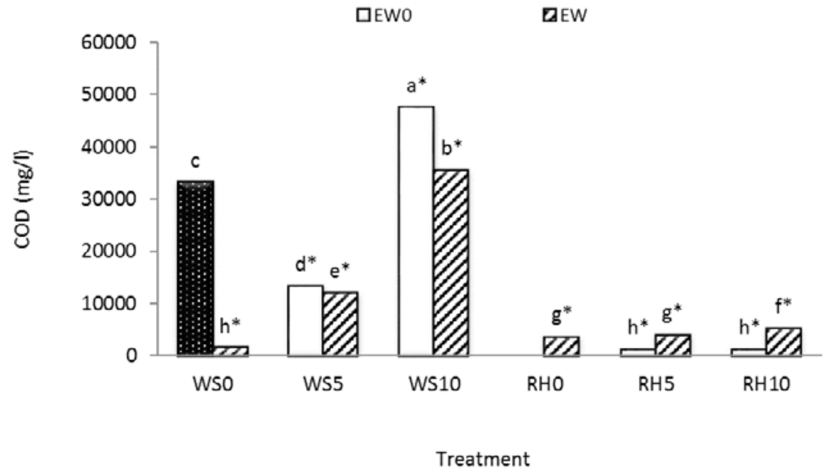

Figure 7. Effects of the treatments on COD of the wastewater

EW0: without vermicomposting; EW: with vermicomposting; WS0: without wheat straw; WS5: 5% v/v wheat straw; WS10: 10% v/v wheat straw; RH0: without rice husk; RH5: 5% v/v rice husk; RH10: 10% v/v rice husk.

Means in the same column followed by the same letter are not significantly different according to DMRT at (P≤0.05).

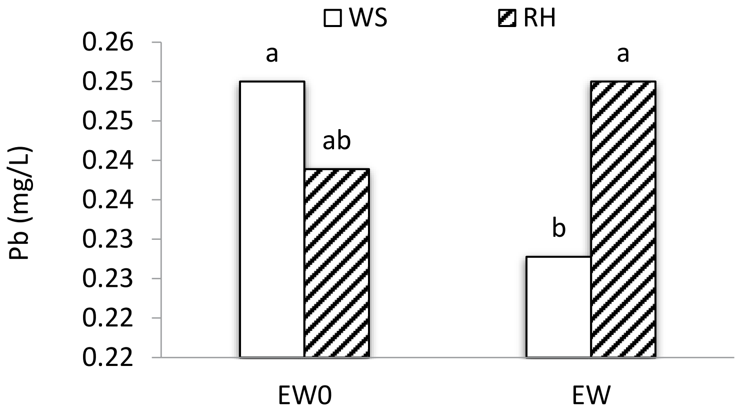

Figure 8. Effects of the treatments on the concentration of Pb in the wastewater

EW0: without vermicomposting; EW: with vermicomposting; WS: wheat straw; RH: rice husk.
Means in the same column followed by the same letter are not significantly different according to DMRT at ($P \leq 0.05$).

Kannadasan [45], earthworms' bodies act as a 'biofilter' and remove the Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), from wastewater by 90%, 80%–90%, respectively by 'ingestion' and biodegradation of organic wastes, heavy metals, and solids from wastewater and by their absorption through body walls. In general, during the vermicomposting process, the earthworms act as a biological filter enhancing the biological treatment of wastewater.

Heavy metals (Ni, Cr, and Pb)

The studied treatments had no significant effect on the content of heavy metals in the wastewater, and interaction effects between bulking agents and earthworms significantly affected only the concentration of Pb in the wastewater. Therefore, it was decided to focus on the changes in Pb affected by the treatments. The results of means comparing bulking agents and vermicomposting showed that vermicomposting in the presence of wheat straw was significantly more successful than the bulking agent of rice husk in reducing the concentration of lead (Pb) in the wastewater, with the lowest concentration of Pb in the wastewater measured in this treatment. However, a completely different result was observed in the absence of earthworms, and the concentration of lead measured in the absence of earthworms in wheat straw treatment was higher than in rice husk (Figure 8), which highlights the role of the type of bulking agent on the efficiency of vermifiltration for removal of heavy metals. The results of Sinha et al. [26] showed that earthworms' bodies act as a 'biofilter' and remove the potentially toxic metals from wastewater by 'ingestion' of these metals from wastewater and their absorption through body walls.

4. Conclusion

The study results showed that vermicomposting and the bulking agents significantly affected the chemical properties of the wastewater. A significant decrease in EC, TDS, TSS, and $N\text{-}NH_3$ of the treated wastewater was recorded in the treatments, which were enriched with earthworms. The concentration of Pb was significantly decreased in WS when it was enriched with earthworms (about an 8% decrease compared to the control), but a reverse status was observed for RC treatment. Although the type of the bulking agent may affect the efficiency of vermifiltration in changing the properties of wastewater, in general, it is concluded that vermifiltration can improve the properties of wastewater to use in agriculture. Our laboratory-scale experiment processing wastewater with earthworms might not fully duplicate large-scale commercial conditions but provides valuable insights into the process, and the changes brought about by earthworm activity.

Ethical Considerations

Compliance with ethical guidelines

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

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Authors' contributions

Conceptualization and Supervision: Mehran Hoodaji; Methodology: All authors; Investigation, Writing-original draft, and Writing-review & editing: All authors; Data collection: Abdol Rasoul Jafarzadeh; Data analysis: Abdol Rasoul Jafarzadeh; Funding acquisition and Resources: Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.

Conflict of interest

The authors declared no conflict of interest.

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