Journal of Environmental Health and Sustainable Development

[DOR: 20.1001.1.24766267.2021.6.3.8.4]

[DOI: 10.18502/jehsd.v6i3.7247]

The Effect of Wastewater Irrigation on the Activity of Soil Microorganisms

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ARTICLE INFO

ORIGINAL ARTICLE

Article History:
Received: 27 May 2021
Accepted: 20 July 2021

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Keywords:
Enzymatic Activity,
Fluorescein Diacetate,
Microbial Population.

ABSTRACT

Introduction: The use of wastewater for irrigation in arid and semi-arid regions of the world is increasing. This study aimed to evaluate the effects of wastewater on the microbial activity of irrigated soils using the enzymatic activity of soil microorganisms.

Materials and Methods: In this study, for soil irrigation, the secondary effluent of the Isfahan municipal wastewater treatment plant was used. As a control, tap water that has no microbial load was also used. Soil samples were collected in two stages, before and immediately after irrigation. All samples were collected in sterile bags, transferred immediately to the laboratory for physicochemical and microbiological tests. Soil samples were analyzed for the amounts of enzymatic activity (Fluorescein Diacetate (FDA) and dehydrogenase), electrical conductivity (EC), oxidation-reduction potential (ORP), and pH.

Results: The EC levels before and after irrigation with tap water was 231.2 and 260.63 $\mu s.cm^{-1}$, respectively, which was significantly different from levels of wastewater-irrigated soil ($P < 0.05$). pH in the two types of used water before and after irrigation was 6-8 and 7-8, respectively. No significant difference was observed in the levels of FDA, dehydrogenase, ORP, and microbial population in samples irrigated with water and wastewaters ($p > 0.05$). It was found that there is a significant relationship between bacterial density and FDA ($P < 0.05$).

Conclusion: The results of the study showed that irrigation with wastewater has no significant effect on the microbial activity of irrigated soil. Because of the short-term wastewater irrigation in the present study, however, further investigation is needed to evaluate the effect of long-term wastewater irrigation on the microbial and physicochemical quality of soil.

Citation: Karimi H, Nikaeen M, Hatamzadeh M, et al. ***The Effect of Wastewater Irrigation on the Activity of Soil Microorganisms.*** J Environ Health Sustain Dev. 2021; 6(3): 1399-406.

Introduction

The use of wastewater for irrigation in arid and semi-arid regions of the world is increasing. The main advantage of wastewater irrigation, in addition to the entry of nutrients into the soil, is the availability of this water source. However, wastewater irrigation may cause soil salinity and may also have adverse health effects [1].

Over the past few years, interest in measuring microbial activity in soil has increased due to the environmental and global changes [2-4]. Various researchers have proposed measuring the enzymatic activity of soil microorganisms as indicators that can be used to determine soil contamination, fertility, safety, and maturity. Among the main reasons for this approach are the close relationship of soil enzymes with organic matter, physical and biological properties of soil, ease of measurement, and rapid response to soil changes [5, 6].

Enzymes in soil microorganisms play a vital role in soil processes such as the food cycle and energy conversion through chemical, physical, and biological reactions [5]. The amount of soil enzymatic activity depends on the manner and intensity of biochemical processes. This activity is also affected by soil and land-use type, the vegetation of the area, and soil management plan. One of the advantages of using enzyme indicators is that measuring the activity of enzymes is simple and requires little cost compared to the other methods of biological analysis. At the same time, it correlates with other soil properties [7].

Enzyme activity can effectively reflect the biological status of the soil. Soil microorganisms are good reflections for soil quality involved in the biogeochemical cycle of carbon, nitrogen, phosphorus, and other nutrients. Since the enzymatic activity is associated with various ecosystem processes, including soil formation, organic matter conversion, and bioremediation activities, finding different physicochemical factors affecting enzymatic activities is of great importance. Therefore, the evaluation of soil microbiological and biochemical properties can be useful in recognizing the main limitations of ecosystems and provide appropriate management strategies to maintain soil stability [7, 8].

The study of soil properties variation during irrigation with treated wastewater has been reported in several studies. One study showed no changes in soil biological and biochemical properties were observed during the three years after irrigation with treated wastewater [9]. Nevertheless, another study showed that the enzymatic activities of the soil increased following irrigation with treated effluent [10]. Another study was conducted to investigate the effect of soil irrigation with mine effluent on enzymatic activities, physiological properties, and the amount of heavy metals. The results showed that the amount of enzymatic activity of soil decreased after irrigation [11].

The study by Adrover et al. [2] showed that irrigation with wastewater has no negative effect on soil properties, and even the amount of organic carbon, enzymatic activities of *beta*-glucosidase, and alkaline phosphatase have improved after irrigation. Also, Azwimbavhi et al. study showed that compared with municipal water, winery wastewater-irrigation significantly increased urease activity in soil, and promoted β-glucosidase activity [12]. Among the enzymatic methods, the fluorescein diacetate (FDA) hydrolysis method measures the potential activity of ester-degrading enzymes. The FDA is used to measure microbial activity in the soil.

Research of scientific databases reveals that there is little information about FDA hydrolysis. The FDA is hydrolyzed by various enzymes that results in the release of fluorescein, which can be measured using a fluorescence microscope and a spectrophotometer [3, 13, 14].

The enzymatic activity of dehydrogenase is considered a general indicator of biological activity because this enzyme has a unique role in the oxidative phosphorylation process and the respiratory metabolism of microorganisms. The aim of this study was to investigate the changes caused by effluent use in the physicochemical and microbial properties of soil. Since Iran is facing water shortages, it can use wastewater as an available water source. However, attention to the changes resulting from irrigation with wastewater has not been paid much attention in Iran. Therefore, this study aimed to evaluate the effects of wastewater on the quality of irrigated soils using the enzymatic activity of soil microorganisms.

Materials and Methods

In this study, the enzymatic activity of dehydrogenase, FDA, number of active microorganisms, changes in EC, ORP, in soils of an experimental field irrigated with secondary treated wastewater were measured in comparison with tap water. Soil sampling was performed before irrigation and after irrigation from November 30, 2016 to May 23, 2017 in weekly or more intervals (based on the precipitation rate) to measure and compare the effect of irrigation with wastewater and tap water[15]. A total of 34 soil samples were collected. In this study, nine soil samples were taken from similar plots (3 samples from each plot) in a depth of 20 cm, mixed, and then tested as a composite sample as described previously. All samples were collected in sterile bags and immediately transferred to the laboratory for chemical and microbiological examinations. Temperature and precipitation were also recorded during the sample collection based on the data of the Isfahan metrological organization.

FDA measurement

To test the FDA activity, 2 g of the sample was mixed in 15 mL of phosphate buffer, and then 0.2 mL of FDA stock solution was added to initiate the reaction. To prepare the control samples, an FDA stock solution was not added to the mixture of soil and phosphate buffer. The mixture was then placed in a shaker incubator for 20 minutes at 30 °C and 100 rpm. The reaction was then terminated by adding 15 ml of 2:1 chloroform/methanol solution. The contents were transferred to a 50 ml centrifuge tube and centrifuged for 3 minutes at 2000 rpm [16]. After centrifugation, the supernatant was filtered by a Watman filter, and the amount of absorption by the filtered liquid was read at 490 nm by a spectrophotometer (HACH, USA). The brand of all chemicals used was Merck, made in Germany.

Dehydrogenase measurement

To perform the dehydrogenase test, 6 g of the soil sample with 0.06 g of calcium carbonate was mixed and transferred to a 100 ml volumetric flask. For each sample in this experiment, two volumetric flasks were used, one of which was labeled with a sample label, the other with a control label. No sample was added to the volumetric control flask. Then 3 ml of distilled water and 3 ml of triphenyl tetrazolium chloride solution (3% TTC) were added to the sample and control containers; after that, 6 ml of distilled water was added to the control containers. It was then incubated for 24 hours at 37 °C. After 24 hours, 10 ml of methanol was added to each volumetric flask. The suspension was passed through fiberglass filters and made up to a volume of 100 ml with methanol, and then the adsorption of solution was measured at 485 nm with a spectrophotometer[16].

Determination of Electrical conductivity (EC), Oxidation-Reduction Potential (ORP)

For ORP, EC, and pH analysis, 20 g of the sample was dissolved in 20 ml of distilled water and placed in a shaker for 20 minutes at 120 rpm. . The pH, ORP, and EC were determined using a pH meter and an electrical conductivity meter (Eutech Instruments, Singapore), respectively[15, 16].

Measurement of bacterial density

Tryptic Soy Agar (TSA) culture medium containing cycloheximide was used to assay the number of the bacterial population in soil. The duplicate culture was performed for each sample, and after the end of incubation time, the number of colonies was counted and reported in CFU/gr.

Statistical analysis

Statistical analysis was performed with SPSS 22. Initially, the normality of the data was assessed. The correlation between the parameters was determined using Spearman correlation analysis. Mann-Whitney test was used to evaluate the difference between physicochemical and microbial parameters in soils irrigated with two types of water. The amount of $p < 0.05$ was considered statistically significant.

Ethical Issue

This study was conducted with the approval of Isfahan University of Medical Sciences, Medical Ethics Committee. Code: IR.MUI.REC.1396.1.193

Results

The average temperature and precipitation at the time of sampling were 21 °C and 90 ml, respectively. The results of the physicochemical and microbial properties of the samples are presented in table 1.

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Table 1: The mean value of analyzed parameters before and after irrigation with treated wastewater (TWW) and tap water (TW).

Parameter	TW		Sig	TWW		Sig	Sign TW/ TWW**
	After	Before		After	Before		
Bacterial density* (CFU/g)	4.9 (3.1)	5.5 (3.3)	0.776	5.44(5.17)	6.38(6.34)	0.784	NS***
FDA(gDM^{-1} h^{-1})	3.13(1.8)	5.67(5.05)	0.199	6(3.7)	8.44(8.11)	0.447	NS
Dehydrogenase (mg TPF g^{-1} DM d^{-1})	5(7.5)	1.67(2.9)	0.5	2.17(1.62)	0.67(1.63)	0.139	Ns
ORP ****	146.75(38.39)	148.22(45)	0.944	142.38(52)	140.33(40.1)	0.928	NS
pH	7-8	6-8	0.566	7-8	6-8	0.694	NS
EC(µS.cm^{-1}) *****	231.2(101.2)	260.63(104.42)	0.565	363.13(81.655)	376.33(208.09)	0.892	0.02

* Bacterial density × 10^6
** Comparison of the physicochemical quality of TWW-irrigated and TW-irrigated plots
*** Not Significant
**** Oxidation-Reduction Potential
***** Electrical Conductivity

TW: Tap Water, TWW, Treated Wastewater

To compare the parameters before and after irrigation and compare the variables based on the type of irrigation, non-parametric tests equivalent to T-test (Mann-Whitney) were used. According to table 1, no significant difference before and after irrigation with wastewater and tap water was observed in any studied variables ($p > 0.05$).

Also, the results showed that after irrigation FDA decreased and dehydrogenase increased (figure 1)

In the present study, the Spearman analysis was used to investigate the relationship between variables (Table 2).

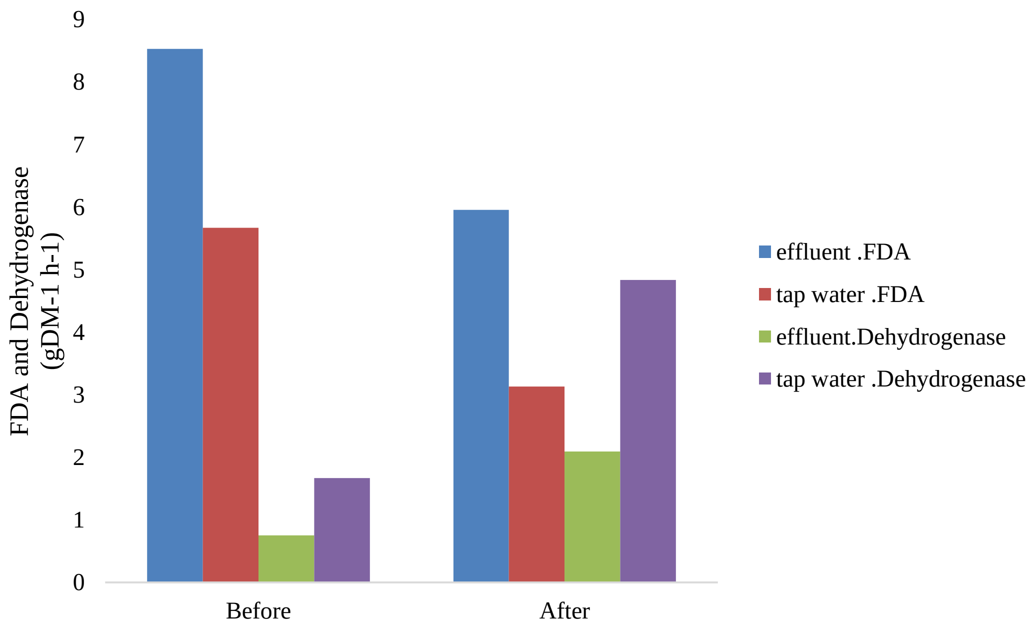

Figure 1: Comparison of enzymatic activities before and after irrigation

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Table 2: Correlation between the analyzed parameters.

Variable	Bacterial density	FDA***	Dehydrogenase	pH	ORP****	EC*****
Bacterial density	1					
FDA	0.23	1				
Dehydrogenase	0.71*	0.6	1			
pH	-0.412*	-0.6**	0.103	1		
ORP	-0.02	0.238	0.132	-0.17	1	
EC	-0.058	0.21	-0.1	-0.247	0.225	1

* Correlation significant at the 0.05 level (2-tailed).
** Correlation significant at the 0.001 level (2-tailed).
*** Fluorescein Diacetate
**** Oxidation-Reduction Potential
***** Electrical Conductivity

Based on the results, it was found that there is a significant relationship between bacterial density and FDA ($P < 0.05$). There was also a reverse correlation between pH with bacterial density and FDA.

Discussion

Table 1 presents the results of the physicochemical and microbial properties of each sample. According to Table 1, no significant difference was observed before and after irrigation in any of the studied variables ($p > 0.05$). However, EC levels before and after irrigation with tap water and wastewater were 231.2 and 260.63 $\mu S.cm^{-1}$, respectively, which was significantly different from irrigated soils with wastewater ($p < 0.05$). In the study of Morugán et al. The EC level of the studied soils increased after irrigation with secondary wastewater treatment effluent. An increase in EC can cause salinity problems in the soil and affect crop productivity [17]. In the present study, the pH of the two types of water used before and after irrigation was in the range of 6-8 and 7-8, respectively (Table 1), which indicates an increase in pH after irrigation. Soil pH affects the solubility of elements, heavy metals, and mineralization of organic matter. Change in soil pH is not easily possible due to the high buffering properties of the soil, especially in calcareous soils.

The soil of the study areas was alkaline, and their pH was between 6 and 8, so a slight rise in pH was recognized after irrigation. In studies to investigate the physicochemical properties of soils irrigated with treated and untreated wastewater, the results showed that pH was significantly higher in soils irrigated with treated wastewater [9, 18]. This was due to the introduction of exchangeable cations in irrigation water, such as Na, Ca, and Mg. Although the influence of pH variations on soil biodiversity has not been studied in the present and mentioned studies, this parameter seems to be an influential factor in determining the number of different species and diversity of soil bacterial communities [19-21].

Fierer Jackson reported that soils with the same pH have similar biodiversity, regardless of climatic conditions [19]. Also, the results of the present study showed that irrigation with wastewater compared to tap water did not affect microbial biomass, enzyme dehydrogenase, and ORP of soils, which is not consistent with the results of some researches. For instance, Adrover et al. mentioned that effluent irrigation over 15 to 20 to 80 years increased the soil organic matter, which developed population and microbial activity [2]. Additionally, Alvarez-Bernal et al. described that irrigation with effluent for 25 years had a significant expansion in the quantity of organic matter in the topsoil [22]. Friedel et al. observed a related development in microbial density and dehydrogenase activity in soils irrigated for a long period with raw wastewater [23].

Further, Alguacil et al. announced that irrigation with effluent increased the phosphorus and organic material [24]. Also, Qiong Liang et al. showed that long-term irrigation with river water significantly increased dehydrogenase, glucosidase, urease, alkaline phosphatase, and arylsulphatase activities in the upstream and midstream soils ($P < 0.05$)[25].

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In all the discussed subjects, the increase in organic content of soil ultimately supervises an expansion in microbial biomass and activity. Besides, in the study of Truu et al., a similar effect of soil irrigation with municipal wastewater treatment was reported on microbial activity [26]. In other words, the results of studies show that irrigation with wastewater has positive effects on microbial activity and biomass due to the easy decomposition of organic matter and nutrients. However, the results of another study showed that industrial effluents not only contain nutrients and organic matter but also contain heavy elements that can remain in the soil for a long time. Therefore, over time, their concentration in the soil reaches toxic levels for soil organisms and reduces the amount of microbial activity [27]. The reason for the inconsistency of the results of the present study with others maybe in part due to the short application period of effluent on the studied soil, and no previously exposure of the field soil with any wastewater.

Dehydrogenase is an intracellular enzyme involved in the microbial metabolism of oxidoreductase. The high association of this enzyme with soil microbial biomass has been widely reported in various literature [23, 28]. In the current investigation, a direct and notable relationship was seen between the dehydrogenase enzyme and microbial community. Adrover et al. examined the activity of the dehydrogenase enzyme and microbial biomass in soils irrigated with treated and untreated wastewater [2].

The prolonged irrigation of clay soils (Vertisols) leads to an increase in microbial population and enzyme dehydrogenase [23]. Also, in soils that contain crop residues on their surface, enzymatic activities have increased [29]. According to table 1, no significant difference was observed before and after irrigation with effluent and tap water in FDA ($p > 0.05$). This enzyme is used to measure microbial activity in the soil. Inconsistent with our results, the study of A.M. Ibekwe showed that the activity of this enzyme in soils irrigated with wastewater had increased significantly compared to control water [30].

Conclusion

This study aimed to investigate and compare the physicochemical and microbial characteristics of irrigated soils with wastewater and tap water. The results of the present study showed that no significant differences were observed in the studied parameters before and after irrigation with wastewater and tap water. In comparison between irrigated samples with water and sewage, only a significant difference was observed in EC. After irrigation with wastewater, the EC level was significantly higher than soils irrigated with water.

Acknowledgment

The authors appreciate Isfahan University of Medical Sciences**.**

Funding

The current study was financially supported by Isfahan University of Medical Sciences (196193).

Conflict of interest

The authors have declared no conflict of interest.

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