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Original Research Article

The Effect of Season and Irrigation with Wastewater on the Growth of *Medicago Sativa* L

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Abstract: The field study was applied in a clay loam soil in Al-Karamah (Al-Hamza Al-Sharqi, Diwaniyah Province) for the period October 1, 2022 to June 20, 2023. The aim of study was to evaluate the use of irrigation with treated wastewater on the *Medicago Sativa* during the summer and winter seasons. The results indicated a significant difference when using treated wastewater during the winter season compared to wastewater during the summer season. It was recorded a significant increase when the plants were exposed to irrigation with wastewater during the winter season for a period of not less than four months, as it gave the best results in the average vegetative and root characteristics. The average number and length of branches, number of leaves, root length, and weight of the fresh and dry vegetative and root group were 18.80 branches/plant, 60.40 cm, 144.63 leaves per branch, 34.08 cm, 72.92 g, 11.36 g, 65.37 g, 9.98 g, respectively, compared to wastewater during the summer season, which gave weak and thin plants that lacked the necessary nutrients for growth. The average vegetative and root characteristics of the plants were the lowest when irrigated with wastewater during the summer season. As for the estimation of the concentration of heavy metals including zinc, copper, cobalt, lead and cadmium in the plant, it did not reach the harmful limit according to the known standards of the Organization (FAO). The samples of the *M. Sativa* irrigated with wastewater during the summer season gave the highest significant increase in the concentration of heavy metals in this plant Zn, Cu, Co, Pb, as they reached 76.43, 62.31, 10.78, 10.78 µg/g dry weight respectively compared to the samples irrigated with wastewater during the winter season. It was also noted that the Cd metal did not have any significant effect in the *M. Sativa* plant samples irrigated with treated wastewater during the summer and winter seasons.

Keywords: Wastewater, Heavy metals, *M. Sativa*, Seasons.

INTRODUCTION

Anthropogenic activities, population growth, and climate change cause a decrease in water quality and quantity and result in huge amounts of wastewater. About 380 billion cubic meters of wastewater are produced globally every year, and this amount can increase to 24% in 2030 and 51% by the year 2050 (Qadir *et al.*, 2020). However, more than 80% of worldwide wastewater is released into the environment without any treatment (Zang *et al.*, 2021). Its release without any treatment led to the precipitation of heavy metals and other toxic elements in soil and plants, as well as an increase in the level of pathogens and microbial threats to human and animal health (Singh, 2021). Competition between different sectors of water users is increasing, the agricultural sector is the main consumer of water. Moreover, there is a scarcity of resources of fresh water in many regions of the world is driving the use of new, non-conventional water sources such as brackish and treated wastewater (Libutti *et al.*, 2018). Irrigation of field crops with wastewater is widespread in areas suffering from water scarcity due to water shortages and inadequate water resources (Jesse *et al.*, 2019).

Wastewater consists of a complex mixture of minerals and organic materials in various forms, including large and small suspended and colloidal

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particles. Wastewater also contains some toxic elements such as Arsenic, Cadmium, Chromium, Lead, Copper, Zinc, Mercury, and others (FAO, 1992). Also among the organic materials found in these waters are pesticides, carbohydrates, fats, proteins, industrial detergents, and complex nitrogenous compounds (Cizmas *et al.,* 2015). These toxic elements have serious effects on public health (Wu *et al.,* 2015). The chemical composition of wastewater is more diverse and concentrated and contains some different acids, alkalis, chemical pollutants, oils, coarse solids, and other inorganic components such as Calcium, Sodium, Potassium, Chlorine, Phosphate, Sulfur, Bicarbonate, and Ammonium salts (Lim *et al.,* 2010). Water-soluble organic substances deplete oxygen levels in water and give an unpleasant taste and odor to water supplies, in addition to toxic substances that can affect the food chain and public health (Hashem and Qi, 2021).

Alfalfa *M. sativa* L. is considered a perennial herbaceous forage legume that grows all over the world, which has the largest agricultural area, and is considered high-quality fodder for all categories of livestock species (Shi *et al.*, 2017). It is distinguished by its high ability to adapt of different types of soils, including acidic and saline soils that are poor in nutrients. It contributes to soil fertility by providing high levels of nitrogen in the soil (Lei *et al.,* 2017; Kebede *et al.,* 2017). It can also be grown in light-textured conditions such as sandy, silty loam, and clay loam, which are higher than heavy-textured soils (Mbarki *et al.,* 2018). Alfalfa is considered one of the most important crops for sustainable agriculture due to its enhancement of soil fertility, ability to feed livestock in mixed production systems, rate of nitrogen fixation, and ability to reduce gas emissions greenhouse (Kulkarni *et al.,* 2018). The study aims to estimate the effect of using treated wastewater irrigation on the survival and growth rate of the *M. sativa* plant in the Al-Karamah, Al-Hamza Al-Sharqi for the winter and summer seasons and to measure the concentration of some heavy metals in treated samples.

MATERIALS AND METHODS

Field experiment

The field study was applied in a clay loam soil in the Al-Karamah (Al-Hamza Al-Sharqi, Diwaniyah Province) for two seasons of growth, winter and summer, for the period 1/10/2023 to 20/6/2022.

Planting method

Seeds were planted in the form of 10-5 m long panels in October until 2/20/2023 in winter and planted in March until 6/20/2023 in summer. After a period of no less than four months of growth, both winter and summer season plants irrigated with drainage water for the Jet plant were uprooted and the following experimental measurements were taken for the green and root group:

1- Number of branches / plant
2- Plant height (cm): The height was measured the plant from the area of contact of the stem with the roots to the growing tip of each plant from the ten randomly selected plants from each field irrigated with treated wastewater during the winter and summer seasons.
3- Number of leaves (leaf/plant^{-1}): according to the total number of leaves for each plant specified from each treatment.
4- Root length (cm): the average measurement of the longest root was taken from the ten selected plants from each treatment.
5- Number of main roots according to the average number of main roots for every ten plants from each field.
6- Fresh and dry weight of the vegetative group (g): Ten plants were cut from each field irrigated with treated wastewater from the area of contact of the roots and the average fresh weight was taken and then they were dried by placing them in an electric oven at a temperature of 70°C for 48 hours and the dry weight was taken when the weight was fixed and its average was recorded.
7- Fresh and dry weight of the root system (g): Ten plants were separated from each field irrigated with treated wastewater during the summer and winter seasons and their average fresh weight was taken. Then they were dried by placing them in an electric oven at a temperature of 70°C for 48 hours and the dry weight was taken when the weight was fixed and its average was recorded.

Estimation of the concentration of some heavy metals in plant

Extraction of some heavy metals from the plant

The concentration of five heavy metals in the experiment were extracted and measured including zinc, copper, lead and cadmium. the plants taken from the summer and winter harvests and exposed to irrigation with wastewater, and each metal had ten replicates. The method was followed (Jackson *et al.,* 1958):

The plant samples were dried using an electric oven at a temperature of 70°C for 48 hours until the weight was fixed and they were ground well using a ceramic mortar. Take 0.5 g of the ground plant material and put it in a 25 ml test tube. Add 5 ml of concentrated nitric acid and leave it for half an hour. Then, Add 2.5 ml of 62% perchloric acid and leave it for 10 minutes, then heat it from 20 to 60 °C for 30 minutes. After that, increase the temperature to 140 °C for 60 minutes, then increase the temperature to 210 °C. Heating continued until white steam appeared in the test tube and about 2.5 ml of the solution remained. Finally, take the remaining solution and put it in a 50 ml volumetric flask and complete the volume with distilled water free of ions and it became ready for measurement using an atomic absorption spectrophotometer type (AA-7000) SHIMDZU-Japan.

Statistical analysis

1-The study experiments were designed according to a completely randomized design (C.R.D.) and the results of the study were analyzed using analysis of variance and the means of the coefficients were compared according to the Revised Least Significant Difference test (R.L.S.D.) at the probability level of 0.05(Al-Rawi And Khalaf Allah, 2000). The ready-made statistical analysis program Genestat (2007) was used to analyze the results.

RESULTS AND DISCUSSION

Effect of irrigation with treated wastewater and seasons on vegetative and root trails of *M.Sativa* L.

The results showed the effect of using treated wastewater during the winter season compared to wastewater during the summer season. The results showed a significant increase when the plants were exposed to irrigation with wastewater during the winter season for a period of not less than four months, as it gave the best results in the average vegetative and root trails (Figure 1). The average number and length of vegetative branches, number of leaves, root length, and weight of the fresh and dry vegetative and root group were 18.80 branches/plant, 60.40 cm, 144.63 leaves per branch 34.08 cm, 72.92 g, 11.36 g, 65.37 g, 9.98 g, respectively, compared to wastewater during the summer season, which gave weak and thin plants that lacked the necessary nutrients for growth. The average vegetative and root trails of plants were the lowest at irrigation with water during the summer season. The average number and length of green branches, number of leaves, root length, and weight of the green and root mass, fresh and dry, reached 10.20 branches/plant, 44.00 cm, 72.80 leaves/branch, 22.00 cm, 42.74 g, 5.36 g, 41.81 g, 5.04 g, respectively.

The significant decrease in the studied vegetative and root characteristics of the plant grown during the summer season as a result of irrigation with water can be attributed to the increase in the content of this water of heavy metals, which accumulated in the soil increases the plant's ability to absorb them and may be included in the toxic fields, which are among the most dangerous toxic substances affecting the soil and plant growth (Alzoubi *et al.*, 2014). Our results agree with (Al-Hamdani *et al.*, 2016) as they explained that exposing barley plants to treated wastewater led to a significant decrease in the average plant height by 35.14% compared to irrigation with fresh river water. This is what scientific research and studies have proven that treated wastewater destroys soil fertility due to the heavy toxic metals it contains (lead, nickel, cadmium and chromium) that lose the soil fertility and eliminate the vegetation cover (Sushil *et al.*, 2019).

The results of the study confirmed by (Shaqirat, 2012) that irrigation with treated wastewater is harmful to public health and agricultural lands. Other similar results were reached by (Abowei, 2010) who explained the reason for the decrease in values in the summer and during the month of (October, November) when temperatures rise, which causes an increase in water evaporation and thus the rapid deposition of mineral salts. The reason is also attributed to (Solomon, *et al.*, 2009) in the decrease in vegetative and root characteristics during the hot months (October and November) and an increase in the cold months (December, February) due to the decrease in oxygen solubility in the hot months and the increase in its solubility during the cold months, which caused a decrease in the decomposition of organic materials. The results were supported by (Wetzel, 2001) that the reason for the low values in the summer is explained by the decrease in oxygen solubility due to the increase in temperature and its consumption in the organic decomposition process or due to the increase in the photosynthesis process, which increases the activity of microorganisms.

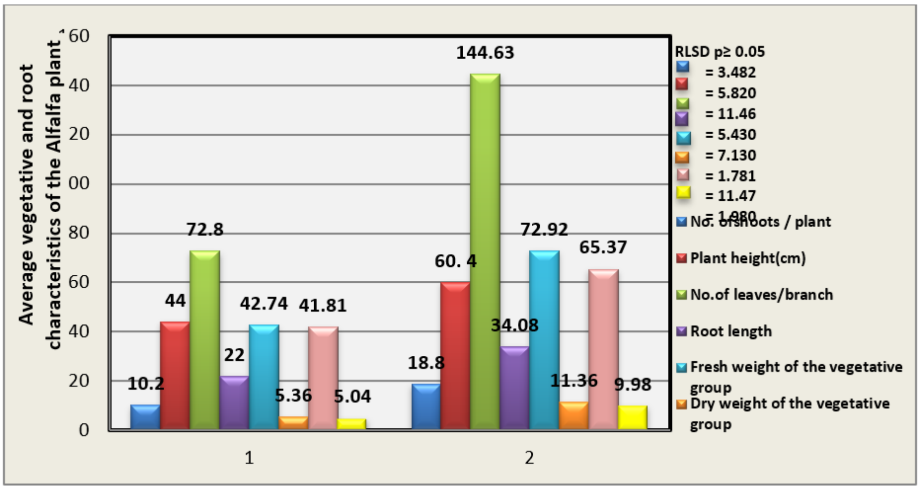

Figure (2) Effect of irrigation with treated wastewater and seasons on the vegetative and root trails of *Medicago Sativa* L.

Effect of irrigation with treated wastewater and seasons on the average concentration of heavy metlas in *M. Sativa* L. plant

Figure (2) shows the seasonal changes (summer and winter) in the averages of trace metals in *M.Sativa* L. (whole plant tissues). The results of the statistical analysis at a significance level of 0.05 p ≥ for the concentrations of heavy metals in the samples of the plant grown during the summer season showed that there is a high significant increase in the concentration of heavy elements, as zinc gave a high significant effect compared to all the studied trace elements, as it reached 76.43 micrograms/gram. Dry weight as a maximum in the summer of 2023 and 70.56 µg/g dry weights as a minimum during the winter season of 2023. This may be attributed to the long-term irrigation with wastewater and the continuous use of inorganic fertilizers (Vanita *et al.*, 2014). The results of the same plot also showed that the summer season has a significant effect on copper, cobalt and lead, reaching 10.78, 10.78, 62.31 µg/g dry weight as a maximum in the summer of 2023, respectively, and 55.58, 6.23, 6.65 micrograms/gram dry weight as a minimum during the winter season of 2023, respectively.

The reason of copper pollution is attributed to various human activities such as excessive addition of chemical fertilizers and low-quality irrigation water (Bhatti *et al.*, 2016). Among the heavy metals studied in plant samples grown during the summer and winter seasons and exposed to sewage water is cadmium. However, this element was not detected in the studied plant sample and no concentration of this element was recorded during the winter season, and the reason is unknown, but it is believed that the reason is likely attributed to the use of irrigation water Cadmium-free groundwater and chemical fertilizers. The study area may also be far from any source of this element, such as industrial processes, heavy traffic, or the use of any type of wastewater in irrigation, which is likely to be contaminated with cadmium (Salem and Alwalayed, 2019). Cadmium is also considered one of the most dangerous heavy metals in terms of environmental toxicity, as it shows harmful effects on soil suitability, plant metabolism, biological activity, and human and animal health (FAO, 2001 /WHO). The results are consistent with both researchers. (FAO, 1992; Lim *et al.*, 2010; Wu *et al.*, 2015 and Hashem and Qi, 2021).

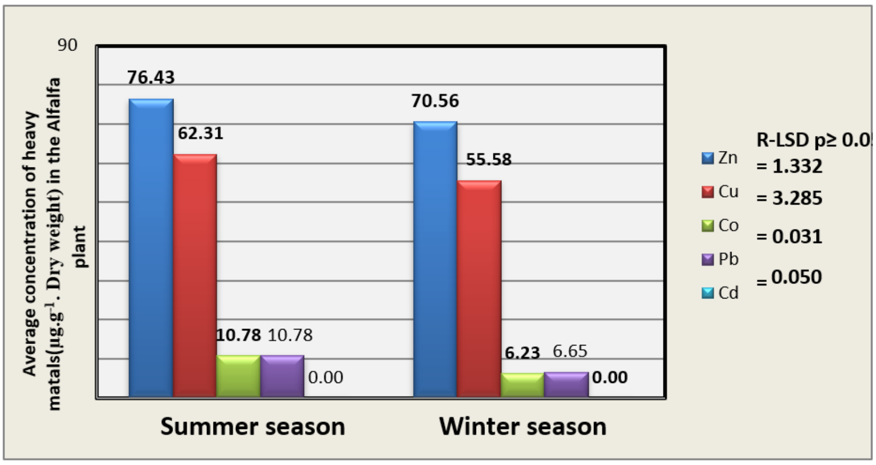

Figure (2) Effect of irrigation with treated wastewater and seasons on the average concentration of heavy metals in *M. Sativa* L.

The reason may be attributed to the high content of untreated wastewater of heavy metals when temperatures increase, which increases the amount of salts which negatively affects plant growth through their osmotic and competitive effects (Al-Dulaimi, 2002). The reason for the superiority of winter season plants over irrigated plants during the summer season, which gave weak and thin plants lacking nutrients is due to the fact that the nutrients of treated wastewater lead to an increase in the crop and improve its quality even without using a limited amount (FAO, 2000).

The Food and Agriculture Organization of the United Nations (FAO) also indicated that reusing treated wastewater to irrigate farms and economic crops is an important source that they can rely on in drought years and achieve savings in fresh water and fertilizers, thus increasing production. This is what was confirmed by (Nofal, 2013) that irrigating crops with reused water encouraged them to use the resulting water to irrigate the most important field or fodder crops such as millet, barley and sorghum. It also greatly improves the condition of the plant in terms of increasing its greenery, growth and production. Al-Hadith, (2011) and Al-Hiti, (2009) explained the reason for the decrease in the growth of freshwater irrigated plants compared to plants irrigated with sewage water during the winter season due to the lack of organic matter that contributes to reducing the salinity of irrigation water because of its role in improving the chemical properties of the soil by regulating the degree of soil reaction to a degree that tends towards acidity due to the release of organic acids by microorganisms that help in the dissolution and availability of nutrients. Also, increasing the acidity of the soil (PH) led to an increase in metals such as zinc, copper, lead, manganese and iron.

Similar results were reached by (Cajuste *et al.*, 1991) on the use of untreated sewage water in irrigating alfalfa crops. It was found that zinc was the most absorbed element by alfalfa plants and that the plant content of nickel, lead, and copper was high and that the accumulated amount of chromium and lead was higher than the known values and that it led to a reduction in the average growth of the general plant and that it may pose a risk to human health. Studies were in agreement with Mapanda *et al.,* (2005) indicated that there was an increase in the concentration values of heavy elements such as copper, zinc, cadmium, lead, and nickel in soil irrigated with sewage water compared to normal irrigation water. The reason for the accumulation of heavy metals during the summer is due to the intensity of sunlight and the length of lighting (Hussein, 2008).

The results showed that the concentration of Zn, Cu, Co, and Pb was lower during the winter than during the summer, due to the fact that the concentration of heavy elements is lower in the rainy season than in the dry season. This is due to the fact that most of the suspended materials that are not attached to the soil or organic materials are washed through the soil during heavy rainy seasons and are released through channels and sewers to the vast lands adjacent to the flood area (Rahman *et al.,* 2012). The result is agreed with Salem and Alwalayed, (2019) that the average concentration of heavy metals (Cr, Cu, Cd, Mn, Zn, Ni and Fe) increased during the summer compared to their decrease during the winter. The results are also agreed with (Khair Allah and Al-Khfaji, (2017) that showed Lead and cobalt concentrations in the tissues of the whole *C. Demersum* plant are highest in summer and lowest in winter.

CONCLUSIONS

The study concludes that irrigation with treated wastewater during the winter and summer seasons was the main source of heavy metals found in the samples of the plant and most likely they also originated from the original soil or from added fertilizers or other treatments applied over the years due to the presence of low concentrations of heavy metals. The Co and Pb were found in all plant samples even with the samples irrigated with wastewater during the winter season, but they were less than the international limits recommended by the Food and Agriculture Organization, as the samples in all sites irrigated with wastewater and taken from the irrigated summer and winter crops lacked the presence of the metal Cd. One of the most important recommendations reached by the research results that irrigation with regular water is one of the most important obstacles to reuse, and we can mitigate and address this by placing restrictions on irrigation with fresh river water and instead using treated irrigation water. Although wastewater treatment processes are costly due to the costs of operation, maintenance, construction, collection and treatment, they have economic and health benefits for humanity and environmental degradation, and they reap greater benefits from marketing agricultural products irrigated with treated wastewater.

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