Effects of treated wastewater reuse in irrigation on germination, biomass, and antioxidant enzyme response of onion (*Allium cepa*)

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ABSTRACT

The present study was conducted on a variety of onion (*Allium cepa*) to evaluate the effects of treated wastewater on various physiological, biochemical, and enzymatic parameters. The experiment involved planting seeds in pots and irrigating them with either treated wastewater from the Guelma city treatment plant (Algeria) or regular water as a control (Ctrl). Samples were collected at three stages: cotyledon drop (CD), five leaves (5-L), and bulb formation ((BF). The parameters measured included germination rate, biomass of leaves and roots, total protein, and proline levels in roots, stems, and leaves, as well as the enzymatic activity of catalase (CAT) and guaiacol peroxidase (GPX). Results showed that treated wastewater irrigation led to an increase in biomass. Germination rates were positively impacted, with a prolonged germination period observed. Proline and total protein levels were higher in treated plants, indicating an adaptation to abiotic stress. Additionally, enhanced specific activities of guaiacol peroxidase and catalase suggested a high antioxidative capacity in onions.

Keywords: Treated wastewater, irrigation, onion, Algeria, stress

1. INTRODUCTION

Water stress and climate change are critical global issues that severely threaten agricultural productivity and food security[1]. The increasing scarcity of freshwater resources necessitates the exploration of alternative water sources for irrigation. Treated wastewater has emerged as a viable option due to its abundance and potential to reduce the demand on freshwater supplies[2]. However, the reuse of treated wastewater in agriculture can pose significant risks if its quality does not adhere to the standards established by the World Health Organization (WHO)[3] and the Food and Agriculture Organization (FAO)[4]. The primary concern with using treated wastewater for irrigation is the presence of various pollutants that can be harmful to both plants and consumers[5]. These pollutants include heavy metals, organic compounds, and residual chemicals that can accumulate in the soil and be taken up by plants, potentially entering the food chain[6]. Furthermore, the presence of pathogenic bacteria in treated wastewater is a substantial risk factor for human health, as these pathogens can contaminate crops and pose a direct threat to consumers[7]. Given these potential hazards, it is imperative to conduct comprehensive evaluations of the impact of treated wastewater on agricultural crops. Such studies should encompass a wide range of physiological, biochemical, and enzymatic parameters to provide a thorough understanding of how plants respond to wastewater irrigation. This knowledge is essential for developing guidelines and practices that ensure the safe and sustainable use of treated wastewater in agriculture. In this study, we focus on the impact of treated wastewater on the growth and development of onion (*Allium cepa*), a widely cultivated vegetable crop. Onions are not only a staple in many diets but also serve as a model for studying plant responses to abiotic stress due to their sensitivity to environmental conditions[8]. By irrigating onion plants with treated wastewater from the Guelma city treatment plant in Algeria and comparing them to plants irrigated with regular water, we aim to assess the effects on various physiological, biochemical, and enzymatic parameters. Specifically, we measured germination rate, biomass of leaves and roots, and levels of total protein and proline in roots, stems, and leaves. Additionally, we evaluated the enzymatic activities of catalase (CAT) and guaiacol peroxidase (GPX), which are indicative of the plants' antioxidative capacity[9]. The results of this study will provide valuable insights into the potential benefits and risks associated with the use of treated wastewater for irrigation and contribute to the development of safe agricultural practices.

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2. MATERIALS AND METHODS

A sowing in pots was carried out in the experimental greenhouse at the University of Annaba. Two different irrigation methods were chosen: one using treated wastewater (TWW) from the Guelma sewage treatment plant, and the other using regular water as a control (Ctrl).

2.1 Experimental set up

As shown in Figure 1, plastic pots with a depth of 25 cm were used to ensure the rooting depth recommended by the FAO (2003)[4]. A 5 cm layer of gravel was added to ensure drainage, and the pots were filled with soil. The growth experiment was conducted under semi-controlled conditions in a growth chamber with 10 hours of lighting and a temperature of 20°C±3°C (Figure 1).

Figure 1. Experimental set up of *Allium cepa* culture.

2.2 Irrigation and water analysis

Treated wastewater used in irrigation were collected periodically (twice a week) for physicochemical and microbiological analysis using methods and protocols established in Table 1.

Table 1. Methods references of physicochemical and bacterial parameters analysis.

Parameter	Method	Unit	Reference
pH, Electrical conductivity (EC).	Multiparameter analyser (Consort™ C3010). Multiparameter analyser (Consort™ C3010).	- µS	- -
Total suspended solids (TSS)	Filtration, drying at 106°C and determination by differential weighing.	mg/L	Reference[10]
Chemical oxygen demand (COD)	By a silver catalyst at 150°C and then titrated with a solution of iron (II) sulphate and ammonium.	mg/L	Reference[10]
Biochemical oxygen demand (BOD_5)	Using an oximeter equipped with a probe, during 5 days of incubation at 20°C.	mg/L	Reference[10]
Nitrate (NO_3) and nitrites (NO_2)	Molecular absorption spectrometry method.	mg/L	Reference[10]
Orthophosphates (OP)	Spectrometric method at λ=700 nm.	mg/L	Reference[11]
Enteric bacteria (MF, FS, TC, and FC)	Inoculation in liquid medium and determination by the MPN method.	U/100 mL	Reference[12]

2.3 Germination test

The seeds are watered every other day with tap water (control group) and with treated wastewater (treated group) in incubation chambers in the dark at 25°C for 34 days. The germination percentage is recorded every 48 hours to establish a germination curve over time for the control and treated plants. The germination rate is determined by the T50 (time required for 50% of the seeds to germinate) Bedouh et al.[13].

2.4 Biomass measurement

A random sampling of 6 plants per pot was carried out each month. The leaves (leaf biomass) of the different sampled plants are cut at the base and weighed using a precision scale. For each sample, the underground part of the different plants is also weighed separately to track their development depending on the type of water used for irrigation.

2.5 Total protein, proline, CAT, and GPX analysis

The total protein was determined using[14], employing Coomassie blue (G250, Merck) as the reagent and bovine serum albumin (BSA, Sigma) as the standard protein. Absorbance was measured at 595 nm.

Proline is quantified using the technique of[15]. It is a spectrophotometric method using a wavelength of 528 nm.

Catalase activity was measured using the method of[16]. The decrease in absorbance at 240 nm was recorded for 1 minute, using a linear molar extinction coefficient (ξ) of 39.400 cm^{-1} M^{-1}. The reaction mixture, with a final volume of 3 mL, contained 100 µL of crude enzyme extract, 50 µL of 0.3% hydrogen peroxide (H_2O_2), and 2.8 mL of Na-K buffer (50 mM, pH 7.2). Catalase activity was expressed in nmol/min/mg protein.

As well, Guaiacol peroxidase activity was determined using the method of[17]. The reaction was initiated by the addition of hydrogen peroxide, and GPX activity was expressed in nmol/min/mg protein.

2.6 Statistic and calculation

Statistical analyses were performed using GraphPad Prism V7.02 software. The data are represented by the mean plus or minus the standard deviation (m±s). One-way ANOVA was conducted, followed by a comparison analysis test (*t*-Test' for normally distributed and Mann-Whitney Test' for non-normally distributed data). Difference was considered statistically significant when $p<0.05$.

3. RESULTS AND DISCUSSION

As showed in Table 2, the water body exhibits moderate organic pollution and significant nutrient levels, particularly nitrates. This could lead to issues like eutrophication, which can deplete oxygen levels and harm aquatic life. Measures to reduce nutrient and organic matter input are recommended[18]. On the other hand, Mesophilic flora, Total coliforms, Faecal coliform, and Faecal streptococci levels suggest significant microbial contamination. High levels of coliforms and streptococci are indicators of fecal contamination, posing a risk to human health and indicating poor water quality[19].

Table 2. Methods references of physicochemical and bacterial parameters analysis.

	T/°C	**pH**	**EC µS**	**TSS mg/L**	**BOD_5 mg/L**	**COD mg/L**	**NO_2 mg/L**	**NO_3 mg/L**	**OP mg/L**	**MF U/100 mL**	**TC U/100 mL**	**FC U/100 mL**	**FS U/100 mL**
Min	11.8	7.1	960	1.3	6.25	10	0.05	5.3	0.1	28500	980	320	240
Max	23.9	7.5	2640	12	23	43.2	0.9	17.7	1.35	52000	1780	1080	430
Mean	5.8	13.05	1574	5.8	13.05	24.63	0.51	10.3	0.56	38520	1408	618	326

Figure 2 represents the effect of treated wastewater on the rate and speed of germination. Observation of the curves shows that treatment with treated wastewater (EUT) causes an extension of the germination period, ranging from a few hours to a day and a half. Regarding the germination rate, the plants treated with EUT exhibit a positive effect on the germination power of onion seeds. According to Reference[20], the mechanisms of high catalase and peroxidase capacities in the cotyledons contribute to detoxification and the reestablishment of thermodynamic equilibrium with the environment.

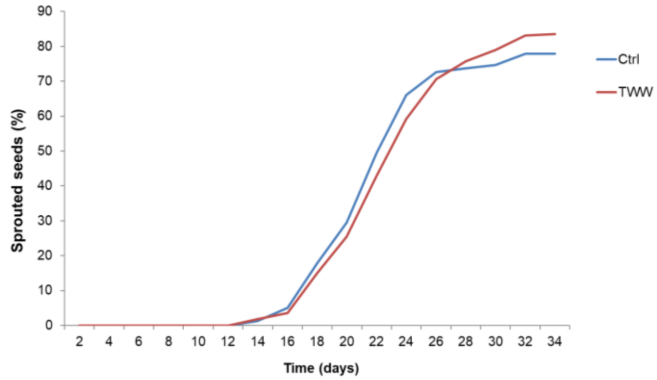

Figure 2. Effect of treated wastewater on the germination rate and speed.

The results in Figure 3a show that treatment with treated wastewater does not seem to significantly modify the biomass of the underground part during the first three months. The highest values are observed after three months of treatment. Figure 3b shows that onion plants exhibit an increase in leaf biomass when irrigated with treated wastewater (EUT). An increase of about 24.7% is recorded after the third month.

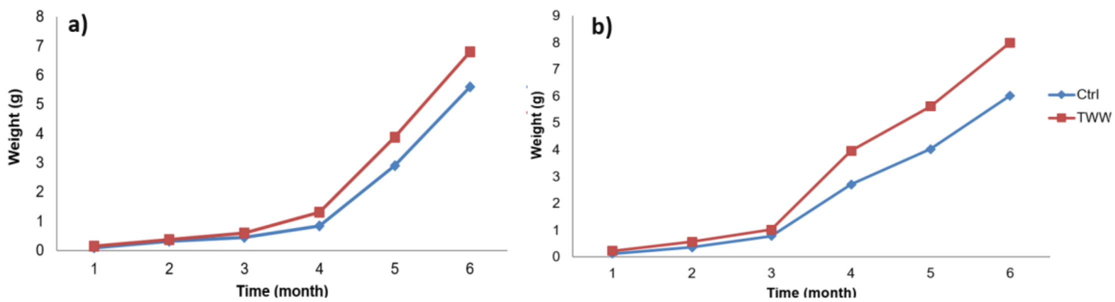

Figure 3. Effect of treated wastewater on (a) Underground biomass and (b) Leaf biomass.

Our results are consistent with those of References[21,22], who observed an increase in biomass in forage plants when irrigated with treated wastewater (EUT). *Allium cepa* appears to respond favourably to irrigation with treated wastewater due to better assimilation facilitated by nutrient inputs, especially nitrogen compounds, which induce significant photosynthetic production in the leaves[23].

The results in Figure 4a show that the treated wastewater (EUT) used during our experiment causes a significant increase in total proteins in the leaves only at the (CC) stage of the treated plants. As shown in Figure 4b, the proline content in the leaves of treated plants is significant following irrigation with treated wastewater. A significant increase (*) was recorded at the (CC) stage, and a highly significant increase (**) at the (5F) stage. This can be explained by the presence of xenobiotics in the tissues, which stimulates the protein synthesis of numerous enzymes, including those involved in detoxification, particularly phytochelatins[24].

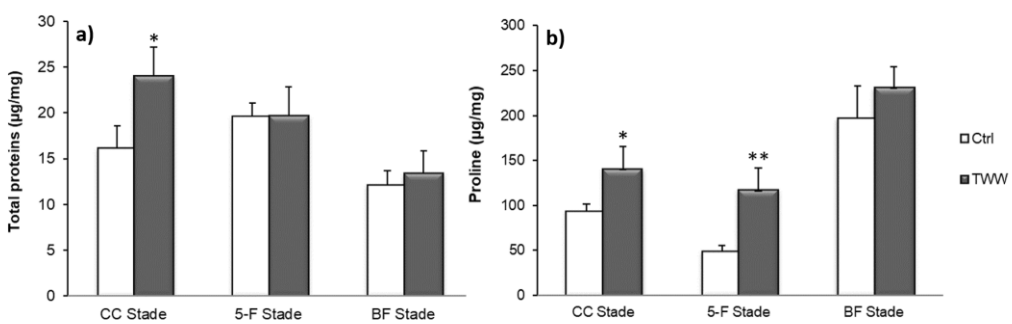

Figure 4. Effect of treated wastewater on (a) total proteins and (b) proline.

Figure 5a shows the evolution of guaiacol peroxidase concentrations in leaves. We observe that treatment with treated wastewater stimulates GPX enzymatic activity, with a significant increase (*) observed at the CC and BF stages. Figure 5b represents the variation in catalase quantity in onion leaves. The catalase levels are significantly higher in plants irrigated with treated wastewater compared to the controls. This increase is significant at the CC stage and highly significant at the 5-F stage. The activity of enzymatic biomarkers is sensitive and responds spontaneously to the presence of pollutants. Tolerance to xenobiotics in *Allium cepa* results in the activation of antioxidant response in the root system, which subsequently extends to the foliar level. The values of specific activities of guaiacol peroxidase (GPX) and catalase (CAT), indicators of stress in plants, demonstrate a strong antioxidative capacity in onions. It can be explained by the increase in protein synthesis and the stimulation of enzymatic oxidation of glutathione, leading to oxidative stress that induces peroxidases[25]. This activity may represent a response of these plants to oxidative stress likely caused by the accumulation of xenobiotics at the cellular level[26].

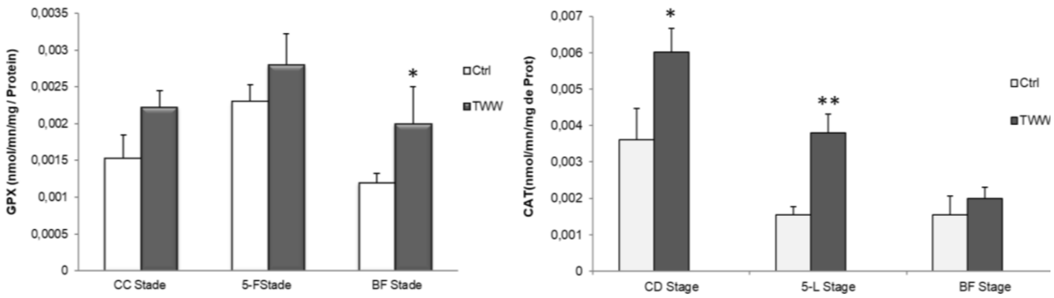

Figure 5. Effect of treated wastewater on guaïgol peroxidase and catalase.

4. CONCLUSION

A trial on a variety of onion "*Allium cepa*" was conducted to verify the effects of treated wastewater on some physiological, biochemical, and enzymatic parameters of this plant. Results show that the variety of onion (*Allium cepa*) seems to respond favourably to the use of treated wastewater for its irrigation due to better assimilation thanks to the nutrients provided by the irrigation water, which results in positive effects on protein and biomass. Concerning germination, treatment with treated wastewater has a positive effect on the rate of removal of grains and a lengthening of the period of this one. The activity of enzymatic biomarkers is sensitive and responds spontaneously to the presence of the pollutant. The tolerance to xenobiotics in *Allium cepa* is manifested by the activation of the antioxidant response in the root system, which subsequently triggers in the foliar level. The values of the specific activities of guaiacol peroxidase (GPX) and catalase (CAT), indicators of stress in the plant, show a high antioxidative capacity in the onion.

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