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Mini Review

# Sanitary Problems and Trace Metals Bioaccumulation during Drip Irrigation with Treated Wastewater in Okra

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## Abstract

Water scarcity, mainly in arid and semi-arid zones, has encouraged efforts to adopt non-conventional waters for food production and agricultural development. Treated Wastewater (TWW) is one of the most continuously produced, accessible, and inexpensive water resources, with great potential for use in irrigation. The aim of this study was to investigate the combined effect of drip irrigation methods (surface *vs.* subsurface at 15 cm depth) and water quality (borehole water *vs.* treated wastewater) on soil water content, morphological and physiological traits, and nutrient content in the plant and fruit of okra grown in the Nabeul region of north-west Tunisia, a semi-arid zone. The results indicated that Okra yield was significantly affected by water quality rather than irrigation technique. Irrigation with TWW significantly increased the Okra plant height; leaf number; total fruit/m²; final yield and leaf area compared with borehole water. Nutrient levels (Fe, Cu, Zn, and Mn) in Okra plant parts (root, stem, leaf, and fruit) were also significantly increased with TWW in subsurface drip irrigation. Apart from these increased parameters were related to better soil moisture and increased available nutrients in the root zone.

In terms of bioaccumulation, apart from Ni, the elements analyzed (Zn, Mn, Cu, Pb, Co, Cd) in plant and fruits showed higher values in treated wastewater than in borehole water with fruit values exceeding the toxicity threshold for Cd and Pb. Furthermore, the increase in the content of trace metal elements analyzed in the fruit following irrigation with TWW did not affect the human Health Risk Index (HRI), which remained below 1 for all treatments. Furthermore, with regard to bacterial and fungal contamination of fruit, we note that although these parameters are significantly higher in Irrigation with TWW, they remain below the toxicity thresholds. Thus, the long-term effect and continuous monitoring of the water and fruit quality in wastewater-irrigated areas to take remedial actions for sustainable agriculture development and protect ecosystems are recommended.

## Introduction

Climatic constraints, population growth, and socio-economic development have led to a constant increase in water demand [1]. Irrigation has become a necessity, even during the rainy seasons. The Tunisian agricultural sector alone accounts for 80% of all available resources. Faced with the growing needs of irrigated agriculture, water resources are becoming increasingly scarce and insufficient, leading to environmental problems and a decline in socio-economic activity [1]. In addition, climate constraints and socio-economic development in the country have led to an ever-increasing demand for water, putting pressure on the exploitation index of renewable natural resources, which varies from 25% to 50% throughout the country. With such a ratio, the country can experience local or cyclical tensions [2,3]. In this tense context, the re-use of treated wastewater in irrigation to preserve good quality water for drinking is considered a necessity [4]. Treated wastewater re-use has become an integral part of the national strategy to mobilize all water resources in the most Mediterranean region [5]. However, the development of the reuse of this additional resource differs from country to country, depending on the water resources and socio-economic conditions of each country. Where some countries still practice the spreading of raw wastewater, other rich countries such as Saudi Arabia, the United Arab Emirates, Kuwait, and the state of California treat

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their water to a very advanced degree, enabling them to reuse it without restriction. Tunisia, on the other hand, has adopted an intermediate and evolutionary approach since the 60s, where wastewater undergoes secondary treatment and is used for restrictive irrigation. The results of these approaches and experience in reusing wastewater in agriculture place Tunisia among the leading countries after Israel in the Mediterranean region in terms of sanitation, wastewater treatment, and reuse of treated wastewater in irrigation [6]. This resource contributed by 6.3% in 2010 and will contribute 12.4% by 2030. It represents a significant proportion of groundwater resources, equivalent to around 30%. This orientation towards alternative water resources is common practice in Tunisia, which began with the country's first experiment in agricultural reuse in 1965. Nevertheless, despite this long experience in wastewater recycling, progress in this sector remains below expectations, with a reuse rate of just over 20% and very modest growth in irrigated areas. This slow progress is largely due to the quality of the water supplied by the Wastewater Treatment Plant (WWTP). Indeed, wastewater treatment systems are often outdated and undersized, which means that they operate with a hydraulic and organic overload. In irrigation, the use of treated wastewater that does not comply with current reuse standards presents pollution risks. To eliminate, if not reduce, the risks of environmental damage, the choice of irrigation technique and water supply method must be based on water quality, while taking into account other factors relating to the soil-plant-atmosphere continuum.

Reusing treated wastewater is not a new concept. By freeing up freshwater resources for domestic supply and other priority uses, reuse contributes to water and energy conservation and improves quality of life. Wastewater can have positive agronomic results, especially when properly planned and controlled. In addition, the fertilizer content of wastewater, particularly nitrogen, potassium, and phosphorus, improves the production potential [7-9] and saves on the cost of mineral fertilizers [10,11]. The use of treated wastewater can also prevent eutrophication and avoid algae growth in enclosed bodies of water, such as lakes and ponds [9]. However, such a practice can also have undesirable effects, as it can bring in organic, mineral, or biological micropollutants and trace metals [12] that can have deleterious effects on the agronomic and sanitary quality of the soil [13-17] the crops [3], as well as on the quality of groundwater due to the percolation of excess nutrients, pathogens, and salts [18,19]. Its trace element content and pathogen composition can pose a risk to groundwater and plant health quality and adversely human health [20].

The quality of treated wastewater is a crucial parameter in wastewater reuse in agriculture, as it must meet standards to safeguard the environment, and human health and be suitable for soil and plants [21]. Among the quality parameters to be taken into account are trace metal composition and bacteriological quality of these waters [22]. State that environmental pollution by heavy metals is becoming more and more of a problem due to the rapid growth of agriculture and inadequate waste disposal. A further effort to improve wastewater quality as well as to propose other irrigation techniques and practices promoting its reuse in irrigation is mandatory. In the same vein [23], states that it is essential to choose appropriate irrigation strategies and methods to solve certain problems linked to the reuse of domestic wastewater in agriculture. If the use of wastewater can be combined with appropriate irrigation methods, the problems of health, pollution, and water crisis could be overcome [13,16,24]. Among these practices [25], argues that drip irrigation is the safest technique, as it allows irrigation water to be applied directly to the root zone. Moreover, micro-irrigation appears to be a solution for conserving water, protecting users, and reducing weed germination and growth [19]. In general, drip irrigation can be applied to the surface of the soil, but recently it has been extended to the subsoil. However, while subsoil irrigation is best known for conventional water, there are few studies involving treated wastewater [26-28]. The main results show that subsoil irrigation improves yields in relation to better water availability than surface drip irrigation [29]. Statistical testing revealed that compared with the surface drip irrigation, the subsurface leaky irrigation system increased leaf area (27%), height (13%), trunk diameter (8%), root length (9%), root fresh (30%) and dry weight (31%), and canopy fresh (25%) and dry weight (31%) [29]. The same authors suggest that water quality was more effective than irrigation systems for the uptake of elements in the leaves and the vegetative growth of Maclura pomifera.

With respect to this, treated wastewater represents a considerable contribution in fertilizing elements for the plant, but its composition in trace elements and pathogens poses a risk to human health and may hinder its reuse. The idea is to check whether the irrigation technique could bring an improvement and preservation of the contamination of Okra fruits by trace metallic elements and pathogens contained in this treated wastewater. Thus, this study came to investigate the impacts of treated wastewater Re-use on the soil, the plant, as well as the associated health risks with regard to bacterial (total aerobic mesophilic flora) and fungal (fungal flora) contamination of fruit and heavy metal bioaccumulation in the plant under surface and subsurface drip irrigation at 15 cm depth, referring to borehole water.

## Methodology

The trial was conducted at INRGREF's experimental plot in Nabeul over two seasons. The site is located at, 32° 37' 36" N and 10° 42' 22" E and has an elevation of 25 m above mean sea level, and is located in the upper semi-arid bioclimatic zone. The annual precipitation and evapotranspiration measured over 28 years were 437.5 mm and 1355 mm respectively. Thus, although the site is in an upper semi-arid bioclimatic stage, it presents a pronounced water deficit over a long period of the year (from March to October) with values exceeding 150 mm in July, hence the need to irrigate during this period. The site's soil is mostly sandy loam with a low content of carbon and a bulk density (Bd) of about 1,4 and 1,5. The soil has a basic pH ranging from 8 to 8.4. Soil EC values range from 1.46 to 1.12 dS/ m in the top 30 cm. The experimental protocol includes two qualities of water that differ in their compositions (Table 1): Treated Wastewater (TWW) and borehole water (BHW).

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**Table 1:** Some physico-chemical parameters of treated wastewater and borehole water used in the trial.

| Water Quality | pH | CE | Na | P | K | Zn | Fe | Mn | Cu | Cd | Pb | Co | Ni |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mS/cm | | (mg/l) | | X $10^{-4}$ | | | (mg/l) | | | | |
| TWW | 7.25±0.2 | 3.35±0.22 | 487±54 | 5.05±1.7 | 42.7±11.1 | 144±90 | 2348±1370 | 120±140 | 84±54 | 72±32 | 349±142 | 114±99 | 137±121 |
| BHW | 7.4 | 2.77 | 325 | - | 8.4 | 300 | 300 | 800 | - | - | - | - | - |
| Standards NT. 106.03 | 6.5 -8.5 | 7 | - | - | - | 5 | 5 | 0.5 | 0.5 | 0.01 | 1 | 0.1 | 0.2 |

For both types of water, two irrigation techniques were used: surface drip irrigation and subsurface drip irrigation at 15 cm depth. In all four treatments, three replicate each. Experiment plots were seeded with local seed of Okra at 35 kg/ha with 95 cm row-to-row distance and 30 cm between plants. Plant height and growth trends were assessed on multiple dates. Likewise, fruit yield was monitored and weighed for fresh and dry matter in different pickings with precision balance.

Irrigation dose was calculated on the basis of the climatic parameters of the unit recorded weekly and on the basis of the cropping coefficients (Kc) of Okra relating to the different vegetative stages mentioned by [30]. The time of irrigation was calculated to give the same dose for both kinds of water taking into account the flow rate for each. For the two campaigns, the water dose applied was 3641 and 3563 m3/, respectively. Soil water content was measured gravimetrically at various stages of growth. Two soil samples were taken for each measure to a depth of 40 cm. The first one was done just before irrigation and the second one about five hours after irrigation. Soil sampling was carried out using a metal auger. Three cores for each treatment were taken and an average sample was used for the determination of the soil water content using gravimetric method. The fresh soil sample collected was weighed and dried at a temperature of 104 °C to determine the Soil Water Content (SWC) using the formula below:

$$SWC = Bulk\ density * \frac{\text{Fresh soil Weight} - \text{Dry soil weight}}{\text{Dry soil weight}}$$

Plant sampling was carried out on fruit throughout the production cycle and on the entire plant at harvest on a yield surface of 3.42 $m^2$ (2.85 m x 1.2 m). At harvest, the entire plant was collected. Roots and shoots were separated and washed with distilled water, and dried in the oven at 70 °C up to constant weight. In the same way, the fruits were washed and dried after each harvest. After drying, the samples were ground and then subjected to acid digestion using a mixture of acid ($HClO_4$, $HNO_3$) then trace metals were determined by atomic absorption (PEKIN-ELMER model 3110), exchangeable Na and K by flame photometry (PFP7/C Research flame photometer, JENWAY, stone, UK) and Phosphorus in plants was determined by the Vanadate method, all on the same filtrates obtained following nitro-perchloric digestion. Growth, yield of okra, and nutrient content were subjected to analysis variance ANOVA using SPSS software (version 20). Treatment means were separated using Duncan's Multiple Range Test at (p 0,05).

## Results and discussion

These results from our study showed that the Okra yield in TWW-irrigated plots was somewhat significantly higher than that with BHW-irrigated plots. The average weight of Okra fruit in the treated wastewater treatment was consistently higher than that in the BHW irrigation, providing evidence that better yields may be attainable when treated wastewater is used for irrigation, a result consistent with previous work [18]. Whereas, in other works on the yield of corn, opposite results have been found [26]. Point out that the decrease in the yield of corn produced in subsurface drip irrigation under TWW compared to fresh water is explained by the decrease in the average rejection of emitters when TWW was used. All things considered, our results advised that water quality was more effective than irrigation technique for the uptake of elements and yield production of *Abelmoscus esculentys* L. Similar conclusions have also been put forward by [29] on Osage orange. Moreover, in agreement with the finding of [29], TWW significantly increased the plant height of okra compared with borehole water, particularly with subsurface irrigation. Similarly, leaf number, total fruit/$m^2$, final yield, and leaf area were also significantly higher in TWW irrigation; nonetheless, the diameter at the collar of okra remained unchanged in all treatments. The use of TWW to irrigate orchards was advised by [31] since it is a rich source of N, P, and K, which contributes to increasing the leaf area and biomass production. Nevertheless, it is important to consider that various other factors could have influenced the outcomes observed. The Observed difference in crop yields could be linked to the higher water content in soil irrigated by subsurface drip irrigation with treated wastewater. Some of the observed differences in yields and nutrient absorption are the direct result of the difference in water nutrition between irrigation techniques. In subsurface irrigation due to the reduction of losses and evaporation, creating more favorable conditions and bringing nutrients to the root zone, the plant growth parameters, mineral nutrition, and water use efficiency increased in plants compared to the surface method. These results are consistent with those reported by other researchers [32]. Another explanation for these differences is that TWW showed a relatively higher nutrient content than borehole water, making it a fertilizing agent of interest for farmers. The implication of higher nutrient content in TWW than in BHW is that soil stability in TWW-irrigated crops will increase, making it more reliable for long-term crop cultivation, while positively influencing crop growth compared to conventional-irrigated fields. With the agricultural use of wastewater being part of appropriate nutrient management in the region, fewer nutrients would be lost to the environment, reducing environmental pollution such as coastal marine areas. These results on Okra clearly support that TWW can be considered as an additional source of nutrients for crops, as suggested by other studies [18,33,34]. Moreover, in this study, it was noted that the level of element nutrition (Fe, Mn, Zn, and Mn) in the various parts of the plant increased

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significantly in subsurface drip irrigation with TWW. Hence, wastewater might be considered of interest for ferti-irrigation to benefit crop growth and reduce fertilizer dependency [10]. Our findings corroborate with previous research [35], highlighting the potential role of wastewater in nutrient supply. Moreover, in many arid and semi-arid bioclimatic regions, the lack of nutrient supply and fertilizers, add to this, soil degradation, and poverty have been pointed as the causes of a low crop yield [36]. Therefore, treated wastewater can be considered a fertilizer input for crop production. However, particular attention should be paid to their composition due to the presence of heavy metals and microbial contaminants.

In addition, given their nutrient content, treated wastewater significantly increased nutrient levels (Na, P, K) in the different plant parts. The highest sodium levels were found in the roots, with an overall average over the two-year study period for all treatments of 1.095% and 1.27% for the BHW and TWW, respectively. Similarly, no effect of dripper depth on Na+ content in the various plant parts was recorded. In addition, potassium was the most absorbed element, particularly in fruit, with higher values in treated wastewater. Similar values of K content in okra fruits were also reported by [24] and [37]. In contrast to Na content and in agreement with the latest work cited by [25] and [38]; the lowest K+ contents are recorded in the roots. We can therefore stipulate that the high sodium levels recorded in the roots compared with the rest of the plant show that the roots act as a barrier preventing sodium from accumulating in the leaves and fruits. Regarding Phosphorus, it's the least absorbed element in the different parts of the plant, with levels in the order of 0.04 to 0.098% in the roots and aerial part, respectively. These low P levels are either related to the low phosphorus levels in the treated wastewater and trial soil or to the intrinsic and physiological properties of the plant. Indeed, comparable P values were recently obtained under the same conditions of reuse of treated wastewater on okra by [39].

Monitoring whether the choice of irrigation technique could reduce or mitigate the toxic effect of trace metal in soil-plant systems is under-treated in the literature. However, the few data show that in the case of subsurface drip irrigation, the contamination of soil surface and product is minimal [40]. In this study, seven heavy metals (Zn, Mn, Cu, Pb, Ni, Co, Cd) were analyzed at plant and fruit levels. Although the contents of these elements in the treated wastewater complied with the Tunisian standards of reuse NT 106.03, levels of certain trace metals were 10 times higher than the safety limits set by the Codex Alimentarius Commission [41] were observed in the various compartments of the Okra, especially in the edible part. These results are in agreement with those obtained [42] on Brassica oleracea var. Italica, and B. oleracea var. Gemmifera and who showed that municipal TWW increased significantly the heavy metal content in the dry matter of the roots as follows: in Brussels sprouts, Cd varied from 0.0083 to 0.78, Co 0.029 to 3.38 and Ni from 4.83 to 7.27 mg/g, respectively, and in Broccoli Ni varied from 4.20 to 10.13 mg/g. TMWW also increased the accumulation of Fe in the roots of Broccoli from 379.5 to 1022.0 mg/kg. However, the levels of the heavy metals in the edible plant parts (heads and sprouts) were very high, varying as follows: in Broccoli Ni 3.91-4.15 mg/g, and Pb 9.82–10.40 mg/g, while in Brussels sprouts Cd 0.8 - 1.17 mg/g, Co 2.35 - 2.70 mg/g, and Ni 5.70-6.17 mg/g. Moreover, in an investigation of nine sites in the Helwan – El Saff area, Cairo, and Giza governorates [43], it was found that all studied heavy metals increased in soil irrigated with wastewater as compared with the soil irrigated with Nile water. The values of the contamination factor were in the order: Co > Cr > Zn > Mn > Fe > Cu > Pb. In terms of bioaccumulation, the contamination factor (CF) calculated in relation to the control shows that, apart from Ni which was blocked at the root level, the CF displays values greater than 1 for the rest of the elements (Cd, Pb, Cu, Mn, Co, and Zn) showing an effect of TWW irrigation on the bioaccumulation of these traces metal in fruits. Of these trace metals and independently of the irrigation technique, Cd and Pb contents were found at values above the toxicity limits of 0.56 mg/l and 6.18 mg/l, compared with 0.15 mg/l and 4.31 mg/l in borehole irrigation, respectively.

Soil analysis showed that irrigation with TWW led to bioaccumulation of trace metals in the soil, particularly on the upper surface. This is in line with previous studies [43-45]. Comparing surface with subsurface irrigation, it was found that subsurface irrigation resulted in bioaccumulation of certain trace metallic elements in the soil, such as Iron and Ni, which showed contamination indices greater than 1. On a plant scale, it seems that although trace metal element levels are below toxicity limits in treated wastewater, relatively large quantities of Zn, Cu, and Fe are absorbed and found in the various parts of the okra plant, which is in line with values recently reported by [46]. The translocation factors for these elements from soil to roots and from roots to aerial parts are >1 [47], indicating that these elements are extracted from the soil, absorbed by the roots, and transported to the leaves and then to the fruits. In contrast to these elements, translocation factors were <1 for Nikel. Similarly, translocation factors from roots to aerial parts and from aerial parts to fruit were also <1. The distribution of trace metals in the plant shows a high concentration of iron in the fruit, particularly in the aerial part. On the other hand, Enrichment factors for the essential elements (Fe, Cu, Zn, and Mn) were greater than 1 with subsurface localized irrigation with treated wastewater. Finally, irrespective of irrigation technique, the human Health Risk Index (HRI) calculated according to EPA [48], shows that these values are less than 1 for both water qualities. This suggests that after two campaigns of TWW irrigation in compliance with Standard NT 106.03, the quality of the fruit harvested poses no health risk to consumers, at least in the short term. However, these results have yet to be consolidated after two irrigation seasons, and the long-term effect of TWW irrigation needs to be investigated. On the other hand, we have noted that particularly for Cd and Ni, the HRI values are the highest compared to the other elements. Hence, particular care should be taken against these two elements, which appear to have a higher toxicity disposition.

Furthermore, with regard to bacterial (total aerobic mesophilic flora) and fungal (fungal flora) contamination of fruit, we have shown that although these parameters are

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significantly higher in Irrigation with treated wastewater, they remain below the toxicity thresholds cited [14]. Similar results on eggplant mentioned that eggplant fruits irrigated with TWW present the same bacteriological quality as those irrigated with well water [49]. On olives, it was found that olive trees irrigated with TWW had good bacteriological quality, which was largely due to the conditions under which TWW was used, and to the fact that the date between irrigation cessation and harvest was respected [50]. Similarly in a study on tomatoes [51], it was found that the bacteriological quality of tomatoes was very good even when industrial water was used which is explained by the precise control of treatments and good agricultural practices. Depending on the irrigation technique [52] showed that with increasing lateral depth, fecal coliform levels decreased at the soil surface. Additionally, it was stated [40] that with regard to health problems, less contact was generated between TWW and the workers or the aerial plant parts, and that dripper line depth at 15 cm provides better microbiological qualities of Tomato [53-64].

## Conclusion

Water scarcity is now a reel fact of life that is beginning to affect even European countries, where one in ten Europeans is affected by "water stress". In August 2022, the European Commission drew up an unambiguous statement: the Old Continent risks running out of water in the next few years if nothing is done. It called on European countries to act by recycling more wastewater.

This work, like many others, supports the reuse of treated wastewater for irrigation. Indeed, this water represents an additional supply of water and elements which, when properly used, can represent an alternative to conventional water, thus saving on good-quality water and reducing the pressure on the latter. However, their use should be rational and accompanied by certain measures and precautions. It is important that users should be informed of the chemical and bacteriological composition of these waters and advised to take account of this water quality when choosing the crops to be grown. Around the world, the level of treatment of these waters varies from one country to another, ranging from raw water, which in irrigation causes serious problems for the environment and human health, to tertiary water, which has proved to be risk-free, even in market gardening. Thus, the reuse of these waters depends on their sanitary and chemical quality. In addition to the treatment techniques downstream of the chain, other measures such as the choice of crops and agricultural practices as well as irrigation techniques to minimize if not eliminate the risks related to pathogens and trace metal elements in the water are developed. Our work is an attempt to investigate the effectiveness of subsurface drip irrigation by secondary treated wastewater to eliminate health risks and metal contamination on the vegetable crop Okra. The results showed that although underground irrigation could reduce the extent of microbial contamination of fruits by less contact between water and the plant, the effect on the attenuation of bioaccumulation of trace elements is not obvious. Moreover, despite the fact that the calculation of the HRI remains less than 1, precautions must be taken into account when the cumulative effect of these elements, especially in long-term irrigation. The challenge remains how to take advantage of the fertilizing elements in TMEs without running the risk of contamination by TMEs. We believe that the best solution is either to allocate this water of lower quality (secondary water) to fodder and arboreal crops, which have shown significant results without major risks for humans or to eliminate these heavy metals at the level of the treatment plants.

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