

Effect of treated wastewater irrigation on physiological and agronomic properties of beans *Vicia faba*

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Abstract—The current study investigated the effect of two doses (50%, and 100 %) of treated wastewater (TWW) on biometric and physiologic parameters of *Vicia faba* beans after 40 days of exposure.

Our data showed a decrease in shoots and roots length and weight in plants amended with TWW. Moreover, a significant decrease in Chlorophyll 'a', 'b' and carotene content was observed in plants irrigated with 100% of TWW.

These findings provided new insights on TWW reuse which can cause different types of stress as it may affect the development of cultivated crops.

Keywords—Treated wastewater, *Vicia faba*, growth, chlorophyll, carotene.

I. INTRODUCTION

Tunisia is a country where the agricultural sector is a priority representing its most important natural resource. However, the annual rainfall is very irregular both in space and time and almost this country belongs to arid and semi-arid climate [1]. In arid and semi-arid regions, variations in rainfall accompanied by successive periods of drought generate undesirable impacts on water availability [2]. In Tunisia, the reuse of treated wastewater (TWW) is part of freshwater resources mobilization strategy and sustainable development of water resources [3]. Nevertheless, once properly treated, wastewater could replace freshwater and decrease this pressure on natural resources to be conserved for other purposes. However, from a quantitative point of view, wastewater is a source

of water always available. Indeed, TWW can ensure the balance of the natural water cycle and preserve resources by reducing harmful discharges into the natural environment [4]. On the other hand, and as has been reported by numerous study the TWW reuse could cause harmful effects on soils and even on living beings such as plant, invertebrates and microorganisms [5,6,7,8,9].

In the end of the past century the use of microbial, animal and human cell culture for toxicity evaluation [10,11,12] have been replaced using animal and vegetable bioindicators. Numerous international studies supported by United Nations Environment Program (UNEP), World Health Organization (WHO) and US Environmental Protection Agency (US-EPA) have validated plant-based bioassays for toxicity monitoring [13,14,15].

Vicia faba is commonly used as a model for cytological, physiological and toxicological studies [16,17,18,19] for many reasons such as its availability around the year, easy to cultivate and does not require sterile conditions. This plant could be used for toxicity assessment of various contaminants in soils i.e. heavy metals, aromatic compounds, pesticides etc.

Vicia faba is one of the oldest domesticated food legumes. Its importance in terms of food and agriculture is reflected by the occupied area worldwide (3.6 million hectares) in more than 50 countries and gives a total production of 4 million tons per year.

Many studies have investigated the effect of TWW on agronomic and physiological aspect of numerous vegetables and improve that those effluent constitute a

reliable source of nutrients i.e. (nitrogen, phosphorus, potassium) and organic matter that enhance soil fertility and productivity [20,21,22,23,24]. However, the chemical composition of TWW could influence vegetable growth, uneven fruit maturity and quality and quantity of yields due to the potential presence of heavy metals, surfactants and pharmaceuticals [25]. The metallic and salt stress have gained an increasing attention, and this was reported by several studies [26,27,28,29,30] which founded that growth factors and physiological properties of many plants are affected when exposed to wide varieties of contaminants that can potentially exist in TWW.

In this context, the current study was, therefore, carried out to evaluate the effect of municipal TWW irrigation on agronomic (growth dynamic properties) and physiological properties (chlorophyll and carotenoid contents) of *Vicia faba* plants.

II. MATERIALS AND METHODS

2.1. Soil sampling:

The soils used for this research were collected from an organic farming plot in the region of Chott-Mariem. The soils were sampled from the depth of 0-15 cm. Before use, samples were air-dried and crushed to pass a (<2 mm) screen.

2.2. Water sampling:

Secondary TWW have been collected in glass bottles from wastewater treatment plant of Northern Sousse, Tunisia, managed by the National Office of Sanitation (ONAS).

2.3. Experimental design:

Dry Certified seeds of beans (*V. faba* *Aguadulce*) obtained from local production were germinated on moistened filter paper at 22°C, when the primary roots were about 2–3 cm long, the seedlings were transplanted in the containers containing 1 kg of soils. Before transplantation soils were moistened with deionized water (Control), diluted TWW (50%) and TWW (100%) brought to 70 % of its holding capacity and this was maintained during the experimentation, five replicates per condition were used.

2.4. Growth measurements:

Five replicates were taken for each treatment were used to calculate the mean of each measurement. Plants were collected after 40 days of exposure to three conditions including control one. The measurements taken were the following:

- Length of the root and shoot system.
- Fresh weights of the root and shoot system
- Number of nodes.

2.5. Chemical content: Photosynthetic pigments

For this purpose, 1g of fresh leaves, was extracted by grinding in a mortar using 20 ml 80% acetone, with small amount of pure (Silica Quartz), and 0.5 g calcium

carbonate to equalize the cellular sap acidity. The extract was filtered and collected in Eppendorf's tubes.

The optical density (DO) of the extract was measured at wave lengths 663, 645, and 440.5 nm [31] to estimate chlorophyll 'a' and 'b', and carotenes respectively, using a Spectrophotometer (VWR-UV-3100-PC) and a vitreous cell (thickness of photo route 1 cm). Three replicates were used for each treatment, and the amount of pigment present in each sample was calculated according to the following equations:

- mg Chlorophyll "a" / g-tissue

$$= 12.7.DO_{663} - 2.69.DO_{663} * v/(w.1000)$$

- mg Chlorophyll "b" / g-tissue

$$= 29.9.DO_{663} - 4.68.DO_{663} * v/(w.1000)$$

- mg Carotenoids / g-tissue

$$= 46.95.DO_{440.5} - (DO_{440.5} - 0.268 * \text{Chlorophyll "a"} + \text{"b"})$$

Whereas, W, the fresh weight by grams for extracted tissue; V, the final size of the extract in 80% acetone; DO, optical density at specific wave length.

2.6. Statistical analysis:

The non-parametric Mann–Whitney U-test was used to compare the data from plants exposed to 50% and 100% of TWW with data from the control soil (irrigated with deionized water).

III. RESULTS

The difference of shoots and roots weight of *V. faba* beans after 40 days of exposure to 50% and 100% of TWW is given in **Figure 1 a and b**. Results showed a significant variation between treatments. Indeed, shoots weight (**Fig.1a**) decreased significantly comparing to control plants irrigated with fresh water with a value of $17,93 \pm 0,75$ g.

However the root weight (**Fig.1b**) increased significantly in plants exposed to soils irrigated with diluted TWW ($8,16 \pm 0,23$ g). In contrast, a slight decrease was recorded in root's weight of plants exposed to 100% which reaches $7,6 \pm 0,2$ g.

On the other hand, **Figure 2 a and b** illustrate the length variation of faba bean's plant shoot and root after 40 days of exposure to 50% and 100% of TWW.

Accurately to weight variation, the length of shoots (**Fig.2a**) decrease progressively with TWW dose to reach $51,33 \pm 4,6$ mm in plants exposed to 100 % of TWW.

Thus, roots length (**Fig.2b**) decreased significantly in plants exposed to 50 % of TWW and reach $15,66 \pm 1,15$ mm which represents approximately 50% of control mean ($28 \pm 3,46$ mm).

Finally, the number of nodes in *V.faba* plants after exposure to TWW are shown in **Figure 3**, whatever, the number of nodes didn't show any significant changes between treatments, it's almost the same in all the experiments.

Figure 4 a and **b** reported the effect of 40 days of irrigation with two doses (50% and 100%) of TWW on chlorophyll "a" and "b". It was noticed that chlorophyll "a" content decrease significantly in the plants exposed to 50% of TWW to reach $0,6 \pm 0,21$ mg/g-fresh tissue compared to control plant where the content was $1,37 \pm 0,04$ mg/g-fresh tissue.

However, the concentration of chlorophyll "b" (**Fig.4b**) decreased by the increasing of TWW dose reaching its lowest $0,61 \pm 0,09$ and $0,77 \pm 0,03$ mg/g-fresh tissue when exposed respectively to 50% and 100% of TWW.

By following carotenoid content after exposure of bean plants to TWW it appears from results illustrated in **Fig.5** that TWW irrigation inhibits the carotenes formation and this was clear in plants exposed to 100% of TWW where means reach its minimum at $0,253 \pm 0,001$ mg/g-fresh tissue.

IV. DISCUSSION

In our study, TWW application on *Vicia faba* beans for 40 days was assessed through the measurement of biometric parameters which were modified after exposure to 50% and 100% of TWW.

Under exposure to numerous pollutants that can reach soils through TWW reuse in irrigation [32,33,34,35], plants can be subject to different types of stress mainly metallic and salt stress.

Globally, a decrease of the length and weight of the shoots and roots was recorded by increasing TWW dose. This decrease could be related to the high amount of salts present in TWW as reported by [36,37,38,39,40,41] who assessed the effect of salts on different plants and they found that it could cause several changes through negative effects on photosynthesis process, changes in enzymatic activity, decrease on the carbohydrates level and growth hormones.

Otherwise, metal content in TWW can exert an inhibitory effect on growth parameters, then they are strongly poisonous to the metabolic activities. However, an exceeded dose of heavy metals such as (Cd, Zn, Pb, Cr...) could cause phytotoxicity and this was proved by many authors [42,43,44,45].

Interestingly, chlorophyll is a clue element for plant's life which contributes to ATP production from the sun's light energy, it is indeed a good biomarker to assess plant's state under stress or exposure to toxics.

Our results regarding a decrease in chlorophyll 'a', 'b' are in concordance with several studies [46,41] which reported that salinity lead to the decrease of chlorophyll rates in barely and beans plant. The second factor that may influence photosynthetic process in *V.faba* beans is the trace elements uptake and this was by inhibiting

chlorophyll biosynthesis and reducing the activity of enzymes involved in CO₂ fixation [47,48,45].

Indeed, in stressed plants the carbon metabolism seems to be inhibited, then, in general the amount of amino acids was lower than in normal plants as proved by Gadallah, 1999 and this was due to the inhibition of amino acids incorporation into proteins under salt/metal stress.

As a part of national strategy to face water scarcity and to save freshwater resources, TWW constitute a sustainable way to manage water resources in the Mediterranean arid and semi-arid regions. But, like every strategy with all its positive effects (i.e. natural fertilizer, source of nutrients, availability), it presents many undesirable effects mainly (heavy metals, high amount of NA⁺ and Cl⁻ cations, pathogens...).

However, this can obviously affect normal development of plants and functional properties of soils receiving this non-conventional water. Moreover, many studies have been assessed the effect of TWW reuse on soils, plants and soil organisms [50,51,52,53,54,55] and in most cases they found that those effluent modify the physicochemical properties of soils, and physiological properties of plants and other organisms.

V. CONCLUSION

Our study showed that TWW reuse affect strongly growth and physiological parameters of *Vicia faba* beans, and this by decreasing shoots and roots length and weight than chlorophyll 'a' and 'b' content. Results also highlighted the effect of TWW on carotene content which decreased after 40 days of exposure.

Overall, TWW reuse as an alternative to save freshwater resources, it could be a good way ensuring the transfer of nutrients, organic matter and minerals in soils.

But, if these effluents are not subject to a periodic control, they can be a source of pathogens and potentially hazardous chemical substances (salts, heavy metals and surfactants), accumulated in soils, then as a result unfavorable effects on crop quality and productivity.

ACKNOWLEDGEMENTS

An acknowledgement section may be presented after the conclusion, if desired.

REFERENCES

- [1] CGRE, (1991).84-90, Bulletins mensuels et annuels, Centre de Gestion des Ressources en eau de l'INAT, INAT, Tunisie.
- [2] Khouri, N. Kalbermatten, J.M. and Bartone, C.R., (1994). The reuse of wastewater in agriculture: A guide for planners. In Khouri, N. Kalbermatten, J.M. and Bartone, C.R. Water and sanitation.

- [3] Al Atiri R., Rezgui, F., Bel Hassen, M.A., (2002). Réutilisation des eaux usées. Cas de la Tunisie. In : Forum de la gestion de la demande en eau. Rabat, Maroc, mars 2002.
- [4] Bouchet, C., (2008). Recyclage et réutilisation des eaux usées : où en sommes-nous ? L'eau, l'industrie, les nuisances, n 308, p. 33-42.
- [5] Yadav RK, Goyal B, Sharma RK, Dubey SK et Minhas PS., (2002). Post-irrigation impact of domestic sewage effluent on composition of soils, crops and groundwater—A case study. *Environmental International* 28 : 481–486.
- [6] Rattan R.K., Datta S.P., Chhonkar P.K., Suribabu K., Singh A.K., (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. *Agriculture, Ecosystems and Environment* 109, 310–322.
- [7] Solis C., Andrade E., Mireles A., Reyes-Solis I.E., Garcia-Calderon N., Lagunas-Solar M.C., Pina C.U. et Flocchini R.G., (2005). Distribution of heavy metals in plants cultivated with wastewater irrigated soils during different periods of time. *Nuclear Instruments and Methods in Physics Research B* 241, 351–355.
- [8] Abbas, H., Nasr, R., & Seif, H. (2006). Study of waste stabilization pond geometry for the wastewater treatment efficiency. *Ecological Engineering*, 28(1), 25-34.
- [9] Herpin U., Gloaguen T. V., da Fonseca A. F., Montes C. R., Mendonça F. C., Piveli R. P., Breulmann G., Forti M. C. Et Melfi A. J., (2007). Chemical effects on the soil-plant system in a secondary treated wastewater irrigated coffee plantation- A pilot field study in Brazil. *Agricultural Water Management* 89, 105 – 115.
- [10] Battersby, N. S. (2000). The biodegradability and microbial toxicity testing of lubricants—some recommendations. *Chemosphere*, 41(7), 1011-1027.
- [11] Parng, C., (2005). In vivo zebra fish assays for toxicity testing. *Curr. Opin. Drug Disc. Dev.* 8, 100–106.
- [12] Wilsnack, R., Meyer, F., Smith, J., (1973). Human cell culture toxicity testing of medical devices and correlation to animal tests. *Artif. Cell. Blood Substit. Biotechnol.* 1, 543–562.
- [13] Grant, W. F. (1999). Higher plant assays for the detection of chromosomal aberrations and gene mutations—a brief historical background on their use for screening and monitoring environmental chemicals. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 426(2), 107-112.
- [14] Rizzo, L. (2011). Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. *Water research*, 45(15), 4311-4340.
- [15] Iqbal, M. (2016). *Vicia faba* bioassay for environmental toxicity monitoring: a review. *Chemosphere*, 144, 785-802.
- [16] Feng, S., Wang, X., Wei, G., Peng, P., Yang, Y., & Cao, Z. (2007). Leachates of municipal solid waste incineration bottom ash from Macao: Heavy metal concentrations and genotoxicity. *Chemosphere*, 67(6), 1133-1137.
- [17] Yi, M., Yi, H., Li, H., & Wu, L. (2010). Aluminum induces chromosome aberrations, micronuclei, and cell cycle dysfunction in root cells of *Vicia faba*. *Environmental Toxicology: An International Journal*, 25(2), 124-129.
- [18] Giorgetti, L., Talouizte, H., Merzouki, M., Caltavuturo, L., Geri, C., & Frassinetti, S. (2011). Genotoxicity evaluation of effluents from textile industries of the region Fez-Boulmane, Morocco: a case study. *Ecotoxicology and environmental safety*, 74(8), 2275-2283.
- [19] Shahid, M., Pinelli, E., Pourrut, B., Silvestre, J., & Dumat, C. (2011). Lead-induced genotoxicity to *Vicia faba* L. roots in relation with metal cell uptake and initial speciation. *Ecotoxicology and environmental safety*, 74(1), 78-84.
- [20] Meli, S., M. Porto, A. Belligno, S. A. Bufo, A. Mazzatura, and A. Scopa. (2002). Influence of irrigation with lagooned urban wastewater on chemical and microbiological soil parameters in a citrus orchard under Mediterranean condition. *Science of the Total Environment* 285: 69–77.
- [21] Rusan, M. J. M., Hinnawi, S., & Rousan, L. (2007). Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination*, 215(1-3), 143-152.
- [22] Kiziloglu, F. M., Turan, M., Sahin, U., Kuslu, Y., & Dursun, A. (2008). Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (*Brassica oleracea* L. var. botrytis) and red cabbage (*Brassica oleracea* L. var. rubra) grown on calcareous soil in Turkey. *Agricultural water management*, 95(6), 716-724.
- [23] Bedbabis, S., Ferrara, G., Rouina, B. B., & Boukhris, M. (2010). Effects of irrigation with treated wastewater on olive tree growth, yield and leaf mineral elements at short term. *Scientia Horticulturae*, 126(3), 345-350.
- [24] Libutti, A., Gatta, G., Gagliardi, A., Vergine, P., Pollice, A., Beneduce, L., ... & Tarantino, E. (2018). Agro-industrial wastewater reuse for irrigation of a

- vegetable crop succession under Mediterranean conditions. *Agricultural Water Management*, 196, 1-14.
- [25] Pedrero, F., Kalavrouziotis, I., Alarcón, J. J., Koukoulakis, P., & Asano, T. (2010). Use of treated municipal wastewater in irrigated agriculture—Review of some practices in Spain and Greece. *Agricultural Water Management*, 97(9), 1233-1241.
- [26] Rusan, M. J. M., Hinnawi, S., & Rousan, L. (2007). Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination*, 215(1-3), 143-152.
- [27] Kaymakanova, M. (2009). Effect of salinity on germination and seed physiology in bean (*Phaseolus vulgaris* L.). *Biotechnology & Biotechnological Equipment*, 23(sup1), 326-329.
- [28] Khalid, S., Shahid, M., Dumat, C., Niazi, N. K., Bibi, I., Gul Bakhat, H. F. S., ... & Javeed, H. M. R. (2017). Influence of groundwater and wastewater irrigation on lead accumulation in soil and vegetables: Implications for health risk assessment and phytoremediation. *International journal of phytoremediation*, 19(11), 1037-1046.
- [29] Tabassum, R. A., Shahid, M., Dumat, C., Niazi, N. K., Khalid, S., Shah, N. S., ... & Khalid, S. (2018). Health risk assessment of drinking arsenic-containing groundwater in Hasilpur, Pakistan: Effect of sampling area, depth, and source. *Environmental Science and Pollution Research*, 1-12.
- [30] Shahid, M., Shamshad, S., Farooq, A. B. U., Rafiq, M., Khalid, S., Dumat, C., ... & Niazi, N. K. (2018). Comparative effect of organic amendments on physio-biochemical traits of young and old bean leaves grown under cadmium stress: a multivariate analysis. *Environmental Science and Pollution Research*, 1-12.
- [31] Smith, J. H., & Benitez, A. (1955). Chlorophylls: analysis in plant materials. In *Modern Methods of Plant Analysis/Moderne Methoden der Pflanzenanalyse* (pp. 142-196). Springer, Berlin, Heidelberg.
- [32] Salgot, M., Vergés, C., & Angelakis, A. N. (2003). Risk assessment in wastewater recycling and reuse. *Water Science and Technology: Water Supply*, 3(4), 301-309.
- [33] Conkle, J.L., White, J.R., Metcalfe, C.D., 2008. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. *Chemosphere* 73, 1741–1748.
- [34] Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z., Zhu, Y.G., (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environ. Pollut.* 152, 686–692.
- [35] Ibekwe, A.M., Gonzalez-Rubio, A., Suarez, D.L., (2018). Impact of treated wastewater for irrigation on soil microbial communities. *Sci. Total Environ.* 622–623, 1603–1610.
- [36] Hamada, A. M. (1995). Alleviation of the adverse effects of NaCl on germination, seedling, growth and metabolic activities of maize plants by calcium salts. *Bull. Fac. Sci. Assiut Univ*, 24, 211-220.
- [37] Misra, A. N., Sahu, S. M., Misra, M., Singh, P., Meera, I., Das, N., ... & Sahu, P. (1997). Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. *Biologia plantarum*, 39(2), 257-262.
- [38] Dantas, B. F., Ribeiro, L. D. S., & Aragão, C. A. (2005). Physiological response of cowpea seeds to salinity stress. *Revista Brasileira de Sementes*, 27(1), 144-148.
- [39] Mazher, A. A., El-Quesni, E. F., & Farahat, M. M. (2007). Responses of ornamental and woody trees to salinity. *World J Agric Sci*, 3, 386-395.
- [40] Memon, S. A., Hou, X., & Wang, L. J. (2010). MORPHOLOGICAL ANALYSIS OF SALT STRESS RESPONSE OF PAK CHOI. *Electronic Journal of Environmental, Agricultural & Food Chemistry*, 9(1).
- [41] Qados, A. M. A. (2011). Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). *Journal of the Saudi Society of Agricultural Sciences*, 10(1), 7-15.
- [42] Di Toppi, L. S., & Gabbriellini, R. (1999). Response to cadmium in higher plants. *Environmental and Experimental Botany*, 41(2), 105-130.
- [43] Wojcik, M., & Tukiendorf, A. (2004). Phytochelatin synthesis and cadmium localization in wild type of *Arabidopsis thaliana*. *Plant Growth Regulation*, 44(1), 71-80.
- [44] Mohanpuria, P., Rana, N. K., & Yadav, S. K. (2007). Cadmium induced oxidative stress influence on glutathione metabolic genes of *Camellia sinensis* (L.) O. Kuntze. *Environmental Toxicology: An International Journal*, 22(4), 368-374.
- [45] Guo, J., Dai, X., Xu, W., & Ma, M. (2008). Overexpressing GSH1 and AsPCS1 simultaneously increases the tolerance and accumulation of cadmium and arsenic in *Arabidopsis thaliana*. *Chemosphere*, 72(7), 1020-1026.
- [46] Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental chemistry letters*, 8(3), 199-216.

- [47] Tort, N., & Turkyilmaz, B. (2004). A physiological investigation on the mechanisms of salinity tolerance in some barley culture forms. JFS, 27, 1-16.
- [48] De Filippis, L. F., & Ziegler, H. (1993). Effect of sublethal concentrations of zinc, cadmium and mercury on the photosynthetic carbon reduction cycle of *Euglena*. Journal of Plant Physiology, 142(2), 167-172.
- [49] Gadallah, M. A. A. (1999). Effects of proline and glycinebetaine on *Vicia faba* responses to salt stress. *Biologia plantarum*, 42(2), 249-257.
- [50] Klay, S., Charef, A., Ayed, L., Houman, B., Rezgui, F., (2010). Effect of irrigation with treated wastewater on geochemical properties (saltiness, C, N and heavy metals) of isohumic soils (Zaouit Sousse perimeter, Oriental Tunisia). *Desalination* 253, 180-187.
- [51] Hidri, Y., Fourti, O., Eturki, S., Jedidi, N., Charef, A., Hassen, A., (2014). Effects of 15-year application of municipal wastewater on microbial biomass, fecal pollution indicators, and heavy metals in a Tunisian calcareous soil. *J. Soils Sediments* 14, 155-163.
- [52] Carlos, F.S., Schaffer, N., Andreazza, R., Morris, L.A., Tedesco, M.J., Boechat, C.L., Camargo, F.A. de O., (2018). Treated Industrial Wastewater Effects on Chemical Constitution Maize Biomass, Physicochemical Soil Properties, and Economic Balance. *Commun. Soil Sci. Plant Anal.* 49, 319-333.
- [53] Elfanssi, S., Ouazzani, N., Mandi, L., (2018). Soil properties and agro-physiological responses of alfalfa (*Medicago sativa* L.) irrigated by treated domestic wastewater. *Agric. Water Manag.* 1-10.
- [54] Lüneberg, K., Schneider, D., Siebe, C., Daniel, R., (2018). Drylands soil bacterial community is affected by land use change and different irrigation practices in the Mezquital Valley, Mexico. *Sci. Rep.* 8, 1-15.
- [55] Pan, M., Chu, L.M., (2018). Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater irrigation areas in the Pearl River Delta region, southern China. *Sci. Total Environ.* 624, 145-152.

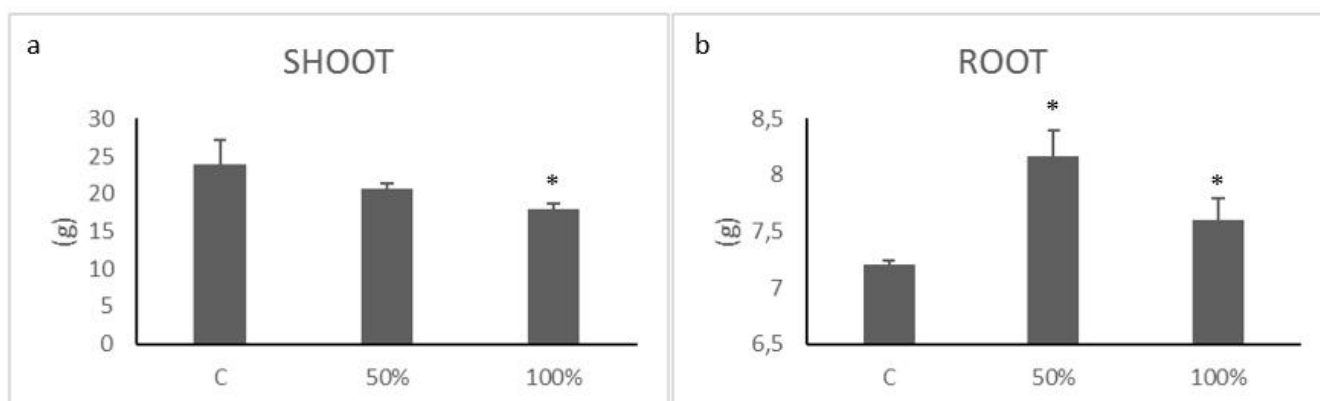


Fig.1: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on *Vicia faba* (a) shoots and (b) roots weight after 40 days of exposure. Results represent the Mean \pm SD of at least 5 replicates. (*) Statistically significant differences ($p < 0.05$) comparing to control.

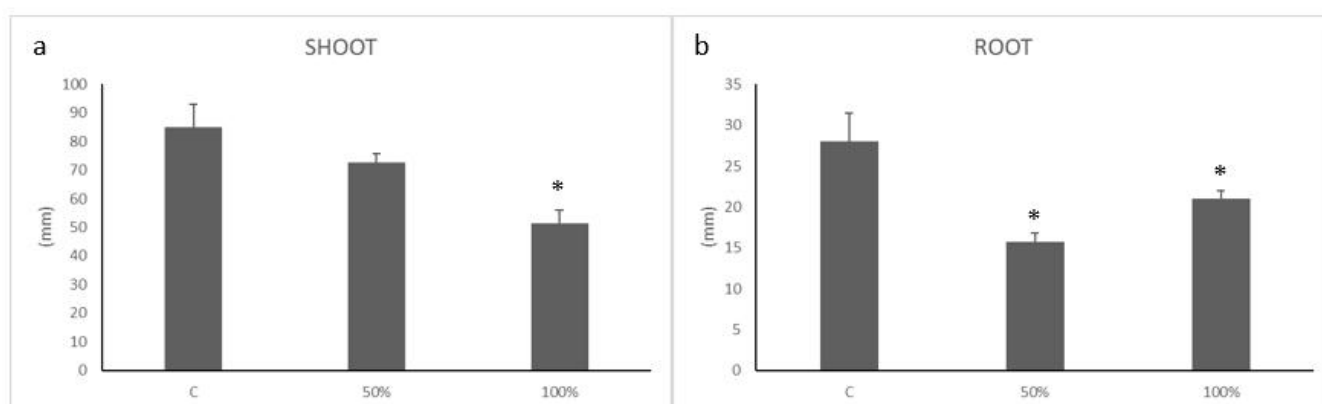


Fig.2: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on *Vicia faba* (a) shoots and (b) roots length after 40 days of exposure. Results represent the Mean \pm SD of at least 5 replicates. (*) Statistically significant differences ($p < 0.05$) comparing to control.

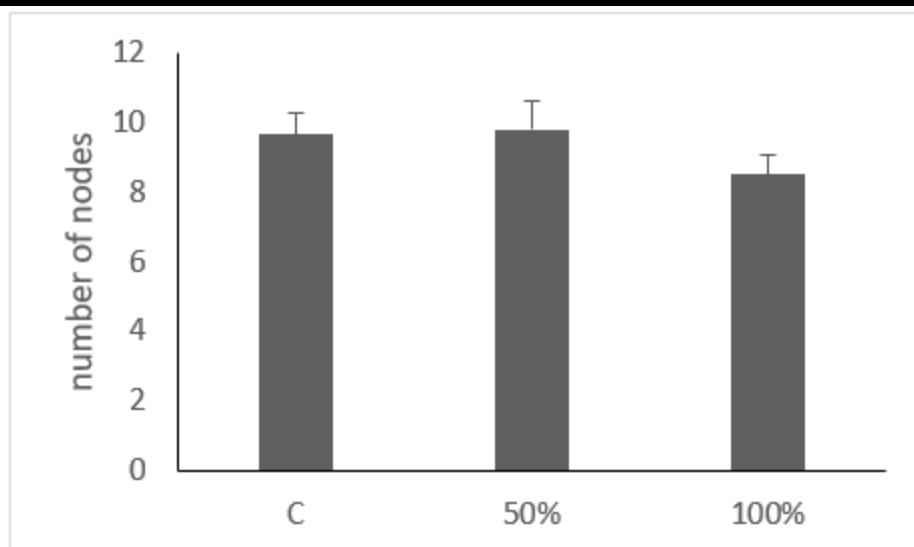


Fig.3: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on the number of nodes of *Vicia faba* after 40 days of exposure. Results represent the Mean \pm SD of at least 5 replicates. (*) Statistically significant differences ($p < 0.05$) comparing to control.

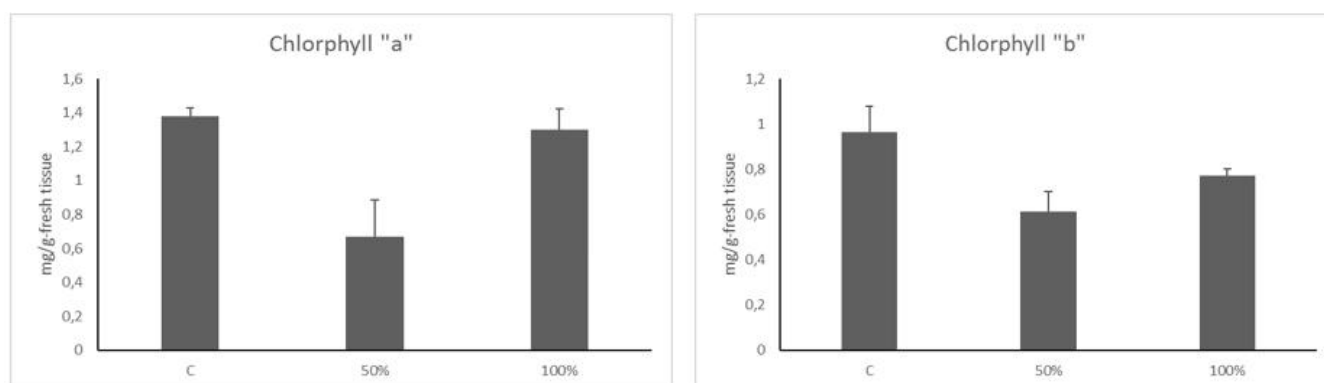


Fig.4: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on (a) chlorophyll 'a' and (b) chlorophyll 'b' content of *Vicia faba* after 40 days of exposure. Results represent the Mean \pm SD of at least 5 replicates. (*) Statistically significant differences ($p < 0.05$) comparing to control.

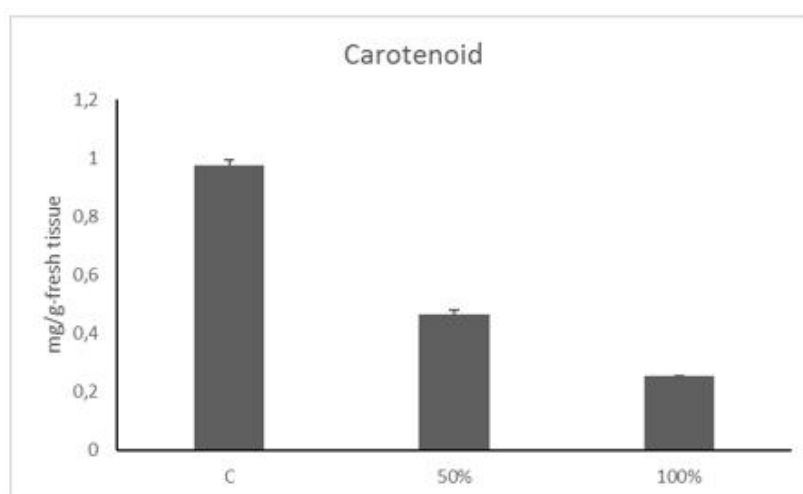


Fig.5: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on carotene content of *Vicia faba* after 40 days of exposure. Results represent the Mean \pm SD of at least 5 replicates. (*) Statistically significant differences ($p < 0.05$) comparing to control.