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# DETERMINATION OF ANTIOXIDANTS AND TOTAL POLYPHENOLS IN CABBAGES (BRASSICA OLERACEA) IRRIGATED WITH WASTEWATER CONTAMINATED WITH TOXIC METALS

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**Abstract:** Water is a natural resource essential to all kinds of life. The reuse of treated wastewater (TWW) for irrigation is a possibility with challenges to be clarified at scientific level, in particular, its use in irrigation activities due to the eventual contamination of vegetables, like cabbages *(Brassica oleracea*), by toxic metals and other compounds harmful to humans present in these waters. On the other hand, the use of TWW for vegetable irrigation may allow significant water saving and also providing the cultivated species with nutrients, which may dispense the use of fertilizers. In this work, vegetables such as *Brassica oleracea* (cabbage), were planted in a greenhouse with a controlled environment (temperature and humidity) located at ISEL campus. Part of the vegetables was irrigated with tap water, and another part with TWW from two wastewater treatment plants (WWTP) located in Lisbon´s District. One of the WWTP receives only domestic wastewater (A), whilst the other one receives industrial and domestic wastewater (B). The antioxidant activity of the vegetable species as well as total content of polyphenols and flavonoids present in plant extracts were determined in order to assess the impact of using TWW contaminated with toxic metals in their irrigation.
**Keywords:** Treated Urban Wastewater, *Brassica Oleracea,* Toxic Metals

## INTRODUCTION

Burning fossil fuels as coal, oil and gas are the largest contributors to global climate change, accounting for over 75 per cent of global greenhouse gas emissions which blanket the Earth and trap the sun's heat, leading to global warming and climate change at a global scale. These anthropogenic activities, followed by rainforest destruction, are leading the world to faster warming, which, in turn, is changing weather patterns and disrupting nature´s balance, posing many risks to humans and all other forms of life on Earth, as well as global ecosystems. Rising earth´s surface temperature, more severe storms, ocean warming leading sea levels to rise, loss of millions of plant and animal species, food scarcity, poverty and people displacement are the most representative consequences and effects of climate change. In addition, one of the most prominent consequences is the increasing decline in water availability each time in more regions of the planet (Bisselink *et al*, 2020; WMO,2021). Water shortages in already water-stressed regions is leading to an increased risk of agricultural droughts affecting crops. In fact, the use of freshwater resources worldwide points out agriculture as the largest user, with irrigation accounting for about 70 percent of global water usage (FAO, 2020). This reality is leading to water conservation policies in many countries, banning urban irrigation with municipal tap water, and decimating several agricultural and horticultural crops. Among the strategies to deal with scarce water resources, the use of alternative irrigation water sources like rainwater, air conditioning condensates, residential greywater, or treated wastewater (TWW) are becoming potentially viable sources to lessen urban and agriculture irrigation dependence on limited water resources.

In particular, the reuse of TWW for this purpose has some challenges and issues that need to be addressed from a scientific point of view (Pedrero *et al*., 2010; Vergine *et al*. 2017 a). In fact, TWW may contain pollutants, such as toxic metals, which affect soil, groundwater, and irrigated vegetables since they are not completely removed during water treatment and not mandatorily monitored at the discharge of domestic WWTPs, as specified in European Council Directive 91/271/EEC of 21 May 1991. However, Portuguese legislation establishes emission limit values for toxic metals in wastewaters which are not considered in the Directive 91/271/EEC. In the particular case of lead (Pb) and cadmium (Cd), the limit values are 1.0 mg/L and 0.2 mg/L, respectively.

Heavy metals, in excessive doses induce both oxidative and genotoxic stress response, leading to cytotoxicity and damage to different cellular components, including proteins, membranes, and nucleic acids, therefore, generating typical abiotic stress response in plants, by increasing the formation of reactive oxygen species (ROS), which renders antioxidants incapable of defense against growing amounts of free radicals (Dutta *et al*, 2018; Hsu & Guo, 2002). The presence of toxic metals in irrigation water can affect the quality of vegetables in two ways: by increasing the concentration of metals in the vegetable which is unfit for human consumption and/or by modifying or changing its composition in bioactive compounds, such as polyphenols, which are important for a healthy diet (Zhang *et al*, 2017; Sharma *et al*, 2020). Polyphenols are bioactive natural phenolic compounds derived from plant-based food, in particular fruits, vegetables, tea, and cereals, with significant positive effects on human health. In fact, polyphenols have a significant health impact, due to their antioxidant activity (Rathod *et al.,* 2022, Rathod *et al.,* 2021).

Oxidative stress is a relevant process whenever reduced antioxidant protection exists or the production of reactive oxygen species during cellular respiration is significant. These conditions often lead to cells and macromolecules (such as proteins or DNA) damage. Consequently, several chronic conditions may result, namely, hypertension, general inflammations, cancer, heart failure, diabetes, arthritis, neurological disorders, and several degenerative diseases, among other conditions (Inanli *et al.,* 2020, Yan *et al.,* 2020).

The bioactivity of these phytochemical substances is attributed to their structure, in particular the presence of phenol units that have attached functional groups, as these substances can effectively promote health by improving several diseases and disorders conditions, including immunomodulatory effects on different of cancer cells (Farhan, 2023, Chimento *et al.,* 2023). Consequently, there has been recent growing interest in the potential of these substances in therapeutic and pharmacological sectors, as well as in the food industry, since polyphenols can be used as bio-preservative in food and beverages, thus leading to a significant activity on what the inhibition of oxidative stress it concerns (Krawczyk *et al.,* 2023, Machado *et al.,* 2023).

Polyphenols can be organized in two major classes: flavonoids and phenolic acids. Presently, about 8000 polyphenolic substances are identified with half of this number being flavonoids only (Rathod *et al.,* 2023, Abbas *et al.,* 2017, Cheynier, 2005). Phenolic acids, which include, caffeic acid, *p*-coumaric acid, ferulic acid, vanillic acid, or benzoic acid, exhibit significant benefits on human health such as modulation and enhancement of immunity and human defence mechanisms, slowing cells oxidative stress, hypertension control, anti-inflammatory and anti-tumoral effect, anti-microbial resistance, cardiovascular system protection and antidiabetic potential (Hedayati *et al.,* 2023, Kumar & Goel, 2019). Flavonoids, which include, kaempferol, quercetin, anthocyanin, on the other hand, besides contributing to the colour observed in some fruits and vegetables, also exhibit several important health benefits such as, osteoporosis prevention gastrointestinal health, anti-inflammatory, anti-tumoral, anti-obesity, anti-viral and anti-diabetic effects (Chagas *et al.,* 2022, Hsiao *et al.,* 2020).

More recently, polyphenols compounds were tested against SARS-CoV-2 infection. Due to the spread of the pandemic, functional foods and active substances have acquired significant importance. Anti-viral potential of several polyphenols was tested, including quercetin, resveratrol, epigallocatechin gallate, curcumin, ellagic acid, myricetin, and quercetin. The results obtained exhibited important levels of viral activity inhibition as well as a significant reduction in lung inflammation (Alexova *et al.,* 2023, Micek *et al.,* 2023, Milton-Laskibar *et al.,* 2023).

Cabbage is one of the most popular vegetables in human diet due to its rich nutritional profile and availability throughout the year. In addition, several studies show that cabbage is rich in antioxidants that reduce the risk of developing chronic diseases (Moreb *et al*. 2020).

The main goal of the present investigation relies on the assessment of whether the water from the wastewater treatment plant (WWTP) can be used for irrigating agricultural fields with negligible effects to human health and the environment, even when contaminated with toxic metal with the highest concentration level of emission allowed by the Portuguese legislation, in particular the Annex XVIII of Decree-Law 238/96). This scenario will certainly promote a sustainable use of this resource as an alternative to its discharge into the environment, ensuring the circularity of water and consequently a more circular economy.

## METHODOLOGY

### CULTIVATION CONDITIONS

In this work, vegetables such as *Brassica oleracea* (cabbage) were planted in eight boxes in the interior of a greenhouse with a controlled environment (temperature and humidity) located at ISEL (Instituto Superior de Engenharia de Lisboa) campus (Figure 1).

In the first four weeks, each box was

irrigated periodically with 1 L of tap water to induce plants germination and growth. After this period, and according to the irrigation scheme in figure 1, part of the vegetables was irrigated with tap water and another with TWW supplemented with toxic metals with half the emission limit value (ELV) and another with the highest emission limit allowed, by Portuguese legislation in Annex XVIII of Decree-Law 238/96, in order to perform the worst-case scenario analysis. In this case, the concentration of metal which supplement the TWW were 1.0 mg/L for Pb, and 0.2 mg/L for Cd.

One of the WWTP receives only domestic wastewater (A) and the other one receives industrial and domestic wastewater (B), both located in Lisbon´s District. Neither of the TWW contained toxic metals, since according to the TWW characterization provided by the WWTP, lead and cadmium concentrations were below the detection limit. In this regard, TWW was supplemented with the intended levels of toxic metals.

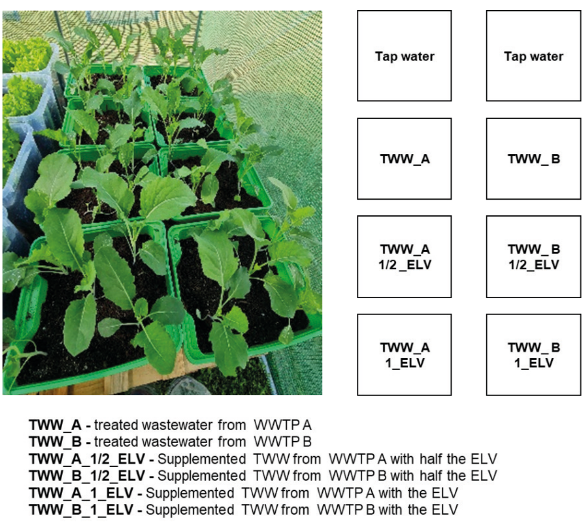


**Figure 1.** Boxes with planted cabbage inside the greenhouse and irrigation scheme.

## SAMPLES PREPARATION

Vegetable samples. cabbage leaves, were weighed and dried for 48 hours in an oven at about 65 °C. After complete drying, grinding was performed in a Retsch S100 mill grinding jars and agate balls for 5 min at 400 rpm. After milling, the diameter of the sample particles was less than 1 µm.

## ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS

The determination of antioxidant activity, total phenolic and flavonoid contents was performed using the plant extracts of *Brassica oleracea* samples obtained by microwave microextraction with methanol (MeOH), at a ratio of 1 g sample per 20 mL of methanol, with strong agitation for 3 minutes at a temperature of 80 °C and a pressure of 100 psi with a power of 100 W. The extract was filtered using a filter paper, and the solvent removed by reduced pressure evaporation in a rotary evaporator. Finally, the residue was dried in a vacuum line until constant weight was obtained.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to determine the radical scavenging activity of the extracts as described in Coelho, *et al*. (2018).

The total content of polyphenols and flavonoids present in the extracts were determined using the Folin Ciocalteu method (Bobo-Garcia, *et al*., 2015) and the aluminum chloride method (Coelho, *et al.,* 2018) respectively, to infer if the presence of toxic metals affected these important parameters of vegetable's food quality.

## RESULTS

The cabbage growth was monitored and documented with photographs each time the irrigation was performed. Comparing the cabbages growth in different photographs it was possible to conclude that there was an identical growth in all boxes while they were irrigated with tap water (Figure 1). On the other hand, after a few weeks of watering cabbages with the different types of water a clear differentiation in growth was observed (Figure 2).

Figure 2 clearly shows two types of growth: a smaller growth rate, evidenced by the cabbages on the left and right watered with tap water and TWW contaminated with the ELV content, respectively; and faster growth rate, evidenced by cabbages in the middle of the planting box, watered with TWW and TWW contaminated with half the ELV.

**Figure 2.** Growth of the plants 7 weeks after the first irrigation with TWWs.

(From left to right: potable water, TWW, TWW_1/2_ELV and TWW_1_ELV)

The positive effects of TWW on plant growth could be due to the presence of nutrient available for uptake as demonstrated in a study with lettuce (Vergine *et al*., 2017 b). In fact, the results from TWW analysis, provided by both WWTP showed that water samples contained nitrogen in the form of nitrates and ammonium. In WWTP A ammonium and nitrates content exhibited an average of 13.7 mg/L $NH_4^+$ and 3.5 mg/L N respectively, whilst in WWTP B these values were 53,9 mg/L $NH_4^+$ and 1 mg/L N respectively.

In figure 2, the lower growth of the cabbages on the right appears to be due to the higher content of toxic metals in the irrigation water, whereas in the case of the cabbages on left, this effect could be related to the lack of nutrients.

Metals (Hsu & Guo, 2002) and deficient of mineral nutrient (Tewari, *et al*., 2021) can cause oxidative stress by increasing the formation of reactive oxygen species, which renders antioxidants incapable of defense against growing amounts of free radicals (Hsu & Guo, 2002).

The total polyphenol content, expressed in mmol of gallic acid equivalent /g of extract, during 4 months of irrigation is shown in figure 3.

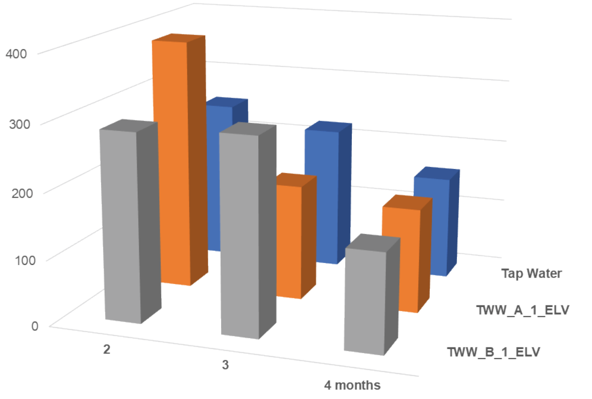


**Figure 3.** Total polyphenol content in cabbage during 4 months of irrigation.

Figure 4 shows the flavonoid content, expressed in mmol Catechin /g of extract, during the same irrigation time.

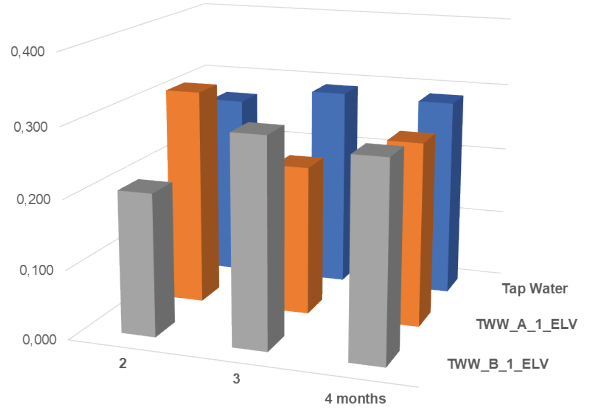


**Figure 4.** Flavonoid content present in cabbage during 4 months of irrigation.


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Figure 5 shows DPPH values, expressed as Trolox-equivalent antioxidant capacity (TEAC) in µg TEAC/mg FW (Fresh Weight - extract), that the cabbage leaves showed during 2, 3 and 4 months of irrigation.

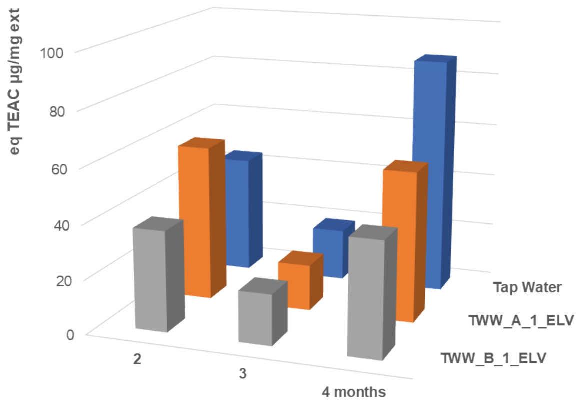


**Figure 5.** DPPH values present in cabbages during 2, 3 and 4 months of irrigation.

## CONCLUSIONS

Total polyphenol content in cabbages irrigated with tap water as well as with wastewater, exhibited a similar variation profile, with a decrease its concentration through the several months of irrigation.

In fact, cabbages will produce antioxidant compounds as a reaction to oxidative stress caused by the presence of toxic metals in irrigation water or a lack of nutrients. On the other hand, their content will naturally decrease to reduce the oxidative stress.

This observation is evidenced by the increase of the antioxidant activity after 4 months of irrigation, when the content of total polyphenols is lower, thus leading to an increase in antioxidant activity.

When determining the antioxidant activity by the DPPH method, the high or low presence of compounds with antioxidant properties is evaluated. When the presence of these compounds is low, this corresponds to a high antioxidant activity since there is a reduced amount of these compounds available to oxidize the DPPH radical.

It is clear that the total content of phenolic complexes, as well as the flavenoid content, remains the same regardless the type of water used to irrigate the cabbages (figure 3 and 4).

We can also conclude, according to figure 5, that the antioxidant activity by the DPPH method does not differ significantly when the cabbage is watered with tap water or treated wastewater from the two WWTP (A and B) supplemented with lead and cadmium.

In this way, and based on the results obtained, we can conclude that the use of TWW in watering vegetable gardens does not affect the antioxidant activity of the vegetables. Furthermore, this possibility can be considered as a sustainable solution for saving water resources, and a feasible alternative irrigation water source, bearing in mind the worldwide water shortages, which pose a serious risk for agricultural droughts.


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