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Physiological and Biochemical Responses of Maize (*Zea mays* L.) to the Application of Re-Treated Urban Wastewater Using Wood Waste Biochar

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ABSTRACT

The present work aimed to evaluate the effect of re-treated urban wastewater using wood biochar on the development of maize (*Zea mays* L.) plants. Maize seeds were sown in plastic pots, containing agronomic soil, and watered with treated wastewater (TWW) before and after re-treatment with wood waste biochar. Before re-treatment, results indicated that the application of TWW at 75% significantly enhanced the maize growth, in terms of plant height, shoot fresh (SFW) and dry (SDW) weight compared to control (natural water). In turn, the application of TWW at 100% showed the opposite effects. In fact, the total chlorophyll content and relative water content (RWC) were significantly decreased by 20% and 4%, respectively, compared to control plants. Furthermore, TWW at 100% significantly ($p < 0.05$) induced an accumulation of oxidative stress markers (MDA, H_2O_2). The non-enzymatic antioxidant process (total polyphenols and flavonoids) and the enzymatic antioxidant activity (CAT and APX) were also interestingly increased. The obtained negative correlation between maize growth and the accumulation of oxidative stress markers could explain the showed reduction in maize growth under 100% TWW. However, this effect seems to be alleviated in maize plants when they were watered with TWW re-treated with biochar, indeed, a significant improvement was marked in plant height, SFW, SDW, total chlorophyll content and RWC by 44%, 106%, 176%, 38% and 12%, respectively, compared to maize under 100% TWW. The finding suggests that the use of TWW diluted or re-treated by wood biochar could be a relevant approach to valorize TWW in agricultural purposes.

Keywords: treated wastewater, biochar adsorption, plant growth, oxidative stress, antioxidants, *Zea mays* L.

INTRODUCTION

The World Bank predicted that the treated wastewater (TWW) volume increased from 666 million m^3 in 2014 to 900 million m^3 in 2020 [Ortega-Pozo et al., 2022]. Within the fast-growing population worldwide, the demand for freshwater (FW) should increase, in parallel the quantity of wastewater has increased and the use of wastewater resources has also grown significantly during the past few decades. Thus, there is a need to think together within all scientific disciplines to manage the overproduced TWW, to preserve the FW and to confront the global water crisis, where two-thirds of mankind will face water scarcity by 2025 [Ungureanu et al., 2020].

In this context, TWW as an alternative source of water, has become a typical practice worldwide including arid and semi-arid countries which can help reduce high-FW usage for agricultural purposes that consumes more than 70% of FW [El Moussaoui et al., 2019]. Furthermore, TWW can reduce the need for chemical fertilizer, for example, 100% of the phosphorus and potassium needs for maize crops would be met by the reuse of treated urban wastewater in nations like Saudi Arabia, Brazil, and Poland, according to Chojnacka et al. [2020]. However, if not treated properly, wastewater has been proven to have negative impacts on soil, groundwater, plants and their consumers [Chaoua et al., 2019; Ungureanu et al., 2020]. Recently, Merbouh et al., [2022] proved that the organic pollution in urban wastewater effluent was the main cause of the pollution in a natural river (Oued *Nfifikh*, Morocco).

For this purpose, because of its adsorption properties, biochar has been proposed as an eco-friendly strategy for wastewater treatment. Biochar can be used in a simple design as a natural potential filter to remove organic and inorganic pollutants including total suspended solids from various types of wastewaters, like municipal and industrial types [Hu et al., 2022; Xiang et al., 2020]. Even for highly salty wastewater, forestry wood biochar was effective in removing numerous potentially toxic elements [Sun et al. 2019]. The forestry wood is the most material used to produce biochar, through thermochemical conversion in a zero- or low-oxygen environment [Braghiroli et al., 2018; Xia et al., 2020].

In this perspective, the present work aimed to evaluate the effect of TWW re-treated with wood waste biochar on the growth of maize (*Zea mays* L.) plants and their oxidative stress responses. In fact, the highest vulnerability of maize crops to the water stress, which was noted recently in Morocco, left their production decreased by 70% in 2022 compared to 2018, according to the Food and Agriculture Organization [FAOSTAT, 2023].

MATERIALS AND METHODS

Water sampling, analysis and re-treatment

The treated urban wastewater used in this study was collected from the outlet of a local wastewater treatment plant in Beni Mellal city, Morocco, which is discharged directly into the wild (32°21′36″ N 6°24′19″ W; 462 m of altitude; Day River) (Fig. 1).

Before and after re-treatment, a composite sample of this TWW was filtered through a sieve of Ø 5 mm and analyzed immediately to determine electrical conductivity (EC), pH, temperature, dissolved oxygen, and total dissolved solids by a calibrated multiparameter (AZ80031). Then, the samples were stored at 4 °C to serve all physicochemical analyses, in terms of total suspended solids, chemical oxygen demand, biological oxygen demand, total Kjeldahl nitrogen as well as the content of orthophosphates and chloride, according to standard guidelines of American Public Health Association [APHA, 2017] and Rodier et al. [2009] (Table 1).

The biochar application was made as follows: The homogeneous powder of wood waste biochar was filtered through 0.5 mm sieve and used directly to re-treat TWW (1/1000; w/v) for 24 h in

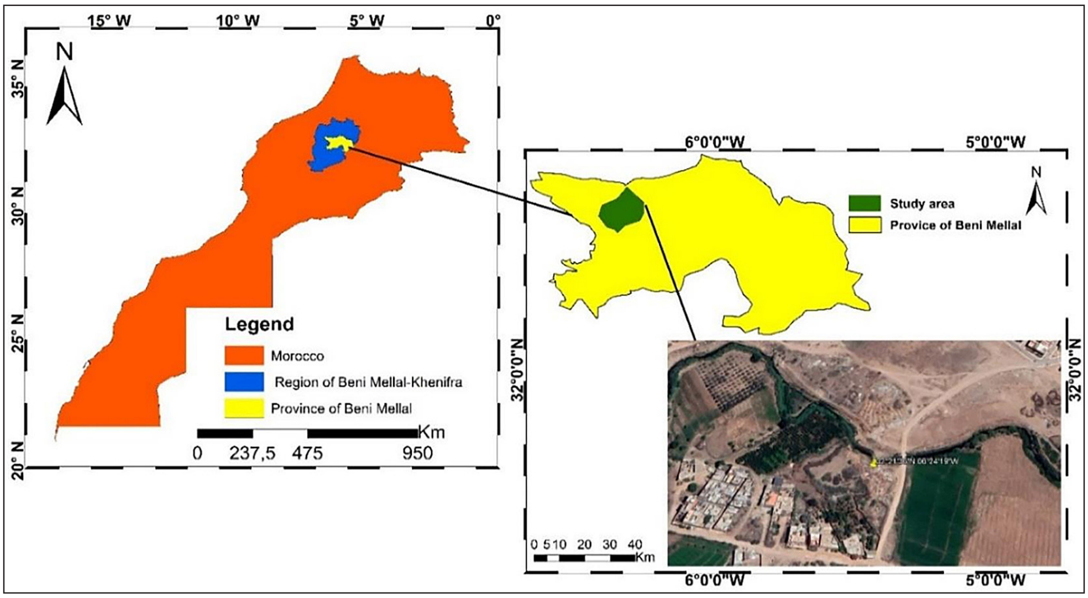

Figure 1. Study area

Table 1. Physico-chemical characteristics of natural water (NW), treated wastewater (TWW) and TWW after re-treatment (TWW-r). Data are means of three replicates ± standard errors

Physico-chemical parameters	NW	TWW	TWW-r	GMLD
Temperature (°C)	19.54 ± 2.97	15.23 ± 0.74	20.91 ± 1.05	30.00
pH	7.56 ± 0.23	8.02 ± 0.10	8.00 ± 0.23	5.50–9.50
EC (μs cm^{-1}) at 20 °C	621.89 ± 10.49	1587.44 ± 10.98	1574.44 ± 7.47	2700.00
TDS (ppm)	314.33 ± 5.49	807.11 ± 31.00	749.56 ± 38.08	-
DO (mg L^{-1})	5.34 ± 0.24	4.56 ± 0.24	3.53 ± 0.57	-
Cl^- (mg L^{-1})	nd	136.08 ± 2.37	127.80 ± 2.05	-
TSS (mg L^{-1})	nd	76.81 ± 3.81	48.27 ± 2.08	100.00
COD (mg O_2 L^{-1})	nd	512.53 ± 26.08	144.48 ± 34.95	500.00
BOD_5 (mg O_2 L^{-1})	nd	138.33 ± 19.65	41.33 ± 6.84	100.00
TKN (mg L^{-1})	nd	196.00 ± 16.17	149.33 ± 16.83	40.00
PO_4^{3-} (mg g^{-1})	nd	5.78 ± 0.23	5.42 ± 0.15	-
BOD_5/COD	nd	0.27 ± 0.05	0.33 ± 0.12	-

Note: BOD_5 – biological oxygen demand in a 5-day; Cl^- – chloride; COD – chemical oxygen demand; DO – dissolved oxygen; EC – electrical conductivity; GMLD – the general maximum limits for the discharge into surface water or groundwater in Morocco (Moroccan standards 2018); nd – not determined; pH – the potential of hydrogen; PO_4^{3-} – orthophosphates; TDS – total dissolved solids; TKN – total Kjeldhal nitrogen; TSS – total suspended solids.

dark at 25 °C under shaking (300 rpm), finally, the homogenate was filtered by a filter paper [El Mrabet et al., 2020; Huggins et al., 2016], then used for maize irrigation.

Experimental design

The seeds of maize (*Zea mays* L. var. Mas 78.T; 100 seeds = 28.66 g) used in this experiment were obtained from a commercial supplier in Beni Mellal city, Morocco. Indeed, the uniform and healthy seeds were first disinfected on the surface by immersion in 0.5% of sodium hypochlorite for 30 min, rinsed several times with distilled water (DW) and then soaked in DW for 8 h. Disinfected maize seeds were then sown in plastic pots (18 cm/12 cm; diameter/height), containing 2 kg of clayey agricultural soil collected from an agricultural field from the same study area (Fig. 1), with the following characteristics: pH 7.23 ± 0.1 and EC at 20 °C 89.28 ± 7.49 μs cm^{-1} tested in 1/5 ratio (w/v) [Kim et al., 2016], A PHSJ-3F pH meter and a DDS-12DW Benchtop conductivity meter were used to measure the pH and EC, respectively.

One week after seedlings emergence, five uniform seedlings were maintained in each pot. Pots were then divided into four treatments as presented in Table 2: i) plants watered with natural water (NW) (T0; control), ii) plants watered with 75% of TWW (T1), iii) plants watered with 100% TWW (T2) and iv) plants watered with TWW re-treated with wood biochar (T3). A preliminary experiment using different TWW concentrations showed that 100% TWW had a toxic effect while 75% TWW had the best effect in improving maize (*Zea mays* L.) plant growth. Consequently, 75% and 100% TWW were chosen as benefic and toxic concentrations, respectively.

Natural water (NW) was used as control and for TWW dilutions. This NW was obtained from a local spring called Ain Asserdoune and classified as drinkable according to Barakat et al. [2018]. The pot experiments were performed from the 4th April to the 5th May 2022 at the Polydisciplinary Faculty of Beni Mellal, Morocco (32° 20′ 22″N, 6° 21′ 39″W, 503 m of altitude) using a randomized design, with three replicates per treatment under natural environmental conditions (ambient climate of spring). The climate is a Mediterranean climate characterized by temperate winter (0–4 °C in January), hot and dry summer (up to 40 °C in August), with an average annual rainfall of

Table 2. Composition of irrigation water

Treatments	NW (%)	TWW (%)	TWW-r (%)
T0	100	0	0
T1	25	75	0
T2	0	100	0
T3	0	0	100

Note: NW – natural water; TWW – treated wastewater; TWW-r, TWW – after re-treatment with wood biochar.

350–400 mm and a temperature of 18 °C [Barakat et al., 2018]. The irrigation was carried out regularly to maintain soil water content at 80% of field capacity. Treatments were applied for 4 weeks, then some agro-physiological, biochemical and enzymatic parameters were assessed.

Determination of plant growth

After 4 weeks of treatments, the plant height was measured using a ruler graduated to centimeters and millimeters. Thereafter, the plants were carefully detached from the pots. Then, the shoot parts were separated from the harvested plants to determine their fresh weight (SFW). Regarding the shoot dry weight (SDW), it was measured after drying the shoot parts at 85 °C in an oven for 48 h.

Determination of relative water content (RWC)

RWC was measured according to El Moukhtari et al. [2021]. Around 100 mg of fresh leaf was sliced into small pieces measuring 10 mm diameter and weighed to determine their fresh weight (*FW*). Pieces were then floated in distilled water for 6 h and their turgid weight (*TW*) was measured. The dry weight (*DW*) of the pieces was determined after drying them in an oven (70 °C for 24 h). Finally, *RWC* was calculated as follow:

$$RWC(\%) = \frac{(FW-DW)}{(TW-DW)} \times 100 \qquad (1)$$

Determination of photosynthetic pigment content

Photosynthetic pigments were extracted following the method of El Moukhtari et al. [2023] which was adopted from Arnon [1949]. Firstly, 50 mg of fresh leaf tissue was ground at 4 °C in 1 mL of acetone (92%; v/v), and centrifuged at 10 000 rpm for 10 min at 4 °C. The resulting supernatant was recovered and the volume was made up to 2 mL using 80% acetone. Then, by using a UV–VIS absorption spectrophotometry (DLAB, SP-UV1000, China), the optical density (*OD*) was determined at 663 nm, 645 nm and 480 nm against 80% acetone as a blank. The content of total chlorophyll (Chl) and carotenoid (*Car*) were calculated by the formula given by D'souza and Devaraj [2013]:

$$\text{Total Chl (mg g}^{-1}\text{)} = \\ [(20.2 \times OD_{645}) + (8.02 \times OD_{663})] \times (V/1000 \times Wt) \qquad (2)$$

$$Car\ (\text{mg g}^{-1}) = OD_{480} + \\ (0.14 \times OD_{663} - 0.638 \times OD_{645}) \times (V/1000 \times Wt) \qquad (3)$$

where: V – volume (2 mL); Wt – weight (50 mg).

Determination of oxidative stress markers

Malondialdehyde content (MDA) in maize leaf was evaluated following the Thiobarbituric acid (TBA) method [Heath and Packer 1968]. Briefly, 100 mg of FW of leaves was mixed in 1.5 mL of Trichloroacetic acid (TCA) (5%). The mixture was centrifuged at 12 000 rpm for 15 min at 4 °C, and then 0.5 mL of the resulting supernatant was added to 1.5 mL TBA prepared in TCA (20%). The homogenate was incubated at 95 °C for 25 min and after cooling down the OD was measured at 532 nm and 600 nm. MDA content was calculated using its extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as ηmol g^{-1} FW.

The hydrogen peroxide (H_2O_2) content was determined according to Hossain et al. [2010]. Around 500 mg of fresh leaf matter was ground at 4 °C using 5 mL of 50 mM sodium phosphate buffer (pH 7.8). The resulting homogenate was then centrifuged at 12 000 rpm for 5 min at 4 °C and to 3 mL of the resulted supernatant, 1 mL of 0.1% $TiCl_4$ (prepared in 20% H_2SO_4) was added. After 10 min of incubation at room temperature, the absorbance was measured at 410 nm and the concentration of H_2O_2 was calculated using a standard curve prepared using different concentration of commercial H_2O_2 and expressed as μmol mg^{-1} FW.

Regarding the electrolyte leakage (*EL*) determination, the maize leaves were washed 3 times with deionized water, and then divided into small pieces and placed in closed test tubes containing 10 mL of deionized water. The tubes were then shaken for 24 h at 25 °C in the dark to liberate all the electrolytes in the solution, and the first electrical conductivity (EC_1) was measured. Then, these tubes were placed in autoclave at 121 °C for 20 min to measure the second *EC* (EC_2) at 25 °C [Ghoulam et al. 2002]. EL was calculated by the following formula:

$$EL(\%) = \left(\frac{EC_1}{EC_2}\right) \times 100 \qquad (4)$$

Determination of non-enzymatic antioxidant activities

To determine the non-enzymatic antioxidant activity, 100 mg of FW of leaves was mixed in 1 mL of methanol (80%) at 4 °C using mortar and pestle. Then, the mixture was centrifuged at 12

000×g at 4 °C for 20 min and the supernatant was recovered and used to determine the content of total polyphenols and flavonoids [El Moukhtari et al. 2022]. The total polyphenols were measured according to the Folin-Ciocalteu method [Singleton and Rossi 1965]. Indeed, 250 µL of FC reagent was added to 50 µL of the obtained supernatant and the volume was adjusted to 5 mL with distilled water. After incubation for 3 min at room temperature, the volume was adjusted to 6.5 mL with Na_2CO_3 (20%) and the mixture was incubated at the dark for 1 h at room temperature. Then, at 725 nm, OD was read and the total polyphenols content was calculated and expressed as mg gallic acid equivalents g^{-1} FW.

The flavonoids content was determined by the method of Chang et al. [2002], where, 100 µL of the resulted supernatant was added to 300 µL of methanol (95%), 20 µL of 10% aluminum chloride ($AlCl^3$), 20 µL of potassium acetate (1 M) and 560 µL of distilled water. After 30 min of incubation at room temperature, the flavonoid content was determined after measuring the OD at 415 nm and referring to a standard curve prepared from different concentrations of quercetin. The amount of flavonoid was represented as mg quercetin g^{-1} FW.

Determination of enzymatic antioxidant activities

For enzyme extraction, 0.5 g of fresh leaf was ground in 5 mL of sodium phosphate buffer (pH 7.8). The extract was centrifuged at 12 000 rpm for 10 min at 4 °C and the resulting supernatant was recovered as well as used for determination of the catalase (CAT) and ascorbate peroxidase (APX) activities. CAT (CAT, EC 1.11.1.6) was assessed according to Ramzan et al. [2021] with slight modifications. In fact, to 50 µL of the enzymatic extract, 500 µL of 0.1 M of potassium phosphate buffer (pH 6.5), 250 µL of distilled water and 200 µL of H_2O_2 (75 mM) were added. The decrease in OD was monitored at 240 nm for 2 min at 25 °C. CAT activity was calculated using the extinction coefficient of H_2O_2 of 36 mM^{-1} cm^{-1} and expressed as enzymatic unit (EU) min^{-1} mg^{-1} protein.

For ascorbate peroxidase (APX, EC 1.11.1.11), the method of Nakano and Asada [1987] was adopted. The reaction mixture of 2 mL contains 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2, 0.1 mM EDTA and 0.1 mL of the enzymatic extract. The APX activity was expressed as EU min^{-1} g^{-1} protein. The Bradford [1976] method was adopted to determine the protein content in the enzymatic extract of maize plant.

Statistical analysis

The results shown represent means ± standard errors (SE) of three replicates. Data were statistically analyzed using IBM SPSS software (version 26.0), where the test of one-way analysis of variance (ANOVA I) was applied. The means of the obtained data were compared by using a post-hoc Tukey's HSD test at $p < 0.05$. In addition, principal component analysis (PCA) was carried out by the XLSTAT software version 2018 (Addinsoft, Paris, France).

RESULTS

Plant growth responses

The effects of treated wastewater (TWW) before and after re-treatment with wood biochar on plant growth, in terms of plant height, shoot fresh and dry weight are summarized in Figure 2. The results indicated that the fertilization of maize plants with 75% of TWW (T1) induced a slight increase in plant height, as compared to control plants (T0). However, when these plants were watered with 100% of TWW (T2), plant height were significantly ($p < 0.05$) reduced by 9%, as compared to those watered with T0. Interestingly, when TWW was re-treated with wood biochar (T3), their application had a significant effect on plant height. Indeed, T3 increased plant height by 31% relative to T0 and by 44% relative to plants watered with T2. Additionally, the presented results revealed that T1 has no significant ($p < 0.05$) effect on either shoot fresh (SFW) or dry (SDW) weight when compared to T0. In contrast, T2 proved the worse effect that reached 4% and 32% of decreasing for SFW and SDW, respectively, than T0. However, T3 induced a significant ($p < 0.05$) increase in both SFW and SDW reached 106% and 176%, respectively, relative to T2.

Photosynthetic parametrs and water nutrition

Total chlorophyll (Chl) was slightly increased in the maize plants irrigated with 75% TWW (T1) with an increment rate of 2% relative to control plants (T0) (Table 3). However, the substitution of natural water with 100% TWW (T2) decreased

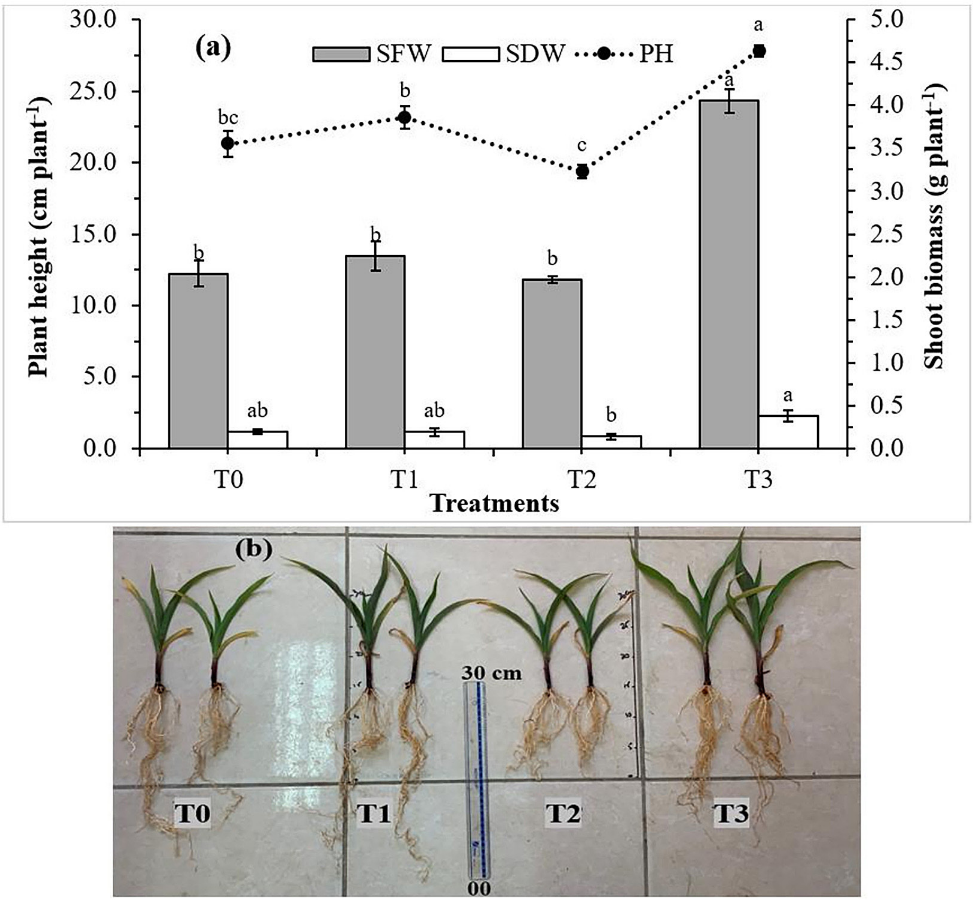

Figure 2. Effect of irrigation with TWW before and after re-treatment with wood biochar on plant height (PH), shoot fresh (SFW) and dry (SDW) weight and phenotype (b) of maize plants. Values are means of three replicates ± standard errors and the different letters show a significant difference at $p < 0.05$. T0, control; T1, TWW at 75%; T2, TWW at 100%; T3, TWW re-treated with wood biochar

significantly ($p < 0.05$) total Chl by 20%. In contrast, re-treating TWW with wood biochar (T3) retracted the negative effect of T2 on maize plants and significantly increased total Chl with an increment rate of 38%. However, a significant increase in carotenoid content (Table 3) was recorded for the maize plants watered with T2 than all other treatments (1.3-fold higher than T0). Regarding leaf water status, the results presented in Table 3 showed that the leaf relative water content (RWC) was significantly decreased from 83 to 79% when T0 was substitute by T2 for maize irrigation. However, this water status increased considerably by 4% and 10% in maize under T1 and T3, respectively, than those under T2.

Oxidative stress indicators

The oxidative stress response of the maize irrigated with TWW before and after re-treatment are presented in Figure 3. Indeed, the malondialdehyde (MDA) content increased in maize under 75% TWW (T1) than control (T0), and reached significantly ($p < 0.05$) their maximum value (7.98 nmol g^{-1} FW) under 100% TWW (T2) (Fig. 3a). However, when TWW was re-treated with wood biochar (T3), an interesting positive effect was observed regarding MDA content. Where, MDA content in maize plants irrigated with T3 was 0.51-fold lower than the plants irrigated with T2. For hydrogen peroxide (H_2O_2) content, values were elevated significantly ($p < 0.05$) by 65% in the maize plants irrigated with T2, relative to control plants (Fig. 3b). In contrast, the maize plants irrigated with T3 showed a significant decrease in H_2O_2 contents (22%) as compared to those watered with T2. Similarly, for electrolyte leakage (EL) (Fig. 3c), irrigating maize plants with T1 increased this parameter, and the increase was

Table 3. Effects of irrigation with treated wastewater (TWW) before and after re-treatment with wood biochar on total chlorophyll, carotenoids content and relative water content of maize plants. Results are means of three replicates ± standard errors and the different and small letters show a significant difference at p < 0.05

Treatments	Total chlorophyll	Carotenoids	Relative water content
	(mg g^{-1} FW)	(µg g^{-1} FW)	(%)
T0	0.77 ± 0.03^{ab}	11.16 ± 0.41^{b}	82.74 ± 2.00^{ab}
T1	0.79 ± 0.05^{ab}	10.98 ± 0.66^{b}	82.62 ± 1.77^{ab}
T2	0.62 ± 0.06^{b}	14.63 ± 0.40^{a}	79.09 ± 1.84^{b}
T3	0.85 ± 0.05^{a}	11.12 ± 0.97^{b}	88.53 ± 0.02^{a}

Note: T0, control; T1, TWW at 75%; T2, TWW at 100%; T3, TWW re-treated with wood biochar.

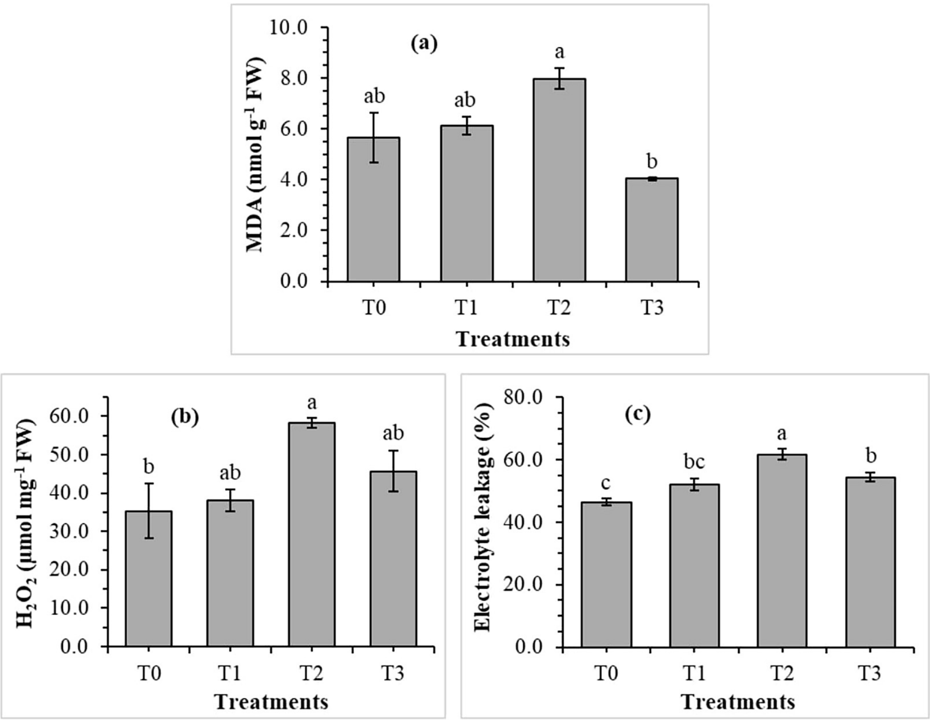

Figure 3. Effect of irrigation with treated wastewater (TWW) before and after re-treatment with wood biochar on malondialdehyde (MDA) (a), hydrogen peroxide (H_2O_2) contents (b) and electrolyte leakage (EL) (c) in maize plants. Values are means of three replicates ± standard errors and the different and small letters show a significant difference at $p < 0.05$. T0, control; T1, TWW at 75%; T2, TWW at 100%; T3, TWW re-treated with wood biochar

more accented ($p < 0.05$) when plants were irrigated with T2. Indeed, as compared to control plants, the increase in EL was 12% and 33%, respectively, for the plants watered with T1 and T2. However, when TWW was re-treated with wood biochar (T3) an enhancement in membrane integrity, reflected by lower EL (12%), was observed compared to the plants irrigated with T2.

Antioxidants response

The results depicted in Table 4 revealed that the contents of non-enzymatic antioxidant molecules, in terms of total polyphenols and flavonoids, were significantly ($p < 0.05$) higher in the maize plants watered with 75% TWW (T1) and the increase was much higher when the maize plants were irrigated with 100% TWW (T2) relative to control plants (T0). Indeed, total polyphenols and flavonoids in the maize plants irrigated with T2 was 1.64 and 1.21-fold higher, respectively, compared to their control plants. However, as expected, both total polyphenols and flavonoids were decreased in the maize plants when irrigated with TWW re-treated with wood biochar (T3). Consequently, relative to T2, total

Table 4. Effect of irrigation with treated wastewater (TWW) before and after re-treatment with wood biochar on total polyphenols, flavonoids contents, catalase (CAT) and ascorbate peroxidase (APX) activities in maize plants. Values are means of three replicates ± standard errors and the different and small letters show a significant difference at $p < 0.05$

Treatments	Non-enzymatic antioxidant content		Enzymatic antioxidant activity	
	Total polyphenols (mg GA g^{-1} FW)	Flavonoids (mg quercetin g^{-1} FW)	CAT activity (EU min^{-1} mg^{-1} protein)	APX activity (EU min^{-1} mg^{-1} protein)
T0	184.64 ± 5.76^{b}	16.07 ± 0.99^{bc}	4.35 ± 1.16^{ab}	43.65 ± 9.92^{a}
T1	223.73 ± 16.96^{ab}	17.65 ± 0.26^{ab}	4.54 ± 0.19^{ab}	45.15 ± 4.32^{a}
T2	302.76 ± 40.39^{a}	19.37 ± 0.72^{a}	6.98 ± 0.99^{a}	69.90 ± 9.58^{a}
T3	248.01 ± 3.61^{ab}	14.32 ± 0.22^{c}	2.45 ± 0.70^{b}	47.62 ± 6.30^{a}

Note: EU – enzymatic unit; GA – gallic acid; T0 – control; T1 – TWW at 75%; T2 – TWW at 100%; T3 – TWW re-treated with wood biochar.

Table 5. Result of one-way ANOVA test for independent variables including naturel water, 75% treated wastewater (TWW), 100% TWW, and TWW re-treated with wood biochar

Parameters	dF	F	*P*-value
PH	3	28.733	0.000***
SFW	3	52.757	0.000***
SDW	3	5.030	0.030*
Total Chl	3	3.759	0.060^{ns}
Car	3	7.436	0.011*
RWC	3	5.798	0.021*
MDA	3	8.270	0.008**
EL	3	15.478	0.001**
H_2O_2	3	4.749	0.035*
Total polyphenols	3	4.974	0.031*
Flavonoids	3	11.651	0.003**
CAT	3	4.815	0.034*
APX	3	2.444	0.139^{ns}

Note: dF, degree of freedom; EL – electrolyte leakage; F – fischer; H_2O_2 – hydrogen peroxide; MDA – malonyldialdehyde; PH – plant height; RWC, –relative water content; SDW – shoot dry weight; SFW, – shoot fresh weight; Total Chl – total chlorophyll; ns – not significant; *significant at 0.05; **significant at 0.01; **significant at 0.001.

polyphenol and flavonoid contents was 0.82 and 0.74-fold lower, respectively.

Regarding enzymatic antioxidant system (Table 4), catalase (CAT) and ascorbate peroxidase (APX) activities were significantly ($p < 0.05$) increased under T1 and the increase was more obvious under T2. Indeed, the CAT and APX activities of maize plants were increased from 4.35 and 43.65 EU min^{-1} mg^{-1} protein under natural water to 6.98 and 69.90 EU min^{-1} mg^{-1} protein under T2, respectively, reflecting an increment rate of 60%, for both antioxidant enzymes. In contrast, the CAT and APX activities were significantly decreased in the maize plants treated with T3. In fact, under T3, the activity of CAT and APX of maize plants decreased by 65% and 32%, respectively, compared to plants treated with T2.

Principal component analysis (PCA) and Pearson's correlation matrix

PCA analyses were conducted to obtain further explanation about the possible correlations between the measured parameters and to know the behavior of maize plants in response to the applied treatments (Fig. 4). The results proved that the variability percent was very high (F1 and F2: 73.46%). Thus, the 100% TWW (T2) showed a highly positive correlation with the oxidative stress markers, including MDA, H_2O_2 and EL and the antioxidant system defense, like the content of total polyphenols and flavonoids and the activity of CAT and APX. This clearly explains the growth reduction in terms of PH, SFW and SDW under this treatment. However, when TWW was re-treated with wood biochar (T3) a significant improvement in maize plants growth was recorded, as evidenced by the highly significant positive correlation observed between T3 and physiological parameters including PH, SFW, SDW, RWC and total chlorophyll.

DISCUSSION

The results of this study recorded that the irrigation of maize plants with concentered TWW (100%, T2) had inimical effect on maize growth. Indeed, as confirmed by the obtained significant

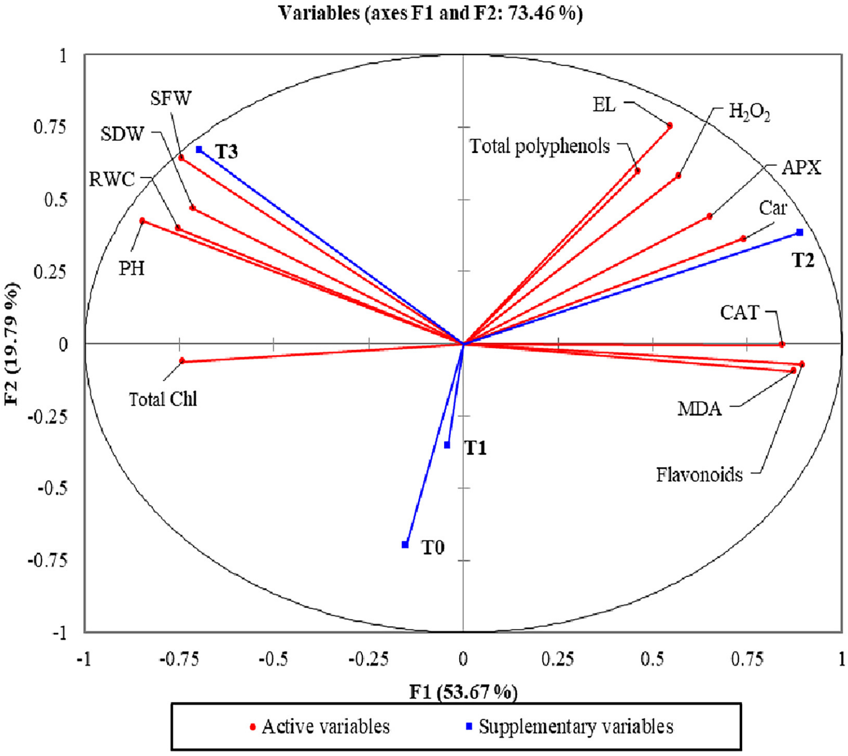

Figure 4. Principal components analysis (PCA) between assessed parameters in maize (*Zea mays* L.) plants irrigated with treated wastewater (TWW) before and after re-treatment with wood biochar for 4 weeks. APX – ascorbate peroxidase activity; Car – carotenoids; CAT – catalase activity; EL – electrolyte leakage; H_2O_2 – hydrogen peroxide; MDA – malonyldialdehyde; PH – plant height; RWC – relative water content; SDW – shoot dry weight; SFW – shoot fresh weight; Total Chl – total chlorophyll; T0 – control; T1 – TWW at 75%; T2 – TWW at 100%; T3 – TWW re-treated with wood biochar

negative correlations as presented in Figure 4, the most evaluated parameters, especially the parameters related to plant growth, water statue and photosynthesis, were significantly decreased in irrigated plant with 100% of TWW. This reaction of maize under T2 may due to the excess nutrients or the presence of toxically elements (salt ions, heavy metals...) dissolved in this type of water, which can at some limit cause remarkable damage to the plants [Bouhadi et al., 2023; Pedrero et al., 2018]. Furthermore, this toxic effect remained below the tolerance limit of maize when irrigated with diluted TWW (TWW at 75%; T1), with minimal oxidative response (Fig. 4). This improvement by T1 was in agreement with the results of Mousavi and Shahsavari [2014], where irrigation of maize with TWW for 3 times against 1 time by natural water gives the best yields for plant height, diameter and grain production (kg ha^{-1}) related to those irrigated only with natural water or 100% TWW. In the same line, Khalid et al. [2023] noted that the wastewater dilution by freshwaters can reduce the pollutants level and therefore their toxic effects. On the other hand, it should also be noted that the TWW reprocessed by biochar (T3) showed the best response for maize growth, with a negative correlation (Fig. 4) with reactive oxygen species (ROS) production (H_2O_2, EL and MDA) and oxidative response (enzymatic and non-enzymatic). This means that the TWW re-treated with wood waste biochar give a good quality water (T3), with little toxic compounds, and that was reflected by the considerable reduction in pollution parameters, such as TSS, BOD_5, COD, Cl^-, NTK and PO_4^{3-} (Table 1).

The negative impact of T2 by decreasing maize plant growth, was supported by the results of Mahmood et al. [2013], who reported that the growth of *Zea mays* plants watered with bio-treated textile wastewater was 35% and 16% lower than control for plumule and radicle, respectively, after 7 days of sowing. In contrast, for alfalfa growth, the results of Elfanssi et al. [2018] showed that the irrigation with wastewater (raw and bio-treated) had a positive effect in three crop seasons. As a physiological response, maize growth was positively correlated (Fig. 4) with the total chlorophyll content and relative water content (RWC). That was similar to Elfanssi et al. [2018] and El Moukhtari et al. [2021] for alfalfa plants, furthermore, the opposite effect was linked to the salinity of applied water. In the same line, in the conducted work, the increasing of maize growth, chlorophyll content and RWC watered by T3 was correlated with salinity reduction in this type of water reflected by TDS, Cl$^-$ and EC reduction (Table 1). It was also noted that the maize watered with T3 showed larger and greener leaves, especially than T2. Overall, TWW with high level of salinity and sodicity would decrease agricultural output and provide a serious environmental risk in terms of soil salinization [Chaganti et al., 2020]. Water quality can be reflected by the behavior of maize plant in terms of oxidative stress markers (Fig. 3). In fact, as reported by Rekik et al. [2017], urban wastewater treated by activated sludge process with a permissible level of heavy metals has an adverse effect in germination and development of alfalfa, sorghum, and fescue at early growth stage (15 day). For example, all species under the effect of this water, registered a decreasing in shoot length, leaf fresh and dry weight, authors also found that plants showed an oxidative stress response, in terms of increasing of free radicals H_2O_2 and MDA. Similarly, the obtained results showed that the maize plants irrigated with T2 had very high levels of MDA, H_2O_2 and EL compared to T0, T1 and those irrigated with T3 (Fig. 3). Indeed, abiotic stresses including salinity, can impact negatively plant growth, resulting in reducing of FW and seedling lengths, in parallel of MDA, H_2O_2 and EL increasing, although in short-time growth. That was proven by El Moukhtari et al. [2021], Fricke et al. [2006] and Lamsaadi et al. [2022a, b] for alfalfa, barley and fenugreek plants, respectively. As antioxidant response (Table 4), enzymatic activity (CAT and APX) increased significantly in the maize irrigated with 100% TWW (T2) than that irrigated with natural water (T0), diluted TWW (T1) and re-treated TWW (T3). As previously proven by Demidchik, [2017], the enzymatic antioxidant

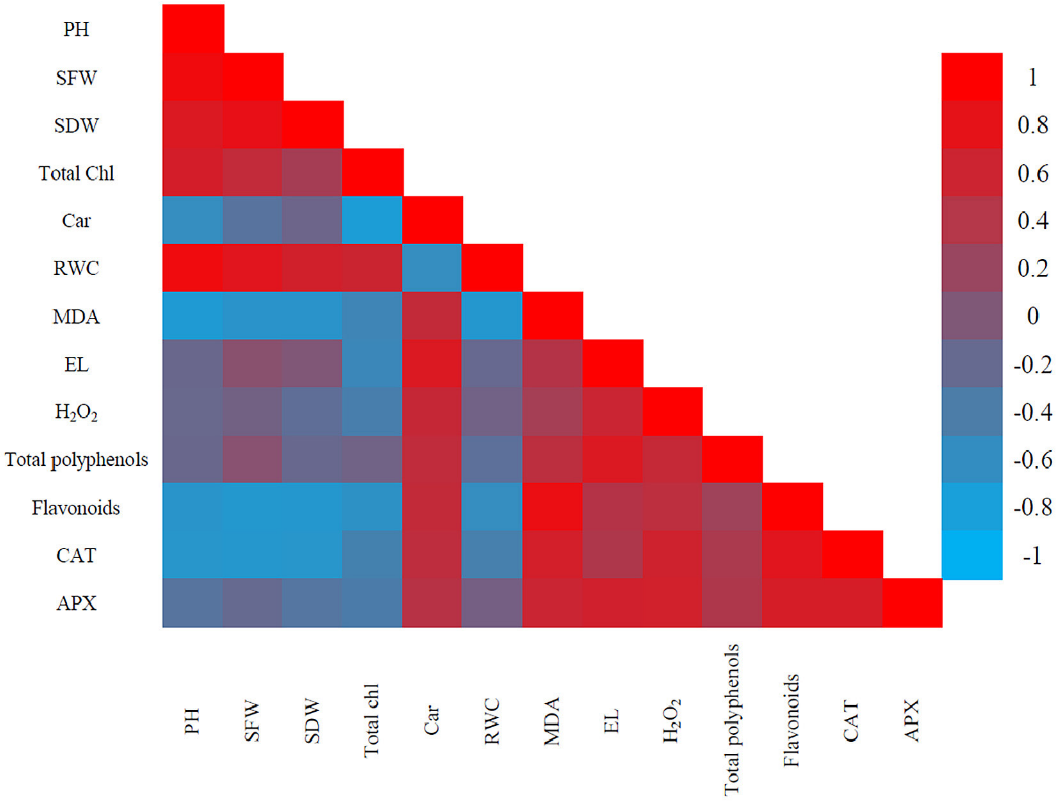

Figure 5. Pearson's correlation matrix between tested parameters in 4 weeks olds plants of maize (*Zea mays* L.) irrigated with treated wastewater (TWW) before and after re-treatment with wood biochar. Positive and negative correlations are shown in red and blue, respectively, and intensity of color is proportional to correlation coefficient. APX, ascorbate peroxidase activity; Car, carotenoids; CAT, catalase activity; EL, electrolyte leakage; H_2O_2, hydrogen peroxide; MDA, malonyldialdehyde; PH, plant height; RWC, relative water content; SDW, shoot dry weight; SFW, shoot fresh weight; Total Chl, total chlorophyll

system play an important role to suppressing the ROS production and maintain the electron transport chain and K^+ flux. This was evident in the conducted study, as indicated by the significant positive correlation between CAT and MDA (r = 0.65; Fig. 5) and H_2O_2 (r = 0.62; Fig. 5), and between APX and MDA (r = 0.59; Fig. 5) and H_2O_2 (r = 0.63; Fig. 5). For non-enzymatic response, the same response was registered, where the maize irrigated with T2 accumulated more polyphenols and flavonoids than that irrigated with T0, T1 and T3. As it is known, the case in which the plant product more of phenolic acids (polyphenols and flavonoids) is when it was under stressful environments [Sharma et al., 2019]. Even for early growth stage, antioxidants (both enzymatic and non-enzymatic) are crucial for plant defense against various abiotic stresses (high/low temperatures, drought, salt, heavy metals...) [Šamec et al., 2021; Taïbi et al., 2016].

In accordance with this finding, marked by TWW quality improvement by wood waste biochar, several studies proved that biochar has the ability to eliminate by adsorbing process, many toxic products in water, which may be encountered in T2 [Enaime et al., 2020; Xiang et al., 2020]. Moreover, biochar as a physical process in cleaning up TWW, was used in the case that many pollutants are usually resistant to chemical and biological degradation during conventional treatment processes. In this study, the wood waste biochar was used to re-treat the treated urban wastewater, previously treated by trickling filter and classified as non-biodegradable (BOD_5/COD < 0.3) [Abdalla and Hammam, 2014]. As a result, quality and biodegradability improvement (BOD_5/COD > 0.3) (Table 1) after biochar application, make the maize plants irrigated by T3 take the positive part of this water (Nitrogen, phosphorus and potassium) without or with a minimal soluble/insoluble contaminant compounds. In fact, the combination of treatment process is practically in the field of wastewater treatment to obtain a TWW safe and reusable [Braghiroli et al., 2018].

CONCLUSIONS

It can be concluded that the random use of TWW at 100% in the discharge area is not recommended and can harm the environment and public health. Likewise, the obtained findings strongly recommend that diluted TWW at 75% and TWW re-treated with wood biochar could be a promising way to irrigate maize under the recent environmental changes marked by the water stress, especially in the Mediterranean area, including Morocco. In fact, the irrigation of maize plants with TWW in diluted form or re-treated using the physical adsorption by wood waste biochar, has shown a positive effect on maize plant growth, photosynthesis pigment, in parallel to a significant reduction in oxidative stress indicators, like MDA and H_2O_2. The latter were positively correlated with a significant stabilization of the activity of both enzymatic and non-enzymatic antioxidant systems. According to the re-treated TWW analysis, the beneficial effect of irrigation with re-treated TWW could be explained by the biodegradability improvement. Indeed, biochar has a high capacity to adsorb several toxic compounds dissolved or not in water, such as, ion salt, heavy metals, hydrocarbons, pesticides, and other toxic products. Moreover, the obtained findings encourage the authors to conduct further studies, in order to determine the effect of wastewater re-treated with biochar in the medium and long term, for a healthy valorization in the agricultural sector. Additionally, since the TWW contains an important level of nutritive elements, the use of TWW at reasonable concentration or after their re-treatment by biochar, could be a promising technique to reduce the chemical fertilizer input.

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