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BIOCHEMICAL AND ANTIOXIDANT RESPONSES OF MATURE RADISH PLANTS (*RAPHANUS SATIVUS* L.) IRRIGATED WITH TREATED WASTEWATER OF SEDRATA REGION (SOUK-AHRAS CITY, NORTHEAST ALGERIA)

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Key words: *Oxidative stress, Biochemical markers, Irrigation, Radish plant, Sedrata region, Treated wastewaters.*

Abstract – This study was devoted to evaluate the effect of abiotic stress due to the contaminants of treated wastewaters of Sedrata region (northeast Algeria) on the mature radish plant cultivated and irrigated under controlled conditions. The used irrigation waters were in line with standards, excluding copper (Cu) showing a slight elevation compared to the recommended limits provided by FAO (0.2 mg/l). Whilst, the treated wastewaters poured into the Oued Charef river feeding Oued Charef dam are all classified from high to very high salinity risk. Also, GPX activity and GSH content were significantly increased in plant treated wastewaters, since the waters of Oued Charef river and dam waters have significantly affected the synthesis of proteins, and carotenoids, production of H_2O_2 and activation of APX and GST in radish hypocotyls. Conclusively, the used waters induced adverse physiological and biochemical effects on this plant, indicating thus the existence of intolerable elements (salts, minerals…etc.) in the plant culture.

INTRODUCTION

Due to the demographic and urban increase, the treated wastewaters are considered as the most efficient way out of the water scarcity, especially in the Mediterranean region threatened by high water scarcity resulting from irregular precipitation and very recurrent dry spells (Barceló and Petrovic, 2011). Here, and as the first country in terms of production and treatment of wastewaters in the maghrebian region in 2009 (only 3-6% of water is reused) (Fatta-Kassinos *et al*., 2016), the Algerian regulations authorize the reuse of treated wastewaters (TWWs) for irrigation purposes, even for culture of consumed raw products, which lead in turn to serious health problems (Bemmoussat *et al.,* 2014). In this regards, several authors (Lubello *et al*., 2004; Pedrero and Alarcón, 2009; Rusan *et al*., 2007) have appreciated the nutritional and fertilizing properties of these waters in providing effective plant growth. In return, the harmful effects on the agriculture quality and consumers health has been well-documented (Ahmad *et al*., 2014; Malchi *et al*., 2014; Manas *et al*., 2009; Prosser and Sibley, 2015) owing to their salinity reaching intolerable thresholds and their toxic element contents (e.g. metallic, drugs) and pathogenic agents, and consequently the agriculture irrigated by treated and well-monitored wastewaters must be consumed after cooking to avoid any risk of toxicity (Al-Lahham *et al*., 2003). Moreover, the biochemical and cellular responses to contaminants are valuable tool in detecting the impact of anthropogenic contaminants (Fernandes *et al*., 2002; Lavado *et al.*, 2006), and thus the plants receiving contaminated waters absorb these hazardous elements which can trigger secondary cellular responses, including oxidative damages following generation of reactive oxygen species (ROS). As a result, plants have enzymatic and non-enzymatic antioxidant systems able to deactivate potential damaging effect of ROS *(Ahmad et al.,* 2019; Apel and Hirt, 2004). Several works have been conducted on the effect of reuse of treated wastewaters on various plant species, including tomato (Christou *et al*., 2017), lettuce (Manas *et al*., 2009) and wheat (Mojiri and Aziz, 2011). Whilst, the effect of treated wastewaters varies considerably according to irrigated plant species, and subsequently this could become a worst-case scenario for root vegetables like carrot and turnip …because of the direct contact between waters and their most consumed fruits, mainly at the raw state (Tekliæ *et al*., 2008). Therefore, the present work focuses on small mature radish plant (*Raphanus sativus* L.) used as a plant species model for many reasons, like yearly culture characterized by small size, short growth period and unequal biomass distribution between its leaves and hypocotyls (underground storage organs (USOs)), and thus these typical characteristics make the radish as an ideal experimental plant for the use in eco- toxicological purposes (Kostka-Rick and Manning, 1993; Sun *et al*., 2010). Additionally, this investigation was undertaken to evaluate the impact of direct irrigation way by treated wastewaters of Sedrata region of Souk-Ahras city (northeast Algeria) and indirect way by the natural environments (river and dam of Oued Charef) on the biochemical and antioxidant properties of mature radish fruits (*Raphanus sativus* L.), which are the main plant part sensible to salinity and the environmental condition changes and may retain more higher (nearly 90%) of absorbed metals than other plant tissues (Kostka-Rick and Manning, 1993; Pourrut *et al*., 2011).

MATERIAL AND METHODS

Sites of sampling and irrigation waters

The wastewater purification station (WPS) of Sedrata region (Souk-Ahras city, north-eastern Algeria) constructed and started in use from 2008 was used to protect and to feed the Oued Charef dam by the purified wastewaters, after having passed through the Oued Krab river which would join to the Oued Charef river. This dam is designed only to cover the irrigation water needs of irrigated perimeter of this region evidenced by high agricultural activity, and thus the treated wastewaters of this region are reused for irrigation purposes. This study was, therefore conducted on three different sampling sites serving as an origin of irrigation water; the first one of treated wastewaters (W1) placed just at the exit of the purification station and before joining to the natural environment, the second one is the Oued Charef river (W2) (nearly 10Km from WPS), and the last one is the Oued Charef dam (W3) (nearly 25Km from WPS, (Fig. 1). Of note, tap water was used as control to compare and to evaluate the quality and effect of these three water types.

Establishment of experimentation sites

The experimental design was performed from February to March, 2019 in testing greenhouse at Mohamed-Cherif Messaadia University of Souk-Ahras by using identical rectangular boxes (20 cm deep / 40 cm long) filled with a finely-prepared mixture of sand and industrial soil (1v: 3v respectively). This substrate has received healthy small-radish seeds (*Raphanus sativus* L.) sown at a depth of 1 cm (20 seeds/box) in a total of 12 boxes (three repetitions for each water type) installed according a fully randomized statistical design. The plant seeds were regularly irrigated by the four water types from twice to three times per week throughout forty (40) days, by which the substrate becomes always humid, and subsequently ideal for the growth of radish plants which are concerned by the lack of water. The different water types were brought only on the day when plants need to be irrigated to ensure the stability of their qualities.

Studied parameters

Properties and quality of irrigation waters

In this study, the quality of the selected waters was monitored in every twenty days during the irrigation period. The sampling standards (filtration using 0.45 μm filter, acidification in 5 mL HCl or HNO_3, and conservation at 4°C (AFNOR/T91E).) have been respected during water sampling campaigns. In addition, the physico-chemical parameters (pH and conductivity) were determined in situ by using WTW Multi parameters 340i sets. The level of nitrates (NO_3^-), nitrites (NO_2^-), ammonium (NH_4^+) were spectrophotometrically evaluated according to the international standards (ISO), since the determination of chloride contents were performed by the Mohr volumetric method. The soluble cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) were estimated by flame photometer and used to determine Sodium-Adsorption-Ratio (SAR). Also, the levels of four heavy metals (Fe, Cu, Zn, Pb) were determined in this study, by atomic absorption spectrophotometer.

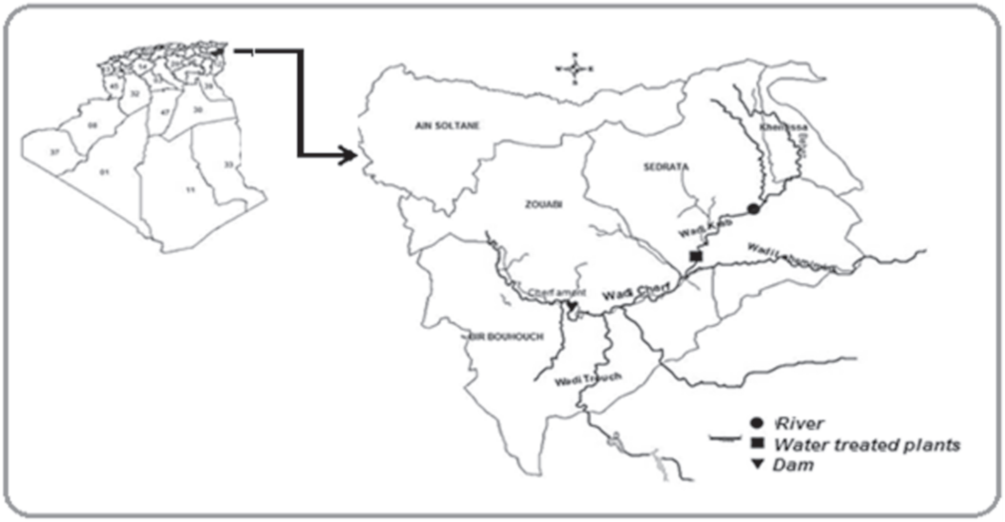

Fig. 1. Geographical location of the study area and the selected distribution stations

Biochemical and antioxidant responses of radish plant

Photosynthetic pigments

The levels of total chlorophyll (Ch a & Ch b) and carotenoids in 1g of fresh leaves of mature radish plant were determined as reported elsewhere (Lichtenthaler and Wellburn, 1983), according to the following equations:

$Chl\ .a= (12, 21.A_{663})-(2, 81\ xA_{646})$

$Chl\ b= (20.12xA_{646})-(5.03\ xA_{663})$

$Chl\ a+b = (8.02.A_{663}+ 20.2.A_{646})$

$CAROT=(1000.A_{470} -3.27_{Chl\ .a}-104_{Chl\ b})/229$

The obtained pigment levels are expressed in mg/g fresh leaf matter.

Total proteins

The level of total proteins in radish fruits were determined as previously reported (Bradford, 1976), using bovine albumin serum as a standard. In brief, 4 mL of Bradford reagent was added to 100 µL of protein extract, then the mixture was agitated slightly (5 to 30 min) and the absorbance was spectrophotometrically measured at 595nm against the blank containing distilled water instead of the extract.

Lipid peroxidation

The malondialdehyde (MDA), a major carbonyl product of lipid peroxidation, was determined according to the previously reported method (Prasad and Saradhi, 1995), by using trichloroacetic acid (TCA) and thiobarbituric acid (TBA) as detection reagents. The absorbance of TBA-MDA complex was spectrophotometrically measured at 532nm and corrected by subtracting the absorbance at 600nm. The extinction coefficient of MDA was 155 $mM^{-1}cm^{-1}$.

Hydrogen peroxide

The level of hydrogen peroxide (H_2O_2) was determined by homogenization of 500 mg of plant tissue in ice bath containing 0.1% of TCA at the concentration of 10 ml/g of fresh matter. The homogenate was centrifuged at 1200g for 15min at 4°C, and then the supernatant was added to 0.5 mL of phosphate buffer (KH_2PO_4/K_2HPO_4; 10mM, pH 7) and 1ml of potassium iodide (1M). The absorbance of the mixture was measured at 390nm, meanwhile the level of H_2O_2 was estimated using calibration curve prepared beforehand (Sergiev *et al.,* 1997).

Evaluation of antioxidant system

Non-antioxidant enzymes

The soluble non-enzymatic antioxidants, including carotenoid contents in radish leaves based on the level of total chlorophyll, and reduced glutathione in radish hypocotyls were determined as previously described (Tanaka *et al*., 1985).

Enzymatic antioxidants

The activities of antioxidant enzymes of superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), catalase (CAT) and glutathione S-transferase (GST) were determined in mature radish hypocotyls.

SOD (EC 1.15.1.1)) was determined according to the colorimetric method described elsewhere (Chen and Zhang, 2015) based on the determination of the photochemical reduction inhibition nitro-blue tetrazolium (NBT). Here, the reagents of the reactional solution were prepared by 100 mM PBS (pH 7.8), 1 mM EDTA-2Na, 130 mM methionine, 750 μM NBT and 20 μM riboflavin, and then 50 μL of the each enzymatic extract sample was added to 1 mL of the reactional solution. In addition, two control samples were prepared by replacement of the enzymatic extract by 50 μL of 100mM PBS (Ph 7,8), and afterwards one control was kept in dark (control 1) and the other one (control 2) with samples was exposed to light (4000 lux) for 10 to 15min. The enzymatic activity of SOD was measured at 560nm and expressed in u/mg of protein, where the unit of SOD activity is defined as the quantity of SOD needed to obtain 50% of NBT reduction inhibition.

GST (EC 2.5.1.18) activity was determined as previously reported (Habig *et al*., 1974). Briefly, each sample was homogenized in 1mL of phosphate buffer (0.1 M, pH 6) with sucrose, and centrifuged at 14000 rpm for 30 min. Thereafter, the supernatant was collected to be used as an enzymatic source, from which 20 μL of enzymatic extract of each sample was added to 1.2 mL of a mixture composed of CDNB (1 mM) and GSH (5 mM). The enzymatic activity was measured by spectrophotometry at wave length of 340nm against blank containing distilled water rather than the extract (ε=9.6 mM^{-1} cm^{-1}). The extract used to measure the enzymatic activities of CAT, APX and GPX in hypocotyls was obtained as reported elsewhere (Loggini *et al*., 1999).

CAT (EC 1.11.1.6) activity was determined following the decrease in H_2O_2 level based on the previously reported method (Cakmak and Horst, 1991) at absorbance wavelength of 240nm.

APX (EC 1.11.1.11) activity was measured as previously reported (Nakano and Asada, 1981), where the reactional mixture is composed of phosphate buffer, sodium ascorbate and hydrogen peroxide. The absorbance was read at λ = 290 nm.

GPX (EC 1.11.1.9) activity was measured at absorbance wavelength of 470nm with extinction coefficient (ε) equals 2470$M^{-1}.cm^{-1}$ (Fielding and Hall, 1978).

Statistical analysis of data display

The quantitative data are displayed through the design of box plots, indicating the minimum (min), maximum (max), median and the mean values. The Student's t test was used for pairwise comparisons (variables vs control), since one-way ANOVA was used for comparisons of multiple variables. Also, bivariate analysis was used to highlight the relationship between the studied parameters, and due to the multi-variability of data in this study, the principal component analysis (PCA) was better used to provide the interesting results. Statistical tests were performed using R statistical software for windows (Ver, 3.6.1), where $p<0.05$ considered as significant (Ihaka and Gentleman, 1996).

RESULTS AND DISCUSSION

Properties and quality of used irrigation waters

In this study, the treated wastewaters (TWWs) and other types of irrigation waters contain two major toxic elements (Fe and Cu) among the four available elements (Fe, Cu, Zn, Pb) dosed in the collected samples during the period of radish irrigation. However, the TWWs have exhibited high iron concentration (0.25 mg/L) which does not always exceed the toxic threshold, and low copper concentration (0.27 mg/L) (Table 1). In fact, the concentration of iron of 0.2 mg/L may cause plant toxicity at long-term exposure, while the copper contents in wastewaters exceed the limit concentration provided by FAO for waters intended for irrigation (Ayers and Westcot, 1985). As compared to several previous studies investigating the phytotoxic effect of copper on radish plants (Chatterjee *et al*., 2006), the found values did not show any obvious effect on this plant species. In this study, the physico-chemical parameters of the used waters showed that pH value meets standards, although this parameter is not a criterion determining the quality of irrigation water because it may be buffered by soil components (Zaman *et al*., 2018). Regarding the electrical conductivity (EC), only the wastewaters W3 have shown the highest EC value (2.34 mS/cm) followed by wastewaters W1 (1.85 mS/cm) and W2 (1.66 mS/cm). According to the classification of USSL (1954), the dam waters W3 are classified in C4 of very high salinity risk, and the other water types are classified in C3 of high salinity risk. Thus, the different water types (W1, W2, W3) used in this study have an electrical conductivity

(EC) ranged between 1.5 mS/cm and 3 mS/cm, which express the level of soluble salts in waters and may have harmful effect on many agricultures, and so they need to be carefully used (Zaman *et al.*, 2018). As reported by Food and Agriculture Organization of the United Nations (FAO), the culture of radish plant may tolerate salinity up to the threshold of 1.2 mS/cm (Ayers and Westcot, 1985), in addition that the potential risk of water sodicity is overall evaluated from the salinity and the sodium-absorption ratio (SAR) (Suarez *et al.*, 2012). The later was found to be low (1.09-2.21 meq/l), and thus the used waters are classified of low sodicity risk (SAR<10) (Allison and Richards, 1954), since the chloride (Cl) determined in the treated wastewaters and the irrigation waters was found to be toxic element. As shown in Table 1, the maximal concentration of chloride ion was observed in the treated wastewaters (3.52meq/l) and the minimal concentration was noticed in the tap waters (2.81meq/l). As previously reported ((Bauder *et al.*, 2011), the chloride concentration ranged between 2 et 4 meq/l has a harmful effect on the sensible species to chloride which infiltrates easily into the soil waters and, then absorbed by plants (Hussain *et al.*, 2010). Indeed, the quality of the studied waters respecting the Algerian and FAO standard limits for the irrigation waters is evidenced by the study period coinciding with the season of winter (February-march, 2019) characterized by a considerable precipitation leading thus to water dilution.

Biochemical and antioxidant responses of radish plant

As indicated in Fig. 2, the treated wastewaters induced marked effect on the level of photosynthetic pigments estimated following the determination of total chlorophyll (Ch a + b) and total carotenoid levels in the radish leaves. Here, the treated wastewaters of the purification station W1 (21.57±1.71 mg/g FW) and, in particular, the waters of Oued Charef river W2 (21.10±0.96 mg/g FW) and the dam waters W3 (24.51±0.21 mg/g FW) have induced a significant decrease in the level of total

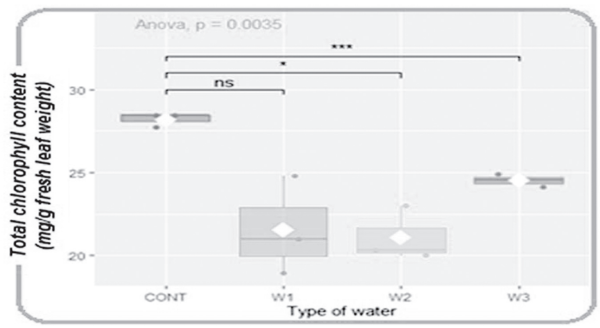

Fig. 2. Effect of irrigation water on total chlorophyll content in fresh radish leaves (mg/g fresh leaf weight).

Table 1. Properties of the studied irrigation waters

Parameter	CONT Tap water	W1 Treated wastewater	W2 Oued Charefriver	W3 Dam of Oued Charef	Algerian standards	FAO standards
pH (unit of pH)	7.08	7.47	7.88	8.33	6.5-8.5	6.5–8.4
EC (mS/cm)	0.77	1.85	1.66	2.34	3	3
Na^+ (meq/l)	1.19	2.08	1.95	2.71	-	9
K^+ (meq/l)	0.02	0.28	0.25	0.38	-	-
Ca^{+2} (meq/l)	2.24	3.03	3.50	2.75	-	-
Mg^{+2} (meq/l)	0.13	0.16	0.29	0.25	-	-
NH_4^+ (mg/l)	<0,064	<0,064	<0,064	<0,064	-	-
Cl- (meq/L)	2.81	3.52	3.33	3.38	10	10
HCO3- (meq/L)	0.35	0.32	0.44	0.57	8.5	8.5
SO42-(mg/L)	25.72	56.59	65.47	64.74	-	-
NO3- (mg/L)	7.65	9.7	8.9	10.4	30	30
P (mg/L)	0.72	1	0.89	1	-	-
Total Fe (mg/L)	0.19	0.25	0.2	0.17	20	5
Cu (mg/L)	0.34	0.27	0.28	0.33	5	0.2
Zn	nd	nd	nd	nd	10	2
Pb	nd	nd	nd	nd	10	5
MnO4 Index (mg/L)	23.9	21.7	17.75	15.7	-	-
SAR (Sodium Absorption Ratio) (meq/l)	1.09	1.64	1.41	2.21	9	9

chlorophyll as compared to control (28.20±0.24 mg/g FW). In contrast, the analysed samples revealed a very highly significant increase in carotenoid levels for waters of stations W2 (6.46±0.20 mg/g FW) and W3 (5.01±0.14 mg/g FW) in comparison with waters of station W1 (3.83± 0.48 mg/g FW) and control. Further, one of the effects of wastewater reuse is the decreased level of plant pigments (Khalid *et al*., 2018), and this might reflect phytotoxic effect related to accumulation of heavy metals (Hashem *et al*., 2013), because the irrigation by treated wastewaters increases the accumulation levels of these elements in radish plant parts (Parveen *et al*., 2013) in the order Fe > Zn > Mn > Cu (Arora *et al*., 2008), alike to those reported by (Belhaj *et al*., 2016) in tomato, lettuce and radishes. The tendency of carotenoid variation was completely opposite to that of chlorophyll. Carotenoid pigments and other non-enzymatic compounds are potential antioxidants (Sharma *et al*., 2012) able to protect cells against singlet molecular oxygen ($^1O_2$) and chlorophyll by reducing the level of MDA (Iqbal *et al*., 2013; Mittler *et al*., 2004). Hence, the higher or lower carotenoid contents in samples irrigated by waters of stations W2 and W3 considered as diluted treated wastewaters, evidenced by carotenoid overproductions to tolerate oxidative stress conditions and compensate the decreased chlorophyll level. Additionally, the correlation matrix shown in Fig. 13 displays a positive correlation between carotenoids and hydrogen peroxide (H_2O_2) indicating thus, the highest needs of treated plants to carotenoids as a defence tool against the abiotic stress and ROS. As shown in figure 4, the treated wastewaters of station W1 have induced a highly significant decrease in protein levels (5.56±1.4 mg/g FW), but no significant effect (t. test: $p>0.05$) has been noticed in waters of W2 and W3 as compared to those in control. Noteworthy, determination of protein levels provide good understanding on the general physiological plant state, and so the low protein level related to the irrigation by treated wastewaters is likely due to the accumulation of toxic elements leading to degradation of soluble molecules (Iqbal *et al*., 2013). This finding does not concord with results of some previous works (Akhkha *et al*., 2019) showing an increase in protein levels in *Calotropis procera* irrigated with treated wastewaters. Regarding the

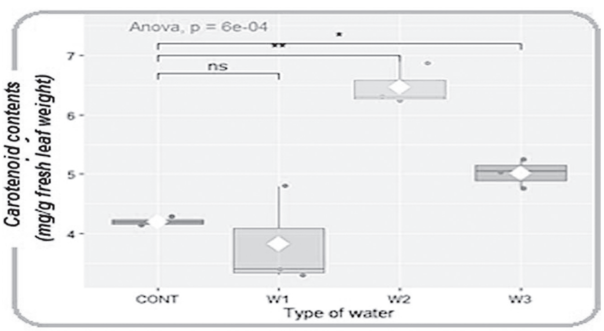

Fig. 3. Effect of irrigation water on carotenoid contents in fresh radish leaves (mg/g fresh leaf weight).

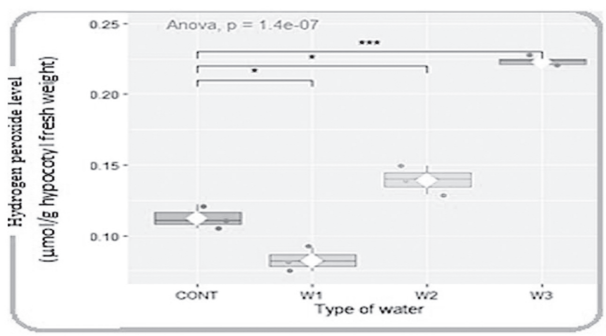

Fig. 5. Effect of irrigation water on hydrogen peroxide (H_2O_2) level in radish hypocotyls (mmol/g hypocotyl fresh weight).

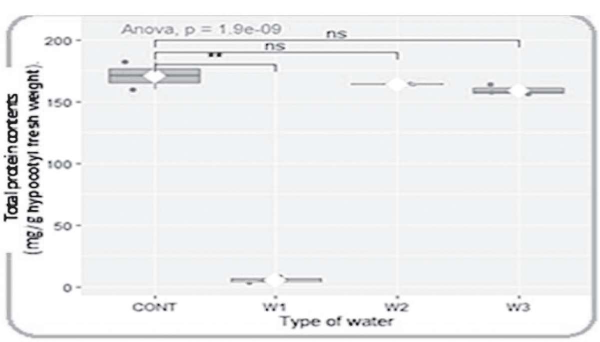

Fig. 4. Effect of irrigation water on total protein contents in radish hypocotyls (mg/g hypocotyl fresh weight).

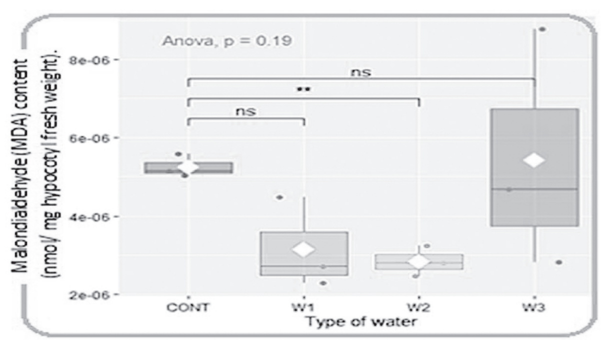

Fig. 6. Effect of irrigation water on malondialdehyde (MDA) content in radish hypocotyls (nmol/ mg hypocotyl fresh weight).

level of hydrogen peroxide (H_2O_2) (Fig.5), we have observed high values in radish hypocotyls irrigated by waters of Oued Charef river (W2) and the dam W3 (0.13±0.005, 0.22±0.002 mmol/g FW successively). ANOVA test revealed a highly significant difference (*p=0.000*) between the studied groups, especially between waters W3 and control, noticing also a very low protein levels (0.082±0.004 mmol/g FW) in only plants irrigated by waters W1(0.082±0.004 mmol/g FW). Interestingly, the production of hydrogen peroxide in plants can be promoted by a wide variety of biotic and abiotic factors (Neill *et al.*, 2002), and this is alike to the case of contaminated wastewaters by pathogens and chemical compounds (Jaramillo and Restrepo, 2017) which may be brought to plants during irrigation. In our study, the high hydrogen peroxide levels could be due to the presence of inorganic salts (saline stress) characterizing the Oued Charef dam (table. 1), and noteworthy radish is a culture sensible to salinity (Shannon and Grieve, 1998). Moreover, the research works carried out on our plant model (*Raphanus sativus* L.) have reported that the high level of hydrogen peroxide (H_2O_2) is related to metallic stress (Biteur *et al.,* 2011) but not saline stress (Noreen and Ashraf, 2009). On top of that, the level of MDA (Fig. 6) was decreased in radish hypocotyls irrigated by waters W1 ($3.1.10^{-6}±6.7.10^{-7}$ nmol/mg FW) and waters of W2 ($2.8.10^{-6}±2.2.10^{-7}$ nmol/mg FW) exhibiting a very high significant difference as compared with control ($5.2.10^{-6}±1.6.10^{-7}$ nmol/mg FW), however, only plants irrigated by dam waters of W3 have reveal a non-significant increase in the levels of lipoperoxidation ($5.4.10^{-6}±1.7.10^{-6}$ nmol/mg FW). It was previously reported (Wyrwicka and Urbaniak, 2018) that the increased lipid peroxidation levels in control can be eventually explained by nutritive element deficiencies considered as stress factors leading to MDA production. Thus, the tissues of radish plant irrigated by the treated wastewaters have shown lesser lipid-oxidative damages than those noticed in control plants owning to their richness of essential nutrients, particularly N, P and K (Tab.1). Actually, the low level of magnesium ions Mg^{2+} (Tab.1) in the control waters compared to other water types can mainly affect the growth of plant roots, and hence, this is the reason why MDA level in control radish roots become eventually high. Furthermore, the

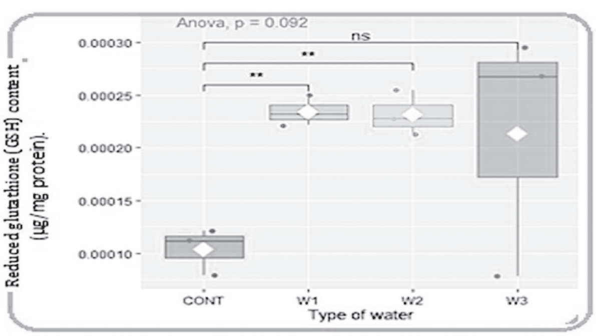

Fig. 7. Effect of irrigation water on reduced Glutathione content (GSH) in radish hypocotyls (mg/mg protein).

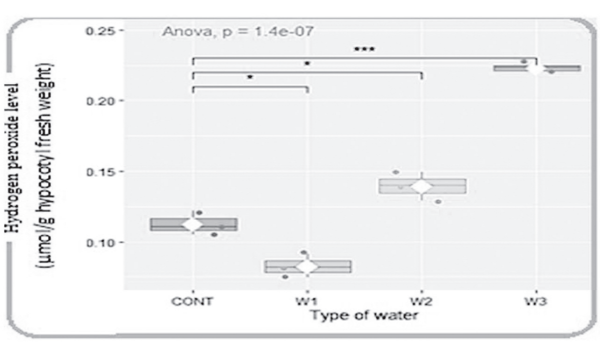

Fig. 8. Effect of irrigation water on the Catalase (CAT) activity in radish hypocotyls (nmol/min/mg protein).

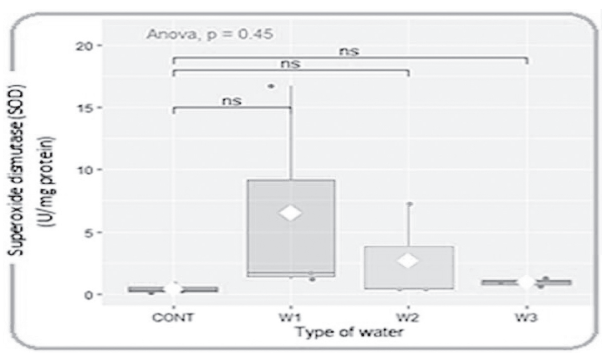

Fig. 9. Effect of irrigation water on the total Superoxide dismutase (SOD) activity in radish hypocotyls (U/mg protein)

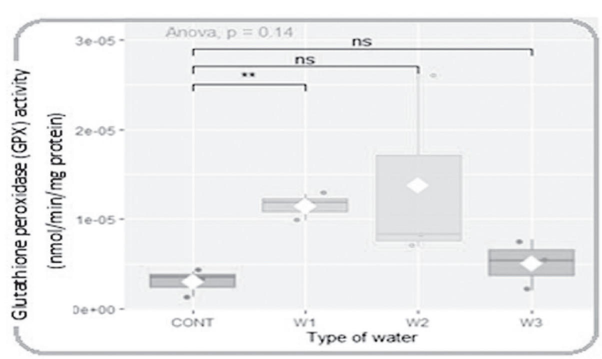

Fig. 10. Effect of irrigation water on the total activity of glutathione peroxidase (GPX) in hypocotyls of radish plant (nmol/min/mg protein)

effect of treated wastewaters and other water types used as diluted waters on radish plant was also evidenced by marked changes in the plant antioxidant defence systems, including enzymatic antioxidants (GST, SOD, CAT, GPX, APX) and non-enzymatic antioxidants (GSH and carotenoids). As indicated in Fig. 7, the reduced GSH tends to accumulate in radish plant in response to all water types compared to control, and notably the maximum level of GSH induced by treated wastewaters was $2.3.10^{-4}$±$8.2.10^{-6}$ mg/mg protein, $2.3.10^{-4}$±$1.2.10^{-4}$ mg/mg protein and $2.1.10^{-4}$±$6.8.10^{-5}$ mg/mg protein, respectively in waters of W1, W2 and W3. Several recent research works have reported that GSH induction is one of effects of wastewater reuse and exposure to heavy metals (Dresler *et al.*, 2019; Elloumi *et al.*, 2016), suggesting thus that the GSH accumulation in treated plants compared to that of control could be somehow, related to the decreased activity of GST, in particular in waters of W1 and W3, with noting that this enzyme uses GSH for glucosinolates biosynthesis in Brassicaceae, like radish plant (Czerniawski and Bednarek, 2018). These data are not in line with those reported by (Fatima and Ahmad, 2005) who have noticed decreased GSH level in *Allium cepa* treated with industrial wastewaters and a mixture of heavy metals. As shown in Figs. 8 – 12, the irrigation of radish culture by treated wastewaters and other water types have no overall significant effect on the activity of antioxidant enzymes, except only GPX activity showing a significantly higher enzyme activity ($1.1.10^{-5}$±$8.6.10^{-7}$ nmol/min/mg protein) than that seen for the other irrigation waters. Similarly, the enzymatic activity of SOD was found very higher in treated wastewater irrigated plants (6.5±5.09 U/mg protein) than that noticed in plants irrigated by waters W2 and W3 (2.7±2.2 et 0.9±0.1 U/mg protein respectively). In addition, the waters W2 induced marked increase in the enzymatic activity of APX ($4.08.10^{-7}$±$1.3.10^{-7}$ nmol/min/mg protein) and GST ($5.2.10^{-5}$±$2.2.10^{-5}$ nmol/min/mg protein) compared to other water types, especially the treated wastewaters W1, which led to obtain minimal values of antioxidant enzyme activities (APX: $1.9.10^{-7}$±$1.3.10^{-8}$ nmol/min/mg protein; GST: $1.5.10^{-5}$±$4.5.10^{-6}$ nmol/min/mg protein). Whilst, catalase activity was found to be less active compared to other enzymes, and obviously was decreased in comparison with control, particularly under effect of dam waters W3 ($2.4.10^{-7}$±$6.7.10^{-8}$ nmol/min/mg protein). The effect of reuse of treated wastewaters on our plant species model (*Raphanus sativus* L.) has been well demonstrated through a significant induction of glutathione peroxide (GPX) as compared with control, and alike to some previous works (Sinam *et al.*, 2011) investigating the effect of wastewaters on some antioxidant enzymes, including GPX. The later serves to eliminate lipid peroxides and to reduce hydrogen peroxide by using GSH to minimize oxidative stress (Noctor *et al.*, 2002), leading thus probably to the decline level of H_2O_2 and MDA, in the face of the significant activation and abundance of GPX and GSH during irrigation by treated wastewaters (W1). This is proved by the correlation coefficient (Fig. 13) showing a strong positive correlation (*r*=0.64) between GPX and GSH at one part, and highly significant negative correlation between GPX (r= -0.80), GSH (r= -0.57) and MDA at the other part. In this regards, it was reported that the increased activity of catalase involved in removing hydrogen peroxide reflects an adaptive response to abiotic stress in plants. Whilst, the decreased catalase activity could be explained by the oxidative stress state (Panda *et al.*, 2003), and

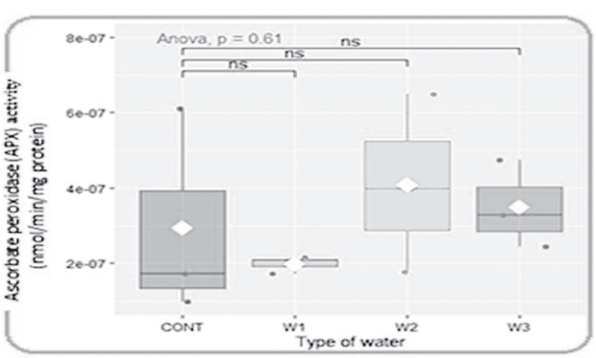

Fig. 11. Effect of irrigation water on ascorbate peroxidase (APX) activity in radish hypocotyls (nmol/min/mg protein)

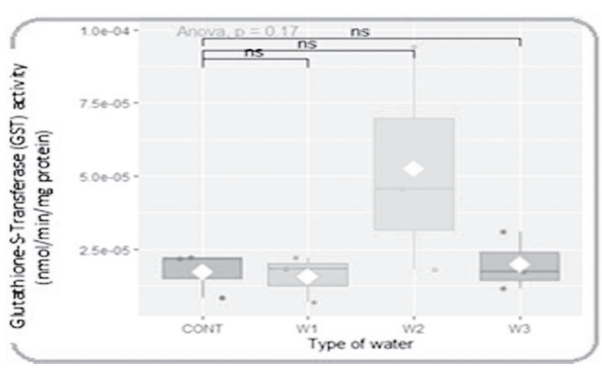

Fig. 12. Effect of irrigation water on Glutathione-S-Transferase (GST) activity in radish hypocotyls (nmol/min/mg protein)

this concords with our findings of waters W3 which induced disruption of the balance CAT-H_2O_2, leading, subsequently to increased level of H_2O_2 and low enzymatic activity of catalase (Figs. 4, 8) (Sun *et al.,* 2010), since the treated wastewater plants W1 did not affect the catalase activity. These contradictory observations may be related to the limited localization of CAT which is present only in peroxisomes (Costa *et al.,* 2010). On the other hand, SOD and APX proved to be more active, especially in radish plants irrigated by waters W2 & W3, so that plants could protect themselves against the oxidative damages induced mainly by superoxide ions (O{) via SOD (Alscher *et al.,* 2002) and H_2O_2 via APX (Bhaduri and Fulekar, 2012), and this is confirmed by the statistical analysis showing a positive correlation (*r*= 0.64) between H_2O_2 and APX. Several previous studies conducted on other plant species have found similar variation in antioxidant makers under metallic stress (Benhamdi *et al.*, 2014; Shri *et al.*, 2009), like the case of GPX, APX and GST largely depend on the availability of

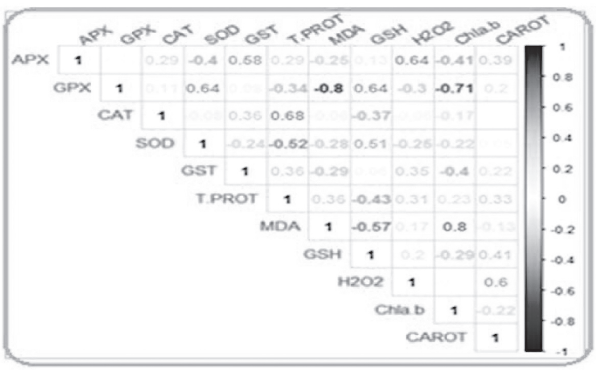

Fig. 13. Inter-variable correlation matrix: Spearman correlation plots of physico-biochemical parameters (11 variables).

GSH and reduced ascorbate (ASC) which are not included in the present study (Rohman *et al.*, 2019; Stavridou *et al.*, 2018). The correlation matrix shown in Fig. 12 showed in return, clear independence between (GST, APX) and GSH (*r=0.06, r=0.13* respectively), while GST and APX are strongly correlated (*r=0.58*), suggesting thus an eventual co-regulation. It's worth noting that 58% of PCA variation is explained only by the two first dimensions (Fig.14a), and the quality of presenting variables expressed in cosine-squared (cos^2) showed that MDA, Chl a+b, GSH and GPX are the most explanatory parameters of this component (increased cos^2). Also, the result showed a very strong correlation (positive correlation for MDA, Chl a+b and negative correlation for GSH and GPX) with the first axis (Dim1) of PCA, exhibiting by itself only more than 30% of variability. This first dimension obviously separates the effect of waters W1 and W2 (negative side) of tap waters (control) (positive side). Hence, the treated wastewaters W1 are characterized by their ability to induce enzymatic activity of GPX, the synthesis of GSH and decrease in MDA and Chl a + b levels (Fig. 14b). This finding is likely explained by the increased chloride level in these waters, because radish plant supports the chloride fertilization only before the vegetation recovering (LIU *et al.*, 2010; Xinju, 2007), since 27.1% of the total variation is explained by Dim2, exhibiting a highly positive correlation with APX, GST and total proteins. This axis slightly expresses a structuration regrouping the effect of waters of Oued Charef river (W2) and Oued Charef dam (W3), unlike to treated wastewaters which have induced the synthesis of proteins, production of hydrogen peroxide and activation APX and GST in

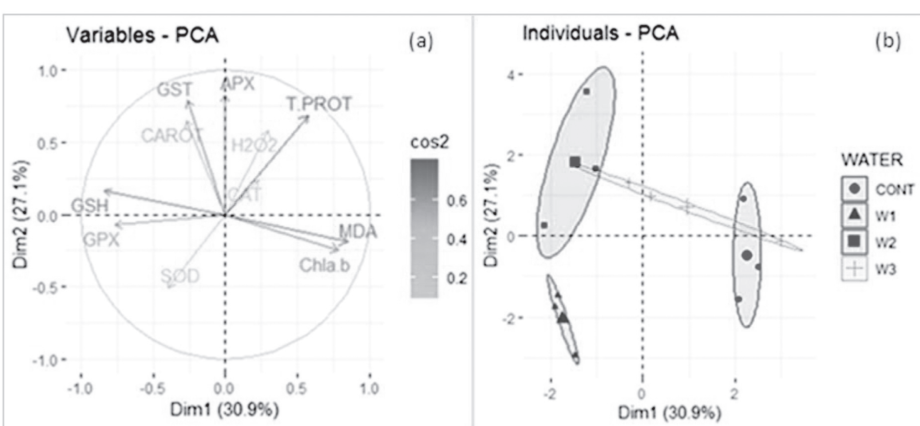

Fig. 14. Contribution of variables and correlation circle of PCA 1-2 (a). Factorial plan 1–2 used to project water groups (b)

radish hypocotyls, and this is probably due to their richness of sulphate ions (SO_4^{2-}) (Table. 1).

CONCLUSION

The obtained results of the present study showed that the treated wastewaters intended for indirect irrigation of perimeter of Sedrata region (Souk-Ahras city, northeast Algeria) provide a quality responding to the recommended standard limits, excluding only copper concentration which exceeds slightly the FAO limits. But, the biochemical analysis have shown the harmful effects of treated wastewaters of radish plants which are mainly evidenced by the decrease in chlorophyll pigments, total protein contents, along with marked increase in the levels of hydrogen peroxide while, plants respond by higher or lower enzymatic activity of GPX, APX and GST with synthesis of reduced glutathione. This indicates the presence of unbearable elements (salts, metals..etc.) in the irrigated cultures of plants like radish. Consequently, monitoring of the oxidative stress level and the antioxidant defence system are helpful as biochemical markers in evaluating the quality of treated wastewaters intended for irrigation, and this could be efficient mainly for the plant species exhibiting high sensibility and hyper-accumulation of contaminants

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Conflicts of interest None.

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