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Impact of treated urban wastewater for reuse in agriculture on crop response and soil ecotoxicity

Dalel Belhaj[1] · Bouthaina Jerbi[2] · Mounir Medhioub[3] · John Zhou[4] · Monem Kallel[2] · Habib Ayadi[1]

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Abstract The scarcity of freshwater resources is a serious problem in arid regions, such as Tunisia, and marginal quality water is gradually being used in agriculture. This study aims to study the impact of treated urban wastewater for reuse in agriculture on the health of soil and food crops. The key findings are that the effluents of Sfax wastewater treatment plant (WWTP) did not meet the relevant guidelines, therefore emitting a range of organic (e.g., up to 90 mg L^{-1} COD and 30 mg L^{-1} BOD_5) and inorganic pollutants (e.g., up to 0.5 mg L^{-1} Cu and 0.1 mg L^{-1} Cd) in the receiving aquatic environments. Greenhouse experiments examining the effects of wastewater reuse on food plants such as tomato, lettuce, and radish showed that the treated effluent adversely affected plant growth, photosynthesis, and antioxidant enzyme contents. However, the pollution burden and biological effects on plants were substantially reduced by using a 50 % dilution of treated sewage effluent, suggesting the potential of reusing treated effluent in agriculture so long as appropriate monitoring and control is in place.

Keywords Antioxidant · Crop plants · Ecotoxicology · Heavy metals · Treated wastewater irrigation

Introduction

Quality fresh water for agriculture is becoming an increasingly scarce resource due to climate change effects (Milano et al. 2012) and increased demand from the agricultural sector (Pedrero et al. 2012; Mesa-Jurado et al. 2012). Hence, wastewater reuse for irrigation represents a sustainable option and an advantageous alternative for the mitigation of the ever-increasing irrigation water scarcity and demand in arid and semiarid regions around the world (Hamilton et al. 2007; Sharma et al. 2007; Angelakis and Durham 2008; Travis et al. 2010). One benefit of such practice is the plant's uptake of wastewater nutrients, and therefore, a reduction in the pollution load that wastewater contributes to the surface water supply (Liu et al. 2005; Chen et al. 2008; Khurana and

Responsible editor: Philippe Garrigues

✉ Dalel Belhaj
dalel_belhaj@yahoo.fr; dalelbelhaj2@gmail.com

Bouthaina Jerbi
bouthainajerbi@gmail.com

Mounir Medhioub
mounirmedhioub@yahoo.fr

John Zhou
Junliang.zhou@uts.edu.au

Monem Kallel
monemkallel@gmail.com

Habib Ayadi
habibayadi62@yahoo.fr

[1] Department of Life Sciences, Laboratory of Biodiversity and Aquatic Ecosystems, Ecology and Planktonology, University of Sfax-Tunisia, FSS, Street Soukra Km 3.5, BP 1171, CP 3000 Sfax, Tunisia

[2] Laboratory of Water-Energy-Environment, University of Sfax-Tunisia, ENIS, Street Soukra Km 3.5, BP 1173, CP 3038 Sfax, Tunisia

[3] Laboratory of Useful Material Valorization (LVMU), University of Sfax-Tunisia, FSS, BP 1171, CP 3000 Sfax, Tunisia

[4] School of Civil and Environmental Engineering, Centre for Technology in Water and Wastewater, University of Technology Sydney, Broadway, NSW 2007, Australia

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Singh 2012). However, depending upon its sources and treatments, sewage effluent may contain undesirable pollutants, and the reclaimed wastewater application may generate undesirable effects on soils, plants, and groundwater resources (Mapanda et al. 2005; Walker and Lin 2008; Drechsel et al. 2010). Among those pollutants, heavy metals are common in urban ecosystem (Li et al. 2009; Xu et al. 2010) and are one of the main anthropogenic toxic chemicals which pose a number of potential environmental and health risks (Singh et al. 2010; Wang et al. 2012; Szkup-Jablonska et al. 2012).

Heavy metal accumulation is known to produce significant physiological and biochemical responses in vascular plants (Mangabeira et al. 2001). The toxicity of heavy metals is generally thought to be due to uncontrolled and excessive production of reactive oxygen species (ROS) which damage cell membranes (Janicka et al. 2008). Removal of ROS is strictly controlled by an assortment of nonenzymatic and enzymatic antioxidant mechanisms in plants. Enzymatic ROS scavenging includes catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), ascorbate peroxidase (APX), and nonenzymatic scavengers, e.g., glutathione (GSH), carotenoids, and ascorbate (ASC) (Srivastava et al. 2009).

The current study focused on the industrial city of Sfax (Central-Eastern Tunisia) known worldwide for their tanneries, pharmaceuticals, and olives mill industries. South Sfax streams in this area receive industrial effluents, urban and domestic sewage, municipal wastes, and agricultural runoff. Furthermore, the significant increase in wastewater volume, mainly in winter season, causes alterations in hydraulics inside the wastewater treatment plants (WWTPs). This may lead to an enhanced toxicity as a result of the remobilization and release of toxic substances from in-sewer deposits (Gasperi et al. 2010). These highly toxic substances in wastewater can negatively affect the purification efficiency of STPs by inhibiting the metabolic processes of the microorganisms in the biological treatment step (Belhaj et al. 2014). In addition, the toxic chemicals in the influent affect the quality of treated wastewater. Once treated, WWTP effluents may be used for irrigation in agricultural land, as is the case in a suburb of Sfax, Tunisia.

The effects of treated wastewater (TWW) irrigation on the physicochemical properties of soil have been studied in detail (Tarchouna et al. 2010; Belaid et al. 2012; Bedbabis et al. 2014); nevertheless, research on the effects of TWW irrigation soil quality and plant on physiology has not been conducted in detail so far. Consequently, it is important to conduct the risk assessment of this site, which is irrigated with water diverted from polluted streams, to ensure that contamination is not adversely affecting the environment.

The present study aims to investigate the short-term effects of both diluted and undiluted TWW, overloaded with toxic metals, compared with fresh water, on the agriculture land and food crops. The impacts of irrigation treatments on plant health and soil including plant growth, photosynthetic pigments, activities of stress responsible enzymes, and ecotoxicological indicators were examined.

Materials and methods

Experimental design

The current study was conducted in winter 2013 in a greenhouse located at the National Engineering School of Sfax, Tunisia.

The statistical design used included three sources of irrigation, freshwater as the control (*C*), TWW (T_1), and diluted TWW (T_2) which is TWW diluted by fresh water (1:1), and three vegetable crops, tomato (*Solanum lycopersicum* L.), lettuce (*Lactuca sativa* L.), and radish (*Raphanus Sativus* L.) with 10 replicates. These vegetables were selected because they differ in edible parts, which are the fruit for tomato, the leaves for lettuce, and the roots for radish.

Seeds of each crop plant under investigation were surface-sterilized with 0.001-M $HgCl_2$ solution for 3 min and washed meticulously with sterilized double distilled water. Afterward, seeds were germinated on wet filter paper in darkness at 25± 2 °C through 1 week.

Previously germinated seedlings under investigation (tomato, lettuce, and radish) were then transplanted into three groups of 10 plastic bottles (2 L) each (Fig. 1) filled with 2.3 kg of sandy clay top soil (73.5 % sand, 17.24 % clay, and 9.26 % loam), collected from a rural area of Sfax (34° 43 N, 10° 41 E). Bottles were irrigated with freshwater in the first week. Afterward, the first group continued to be irrigated by freshwater (control); the second and third groups were irrigated by TWW and diluted TWW collected from the outlet of Sfax WWTP that has an activated sludge treatment process. This plant serves a population of 526,800 and is designed to purify the urban wastewater with a daily average flow rate of 49,500 m^3 day^{-1}. Domestic sources account for approximately 65 % of influent, while industrial sources account for 35 % of influent.

Bottles were maintained in a greenhouse designed as growth chamber programmed for a 12-h photoperiod with photosynthetic photon flux density of 300 μmol m^{-2} s^{-1}, temperature of 24±1/18±1 °C day/night, and relative humidity of 60/70±3 %.

Samples were taken at the vegetative phase (60 days after sowing for tomato plants and 30 days after sowing for lettuce and radish plants). After washing with distilled water, the plants were separate into roots and shoots. Different growth attributes studied were the number of leaves per plant and area of leaves per plant. After measuring fresh weights, plants were oven dried at 70 °C for 3 days, and the root and shoot dry weights were recorded. The oven-dried plant tissues were

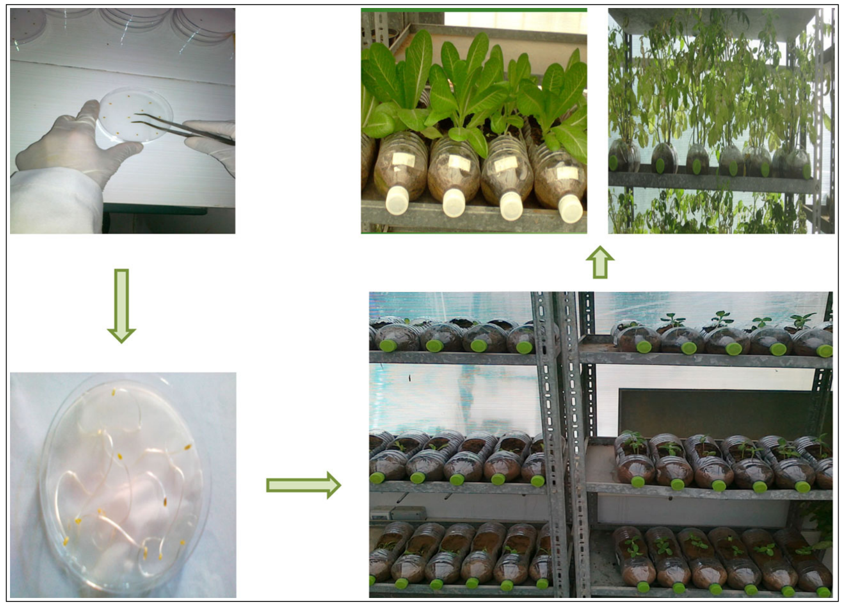

Fig. 1 The experimental design for studying the effects of wastewater reuse on plant health

ground by mortar into a fine powder and passed through a 0.5-mm sieve for further analysis.

Other samples were taken before the flowering phase (100 days after sowing for tomato and 70 days after sowing for lettuce and radish) to assess components of the antioxidant system in leaves (antioxidant enzymes including CAT and POD and antioxidant compounds including reduced GSH and ASC).

After the plants had been harvested, the soil in each bottle was air dried in open plastic ziplock bags at room temperature before the bags were sealed and stored until required at 20± 2 °C. Before analysis, soil was passed through a 1-mm sieve.

Analysis of irrigation waters

Characteristics of each water type used for irrigation are shown in Table 1. The pH and electrical conductivity (EC) were measured using a pH-meter and conductivity meter, respectively. Total nitrogen was determined by the Kjeldahl method. Total phosphorus was measured by direct colorimetric analysis. Chemical oxygen demand (COD) was determined according to the method by Knechtel (1978). Five-day biochemical oxygen demand (BOD_5) was measured using the respirometric method. Macronutrients (Na, Mg, Ca, and K) and heavy metals were determined using atomic absorption spectrometry (Thermo Scientific ICE 3200).

Plant analysis

Photosynthetic pigments and protein content

Fresh leaves (about 0.5 g) were homogenized using a pestle and mortar in 5 mL of 95 % (*v*/*v*) ethanol. The homogenate was filtered through pre-ashed filter paper and made up to 25 mL with 95 % ethanol. The filtered solution was used for chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid estimation. Absorbance at 470, 665, and 649 nm was measured using a method described by Lou et al. (2004). The pigment contents were calculated using the following equations (Lichtenthaler and Wellburn 1983):

$$Chl\ \ a = 13.95(A_{665}){-}6.88(A_{649}) \tag{1}$$

$$Chl\ \ b = 24.95(A_{649}){-}7.32(A_{655}) \tag{2}$$

$$Carotenoid = \frac{1000(A_{470}){-}2.05(Chl\ \ a){-}114.8(Chl\ \ b)}{245} \tag{3}$$

Chlorophyll and carotenoid contents were expressed as micrograms per gram dry weight (DW) of leaves

Heavy metal contents

Both dry shoot and root samples (0.5 g) for each plant were dry-ashed at 450 °C and digested in three replicates to minimize error, with 1 M HNO_3. Plant digests were filtered and transferred first into 25-mL volumetric flasks, brought to volume with Milli-Q water, and then into polyethylene flasks for analysis. The metal concentrations were determined directly in the extract solution by means of atomic absorption spectrometry (Thermo Scientific ICE 3200). Depending on the concentration, a flame atomic absorption spectrometer was introduced to measure Zn, Fe, Pb, Mn, and Cr, whereas the Ni and Cu were measured with a graphite furnace atomic absorption spectrometer. The instrument response was periodically checked versus known standards. An air acetylene flame and hollow cathode lamp were used for all samples. Calibration curves were prepared from dilutions of stock

Table 1 Mean values (±standard deviation) of the key properties of irrigation waters used in the study

Parameter	Control (fresh water)	STW effluent (T_1)	50 % diluted STW effluent (T_2)	Tunisian limit[a]	FAO guidelines[b]
pH	6.9±4	8.2±1.2	8±2.8	6.5–8.5	6.5–8.0
EC (mS cm^{-1})	5.92±2.2	9.12±3.7	8.3±4.9	7.0	3.0
COD (mg L^{-1})	–	1210±120	702.3±212	90	
BOD_5 (mg L^{-1})	–	312±85.4	155±76.8	30	
Total P (mg L^{-1})	10.7±5.3	14.3±5.7	8.7±3.0	0.05	
Total N (mg L^{-1})	–	69.7±4.3	38.3±12.3	30	
Fe (mg L^{-1})	6.1±1.3	15.48±3.2	9.0±3.2	5.0	5.0
Mn (mg L^{-1})	2.1±0.4	4.2±0.11	2.6±1.6	0.5	0.2
Zn (mg L^{-1})	2±0.6	6.3±0.9	4.7±1.5	5.0	2.0
Cu (mg L^{-1})	0.2±0.1	1.46±0.3	0.74±0.2	0.5	0.2
Pb (mg L^{-1})	nd	2.08±0.2	1.2±0.3	1.0	5.0
Cd (mg L^{-1})	nd	0.69±0.1	0.31±0.1	0.1	0.01
Ni (mg L^{-1})	nd	0.92±0.3	0.53±0.2	0.2	0.2

n=5

nd not detected

[a] Tunisian standards for wastewater reuse, NT 106.002 (1989)

[b] Wastewater quality guidelines for agricultural use, FAO

solutions, and all analyses were conducted in triplicate to ensure the accuracy of experimental data/results. The recovery rates for all heavy metals from the plant tissues were found to be more than 98.7 % as determined by digesting three samples each from an untreated plant with known amount of metals. The blanks were run in triplicate to check the precision of the method with each set of samples.

Antioxidant enzymes content

Preparation of samples for enzyme extraction followed the method described by Mukherjee and Choudhury (1983). SOD activity was measured in accordance with the method of Dhindsa et al. (1981). One unit of SOD activity was defined as the amount of enzyme required to inhibit 50 % of the initial reduction of nitro-blue tetrazolium chloride at 560 nm under the experimental conditions. CAT activity was estimated by the decrease in absorbance at 240 nm over 1 min as a result of H_2O_2 consumption (Verma and Dubey 2003). POD activity was determined using guaiacol as substrate (Wu and VonTiedemann 2002). One unit of POD activity was calculated by the change in absorbance at 470 nm min^{-1} g^{-1} fresh weights at 25 °C (Klapheck et al. 1990). APX activity was assayed in accordance with the method of Asada (1992) by measuring the decrease in absorbance at 290 nm over 1 min as a result of oxidation of ASC using UV spectrophotometer.

The activities of CAT, POD, SOD, and APX were expressed as enzyme units per gram fresh weight (U g^{-1} FW).

GSH was extracted and measured by the method adopted by Tanaka et al. (1985) and expressed as units per gram FW. Reduced ASC was quantified by the bi-pyridyl method (Knorzer et al. 1996) and expressed as nanomoles per milligram FW.

Soil ecotoxicity analysis

Terrestrial avoidance behavior is proposed as a fast and cost-effective method for assessing toxic effects generated by hazardous chemicals in soils (OECD 2004; Römbke et al. 2005). This rapid and sensitive method is suitable for evaluating the habitat function of soils for soil invertebrates. It is a sublethal test reflecting the bioavailability of a mixture of contaminants in natural soils or contaminants/chemicals in spiked soils. To check whether the studied TWW is suitable for irrigation, the earthworm *Eisenia andrei andrei* was tested (ISO 2007). The worm avoidance assay (adapted from De Silva and van Gestel 2009; ISO 2007) was conducted by means of disposable aluminum containers (30×22×6 cm). Each one was separated into two with a cardboard screen, 150 g of air-dried soil placed on each area and afterward the screen gently removed. In one area, experimental soil from one of the irrigation treatments was placed, and in the other area, second soil (OECD soil) that had not been used in the trial, composed of 70 % sand, 20 % kaolin, and 10 % sphagnum, was used. Each of the 90 containers was watered with 48 mL of tap water and left to absorb the water. Ten adult worms were placed in the center of each container at the junction of the two soils. A cardboard cover was placed on top and the containers conserved. Worms were incubated for 48 h at 26±2 °C in the dark. At the end of the assay, screen was replaced between the two soils and each soil removed separately from the container. Worm numbers in each soil were determined by hand sorting. The worms found on the separating line were counted by locating the direction of the head. For each replicate, the avoidance response (%) was calculated using Eq. 4:

$$NR = [(C - T/N)] \times 100 \quad (4)$$

where NR is the net avoidance response, C is the number of worms in control soil, T is the number of worms in the treatment soil, and N is the total number of worms exposed (De Silva and van Gestel 2009). Avoidance is indicated by a positive response and attraction by a negative response. When avoidance was ≥80 %, the treatment could be considered to have limited or reduced habitat function (De Silva and van Gestel 2009).

Statistical evaluation of the data

Statistical analyses were performed using the SPSS program (version 17.0) to compare treatment effects. Plant, photosynthetic pigment content, heavy metals, and enzyme assay means were compared by analysis of variance and multiple comparison tests (post hoc Fishers least significant differences). Net avoidance response for the earthworms was evaluated using a two-tailed *t* test on combined radish, tomato, and lettuce soil data. Differences were considered significant at $P<0.05$ for all statistical tests.

Results and discussion

Irrigation water quality

The physicochemical parameters and the heavy metal levels of TWW (T_1) and diluted TWW (T_2) used for irrigation purposes as well as the control fresh water (C) were appraised and compared with Tunisian standards for wastewater reuse in crop irrigation. In general, the quality of the T_1 and T_2 was not very good due to dysfunction of the Sfax WWTP. As shown in Table 1, the EC of T_1 and T_2 was found to be above the threshold of 7 mS cm^{-1} defined as the water salinity beyond which severe restrictions for crop irrigation may occur. Treated waste water's high salinity may be attributed to the deterioration of groundwater quality, due to persistent groundwater pumping for irrigation and the consequent salt water intrusion (Werner et al. 2009; Zhao et al. 2015). Thus, both T_1 and T_2 reuse for irrigation purposes may contribute to the amelioration of groundwater over-abstraction and to its qualitative restoration. High contents of BOD_5 and total N were also recorded. This may be ascribed to the fact that Sfax WWTP under various incoming load and residence time during this period which makes the quality of TWW unsteady. The excess of nitrogen may be risky due to possible groundwater pollution. Lubello et al. (2004) reported that in many investigations, a negative effect of high concentrations of ammonia on crop growth was observed. In the bulk of the soil, ammonia goes through nitrification process. Nitrates indeed migrate into the deep soil layers and can be hazardous for shallow groundwater. On the other hand, higher concentrations of COD and heavy metals were also detected in T_1 and T_2. This could be attributed to an increase of illegal discharge of industrial effluents and high loading rate of organic matter (Belhaj et al. 2013; 2014). P, Mn, Fe, Cd, and Ni concentrations in T_1 and T_2 exceeded the limits recommended by the Tunisian Government (NT 106.002 1989) and the Food and Agriculture Organization (FAO) of the United Nations (wastewater quality guidelines for agriculture reuse) for trace metals in irrigation water. In all treatments, P, Mn, and Fe contents in plant tissues (Table 3) are within the safe limit and are not in toxic range (Kabata-Pendias and Pendias 1986). Cd concentration, in both T_1 and T_2, was 6.9- and 3.1-fold higher than the limit permissible level (0.1 mg L^{-1}), respectively. Ni concentrations were 4.6- and 2.65-fold higher than the maximum permissible level in T_1 and T_2. Given the fact that these metals could be accumulated in soil and hence crops with continuous reuse of wastewater in irrigation, therefore, their periodic monitoring should be an important component of wastewater management.

Plant growth, dry matter, and photosynthetic pigment content

A major precursor for increasing plant yield is an increase in biomass production in terms of dry weight mass. Considerable decreases in leaf area in the T_1 and T_2 of shoots and roots were detected in the three studied plants (Table 2); the decreases were positively correlated with the amounts of heavy metals detected in the irrigation waters and was calculated by 52, 49, and 65 % inhibition in leaf area of radish, lettuce, and tomato irrigated with diluted TWW (T_2) as compared with control plants irrigated with fresh water (C). Similar results were reported in studies on *Kandelia candel* and *Bruguiera gymnorrhiza* (Huang and Wang 2010), *Brassica campestris* and *Apium graveolens* (Yang et al. 2011), and *Taraxacum officinale* (Bini et al. 2012a, b). The growth responses are probably due to high metal concentrations that damaged plant roots and inhibited uptake of nutrients, thus inhibiting normal plant growth. The level of reduction differed among the studied plants; the maximum inhibition of the shoot system was detected in radish plants and calculated as 66.4 % inhibition of the FW and 76.8 % of the DW of the control plants. Alternatively, the shoot system of lettuce was less affected, with only 14.3 and 18 % inhibition in FW and DW, respectively, of controls. For the root system, the maximum inhibition in FW was found in lettuce plants, followed by radish and

Table 2 Growth parameters of radish plants (*Raphinus sativus*), tomato plants (*Solanum lycopersicum*), and lettuce plants (*Lactuca sativa*) under three irrigation treatments

Treatment	Character					
	Root system DW (g)	Shoot system DW (g)	Root system FW (g)	Shoot system FW (g)	Number of leaves per plant	Area of leaves per plant (cm^3)
Radish	115.6^{a}	182.4^{a}	191.0^{a}	344.5^{a}	10.3^{a}	161.3^{a}
C	51.2^{b}	43.6^{c}	144.3^{c}	120.8^{c}	6.9^{c}	76.8^{b}
T_1	69.4^{c}	94.3^{b}	149.3^{b}	177.6^{b}	10.0^{a}	122.4^{a}
T_2	4.9	5.1	5.2	5.9	1.1	19.1
LSD						
Tomato	31.6^{a}	201.8^{a}	78.4^{a}	291.3^{a}	11.2^{a}	33.6^{a}
C	12.4^{c}	99.8^{c}	59.7^{c}	211.2^{c}	9.2^{a}	17.1^{b}
T_1	23.7^{b}	153.4^{b}	61.2^{ab}	271.6^{b}	11.1^{a}	30.2^{a}
T_2	4.2	8.1	9.5	12.9	2.0	9.6
LSD						
Lettuce	51.74^{a}	316.3^{a}	91.9^{a}	403.0^{a}	11.2^{a}	181.3^{a}
C	24.2^{c}	259.1^{c}	63.0^{c}	341.0^{c}	8.2^{b}	62.8^{c}
T_1	40.31^{b}	297.4^{b}	81.6^{b}	382.3^{b}	11.0^{a}	145.6^{b}
T_2	6.5	8.5	5.7	13.2	1.0	22.9
LSD						

The letters a, b, and c indicate significant differences between the treatments, based on ANOVA analysis ($P<0.05$). Values are mean±standards deviation ($n=5$)

LSD limit significant difference

afterward tomato, whereas the maximum decrease in DW was detected in tomato plants, followed by radish and then lettuce, which showed the minimum inhibition in root DW in response to heavy metals. This could be attributed to the variable rates of uptake and transport of heavy metals by these three plants (Yang et al. 1995).

Pigment concentration is directly linked to photosynthetic activity, and photosynthetic rates and pigment content, i.e., chl a/chl b ratio, have been found to be correlated (Das et al. 2002).

In accordance with the growth responses, the studied plants showed a significant and progressive decline in chl a, chl b, and carotenoid pigment contents with increase in heavy metal content in the irrigation water (Fig. 2). The decline in photosynthetic amount can be the result of increase of chlorophyll as activity or inhibition of delta-aminolevulinic acid dehydratase (Singh et al. 2012; Aldoobie and Beltagi 2013). In lettuce and tomato, chl b was affected more than chl a by increasing amounts of heavy metals in irrigation water, which resulted in marked increase in the chl a/chl b ratio in plants treated with TWW and diluted TWW, compared with fresh water. Increase in chl a/chl b ratio after heavy metal treatment was reported also by Zengin and Munzuroglu (2005) and Gomes et al. (2011). Subsequent chlorosis progression was described by Ebbs and Uchil (2008). These observations suggest a specific pattern of Chl loss during metal-induced chlorosis, representing either a direct effect of metals on the two Chl pools or an indirect effect via oxidative stress.

Carotenoids are essential components of the photosynthetic apparatus in a wide range of organisms, which participate in the adaptation of plastids to changing environmental light conditions and prevent photooxidative damage of the photosynthetic apparatus by detoxifying ROS (Simkin et al. 2008). Previous studies have reported increase (Drazkiewicz and Baszynski 2005), decrease (Ekmekc et al. 2008), or no change (Mishra et al. 2006) in the content of carotenoids in plants in response to heavy metal stress. In our study, carotenoid content in the three plants decreased significantly at the highest concentration of heavy metals. As a result, the lowest carotenoid content in radish leaves could lead to a diminished capacity to protect photosystems against photooxidation (Di Toppi et al. 2009).

Heavy metal accumulation in plants

The concentrations of Fe, Ni, Cd, Cu, Mn, and Pb (mg kg^{-1} FW) in shoots and roots of radish, tomato, and lettuce are shown in Table 3. Significant increases in the accumulated FE, Mn, Zn, and Cu were detected in both shoots and roots of the three studied plants irrigated with T_1 and T_2 compared with the control plants irrigated with fresh water. In addition, other toxic heavy metals (Ni, Cd, and Pb) were detected only in plants irrigated with TWW. Although, heavy metals accumulated primarily in the roots plants receiving TWW for irrigation. The restriction of metal absorption and translocation to the shoots may be related to the avoidance mechanism in the roots. In this respect, El-Beltagi et al. (2010) found that in plants that are not hyperaccumulators of Cd, roots have been shown to be the major site of phytochelatin synthesis and hence Cd accumulation.

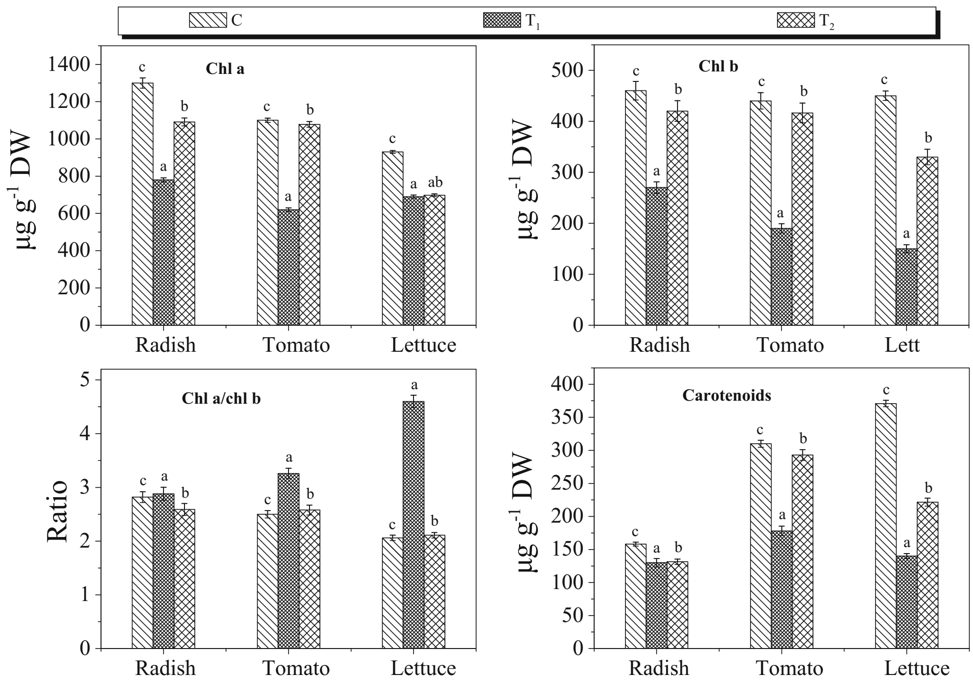

Fig. 2 Effect of wastewater irrigation on photosynthetic pigment contents of radish (*Raphanus sativus*), tomato (*Solanum lycopersicum*), and lettuce (*Lactuca sativa*). The letters *a*, *b*, and *c* indicate significant difference ($P<0.05$) from ANOVA analysis

It was observed that lettuce shoots accumulated much more amounts of all the detected heavy metals as compared with the shoots of radish and tomato plants. Leafy vegetables tend to accumulate high amounts of Cd, Ni, and Pb due not only to their large leaf area and high transpiration rate but also to the fast growth rate of these plants as observed by Weldegebriel et al. (2012). Surprisingly, the results (Table 3) showed that Cd contents in radish plant and Pb contents, in both lettuce and radish, irrigated with T_1 and T_2 surpassed the maximum limit. In these cases, consuming the vegetables may pose health risk due to the high Cd and Pb concentrations (Codex Alimentarius Commission 2001).

Antioxidant defense system

In order to elucidate the response of antioxidant system to heavy metals, activities of antioxidative enzymes were followed. The three studied plants irrigated with wastewater

Table 3 Accumulation of heavy metals in radish (*Raphinus sativus*), tomato (*Solanum lycopersicum*), and lettuce (*Lactuca sativa*) from the application of treated sewage effluent in irrigation

Treatment	Cu		Mn		Fe		Pb		Zn		Cd		Ni	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Radish	0.6^{ab}	0.8^{c}	2.3^{c}	3.2^{c}	20^{c}	21.6^{c}	nd	nd	4.2^{c}	5.3^{c}	nd	nd	nd	nd
C	0.9^{a}	1.2^{a}	4.5^{a}	6.3^{a}	25.8^{a}	28.3^{a}	0.41^{a}	0.8^{a}	6.1^{a}	7.1^{a}	0.21^{a}	0.36^{a}	0.4^{a}	0.62^{a}
T_1	0.72^{ab}	0.97^{b}	3.9^{b}	4.81^{b}	22^{b}	25.3^{b}	0.21^{ab}	0.41^{b}	4.9^{b}	6.7^{b}	0.11^{b}	0.19^{b}	0.28^{b}	0.41^{b}
T_2	0.27	0.12	0.21	0.31	1.00	1.03	0.02	0.10	0.03	0.02	0.01	0.05	0.03	0.10
LSD														
Tomato	0.58^{c}	0.75^{c}	2.28^{c}	3.1^{c}	20.1^{c}	21.3^{c}	nd	nd	4.2^{c}	5.5^{c}	nd	nd	nd	nd
C	0.88^{a}	1.1^{a}	4.38^{a}	6.18^{a}	25.6^{a}	24.7^{a}	0.39^{a}	0.73^{a}	5.8^{a}	7.6^{a}	0.21^{a}	0.35^{a}	0.51^{a}	0.73^{a}
T_1	0.74^{b}	0.87^{c}	3.76^{b}	4.7^{b}	22.2^{b}	24.2^{b}	$0.2a^{b}$	0.39^{b}	4.71^{b}	6.68^{b}	0.11^{b}	0.17^{b}	0.22^{b}	0.47^{b}
T_2	0.04	0.10	0.23	0.31	0.10	0.84	0.02	0.09	0.30	0.23	0.02	0.05	0.03	0.10
LSD														
Lettuce	0.68^{b}	0.87^{c}	2.3^{c}	3.4^{c}	21.6^{c}	22.4^{c}	nd	nd	4.1^{c}	6^{c}	nd	nd	nd	nd
C	0.98^{a}	1.45^{a}	4.8^{a}	6.7^{a}	26.2^{a}	29^{a}	0.43^{a}	0.8^{a}	6^{a}	8^{a}	0.22^{a}	0.37^{a}	0.54^{a}	0.7^{a}
T_1	0.83^{b}	0.96^{c}	3.8^{b}	5.1^{b}	23.1^{b}	26.8^{b}	0.22^{b}	0.42^{b}	5^{b}	6.95^{b}	0.13^{b}	0.2^{b}	0.31^{b}	0.5^{b}
T_2	0.03	0.10	0.23	0.31	1.01	1.20	0.03	0.08	0.30	0.22	0.05	0.04	0.03	0.09
LSD														

The letters a, b, and c indicate significant differences between the treatments, based on ANOVA analysis ($P<0.05$). Values expressed as milligrams per kilogram FW

nd not detected, *LSD* limit significant difference

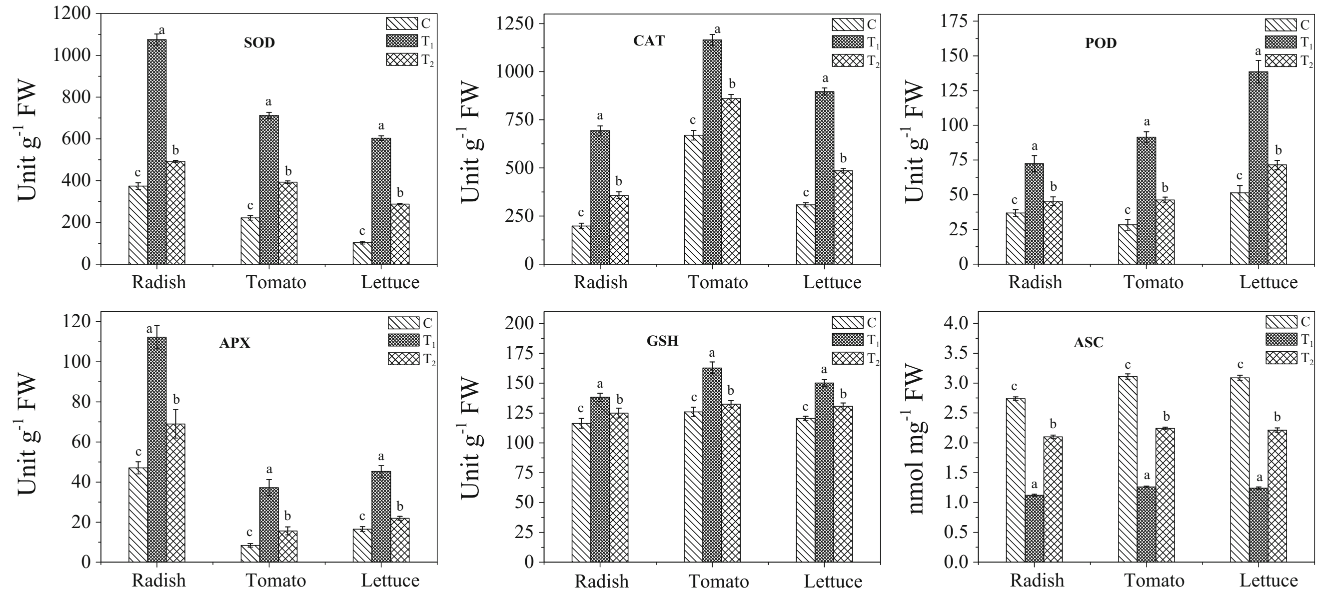

Fig. 3 Effect of wastewater irrigation on antioxidant enzymes (SOD, CAT, and POD) and antioxidant compounds (APX, GSH, and ASC) in radish (*Raphanus sativus*), tomato (*Solanum lycopersicum*), and lettuce (*Lactuca sativa*). The letters *a*, *b*, and *c* indicate significant difference ($P<0.05$) from ANOVA analysis

exhibited higher superoxide dismutases (SOD), enzymes-catalases (CAT), peroxidases (POD), and ascorbate peroxidase (APX) activities compared to the same plants irrigated with fresh water (Fig. 3). Measurement of the activity of antioxidant enzymes (CAT, POD, and SOD) can provide useful information about the stress level in plants. Significant increase in SOD, CAT, and POD activities under heavy metals exposure has been observed in many plant species treated with different metals (Najeeb et al. 2011; Wang et al. 2014). In addition, a significant amount of GSH accumulated in the plants in response to all treatments, with the highest accumulation in plants irrigated with T_1 relative to controls of the same species. The accumulation was the lowest in radish (118 %), intermediate in lettuce (124 %), and highest in tomato (129 %). The induction of the synthesis of glutathione, a substrate in the synthesis of phytochelatins, was observed in plants in response to heavy metals (Petrova et al. 2012). In contrast, the amount of detected ASC decreased in plants irrigated with contaminated wastewater relative to control plants. Rao and Sresty (2000) also reported that the ASC content of roots and shoots of two pigeon pea cultivars showed a significant negative correlation with increasing concentrations of metal ions (Zn and Ni).

Ecological risk evaluation

After irrigation with wastewater, some contaminants may persist in soil leading to high-risk exposure of soil organisms to the product. Among them, the earthworms may be in direct contact and ingest the contaminated soil particles. However, by having sensory tubercles on their body surfaces, depending on the pollutant concentration, they can detect and avoid the contaminated soil. Evidently, the avoidance test is considered a sensitive tool in risk assessment as earthworms detect a wide range of contaminants including polyaromatic hydrocarbons, heavy metals, explosives, crude oil, and pesticides (ISO 2007).

The sensitive response of earthworms, especially to contaminants, makes them one of the most suitable animals for use as bioindicators of soil quality (Reinecke and Reinecke 2004; Lukkari et al. 2005). Species numbers, abundance, and

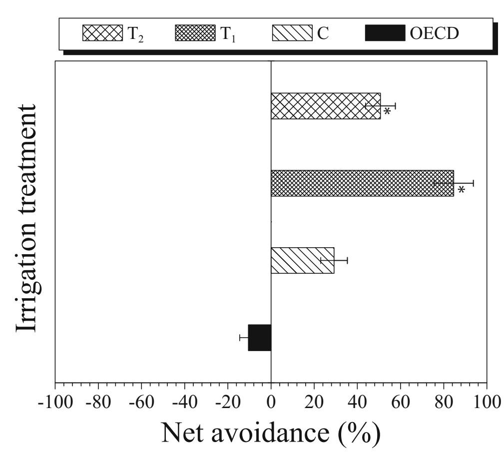

Fig. 4 Net avoidance (%) for earthworms between an OECD soil and soil treated with either fresh water (*C*), treated wastewater (T_1), and diluted treated wastewater (T_2). Avoidance is indicated by a positive response and attraction by a negative response. Each value is the mean (±standard error) of 10 replicates with 10 worms per replicate. *Columns with an asterisk* above them suggest significant difference to an avoidance of zero ($P<0.05$)

biomass can easily give measurable elements. In this study, the net response (Amorim et al. 2005) was used as an intuitive parameter due to its simple scale varying between avoidance of (+1), preference (−1), and no avoidance (0) for the test soil. For all treatments, net avoidance was positive indicating that worms were avoiding the treated soils (Fig. 4). Furthermore, there was no significant difference on worm avoidance due to the plant species grown in the soil (P=0.769) or the irrigation treatment (P=0.542). Indeed, according to the 80 % avoidance criterion for the habitat function proposed by Mangala et al. (2009), soils treated with wastewater T_1, with a significantly avoidance percentages (84.6±9.12 %), presented a limited habitat quality. A clear preference pattern for soil irrigated with diluted wastewater (T_2) and freshwater (C) was observed. Therefore, these soils would be a better environment for these ground-living invertebrates than the soils treated with wastewater (T_1).

Conclusions

In summary, this study showed that the performance of Sfax WWTP was less than satisfactory; hence, the effluent quality was less than desirable. The concentrations of many pollutants such as COD, BOD5, Cd, and Ni in the effluent exceeded the maximum recommended values, hence presenting a pollution problem to the local rivers and agriculture land. The results showed that tomato, lettuce, and radish plants grown in effluent caused harmful effects on their growth and photosynthetic activity. In addition, the plants were found to accumulate higher amounts of Cd and Pb than the permissible limits, thereby presenting that a potential human health risk resulted from the consumption of vegetables irrigated with such wastewater. Besides, a decrease in enzymatic activities in plants depending on the irrigation with TWW was detected. The positive correlation of the stimulation measurements of the antioxidant system with the amounts of heavy metal in irrigation water proved that these two parameters are efficient biomarkers for the degree of water pollution with heavy metals. Indeed, polluted water irrigation was more likely to have direct toxic impact on soil habitat quality after short-term usage, with implications for human health from the consumption of food crops.

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