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Heavy metal accumulation imparts structural differences in fragrant *Rosa* species irrigated with marginal quality water

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ABSTRACT

Wastewater is an alternative to traditional sources of renewable irrigation water in agriculture, particularly in water-scarce regions. However, the possible risks due to heavy metals accumulation in plant tissues are often overlooked by producers. The present study aimed to identify heavy metals-induced structural modifications to roots of scented *Rosa* species that were irrigated with water of marginal quality. The chemical and mineral contents from the experimental irrigation canal water (control) and treated wastewater were below the limits recommended by the Pakistan Environmental Protection Agency (Pak-EPA) for medicinal plants. The experimentally untreated wastewater contained electrical conductivity (EC), chemical oxygen demand (COD), biological oxygen demand (BOD), and heavy metals (Co, Cu, Cd, Pb) that were above the recommended limits. The responses by wastewater-treated *Rosa* species (*Rosa damascena, R. bourboniana, R.* Gruss-an-Teplitz, and *R. centifolia*) were evaluated. The experimental data revealed that treated wastewater significantly increased the thickness of collenchyma (cortex and pith) and parenchyma tissues (vascular bundle, xylem, and phloem) of *R.* Gruss-an-Teplitz. Root dermal tissues (epidermis) of *R. bourboniana* also responded to treated wastewater. *R. damascena* and *R. centifolia* were the least affected species, under the experimental irrigation conditions. Collenchyma and dermal tissues were thicker in *R. damascena* and *R.* Gruss-an-Teplitz under untreated wastewater conditions. In parenchyma tissues, vascular bundles were thicker in *R. damascena* in untreated wastewater conditions, while the xylem and phloem of *R.* Gruss-an-Teplitz were thicker where treated wastewater was applied. In tissues other than the vascular bundle, the differences in anatomical metrics due to the experimental irrigation treatments were greater during the second year of the experiment than in the first year. The contents of metals other than chromium in the roots and stems of roses were below the WHO limits, under all of the experimental irrigation conditions. *Rosa centifolia* contained higher heavy metals content than the other experimental species, and heavy metals content was associated with anatomical changes due to the treatments. We conclude that, under conditions of wastewater irrigation, *R.* Gruss-an-Teplitz was highly resistant; *R. damascena* was moderately resistant while *R. bourboniana* and *R. centifolia* were the most susceptible to irrigation with marginal quality water. This is the first report of plant tissue responses to wastewater irrigation by the experimental species. Regarding the accumulation of heavy metals in rose plant tissues, the results confirm that untreated wastewater must be treated to grow *Rosa* species where water is scarce.

1. Introduction

50–97% of a plant body is water. As such, water is necessary for plant life, but it is unfortunately the most poorly managed reserve in the world (Khurana and Singh, 2012). Severe water scarcity is expected in many countries as the world enters the era of changed climate (Sowers et al., 2011). Anthropogenic (industrial) activities are the major source of exaggerated use of water resources to a crisis level (Rusan et al., 2007; Safi et al., 2007). The irrigation of crop plants is by far the chief user of fresh water (70–90%) in developed and developing countries

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(Ensink et al., 2002; Pedrero et al., 2010) and the demand for fresh water is expected to continue to increase (FAOWATER, 2008). Water of marginal quality or sewage wastewater can be used to irrigate 10% of crops, notably in urban and peri-urban areas (Scott et al., 2000). Increasing wastewater usage in peri-urban agriculture creates extensive economic activity and increases the living standards of poor growers, but also modifies the qualities of environments (Qishlaqi et al., 2008; Murtaza et al., 2003; Talal et al., 2014). Treated and untreated wastewater (UTWW) has been used in agriculture for centuries (Khaleel et al., 2013) in China, Vietnam, and Mexico (Shuval et al., 1986). Wastewater is currently a dormant water source but, under conditions of severe freshwater shortage, in Pakistan, India, Israel, Saudi Arabia, and Jordan, the use of wastewater is common practice (Ensink et al., 2002).

Wastewater is a reliable water source for crops in peri-urban areas. Due to the availability of macronutrients (N, P, K) in wastewater, the application of additional chemical fertilizers can be reduced. Such wastewater used for irrigation may provide plant nutrients where crops are grown in low-fertility soils (Jimenez-Cisneros, 1995). It can increase S, K and Fe concentrations in the soil and it can contribute up to 60% of soil organic matter (Agency for International Development, 2004). The efficient use of wastewater can produce food, create employment, and generate income (Hussain et al., 2006a, b).

Continuous industrial and municipal wastewater application to agricultural lands can result in the accumulation of heavy metals in plants and in soils (Sharma et al., 2007; Gupta and Sinha, 2007; Ali et al., 2012; Du et al., 2014; Chen et al., 2015). Heavy metals Cd and Pb are considered non-essential elements for plants. Excessively high contents of heavy metals in irrigation water attenuate the absorption and transport of essential nutrients by plants; inhibit plant metabolism; damage the membranes of plant cells; disorganize plant structure; weaken the functioning of plant physiology; and ultimately restrain the growth of plants (Xu and Shi, 2000; Long et al., 2003; Zhang et al., 2007). To avoid the contamination of food by marginal quality water, the use of treated wastewater (TWW) was proposed as an alternative for growing ornamentals, fiber crops, oil crops (Saber et al., 2002; Angelova et al., 2004; Hussein et al., 2004), or for the cultivation of medicinal, aromatic, and flower crops such as roses (Zheljazkov and Warman, 2004; Nirit et al., 2006; Darvishi et al., 2010). The oils extracted from plants contain relatively low levels of heavy metals. Where ornamental plants are not used simultaneously as food, the treatment of sewage waste is not necessary (Gori et al., 2000).

In the peri-urban areas of large cities such as Faisalabad, Pakistan, treated and untreated wastewaters are utilized for the production of vegetable crops (Hussain et al., 2006a, 2006b). The resultant vegetable crops contain toxic heavy metals that cause human diseases. Floricultural crops that are not destined for use as food can be more suitable for agricultural operations that depend on (treated and untreated) marginal quality water. All of the ornamental crops that are cultivated commercially in Pakistan are irrigated with fresh or canal water (CW), and therefore no information is available concerning the effect of treated and UTWW on the growth and quality of field grown ornamental crops such as roses. Therefore, the present study was conducted to determine whether or not plant structural changes occur due to metal uptake by the roots of four scented *Rosa* species that were grown using marginal quality water for irrigation in a peri-urban area.

2. Materials and methods

2.1. Field site description, soil analysis and experimental design

The field study was conducted from 3rd January 2014–29th of December 2015 at the Horticultural Research Area (31°25′ N, 73°09′ E, 300 m above mean sea level) at the University of Agriculture, Faisalabad, Pakistan. The climate of the experimental region is semi-arid with scarce rainfall annually. The field soil was clay loam, which was irrigated by untreated sewage wastewater from the university.

Table 1
Soil composition before experiment.
Source: Alloway (1990)

Sr. no.	Characteristics	Unit	Value		IASS*
1	Texture		Clay loam soil		
			00–15 cm	15–30 cm	
2	pH		8.2	8.2	4–8.5
3	EC	dS m^{-1}	2.54	2.49	4
4	Organic Matter	%	1.12	1.18	> 0.86
5	Nitrogen	%	0.041	0.041	–
6	Phosphorus	ppm	10.5	9.5	> 7
7	Potassium	ppm	194	134	> 80
8	Lead	ppm	3.16	3.32	500
9	Cadmium	ppm	0.04	0.05	1.0
10	Nickel	ppm	0.36	0.34	20
11	Zinc	ppm	5.28	3.6	250
12	Copper	ppm	3.04	2.3	100

* International Agricultural Soil Standards.

Before cultivation, 16 sites were selected at random and soil samples were collected (15 cm and 30 cm depth). Composite soil samples were analyzed according to the standard procedures and results are presented in Table 1.

For soil pH, method 21a (U. S. Salinity Laboratory Staff, 1954) was adopted and reading was noted with the help of Jenway pH meter (Model-671P). Electrical conductivity (EC) was determined by method 3a and 4a (U.S. Salinity Laboratory Staff, 1954) with the help of digital Jenway conductivity meter (model 4070). Soil organic content was determined according to standard method proposed by Moodie et al. (1959). For this purpose, one gram soil was weighed and 10 ml 1.0 N potassium dichromate solution and 20 ml concentrated sulfuric acid was added. Then 25 ml 0.5 N ferrous sulphate solution and 150 ml distilled water also added and mixed thoroughly. Titrated this mixture against 0.1 N potassium permanganate solution up to pink end point. For nitrogen determination in soil, mixture was first digested by 0.42 g Se and 14 g of $Li_2SO_4{\cdot}H_2O$ to 350 ml of 100% pure H_2O_2. After digestion of soil sample, nitrogen was determined by kjeldhal method (Jackson, 1962) and final nitrogen was determined by using following formula;

$$N\% = (T \times N \times 1.4)/\text{sample weight}$$

Where T = volume of acid used for titration (ml), N = normality of acid = 0.01 N and sample weight = 0.4 g.

Phosphorus concentration was measured by taking 5.0 g of sieved soil that was extracted by using 0.5 M sodium bicarbonate ($NaHCO_3$) (pH 8.5). Then 5 ml of filtered extract was taken in 100 ml of volumetric flask and 5 ml of color developing reagent (ascorbic acid, ammonium molybdate, antimony potassium tartrate and sulfuric acid) was added and volume was made up to 100 ml with distilled water. Reading was recorded on spectrophotometer (ANA-730) at 410 nm and available P was calculated by standard curve (Watanabe and Olsen, 1965). Potassium was measured by flame photometer (Jenway PEP-7) by adopting method 11a (U. S. Salinity Laboratory Staff, 1954). Metals (Pb, Zn, Cu, Cd and Ni) contents in soil were determined by ammonium bicarbonate-DTPA (AB-DTPA) procedure for extractable metals (Soltanpour, 1985). Calculations involved for metal estimation were;

$$\text{Metal}(\text{mgKg}^{-1}) = \frac{\text{Metals in the extract}(\text{mgKg}^{-1}) - \text{metal ion in the blank}(\text{mgKg}^{-1}) \times A}{\text{Weight of soil}(g)}$$

Where A is total volume of the extract expressed in ml.

The species of fragrant rose and the irrigation water treatment were the fixed factors of the experiment. Two-year-old healthy and vigorous cuttings of four scented rose species (*R. damascena, R. bourboniana, R.* Gruss-an-Teplitz, and *R. centifolia*) were collected from the Floriculture Research Department, Ayub Agricultural Research Institute (AARI), Faisalabad. The cuttings were planted and irrigated with canal water