INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–1262/2019/21–6–1159–1165
DOI: 10.17957/IJAB/15.1006
http://www.fspublishers.org

Full Length Article

Anatomical Changes in Stem of Scented *Rosa* spp. in Response to Heavy Metal Accumulation under Wastewater Treatment

Muhammad Ahsan[1*], Adnan Younis[2], Muhammad Jafar Jaskani[2], Usman Tariq[3], Muhammad Rashid Shaheen[1], Aasma Tufail[4], Javaria Sherani[5] and Fahim Nawaz[6]
[1]*Department of Horticultural Sciences, University College of Agriculture & Environmental Sciences, The Islamia University of Bahawalpur, Pakistan*
[2]*Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan*
[3]*College of Agriculture, Bahaudin Zakariya University, Bahadur Sub-campus, Layyah, Pakistan*
[4]*Division of Science and Technology, Department of Botany, University of Education Lahore, Pakistan*
[5]*Department of Horticulture, Ghazi University, Dera Ghazi Khan, Pakistan*
[6]*Department of Agronomy, Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan*
[*]For correspondence: ahsan.horti@iub.edu.pk; lyconference@bzu.edu.pk

Abstract

Irrigation with marginal quality water has emerged as a potential alternative of water in agriculture mainly water scarce regions around the globe. Most of the cases, the potential hazards of heavy metal accumulation in various plant parts are usually ignored. The current study was conducted to investigate heavy metals induced anatomical changes in the stem of oil bearing and scented *Rosa* species irrigated with treated and untreated wastewater. Chemicals and minerals were in acceptable range in sweet or canal water and treated wastewater (TWW) while higher heavy metal contents [cadmium (Cd), cobalt (Co), copper (Cu), lead (Pb)], electrical conductivity, biological oxygen demand and chemical oxygen demand than permissible levels were found in untreated wastewater (UTWW). Four species of *Rosa viz*., *Rosa damascena*, *R. bourboniana*, *R.* Gruss-an-Teplitz, *R. centifolia* were assessed for their response to wastewater. The results indicated that TWW considerably enhanced parenchymatous tissues like xylem, phloem and vascular bundle and collenchyma (cortex and pith) of *R.* Gruss-an-Teplitz whereas *R. centifolia* produced minimum values. The same trend was also recorded in epidermal thickness during both years of experiment. *Rosa centifolia* had higher metal concentration than other species in stems thereof it was anatomically the most affected. It is proposed that *R.* Gruss-an-Teplitz verified to be highly impervious whereas *R. centifolia* was most vulnerable species for germination under polluted waters. It is also strongly recommended to treat UTWW by some extent to use as an alternative source to fresh water.

Keywords: Marginal quality water; Metal noxiousness; Oil bearing roses; Structural modification

Introduction

Almost half of the international floriculture trade revolves around roses which are most imperative part of all fragrances used in cosmetic industries and herbal medicines (Ahsan *et al*., 2017a). This woody perennial rose plant belongs to family Rosaceae and sub-family Rosoideae which have more than 20,000 cultivars all over the globe (Ahsan *et al*., 2017b). Most of these species are grown for different purposes like indoor and garden plant, cut flowers and making different types of food items (Nybon, 2009). Amongst these species, *Rosa centifolia* is economically most value added and highly profitable species with great capacity to produce essential oil (Ahsan, 2016). It produces more than 650 flowers per plant per year and has ability to produce flower even temperature of above 48ºC (Younis *et al.,* 2013). In Pakistan, it is successfully cultivated in districts of Lahore, Sheikhupura, Qasoor (Pattoki), Faisalabad, Multan, Haiderabad and some areas of Azad Jammu & Kashmir (Younis *et al.*, 2009).

Water is a basic necessity for all organisms especially plants, but unfortunately ranked at the top of most unwisely used natural resource around the globe (Khurana and Singh, 2012). Due to extreme shortage of water, tropical and subtropical countries of the world are entering in period of severe climatic changes (Sowers *et al*., 2011). Due to industrial and others human activities, water resources are dwindling very rapidly to the level of calamity (Rusan *et al*., 2007; Safi *et al*., 2007). Share for freshwater utilization (70-90%) to agricultural crops is

To cite this paper: Ahsan, M., A. Younis, M.J. Jaskani, U. Tariq, M.R. Shaheen, A. Tufail, J. Sherani and F. Nawaz, 2019. Anatomical changes in stem of scented *Rosa* spp. in response to heavy metal accumulation under wastewater treatment. *Intl. J. Agric. Biol*., 21: 1159–1165

increasing day by day in all over the world especially in developing countries (Pedrero *et al*., 2010). Treated and untreated marginal quality water can be used to supply water up to 10% of the crops especially in peri-urban areas (Scott *et al*., 2000). This vast utilization of wastewater in rural areas provides opportunity to boost income and living standards of poor farmers as well as negatively affect the environmental quality (Murtaza *et al*., 2010; Talal *et al*., 2014). Marginal quality water is being utilized for irrigation to agricultural crops for decades (Khaleel *et al*., 2013) in Mexico, Vietnam and China. Wastewater is potential water source but due to extreme scarcity of freshwater in Pakistan, Jordan, India and Kingdom of Saudi Arabia, the utilization of marginal quality water is routine activity (Ensink *et al*., 2002).

Marginal quality water is a consistent irrigation source of irrigation to agricultural crops. Due to macronutrient (NPK) accumulation in water, the extra expenses of artificial fertilizers could be minimized. These types of marginal quality wastewater can be highly beneficial for crops of areas with low soil fertility status (Jimenez-Cisneros, 1995). This may add S, K, and Fe in the root zone and may add 60% organic matter (AID, 2004).

Constant application of municipal and industrial marginal quality water may affect in accrual of heavy metal in different plant parts as well as agricultural lands (Du *et al*., 2014; Chen *et al*., 2015). Metals like Pb and Cd are un-necessary element in plant and if concentrations of these metals remain high in wastewater can reduce plant metabolism, damage plant cell membrane and ultimately reduces the plant growth (Zhang *et al*., 2007). To evade adulteration of food by wastewater, irrigation with low quality water was supposed to as an alternate of ornamental crop plants, essential oil bearing plants, and fiber crops production (Angelova *et al.*, 2004; Hussain *et al.*, 2006) or for the growing of medicinal, aromatic and flower crop plants like rose (Nirit *et al*., 2006; Darvishi *et al*., 2010). Gori *et al*. (2000) reported that as ornamental crop plants are not directly used as food purpose, so requirements for the treatment of marginal quality wastewater is not compulsory.

Marginal quality wastewaters are commonly used for the production of vegetables in peri-urban cities of larger cities like Faisalabad, Pakistan (Hussain *et al*., 2006). The heavy metals accumulated in these vegetables are sources of diseases in humans. Floricultural crops are more likely to grow under these low qualities treated and untreated wastewater due to its not intended for use as food purpose. Most of floricultural crops produced commercially are watered by tube wells/canal water, so there is no data regarding the impact of treated and untreated wastewater on the production of floricultural crops like roses. The current study was carried out to evaluate anatomical modifications arise owed to heavy metal intake by stems of fragrant roses cultivated with treated and untreated wastewater for irrigation in peri-urban areas of Faisalabad.

Materials and Methods

Experimental Area and Design

The current field experiment was carried out during 2014 to 2015 at the research area of Department of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan. Monthly means of minimum and maximum temperatures (°C), relative humidity (%) and total rainfall (mm) of this semi-arid climate are shown in Table 1. The untreated sewage wastewater from university's living hostels of students and employees' colonies was major source of irrigation to this clay loam soil. Before experiment, randomly selected soil samples were collected under the soil depth of 15 cm to 30 cm. These samples were thoroughly mixed and standard procedures were adopted for analysis (Table 2).

Two factors used were scented *Rosa* species (*R. centifolia, R. damascena R. bourboniana* and *R*. Gruss-an-Teplitz) and second irrigation waters [untreated wastewater (UTWW), treated wastewater (TWW) and canal water (CW)]. Healthy and vigorously growing two years old plant cuttings of these species were taken from Horticultural Research Station, Ayub Agricultural Research Institute (AARI), Faisalabad. Pits (0.5 m^2) were made and these rose species were planted (1.22 m) on January 02, 2014 according to two factor factorial with randomized complete block design (RCBD) arrangements and replicated thrice. Irrigation with UTWW, TWW and CW were applied immediately to the cuttings and irrigation continued at regular intervals of 4–7 days depending upon weather condition and season up to completion of the experiment.

Treatment and Analysis of Wastewater

Untreated sewage wastewater was purified in three large plastic vats (placed 2.5' away and 2' below from each other) with water storage capacity of 1500 gallon in three steps *i.e*., primary, secondary and tertiary purification (Pescod, 1992). This conventional and natural purification process improved the chemical and physical qualities of the experimental sewage wastewater (Pescod, 1992; Kiziloglu *et al.*, 2008). The physio-chemical analysis of these waters was carried out by methods described by Eaton *et al*. (2005). With inductively coupled plasma optical emission spectrometry (ICP-OES optima 2100-DV Perkin Elmer), macronutrients and heavy metals concentration was determined at Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan. Analysis results of all water types are presented in Table 3.

Anatomical Study

Anatomical characteristics of stems of roses were done by free hand sectioning technique. About one-centimeter part of healthy growing stem was taken from each plant. Solution of acetic acid containing one-part acetic acid and

Table 1: Average temperatures (°C), relative humidity (%) and rain fall (mm) during growing season 2014 and 2015

Month	2014				2015			
	Mean Temperature(°C)		R.H*	R.F*	Mean Temperature(°C)		R.H*	R.F*
	Daily Max	Daily Min			Daily Max	Daily Min		
Jan	17.3	03.2	69.6	3.8	16.8	03.5	77.6	1.5
Feb	18.4	04.6	62.1	8.0	19.2	08.3	81.0	55.0
Mar	25.9	11.7	58.2	1.5	27.0	13.0	61.2	1.3
Apr	32.7	18.0	59.1	10.5	33.5	19.7	36.7	21.6
May	38.9	23.3	43.3	0.0	39.7	24.4	24.5	4.6
Jun	42.0	27.5	44.1	23.6	39.5	27.9	43.3	67.5
Jul	39.1	27.0	59.2	45.4	37.4	28.6	58.5	4.7
Aug	36.2	26.4	65.0	38.5	35.3	27.2	65.6	114.8
Sep	33.1	23.6	75.0	1635	36.2	25.4	53.7	3.3
Oct	30.2	16.2	64.3	11.5	32.9	21.3	54.0	0.0
Nov	24.8	10.8	73.0	0.0	26.1	11.8	59.4	0.5
Dec	18.8	06.1	81.5	17.2	20.5	08.4	64.5	0.0

* R.H = relative humidity (%); R.F = Rain fall (mm)

Table 2: Soil composition before experiment

Characteristics	Unit	Value		IASS*
		00-15 cm	15-30 cm	
Texture		Clay loam soil		
pH		8.2	8.2	4-8.5
EC	dS m^{-1}	2.54	2.49	4
Organic Matter	%	1.12	1.18	>0.86
Nitrogen	%	0.041	0.041	---
Phosphorus	mg l^{-1}	10.5	9.5	>7
Potassium	mg l^{-1}	194	134	>80
Lead	mg l^{-1}	3.16	3.32	500
Cadmium	mg l^{-1}	0.04	0.05	1.0
Nickel	mg l^{-1}	0.36	0.34	20
Zinc	mg l^{-1}	5.28	3.6	250
Copper	mg l^{-1}	3.04	2.3	100

*International Agricultural Soil Standards Source: Alloway (1990)

three parts ethyl alcohol was used for long term preservation whereas solution of formalin acetic acid (FAA) was used for short term preservation of plant samples. Prepared samples were fixed in FAA solution containing acetic acid 5%, formalin 10%, ethyl alcohol 50% and 35% of distilled water. Sectioning, mounting and staining methods were adopted by the procedure suggested by Sass (1951). Light microscope (Nikon 104, Japan) was used for the viewing and measurement of all the slides.

Statistical Analysis

Computer program STATISTIX (version 8.1) was used to compute analysis of variance (ANOVA). All the significant means were analyzed according to least significant difference (LSD) test at 5% level of probability (Steel *et al.*, 1997).

Results

Untreated wastewater, treated wastewater and canal water analysis showed that EC of untreated wastewater was higher than the standard values recommended by National Environmental Quality Standards (NEQS) for marginal quality wastewater in Pakistan. Elevated concentration of macronutrients (N and Na), heavy metals (Co, Pb, Cu, Cd), chemical oxygen demand (COD) and biological oxygen demand (BOD) were observed in untreated wastewater. Treated wastewater and canal water contained levels within recommended value.

Table 3: Analysis of experimental waters used in the experiment

Parameters	Canal Water	Treated Water	Untreated Water	NEQS**
EC (μ S/L)	1.13	1.44	2.11	1.5
pH	7.42	7.58	8.31	6-9.2
Color	----	Rust Brown	Greyish	--
Turbidity	43	29.12	155	--
Hardness (mg/L)	184	416	536	--
DO (mg/L)	4	2.38	1.36	--
BOD (mg/L)	---	267	432	300
COD (mg/L)	---	481	669	500
TDS (mg/L)	218	1281	1678	2500
SS (mg/L)	0.9	0.15	1.1	--
Total Solids (mg/L)	218	982	1372	--
TSS (mg/L)	24	63	194	400
Chlorides (mg/L)	138	290	436	1000
Cadmium (mg/L)	0.001	0.01	0.013	0.1
Nickel (mg/L)	0.10	0.08	0.12	1.0
Arsenic (mg/L)	ND	0.004	0.005	0.1
Zinc (mg/L)	0.18	2.62	3.48	5.0
Potassium (mg/L)	30.41	17.61	40.73	--
Lead (mg/L)	0.021	0.42	0.66	0.5
Iron (mg/L)	0.32	3.47	4.82	8.0
Cobalt (mg/L)	0.17	0.029	0.079	0.05
Copper (mg/L)	0.05	0.13	0.24	1.0
Chromium (mg/L)	0.04	0.067	0.093	1.0
Calcium (mg/L)	28.1	39.72	54.29	200
Sodium (mg/L)	36.47	178.23	252.77	250
Magnesium (mg/L)	30	47	63	150
Phosphorus (mg/L)	0.39	1.76	2.49	15
Total Nitrogen (mg/L)	4	5.72	8.0	5.0

****NEQS** stands for National Environmental Quality Standards for municipal wastewater of Pakistan; **EC**: Electrical conductivity; **DO**: Dissolved Oxygen; **BOD**: Biological Oxygen Demand; **COD**: Chemical Oxygen Demand; **TDS**: Total Dissolved Solids; **SS**: Settle able Solids; **TSS**: Total Suspended Solids. NEQS source: EPA (2007)

Stem Anatomical Attributes

Cortical cell area (μm^2): The irrigation treatments and experimental species affected stem cortical cell area significantly. The greatest cortical cells were found from *R. damascena* and small values of the of cortical cell area were observed from *R. centifolia* under UTWW or CW (in 2014 and 2015) irrigation during 2014 and 2015. Cortical cells of *R. centifolia* with UTWW irrigation were 73.42% greater than plants watered with CW irrigation during 2014 and 31.07% higher than CW-irrigated during 2015 (Fig. 1).

Epidermal thickness (μm): The species and irrigation treatments affected epidermal thickness to a statistically significant degree in both years of the experiment. The greatest value of epidermal thickness was found under UTWW irrigation in *R.* Gruss-an-Teplitz, while minimum values of epidermal thickness were found from *R. centifolia* under CW irrigation during both

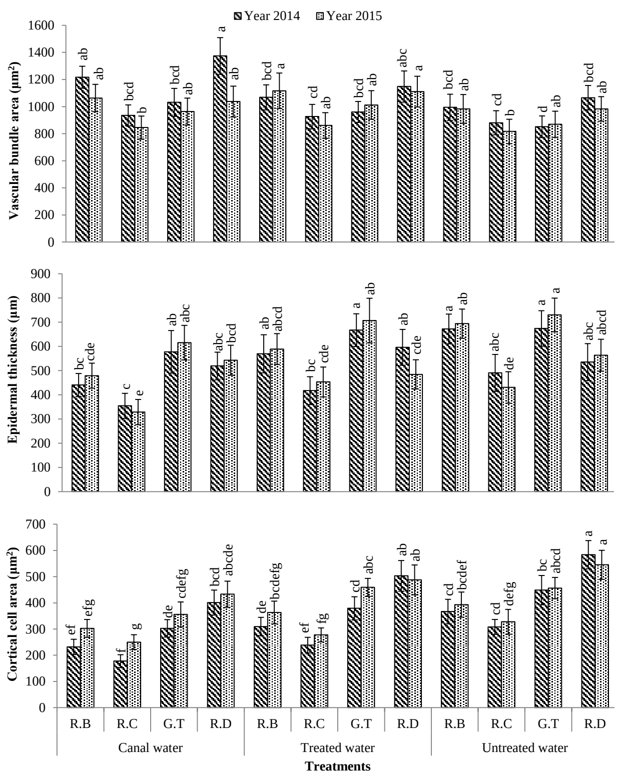

Fig. 1: Stem anatomical parameters (vascular bundles, epidermis, cortex) of roses under different sources of irrigation

RB: *Rosa bourboniana*; RC: *Rosa centifolia*; GT: Gruss-an-Teplitz; RD: *Rosa damascena*

years. This increase in *R.* Gruss-an-Teplitz was 16.75% and 18.62% during 2014 and 2015 respectively. The *R. bourboniana* reacted positively to epidermal thickness under irrigation with TWW (+29.21% in 2014 and +23% in 2015) and with UTWW (+52.59% in 2014 and +44.96% in 2015) (Fig. 1).

Vascular bundle area (μm^2): Thicker vascular bundles were recorded in *R. damascena* irrigated with CW during 2014 than *R. bourboniana* under TWW during 2015 (Fig. 1). The means of vascular bundles were small in *R. centifolia* under UTWW irrigation in both years.

Xylem area (μm^2): *R.* Gruss-an-Teplitz exhibited maximum xylem area with TWW and UTWW during both years of experiment. The xylem was 14.04% smaller in plants of *R.* Gruss-an-Teplitz irrigated with CW during 2014 while the watered with UTWW were 33.96% greater than plants irrigated in 2015 with CW. Minimum xylem area was found from *R. damascena* (2014) and *R. bourboniana* (2015) irrigated with CW. During 2015, the xylem area was greater than in 2014 (under all irrigation treatments). The difference between annual means of the xylem area was observed where UTWW was applied to the plants (Fig. 2).

Phloem area (μm^2): The results indicated that irrigation with UTWW reduced the phloem area in all rose species during both years. In 2014, TWW irrigation enhanced the phloem area of *R. bourboniana* (+14.48%) and *R. centifolia* (+4.75%) and decreased the phloem area of *R.* Gruss-an-Teplitz (-5.96%) and *R. damascena* (-6.96%). The phloem area of all *Rosa* species responded negatively to TWW irrigation during 2015. The greatest phloem area was observed from *R.* Gruss-an-Teplitz under CW irrigation and minimum phloem area was observed from *R. bourboniana* under UTWW irrigation (Fig. 2).

Pith area (μm^2): Highest pith area was found in plants of *R. bourboniana* irrigated with TWW during both years of experiment, while the pith area were relatively low for *R. damascena* when irrigated with CW (Fig. 3). During 2014, *R. centifolia* (-7.44%) and *R.* Gruss-an-Teplitz (-11.55%) responded negatively to irrigation with UTWW. The pith area of other species responded positively in both years of the experiment (Fig. 2).

Discussion

The structure of plant tissues and variation in its size depends on the plant type and condition of pollutants in the water (Melo *et al*., 2017). All pollutants from TWW were noticed within permissible range and the cortex in all roses tended to rise. Earlier experimental reported similar findings that cortical cell area likely to reduce under UTWW treatment (Sridhar *et al*., 2005). It was assumed that nutrient and heavy metals concentration in irrigation water encouraged the exodermises lignification, reduced heavy metal entry into plant parts and enhanced metal tolerance of the plant (Cheng *et al*., 2012). Shi and Cai (2008) and Guo and Feng (2010) revealed that the higher metal concentration of wastewater irrigation rouse cortical development in the plants. It is considered that denser cortical cells in the stem of *R.* Gruss-an-Teplitz and *R. bourboniana* were linked with efficient irrigation water usage, which results by the widely spreading of rose species and the existence of these roses under diverse climatic conditions (Zwieniecki and Newton, 1995). The higher level of salts and heavy metals reduced the cortex in vulnerable varieties of some plant but the stem cortex tends to improve in heavy metal tolerant plant species (Younis *et al.,* 2014).

Increased in epidermal thickness due to the presence of heavy metals is linked with the adsorption of heavy metals in cells, which create an alternate path for the circulation of ions which averts the ions of heavy metals that could translocate to the vascular cells (Gomes *et al.,* 2011). The two species, *R. bourboniana* and *R.* Gruss-an-Teplitz were most resilient for marginal quality water and these species endure heavy load of chemicals due to specific structural changes which might be a main reason for the success of these species under diverse climatic regions (Nawaz *et al.,* 2011). The decrease in the size of the cells for *R. centifolia* showed the vulnerability to the wastewater and could be a sign of meat venomousness, and can specify the heavy metal profusion in marginal quality wastewater

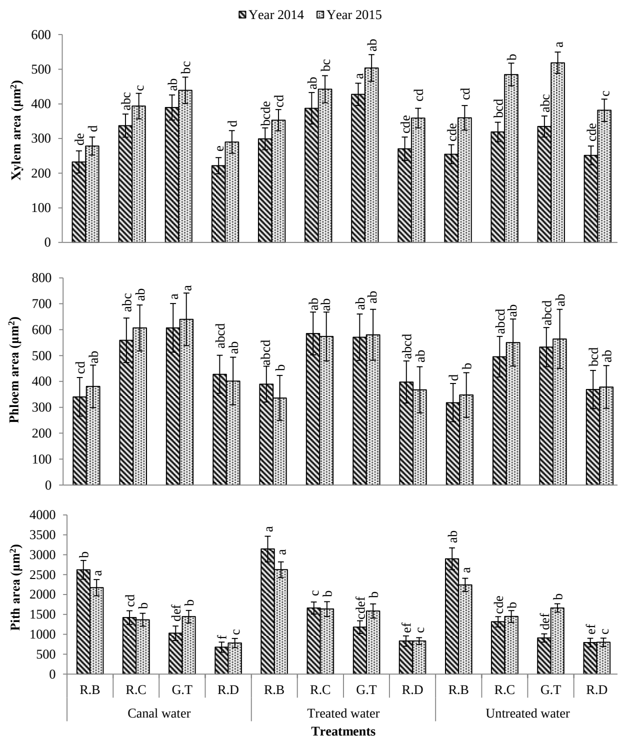

Fig. 2: Stem anatomical parameters (Xylem, phloem, pith area) of roses under different sources of irrigation
RB: *Rosa bourboniana*; RC: *Rosa centifolia*; GT: Gruss-an-Teplitz; RD: *Rosa damascena*

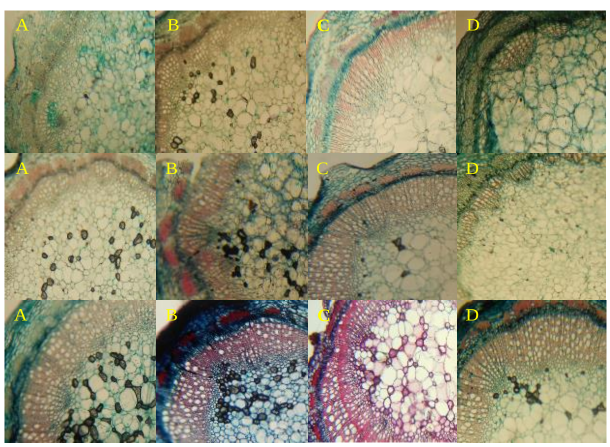

Fig. 3: Transverse sections of stem of different *Rosa* species showed anatomical modifications under different irrigation treatments. Label A is for *R. bourboniana*, B is for *R. centifolia*, C is for *R.* Gruss-an-Teplitz while D is for *R. damascena*. *Rosa* species in row one were irrigated with canal water, whereas species in row two and three were irrigated by treated wastewater and untreated wastewater respectively

(Ogunkune *et al*., 2013).

Vascular bundles, phloem, xylem and pith size of roses were greater under UTWW and TWW, which showed that cell responded contrarily to polluted and unpolluted waters. This contradicts with Hameed *et al*. (2010) and Tyagi *et al*. (2013) that area of the conducting tissues is reported to decrease as level of wastewater contaminants augmented under irrigation water. Aldesuquy (2014) recorded that UTWW and TWW elevated the area of vascular bundles in wheat crops. Sridhar *et al.* (2005) recorded structural modifications of plant tissues associated with metal uptake. Species like *R.* Gruss-an-Teplitz and *R. bourboniana* can upsurge parenchyma cell production that maintained the entry of metallic ions into the stems (Vijayakumar and Udayasoorian, 2007) The reduction of parenchyma cells dismisses surplus ions from xylem and declines noxiousness from the flow of wastewater to aerial plant parts when ions are strenuous (Ogunkune *et al*., 2013). Omosun *et al.* (2008) and Vijayakumar and Udayasoorian (2007) recorded that reducing the parenchyma cells of *Amaranthus hybridus* and *Cenchurus cellaris* prohibited lethal ions entry into the root vessels, but in roses, the discrepancy of parenchyma cells was observed during second year of the experiment. It is assumed that many toxic elements of the wastewater treatment were assured and omitted from parenchyma cells (Ahsan *et al*., 2017b). Akram *et al.* (2002) showed that there was ability in pith cells to store water under stress conditions and *R. bourboniana* contained larger pith size than rose species of the experiment. This pith cells growth showed the adoptability of *R. bourboniana* to extreme environmental conditions.

Conclusion

The application of untreated wastewater modified stem anatomy especially stems of *R. damascena* (cortex and vascular bundles) and *R.* Gruss-an-Teplitz (epidermis, xylem and phloem) that responded superior than the other experimental *Rosa* species. Longer wastewater duration played key role in greater accumulation of metals in plant parts which induced harmful effects on susceptible *Rosa* species. Present study showed that *R.* Gruss-an-Teplitz proved to be extremely resistant species than *R. centifolia* with great vulnerability for growing with TWW and UTWW. It is also highly suggested that the raw sewage untreated municipal wastewater must be treated before its use for irrigation to roses.

References

Agency for International Development (AID), 2004. *Guidelines for Water Reuse*, pp: 180–181. Washington DC

Ahsan, M., 2016. Comparative study of treated and untreated wastewater on morpho- anatomical, chemical and essential oil contents of fragrant Rosa species in peri-urban area. *Ph.D. thesis*. University of Agriculture Faisalabad, Pakistan

Ahsan, M., A. Younis, M.J. Jaskani, M.A. Haneef, A. Tufail, U. Tariq, U.H. Siddiqua, S. Aziz and R. Gull, 2017a. Heavy metal profile of four fragrant and oil bearing *Rosa* species cultivated under treated and untreated wastewater in peri-urban area. *Oxid. Commun.,* 40: 1404–1418

Ahsan, M., A. Riaz, M.J. Jaskani and M. Hameed, 2017b. Physiological and anatomical response of fragrant *Rosa* species with treated and untreated wastewater. *Intl. J. Agric. Biol.,* 19: 13–22

Akram, M., S. Akhtar, I.H. Javed, A. Wahid and E. Rasul, 2002. Anatomical attributes of different wheat (*Triticum aestivum*) accessions/varieties to NaCl salinity. *Intl. J. Agric. Biol.,* 4: 166–168

Aldesuquy, H.S., 2014. Effect of spermine and spermidine on wheat plants irrigated with waste water: Conductive canals of flag leaf and peduncle in relation to grain yield. *J. Stress Physiol. Biochem.*, 10: 145–166

Alloway, B.J., 1990. *Heavy Metals in Soils*. John Wiley Sons, Inc., New York, USA

Angelova, V., R.V. Ivanov, Delibaltova and K. Ivanov, 2004. Bio-accumulation and distribution of heavy metals in fiber crops (flax, cotton and hemp). *Ind. Crops Prod.,* 19: 197–205

Chen, X.W., W.J. Tsz-Fung, W.Y. Mo, Y.B. Man, N.C. Wang-Wai and M.H. Wong, 2015. Ecological performance of the restored south east new territories (SENT) landfill in Hong Kong (2000–2012). *Land Degrad. Dev.,* 1: 1–13

Cheng, H., Y.S. Wang, Z.H. Ye, D.T. Chen, Y.L. Peng and L.Y. Wang, 2012. Influence of N deficiency and salinity on metal (Pb, Zn and Cu) accumulation and tolerance by *Rhizophora stylosa* in relation to root anatomy and permeability. *Environ. Pollut.,* 164: 110–117

Darvishi, H.H., M. Manshouri, H. Sedghi and S.H.M. Jahromi, 2010. Irrigation influence by treated domestic wastewater instead of agronomical water on essential oil yield of basil (*Ocimum basilicum* L.). *Afr. J. Microbiol. Res.,* 4: 475–479

Du, Y., B. Gao, H. Zhou, X. Ju and S. Yin, 2014. Health risk assessment of heavy metals in road dusts in urban parks of Beijing, China. *Proc. Environ. Sci.,* 18: 299–309

Eaton, A.D., L.S. Glescer, E.W. Rice and A.E. Greenberg, 2005. *Standard Methods for the Examination of Water and Wastewater,* 21st Edition. American Public Health Association, Washington, USA

Ensink, J.H.J., W.V.D. Hoek, Y. Matsuno, S. Munir and M.R. Aslam, 2002. *Use of Untreated Wastewater in Peri-urban Agriculture in Pakistan: Risks and Opportunities*, Vol. 64, pp: 1–22. International Water Management Institute, Colombo, Sri Lanka

Environmental Protection Agency (EPA), 2007. *Water Quality Standards.* Government of Pakistan, Islamabad

Gomes, M.P., T.C. Marques, M.O. Nogueira, E.M. Castro and A.M. Soares, 2011. Eco-physiological and anatomical changes due to uptake and accumulation of heavy metal in *Brachiaria decumbens*. *Sci. Agric.,* 68: 566–573

Gori, R., F. Ferrini, F.P. Nicese and C. Lubello, 2000. Effect of reclaimed wastewater on the growth and nutrient content of three landscape shrubs. *J. Environ. Hortic.*, 18: 108–114

Guo, Z.H. and M.X. Feng, 2010. Growth changes and tissues anatomical characteristics of giant reed (*Arundo donax* L.) in soil contaminated with arsenic, cadmium and lead. *J. Cent. South Univ. Technol.,* 17: 770–777

Hameed, M., M. Ashraf, N. Naz and F. Al-Qurainy, 2010. Anatomical adaptations of *Cynodon dactylon* (L.) pers. from the salt range Pakistan, to salinity stress. I. root and stem anatomy. *Pak. J. Bot.,* 42: 279–289

Hussain, S.I., A. Ghafoor, S. Ahmad, G. Murtaza and M. Sabir, 2006. Irrigation of crops with raw sewage: hazard and assessment in effluent, soil and vegetables. *Pak. J. Agric. Sci.,* 43: 97–101

Jimenez-Cisneros, B., 1995. Wastewater reuse to increase soil productivity. *Water Sci. Technol.,* 32: 173–180

Khaleel, R.I., N. Ismail and M.H. Ibrahim, 2013. The impact of wastewater treatments on seed germination and biochemical parameter of *Abelmoschus esculentus* L. *Soc. Behav. Sci.,* 91: 453–460

Khurana, M.P.S. and P. Singh, 2012. Wastewater use in crop production: A Review. *Resour. Environ.,* 2: 116–131

Kiziloglu, F.M., M. Turan, U. Sahin, Y. Kuslu and A. Dursun, 2008. Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (*Brassica olerecea* L. var. Botrytis) and red cabbage (*Brassica olerecea* L. var. Rubra) grown on calcareous soil in Turkey. *Agric. Water Manage.,* 95: 716–724

Melo, H.C., E.M. Castro, A.M. Soares, L.A. Melo and J.D. Alves, 2007. Anatomical and physiological alterations in *Setaria ancep*s, *Stapfex Massey* and *Paspalum paniculatum* L. under water deficit conditions. *Hoehnea,* 34: 145–153

Murtaza, G., A. Gafoor, M. Qadir and M.K. Rashid, 2010. Accumulation and bioavailability of Cd, Co and Mn in soils and vegetables irrigated with city effluent. *Pak. J. Agric. Sci.,* 40: 18–24

Nawaz, T., M. Hameed, M. Ashraf, F. Al-Qurainy, M.S.A. Ahmad, A. Younis and M. Hayat, 2011. Ecological significance of diversity in leaf tissue architecture of some species/cultivars of the genus *Rosa* L. *Pak. J. Bot.,* 43: 873–883

Nirit, B., B.T. Asher, F. Haya, S. Pini, R. Ilona, C. Amram and I. Marina, 2006. Application of treated wastewater for cultivation of roses (*Rosa hybrida*) in soilless culture. *Sci. Hortic.,* 108: 185–193

Nybon, H., 2009. Introduction to *Rosa. In: Plant Genetics and Genomics: Genetics and Genomics of Rosaceae, Crops and Models,* pp: 167–171. Folta, K.M. and S.E. Gardiner (eds.). Springer Science, Business Media, Dordrecht, The Netherlands

Ogunkune, C.O., A.A. Abdulrahman, T.A. Aluko, O.S. Kolawolf, P.O. Fatoba and F.A. Oladele, 2013. Anatomical response of *Amaranthus hybridus* Linn. as influenced by pharmaceutical effluents. *Not. Sci. Biol.,* 5: 431–437

Omosun, G., A.A. Markcon and O. Mbanasor, 2008. Growth and anatomy of *Amaranthus hybridus* as affected by different crude oil concentrations. *Amer.-Euras. J. Sci. Res.,* 3: 7074–7082

Pedrero, F., I. Kalavrouziotis, J.J. Alarcón, P. Koukoulakis and T. Asano, 2010. Use of treated municipal wastewater in irrigated agriculture. Review of some practices in Spain and Greece. *Agric. Water Manage.,* 97: 1233–1241

Pescod, M.B., 1992. *Wastewater Treatment and Use in Agriculture.* Irrigation and Drainage paper 47. FAO, Rome, Italy

Rusan, M.J.M., S. Hinnawi and L. Rousan, 2007. Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination,* 215: 143–152

Safi, M.I., A. Fardous, M. Muddaber, S. El-Zuraiqi, A. Balawenth, L. Al-Hadidi and I. Bashabsheh, 2007. Long term effects of reclaimed water on rose and carnation cut flower crops in soil and soilless media. *J. Appl. Sci.,* 7: 1191–1198

Sass, J.E., 1951. *Botanical Micro Technique*. The Iowa State College Press, Ames. Iowa, USA

Scott, C.A., J.A. Zarazqa and G. Levine, 2000. *Urban-Wastewater Reuse for Crop Production in the Water-short Guanajuato River Basin, Mexico.* Resident Representative, Colombo, Sri Lanka

Shi, G.R. and Q.S. Cai, 2008. Photosynthetic and anatomic responses of peanut leaves to cadmium stress. *Photosynthesis,* 46: 627–630

Sowers, J., A. Vengosh and E. Weinthal, 2011. Climate change, water resources, and the politics of adaptation in the Middle East and North Africa. *Clim. Change,* 104: 599–627

Sridhar, B.B.M., S.V. Diehl, F.X. Hanc, D.L. Monts and Y. Sub, 2005. Anatomical changes due to uptake and accumulation of Zn and Cd in Indian mustard (*Brassica juncea*). *Environ. Exp. Bot.,* 54: 131–141

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics: A Biometric Approach,* 3rd Edition. McGraw Hill Book Co., New York, USA

Talal, A., I. Ismail, J.M. Basahi, H.A. Qari and I.A. Hassan, 2014. Hazardous of waste water irrigation on quality attributes and contamination of citrus fruits. *Biosci. Biotechnol. Res. Asia,* 11: 89–97

Tyagi, K., S. Sharma, S. Kumar and S. Ayub, 2013. Cytological, morphological and anatomical studies of *Ricinus communis* Linn. grown under the influence of industrial effluent-A comparative study. *J. Pharm. Res.,* 7: 454–458

Vijayakumar, P.S. and C. Udayasoorian, 2007. Anatomical response of *Cenchrus celiaris* to the paperboard effluent irrigation. *World J. Agric. Sci.,* 3: 553–557

Younis, A., A. Riaz, A. Iftikhar, M.I. Siddique, U. Tariq, M. Hameed and M. Nadeem, 2014. Anatomical changes induced by NaCl stress in root and stem of *Gazania harlequin* L. *Agric. Commun.,* 2: 8–14

Younis, A., A. Riaz, S. Aslam, M. Ahsan, U. Tariq, F. Javaid, M. Nadeem and M. Hameed, 2013. Effect of different pruning dates on growth and flowering of *Rosa centifolia*. *Pak. J. Agric. Sci.,* 4: 605–609

Younis, A., A. Riaz, M.A. Khan and A.A. Khan, 2009. Effect of time of growing season and time of day for flower harvest on flower yield and essential oil quality and quantity of four *Rosa* cultivars. *Floric. Ornam. Biotechnol.,* 3: 98–103

Zhang, F.Q., Y.S. Wang, Z.P. Lou and J.D. Dong, 2007. Effect of heavy metal stress on antioxidative enzyme and lipid perdoxidat ion in leaves and root of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Chemosphere,* 61: 44–50

Zwieniecki, M.A. and M. Newton, 1995. Roots growing in rock fissures: Their morphological adaptation. *Plant Soil,* 172: 181–187

(Received 10 September 2018; Accepted 31 December 2018)