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Full Length Article

Physiological and Anatomical Response of Fragrant *Rosa* Species with Treated and Untreated Wastewater

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Abstract

The present study assessed the response of physiological and anatomical characteristics of four widely cultivated fragrant *Rosa* species. Water analysis showed that all minerals and chemicals were in permissible level in canal water and treated wastewater, whereas untreated wastewater contained higher EC, biological oxygen demand (BOD), chemical oxygen demand (COD) and heavy metals like Cd, Co, Cu, Pb. There was considerable variations among different *Rosa* species regarding response to wastewater irrigations. Under treated wastewater, *R. bourboniana* showed highest photosynthetic rate, high transpiration rate and maximum chlorophyll contents than other *Rosa* species whereas stomatal conductance of *R. gruss-an-teplitz* was highest under treated wastewater. Leaf anatomical characteristics showed that *R.* Gruss-an-Teplitz under untreated wastewater showed large cortical cell area, vascular bundle area, large spongy cell area and thick midrib while large epidermal thickness of *R. centifolia* was recorded under treated wastewater. Large palisade cell and phloem area and thick leaves (lamina) were found in *R. damascena* under treated wastewater while large metaxylem area of *R. bourboniana* in untreated wastewater. The study showed that treated wastewater was most suitable and desirable irrigation treatment than canal water and untreated wastewater while *R. bourboniana* and *R.* Gruss-an-Teplitz was dominant *Rosa* species regarding physiological characteristics, while all species showed great diversity in leaf tissue architecture under treated and untreated wastewater.

Keywords: Physiology; Anatomy; Municipal wastewater effluent; Roses

Introduction

Rose is one of the most imperative ornamental crops in floricultural industry. It is woody perennial flowering plant which belongs to subfamily Rosoideae and family Rosaceae. Its genus *Rosa* encompasses more than 200 species and 20,000 cultivars, which are distributed globally (Younis *et al.,* 2013). Many of these cultivars and species are cultivated for different sort of purposes and used in various industries e.g. garden and indoor plant, cut flower and making different kind of food stuffs (Nybon, 2009). There are four main fragrant species of roses grown for essential oil production in Pakistan with *Rosa damascena* as top ranked and extensively cultivated in Bulgaria (70–80%), China, Turkey, Russia and India (Nasir *et al.,* 2007). Second one is *Rosa centifolia*, commonly grown in France, Egypt and Morocco. Later, *Rosa bourboniana* and *Rosa* Gruss-an-Teplitz were introduced in France and China respectively (Laurie and Ries, 1950).

Water is the most important need of plants which comprise 50–97% of plant body and also the most poorly managed reserve in the world (Khurana and Singh, 2012). Irrigation is by far the prime user of fresh water (70–90%) in both developed and developing countries (Ensink *et al.,* 2002; Pedrero *et al.,* 2010) with increasing continuously demand (FAOWATER, 2008). Industries and anthropogenic activities are main cause of exaggerated use of water resources to a crisis level (Rusan *et al.,* 2007; Safi *et al.,* 2007).

The use of wastewater for irrigation especially to woody plants and fruit vegetables exerts several anatomical (Ogunkune *et al.,* 2013) and physiological modifications (Sun and Wang, 2005). Singh and Agrawal (2010) reported that photosynthetic rate, stomatal conductance and transpiration rate was high in wastewater irrigated plants than canal water irrigated ones. Petousi *et al.* (2013) observed that high pH of wastewater does not impose negative effects on carnation physiological characteristics. The supply of wastewater improves pigment concentration in leaves with clear increment in chlorophyll content in different plant species which ultimately resulted in increase of photosynthesis rate in plant leaves (Herteman *et al.,* 2011). Due to heavy metals toxicity, stomatal size, epidermal size (Noman *et al.,* 2012),

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xylem and phloem cell size (Mahmood *et al.,* 2005) are altered, while Ogunkune *et al.* (2013) found that wastewater posed severe hazardous impacts on vascular bundle areas of roots and stems of *Amaranthus hybridus* in the form of decreased vascular bundle area. Aldesuquy (2014) showed significant increment in lamina thickness, metaxylem vessel area, xylem area, vascular bundle area and number of closed stomata on both upper and lower epidermis occur under wastewater treatment in wheat plants.

There is no literature available about physiological and anatomical characteristics of *Rosa* species under municipal wastewater irrigation. The present study was therefore carried out to investigate the physio-anatomical attributes of four widely cultivated fragrant *Rosa* species of Pakistan under untreated and treated municipal wastewater irrigation in peri-urban areas.

Materials and Methods

Experimental Site

The experiment was carried out at the Agronomy Research Area of University of Agriculture, Faisalabad (31˚25' N, 73˚09' E and altitude of 300 m above mean sea level) Pakistan, from first week of January, 2012 to January, 2014. This experimental region had a semi-arid climate with less rainfall annually. Soil of this experimental area is clay loam which collects sewage wastewater from the students living hostels of agriculture university and canal water from main canal of the city.

Water Treatment and Analysis

In this experiment, untreated wastewater was treated by natural purification process as discussed by Kiziloglu *et al.* (2008) to improve its physical and chemical quality using conventional method (Pescod, 1992). Water was treated in three large plastic tanks of 1500 gallons water storage capacity in three step process. First tank was filled by sewage wastewater through a pipe and opening of the pipe covered with 6 mm opening sieve as preliminary treatment to block the way of grits and removal of course solid and other materials often found in wastewater (Pescod, 1992). The upper portion of the tanks was kept to remain open for sun light penetration in wastewater as natural treatment process. These three tanks were connected with each other through plastic pipes and adjusted with valves to transfer water from one tank to another. Wastewater in first tank was kept for five days for the purpose of removal of settle able organic and inorganic solids by sedimentation and removal of floating materials by skimming. This water was shifted to second tank placed 2.5' away and 2' below than the first tank, water stayed there for next five days for further sedimentation of smaller particles. Then water was shifted to third tank (which was placed 2.5' away and 2' below than the second tank) and kept there for next 5 days. This water treatment process was completed in 15 days and water in third tank obtained after 15 days of treatment was applied to plants as treated wastewater treatment.

Physico-chemical properties of all irrigation water types were determined by standard methods of wastewater examination proposed by Eaton *et al.* (2005) and all heavy metals and some nutrients like P, K, Na and Ca concentration was determined with the help of inductively couples plasma (ICP-OES) (Optima 2100-DV Perkin Elmer) at Nuclear Institute of Agriculture and Biology (NIAB) Faisalabad.

Soil Analysis

Sixteen soil samples were randomly collected at the depth of 15 and 30 cm into the soil before the start of experiment. Composite soil samples of experimental sites were analyzed according to standard procedures (Table 1).

Experimental Treatments and Measurement of Physiological Anatomical Characteristics of Plants

There were two treatment factors in this experiment i.e., irrigation water and *Rosa* species. Two years old cuttings of four fragrant *Rosa* species like *Rosa centifolia, R. damascena, R. bourboniana* and *R.* Gruss-an-Teplitz were planted during first week of January 2012 and irrigated by canal water, treated and untreated wastewater. Treatments were set according to randomized complete block design (RCBD) with three replications. Data regarding physiological attributes including chlorophyll contents was estimated with the help of chlorophyll meter (CCM-200 plus) after its calibration and average was computed while photosynthetic rate, stomatal conductance and transpiration rate of five randomly selected leaves within each treatment were measured with the help of infra-red gas analyzer (IRGA) (LCi-SD, ADC-Bioscintific UK). For anatomical studies, one cm piece from the leaf center along the midrib was taken. The material was preserved in FAA (Formalin Acetic Acid) solution for fixation, which contained formalin 50%, ethyl alcohol 50%, acetic acid 10% and distilled water 35%. The material was transferred in acetic acid solution (one part acetic acid and three parts ethyl alcohol) for long term preservation. Samples were prepared and fixed in Formalin-Acetic acid-Alcohol (FAA). Dehydration, sectioning staining and mounting procedures was followed according to method described by Sass (1951). Sections were measured by the aid of light microscope. Measurement of each anatomical parameter was calculated in twelve sections in keel region in µm.

Statistical Analysis

Data collected for all parameters were analyzed by performing Fisher`s analysis of variance technique (ANOVA) using Statistica soft 5.5 and treatment means were compared according to least significant difference test (LSD) at 5% level of probability (Steel *et al.,* 1997).

Table 1: Composition of soil before experiment

Soil characteristics	Texture	pH	EC	OM (%)	N (%)	P (ppm)	K (ppm)	Pb (ppm)	Cd (ppm)	Ni (ppm)	Zn (ppm)	Cu (ppm)
00–15 cm	Clay loam soil	8.20	2.54	1.12	0.041	10.50	194	3.16	0.04	0.36	5.28	3.04
16–30 cm		8.20	2.49	1.18	0.041	9.50	134	3.32	0.05	0.34	3.60	2.30
IASS		4-8.5	4.00	>0.86	---	>7	>80	500	1.0	20	250	100

EC= Electrical conductivity, OM= Organic matter

*IASS=International Agricultural Soil Standards, Source: Alloway (1990)

Results

Before experiment, canal water, treated and untreated wastewater was physically and chemically analyzed. Water analysis showed that EC of untreated wastewater was above the standard limit of international irrigation water quality standards (IIWQS) and national environmental quality standards (NEQS) for municipal wastewaters of Pakistan. Untreated wastewater also contained higher biological oxygen demand (BOD), chemical oxygen demand (COD), heavy metals (Cd, Pb, Co, Cu), sodium and nitrogen while treated wastewater and canal water contained all physical and chemical values within permissible limits (Table 2).

Physiological Characteristics of *Rosa* Species

Photosynthetic rate (μ mol m^{-2} S^{-1}): Maximum photosynthetic rate was recorded in *R. bourboniana* followed by *R. centifolia* under treated wastewater treatment while minimum in *R. damascena* under canal water treatment. Mean values showed that *R. bourboniana* gained highest photosynthetic rate among *Rosa* species and treated wastewater attained highest value regarding irrigation water treatments in 2012. During 2013, *R. bourboniana* had highest photosynthetic rate followed by *R.* Gruzz-an-Teplitz under treated wastewater and minimum of it was found in *R. centifolia* under canal water treatment. Mean values showed that *R. bourboniana* and treated wastewater was at the top regarding *Rosa* species and irrigation water treatment respectively (Table 3).

Transpiration rate (mmol m^{-2} S^{-1}): Higher transpiration rate was recorded in *R. damascena* under treated wastewater treatment followed by *R. bourboniana* and *R. centifolia* under same irrigation treatment respectively during 2012 while minimum in *R. damascena* under canal water treatment. Mean values regarding *Rosa* species treatment showed that *R. bourboniana* and *R. centifolia* had highest and lowest transpiration rate, respectively while treated wastewater had maximum of it under irrigation water treatment. During 2013, maximum transpiration rate was recorded in *R. bourboniana* while minimum in *R. centifolia* under canal water treatment. Mean values revealed that treated wastewater showed maximum value and canal water showed minimum regarding irrigation water treatment and *R. bourboniana* attained highest transpiration rate in case of *Rosa* species treatment (Table 3).

Stomatal conductance (mmol m^{-2} S^{-1}): *Rosa* Gruss-an-Teplitz had maximum stomatal conductance under treated wastewater followed by *R. centifolia* under same irrigation water treatment while minimum stomatal conductance was recorded in *R. centifolia* under canal water treatment during 2012. Mean values showed that *R.* Gruss-an-Teplitz and treated wastewater treatment showed high values in *Rosa* species and irrigation wastewater treatments respectively. In 2013, high stomatal conductance was recorded in *R.* Gruss-an-Teplitz under treated wastewater treatment, while minimum in *R. centifolia* under canal water treatment. Mean values showed that treated wastewater and *R.* Gruss-an-Teplitz showed maximum values under irrigation water and *Rosa* species treatment respectively (Table 3).

Chlorophyll contents (mg g^{-1}): *Rosa bourboniana* under untreated wastewater contained highest chlorophyll contents while minimum in *R. damascena* under canal water treatment in 2012. Mean values showed that *R. bourboniana* in *Rosa* species with irrigation of canal water contained maximum chlorophyll contents. During 2013, high value was recorded in *R. bourboniana* under treated wastewater treatment followed by *R.* Gruss-an-Teplitz in same irrigation treatment while minimum in *R. damascena* under canal water treatment. Mean values showed similar trend of highest chlorophyll contents in *R. bourboniana* and lowest in *R. damascena* in *Rosa* species and treated wastewater contained maximum value under irrigation water treatments (Table 3).

Anatomical Characteristics of *Rosa* Species

Cortical cell area (µm^2): *Rosa* Gruss-an-Teplitz was dominated in all irrigation water treatments as compared to other *Rosa* species during both years of experiment (Fig. 1). In 2012, maximum cortical cell area was recorded in untreated wastewater, treated wastewater and canal water treatment with values of 851.75 µm^2, 815.96 µm^2 and 728.64 µm^2 respectively. Cortical cell area (µm^2) of *R. bourboniana* and *R. centifolia* was in medium range and minimum value was recorded in *R. damascena* (373.05 µm^2) in canal water treatment. During 2013, *R.* Gruss-an-Teplitz (721.72 µm^2, 620.13 µm^2 and 618.79 µm^2) in treated wastewater, canal water and untreated wastewater treatment had maximum values, respectively while *R. damascena* (425.75 µm^2) in canal water treatment exhibited minimum cortical cell area.

Epidermal thickness (µm): Results showed that all *Rosa* species showed significant variations but *R. centifolia* was dominant in all irrigation water treatments during 2012

Table 2: Composition of canal water, treated and untreated wastewater utilized in the experiment

Parameters	Canal Water	Treated Water	Untreated Water	IIWQS/NEQS**
EC (µS/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	0.01††
Nickel (mg/L)	0.10	0.08	0.12	0.2††
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	5.0††
Cobalt (mg/L)	0.17	0.029	0.079	0.05†
Copper (mg/L)	0.05	0.13	0.24	0.2††
Chromium (mg/L)	0.04	0.067	0.093	0.1††
Calcium (mg/L)	28.1	39.72	54.29	230††
Sodium (mg/L)	36.47	178.23	252.77	230††
Magnesium (mg/L)	30	47	63	100††
Phosphorus (mg/L)	0.39	1.76	2.49	15†
Total Nitrogen (mg/L)	4	5.72	8.0	5.0†

**IIWQS: International Irrigation Water Quality Standards; NEQS: National Environmental Quality Standards for municipal wastewater of Pakistan; †: Standard value of NEQS; ††: Standard value of IIWQS; 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: Anon. (2007); WHO (1989)

Table 3: Physiological characteristics of *Rosa* species under different irrigations

Rosa species	2012				2013			
	Photosynthetic rate							
	CW	TW	UTW	Mean	CW	TW	UTW	Mean
R.B.	8.62±0.4^{cdef}	11.38±0.5^{a}	10.15±0.8^{abc}	10.05^{a}	8.15±0.5^{bc}	10.54±0.5^{a}	9.72±0.6^{ab}	9.47^{a}
R.C	7.02±0.7^{ef}	10.74±0.7^{ab}	8.95±0.9^{bcde}	8.90^{bc}	6.48±0.6^{d}	10.23±0.6^{a}	8.36±0.6^{bc}	8.36^{bc}
G.T	8.44±0.7^{cdef}	10.35±0.8^{abc}	8.86±0.7^{bcde}	9.22^{ab}	8.21±0.6^{bc}	10.43±0.4^{a}	8.23±0.5^{bc}	8.96^{ab}
R.D	6.77±0.4^{f}	9.44±0.4^{abcd}	7.94±0.5^{def}	8.05^{c}	6.56±0.6^{d}	9.62±0.3^{ab}	7.35±0.6^{cd}	7.84^{c}
Average	7.71^{c}	10.47^{a}	8.97^{b}		7.35^{c}	10.20^{a}	8.41^{b}	
	Transpiration rate							
R.B.	0.788±0.04^{bc}	0.923±0.04^{ab}	0.81±0.04^{abc}	0.84^{a}	0.72±0.05^{d}	0.901±0.04^{a}	0.774±0.02^{abcd}	0.80^{a}
R.C	0.657±0.03^{c}	0.885±0.07^{ab}	0.761±0.04^{bc}	0.77^{a}	0.561±0.06^{e}	0.85±0.07^{abc}	0.742±0.03^{cd}	0.72^{b}
G.T	0.762±0.11^{bc}	0.84±0.04^{ab}	0.802±0.08^{abc}	0.80^{a}	0.713±0.07^{d}	0.799±0.04^{abcd}	0.756±0.04^{bcd}	0.76^{ab}
R.D	0.645±0.02^{c}	0.967±0.07^{a}	0.784±0.05^{bc}	0.79^{a}	0.571±0.06^{e}	0.883±0.04^{ab}	0.726±0.05^{cd}	0.73^{ab}
Average	0.713^{b}	0.904^{a}	0.789^{b}		0.641^{c}	0.858^{a}	0.749^{b}	
	Stomatal conductance							
R.B.	88.78±4.5^{abc}	92.22±4.4^{ab}	90.5±4.5^{abc}	90.50^{a}	85.12±5.7^{cde}	87.98±3.7^{bcde}	86.63±3.3^{bcde}	86.58^{ab}
R.C	76.74±3.7^{c}	101.61±4.9^{a}	86.63±3.8^{bc}	88.33^{a}	66.91±3.5^{f}	97.12±3.0^{ab}	77.3±5.4^{ef}	80.44^{b}
G.T	88.46±5.3^{abc}	102.67±6.1^{a}	96.37±4.5^{ab}	95.83^{a}	81.38±4.9^{de}	102.99±3.7^{a}	92.31±3.1^{abcd}	92.23^{a}
R.D	86.84±7.2^{bc}	99.54±5.2^{ab}	93.49±5.1^{ab}	93.29^{a}	77.71±3.7^{ef}	96.17±5.9^{abc}	85.93±4.9^{bcde}	86.60^{ab}
Average	85.20^{b}	99.01^{a}	91.74^{ab}		77.78^{c}	96.06^{a}	85.54^{b}	
	Chlorophyll contents							
R.B.	34.45±3.0^{abc}	37.23±3.8^{ab}	37.56±3.3^{a}	36.41^{a}	33.61±1.7^{abc}	37.53±2.6^{a}	34.53±2.8^{abc}	35.22^{a}
R.C	29.51±2.5^{bcd}	35.81±3.1^{abc}	32.6±1.6^{abc}	32.64^{ab}	28.3±2.4^{cd}	34.2±1.7^{abc}	33.16±2.4^{abc}	31.89^{ab}
G.T	29.23±2.2^{cd}	34.86±4.1^{abc}	31.1±2.9^{abcd}	31.73^{b}	31.21±1.7^{abcd}	36.1±2.4^{ab}	32.26±2.1^{abcd}	33.19^{a}
R.D	24.76±1.5^{d}	31.11±4.1^{abcd}	29.96±2.8^{abcd}	28.61^{b}	26.75±1.4^{d}	28.96±2.2^{cd}	29.85±2.6^{bcd}	28.52^{b}
Average	29.49^{b}	34.75^{a}	32.80^{ab}		29.96^{b}	34.19^{a}	32.45^{ab}	

- Treatments sharing similar statistical letters are significantly not different from each other
- CW= Canal water; TW= Treated wastewater; UTW= Untreated wastewater
- RB = *R. bourboniana*; RC= *R. centifolia*; GT= Gruss-an-Teplitz; RD= *R. damascena*

and maximum epidermal thickness was recorded under treated wastewater treatment (359.47 µm) followed by untreated wastewater with value of 309.47 µm and minimum in *R. bourboniana* (100.52 µm) in untreated wastewater treatment. During 2013, *R. centifolia* (334.18 µm) in treated wastewater was at the top followed by same *Rosa* species in canal water and untreated wastewater treatments with values of 332.31 µm and 317.03 µm respectively while minimum epidermal thickness was recorded in *R. bourboniana* (117.73 µm) in untreated wastewater treatment (Fig. 1).

Midrib thickness (µm): Results showed that *R.* Gruss-an-Teplitz dominated in all irrigation water treatments than other *Rosa* species (Fig. 1). Maximum midrib thickness during both years was found in *R.* Gruss-an-Teplitz (737.01 µm and 823.61 µm) in untreated wastewater followed by same species (656.08 µm and 702.98 µm) in treated wastewater and *R. centifolia* (570.90 µm and 644.68 µm) in untreated wastewater treatment while minimum value was recorded in *R. bourboniana* (398.47 µm and 431.63 µm) in canal water treatment. All species during 2013 showed comparatively larger midrib thickness than 2012 in all irrigation treatments.

Vascular bundle area (µm^2): *Rosa* Gruss-an-Teplitz contained highest vascular bundle area than other species and maximum value was recorded under untreated wastewater (64924.9 µm^2) followed by same *Rosa* species in treated wastewater (57953.6 µm^2) and canal water (50285.7 µm^2) during 2012, while minimum of it in *R. bourboniana* (15882.6 µm^2) in canal water treatment. During 2013, maximum vascular bundle area was recorded in *R.* Gruss-an-Teplitz (60638.8 µm^2) in treated wastewater treatment followed by same *Rosa* species (57207.9 µm^2) in untreated wastewater treatment while minimum in *R. bourboniana* (21016.3 µm^2) in canal water treatment. Cortical cell area of *R. centifolia* and *R. bourboniana* was in medium range between *R.* Gruss-an-Teplitz and *R. bourboniana* during both years (Fig. 2).

Metaxylem area (µm^2): There was significant variation in metaxylem area in *Rosa* species and *R. bourboniana* was dominant in all irrigation treatments during both years (Fig. 2). Maximum metaxylem area was recorded in untreated wastewater treatment (435.53 µm^2 and 379.8 µm^2) followed by treated wastewater (399.27 µm^2 and 366.05 µm^2) during 2012 and 2013, respectively while minimum in *R. damascena* (85.80 µm^2 and 78.44 µm^2) for untreated

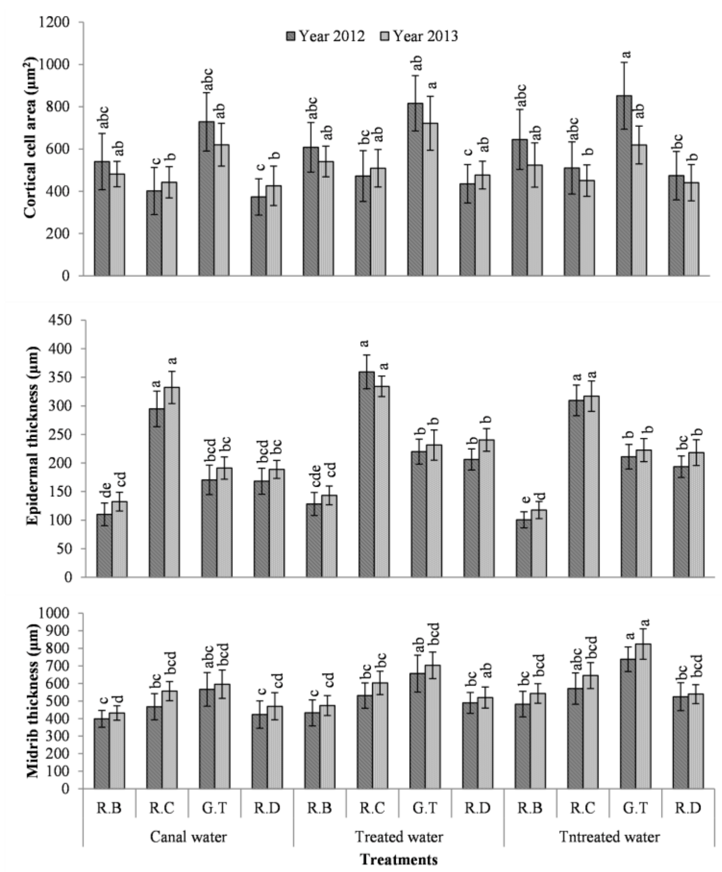

Fig. 1: Leaf anatomical characteristics (cortex, epidermis, midrib thickness) of *Rosa* species under different irrigations
Note: RB= *R. bourboniana*; RC= *R. centifolia*; GT= Gruss-an-Teplitz; RD= *R. damascena*

wastewater treatment. Metaxylem area of *R.* Gruss-an-Teplitz and *R. centifolia* was in medium range between *R. bourboniana* and *R. damascena* in all irrigation treatments during both years of experiment.

Phloem area (μm^2): Results showed that *R. damascena* (236.67 μm^2) in treated wastewater had maximum phloem area followed by *R.* Gruss-an-Teplitz (225.48 μm^2) in same irrigation water treatment during 2012 while minimum value was recorded in *R. bourboniana* (109.62 μm^2) in untreated wastewater. During 2013, *R.* Gruss-an-Teplitz (238.44 μm^2) in treated wastewater obtained highest phloem area followed by *R. damascena* in treated wastewater with value of 223.47 μm^2 while minimum phloem area was recorded in *R. bourboniana* (129.84 μm^2) in untreated wastewater treatment (Fig. 3).

Palisade cell area (μm^2): Results showed that during 2012, *R. damascena* (495.41 μm^2) in treated wastewater obtained maximum palisade cell area followed by *R. centifolia* (409.44 μm^2) and *R. damascena* (406.08 μm^2) in treated wastewater and canal water treatment respectively while minimum of it was found in *R. bourboniana* (136.11 μm^2) for untreated wastewater. During 2013, *R. damascena* (492.2 μm^2) in treated wastewater treatment again showed maximum palisade cell area while minimum value was recorded in *R. bourboniana* (139.68 μm^2) in untreated wastewater (Fig. 3).

Spongy cell area (μm^2): Results showed that *R.* Gruss-an-Teplitz was at the top during both years as compared to other *Rosa* species in all irrigation waters. Maximum spongy cell area was recorded in untreated wastewater (459.44 μm^2) followed by treated wastewater and canal water treatment with values of 386.8 μm^2 and 341.89 μm^2 respectively while minimum of it in *R. bourboniana* (119.44 μm^2) under canal water treatment. During 2013, *R.* Gruss-an-Teplitz in treated wastewater (385.92 μm^2) had maximum spongy cell area followed by same species (355.68 μm^2) in canal water while minimum value was recorded in leaves of *R. bourboniana* (153.68 μm^2) in canal water (Fig. 3).

Lamina thickness (μm): Data showed that *R. damascena* (193.28 μm) in treated wastewater was at the top followed by same *Rosa* species in canal water and untreated wastewater with leaf thickness values of 177.31 μm and 171.01 μm respectively during 2012, while minimum of it was recorded in *R. bourboniana* (104.16 μm) in untreated wastewater. Results during 2013 illustrated that

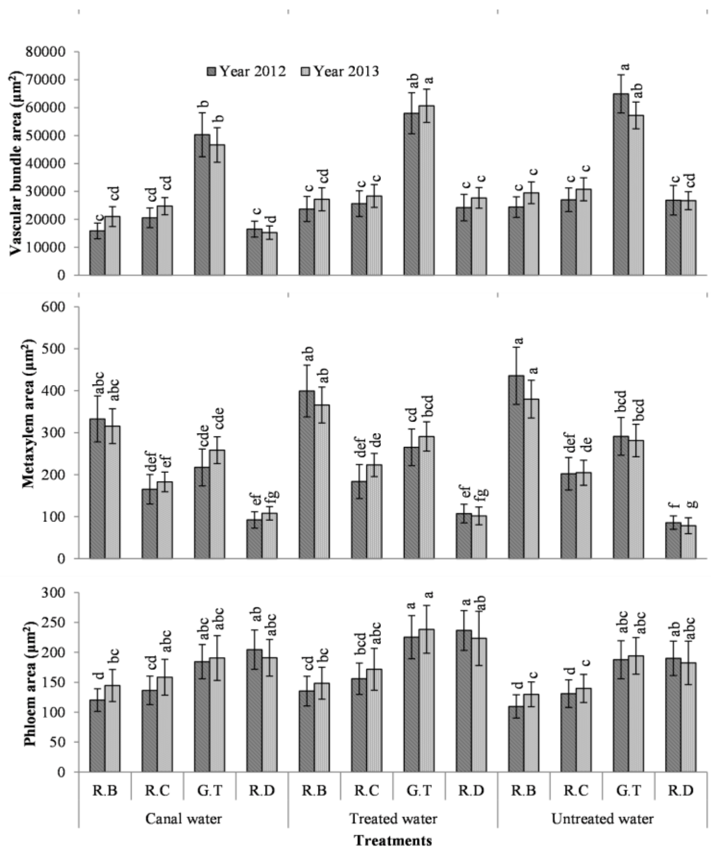

Fig. 2: Leaf anatomical characteristics (vascular bundle, xylem, phloem) of *Rosa* species under different irrigations
Note: RB= *R. bourboniana*; RC= *R. centifolia*; GT= Gruss-an-Teplitz; RD= *R. damascena*

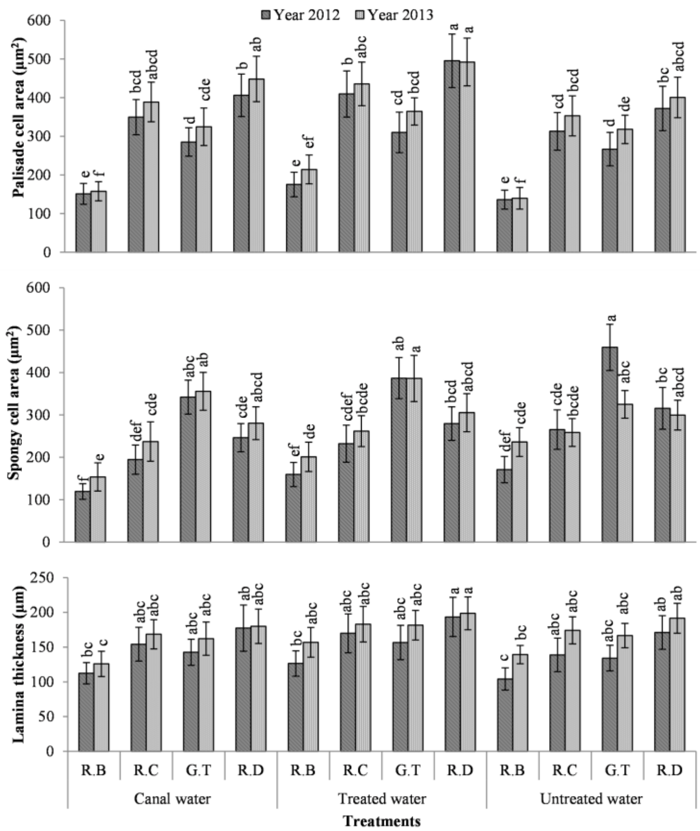

Fig. 3: Leaf anatomical characteristics (palisade, spongy, lamina thickness) of *Rosa* species under different irrigations
Note: RB= *Rosa bourboniana*; RC= *Rosa centifolia*; GT= Gruss-an-Teplitz; RD= *Rosa damascena*

R. damascena (198.56 µm) in treated wastewater was at the top followed by same species (191.42 µm) and *R. centifolia* (183.00 µm) in untreated wastewater and treated wastewater treatment respectively whereas minimum of it was recorded in *R. bourboniana* (125.88 µm) under canal water. All species during 2013 showed comparatively higher lamina thickness than 2012 (Fig. 3). Transverse sections of leaf anatomical characteristics of fragrant *Rosa* species under canal water, treated and untreated wastewaters were presented in Fig. 4.

Discussion

Water used in this experiment was basic in nature as its pH was more than 7 and EC of untreated wastewater was more than standard values set by IIWQS and NEQS for municipal wastewaters of Pakistan while all other minerals and chemicals in treated wastewater and canal water treatment were in permissible range. Untreated wastewater contained high concentration of some minerals and toxic heavy metals (Cd, Pb, Co, Cu) and for this reason its BOD and COD were high (Kakar *et al.,* 2011). Plants were silent sufferers and their response against untreated wastewater reduces growth and physiological disturbance as compared to canal water and treated wastewater treatments.

All physiological parameters considered in this experiment increased under treated wastewater treatment due to optimum concentration of nutrients in treated water as compared to canal water and untreated wastewater treatment. Due to higher concentrations of EC, BOD, COD, Pb, Cd, Co and Na in untreated wastewater, photosynthetic rate, transpiration rate and stomatal conductance were reduced in all *Rosa* species during both years while chlorophyll contents slightly increased in *R. bourboniana* and *R.* Gruss-an-Teplitz during second year of experiment. Cornic (2000) and Flexas and Medrano (2002) reported that due to chemical stress, stomatal closure takes place which limits photosynthesis. Stomatal limitation reduces photosynthetic rate which ultimately results in inhibition of metabolism in plants (Cornic, 2000). Copper and nickel are essential metals for higher plants particularly for physiological processes i.e., photosynthesis (Chatterjee *et al.,* 2006; Mahmood and Islam, 2006) and its toxicity leads to distance in metabolic pathways and damage to

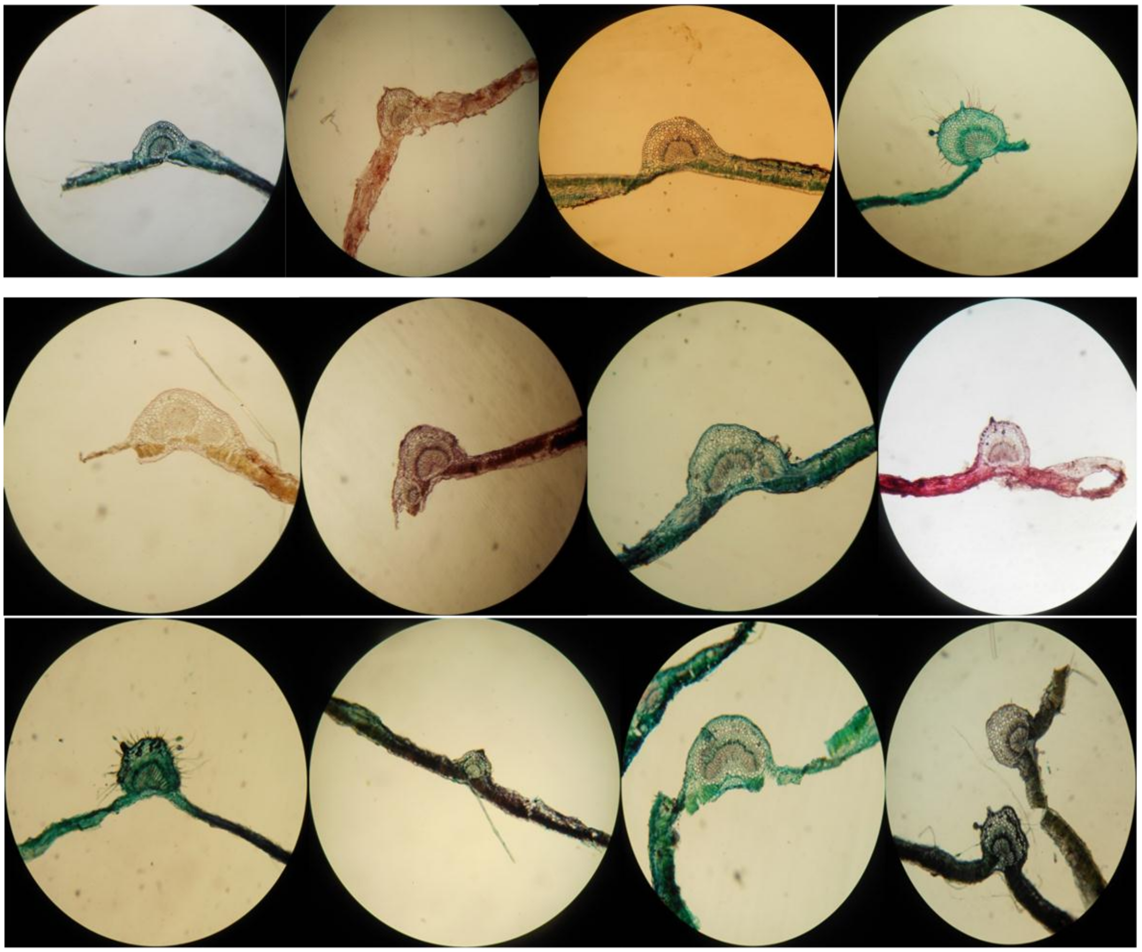

Fig. 4: Transverse sections of leaf anatomical characteristics of *Rosa* species under different irrigation treatments
Note: Row 1= Canal water; Row 2= Treated wastewater; Row 3= Untreated wastewater
Left to right: *R. bourboniana, R. centifolia, R.* Gruss-an-Teplitz, *R. damascena*

macromolecules (Neelima and Reddy, 2002; Singh and Tiwari, 2003; Demirevska-kapova *et al.,* 2004). Kannaiyan (2001) and Saravanamoorthy and Kumari (2007) reported that optimum mineral nutrient status resulted in higher concentrations of chlorophyll in plants and elevated metals and salts concentration in irrigation water reduces the chlorophyll contents in *Rosa* species (Niu *et al*., 2008).

Photosynthesis inhibition in landscape plants was also reported by Azza *et al.* (2007) and Munns and Tester (2008) due to high salt and metal concentration. Singh and Agrawal (2010) found that in *Beta vulgaris*, photosynthetic rate and stomatal conductance were higher in wastewater irrigated sites as compared to ground water irrigated ones and concluded that these positive response of physiological characteristics of the plants at wastewater irrigated site suggest that the concentration of toxic metals may not be up to the extent causing adverse effects on photosynthetic apparatus. Khaleel *et al.* (2013) confirmed the results of this experiment and argued that treated wastewater treatment elevated the chlorophyll contents in *Abelmoschus esculentus*. Enhancement of chlorophyll could be due to enhanced nutrient uptake, synthesis and translocation probably facilitated by optimum availability of some of the beneficiary plant nutrients and also due to reduction in phenol compounds due to the dilution effect. Results of this study are also similar with the findings of Kakar *et al.* (2010) who argued that optimum chemical concentration in irrigation water increased stomatal conductance, transpiration rate and photosynthetic rate while untreated wastewater with high load of contamination strongly reduced the physiological parameters.

Large cortical cell area, epidermal thickness, midrib thickness, vascular bundle area, metaxylem area and spongy cell area in leaves increased in all *Rosa* species as contamination increased in irrigation waters and *R.* Gruss-an-Teplitz produced highest cortical cell area as compared to other species. It could be due to the reason that most of salts and heavy metals in polluted waters were in permissible range to apply for irrigation to plants. Aldesuquy (2014) reported that wastewater increased vascular bundle area and xylem area in leaves of wheat plants. Tyagi *et al.* (2013) reported that anatomical characteristics were reduced as pollution increased in irrigation water treatments. Nawaz *et al.* (2011) showed similar results and argued that *R.* Gruss-an-Teplitz had larger cortical cell area, thick midrib, larger vascular bundles and metaxylem area as compared to other *Rosa* species. Plants shows gradual changes in leaf structure with an upturn in heavy metals concentration (Maruthi *et al.*, 2005; Shanker *et al.*, 2005; Valerie *et al.*, 2006). Optimum concentration of nutrients and heavy metals delayed the entry of metals into the roots by stimulating a lignified exodermis and thereby subsidized to a higher metal tolerance (Cheng *et al.*, 2012).

Conclusion

From this study, it can be concluded that there were substantial variations among *Rosa* species regarding response to wastewater irrigations. Treated wastewater regarding irrigation water and *R.* Gruss-an-Teplitz among *Rosa* species was dominant treatment and plants grown under treated wastewater produced highest values of physiological characteristics while leaf structural features of *Rosa* species showed diversity in their response to treated and untreated wastewater irrigation treatments.

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