

## TREATED AND UNTREATED WASTEWATER IMPARTS MORPHOLOGICAL CHANGES TO SCENTED *ROSA* SPECIES IN PERI-URBAN AREA

Muhammad Ahsan<sup>1,\*</sup>, Adnan Younis<sup>2</sup>, Atif Riaz<sup>2</sup>, Muhammad Jafar Jaskani<sup>2</sup>, Muhammad Qasim<sup>2</sup>, Mansoor Hameed<sup>3</sup>, Aasma Tufail<sup>4</sup>, Muhammad Nafees<sup>1</sup>, Haider Abbas<sup>5</sup> and Usman Tariq<sup>6</sup>

<sup>1</sup>Department of Horticultural Sciences, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan; <sup>2</sup>Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan; <sup>3</sup>Department of Botany, University of Agriculture Faisalabad, Pakistan; <sup>4</sup>Department of Botany, Division of Science and Technology, University of Education Lahore, Pakistan; <sup>5</sup>Department of Agriculture and Agribusiness Management, University of Karachi, Karachi, Pakistan <sup>6</sup>College of Agriculture, Bahadur sub-campus Layyah, Bahaudin Zakariya University, Multan, Pakistan

\*Corresponding author's e-mail: ahsan2065@gmail.com; ahsan.horti@iub.edu.pk

An experiment was conducted to assess the effects of treated and untreated waste water on morphological characteristics of four widely cultivated fragrant *Rosa* species of Pakistan during 2012-2013. Experiment was designed according to randomized complete block design with two factor factorial arrangement. One treatment factor was *Rosa* species and other was irrigation source. All minerals and chemicals present in canal water and treated waste water were in permissible level, whereas untreated waste water contained higher values of EC, biological oxygen demand (BOD), chemical oxygen demand (COD) and heavy metals like Cd, Co, Cu, Pb. Results showed that maximum plant height and number of leaves per branch was recorded in *R. bourboniana* and *R. damascena* respectively under treated waste water and minimum height was found in *R. centifolia* under untreated waste water whereas highest leaf area was recorded in *R. damascena* under canal water and treated waste water during 1<sup>st</sup> and 2<sup>nd</sup> year of experiment respectively. *R. Gruss-an-Teplitz* was dominant species for floral characteristics like minimum days to flower emergence, flower diameter, number of flowers plant<sup>-1</sup> and number of petals flower<sup>-1</sup> under treated waste water whereas thickest flower bud was recorded under untreated waste water. Due to high load of contaminants, untreated waste water was responsible to produced minimum flower numbers in all species. In present study, *R. Gruss-an-Teplitz* was found to be most resistant and suitable rose species to cultivate in peri-urban areas under treated and untreated waste water and it is strongly recommended that untreated waste water must be treated to some extent before application to roses.

**Keywords:** Floral growth, heavy metals, water quality, roses, vegetative characteristics

### INTRODUCTION

It is centuries old practice to use municipal waste water in agriculture, which has lately gained renewed consideration in many areas of the world due to the increasing shortage of irrigation water (Khaleel *et al.*, 2013; Kurian, 2017). Increasing the use of treated and untreated waste water in peri-urban agriculture and even in distant rural areas where its usage originates, increases economic activity and improves the livelihood of poor growers but modifies the quality of environment (Abu Qdais and Al-Widyan, 2016; Murtaza *et al.*, 2010; Talal *et al.*, 2014). The efficient use of municipal sewage water can efficiently increase water resources for irrigation and may prove to be a bonus for agricultural production and for this purpose, reclaimed municipal waste water can be an alternative source of nutrients and water for horticultural and nursery crop production since nutrients are available in a usable form and, in general, do not need any supplementary energy input to make them accessible to plants

(Asgharipour and Azizmoghaddam, 2012; Farahat and Linderholm, 2013).

As agriculture sector in Pakistan is dominated by the farmers of small land holdings and production of conventional crops is no more profitable, therefore the shift from conventional to high value crops and introduction of new alternative crops like flowers cultivation is increasing overtime. In Pakistan, the most important floricultural crops are rose, jasmine, gladiolus, tuberose, carnation, iris, narcissus, lilies and gerbra (Riaz *et al.*, 2008). Among these crops; rose is first ranked high value floricultural crop. It is woody perennial flowering plant of famous plant family Rosaceae. Economically roses are considered as highly viable crop with a share of 65% of total floricultural trade in under developed countries of the world (Janko and Alemu, 2014). There are four important species of roses that are grown for essential oil production that is used in perfumes, medicine, cosmetics and many other products. Top ranked is *R. damascena* which is extensively cultivated in Bulgaria (70-80%), China, Turkey and India while second one is *R. centifolia*, commonly grown in France,

Egypt and Morocco (Nasir *et al.*, 2007). Later come *R. bourboniana* and *R. Gruss-an-Teplitz* which were introduced in France and China respectively (Laurie and Ries, 1950). In peri-urban areas of large cities like Faisalabad, both untreated and treated forms of waste water are used for production of vegetables (Hussain *et al.*, 2006). These vegetables are enriched with heavy metals in waste water that causes human health impairment of humans in peri-urban and urban areas. Ornamental crops can be more appropriate for growing with treated and untreated waste water as they are not consumed and health issues are lesser. Keeping in view the effects of treated and untreated waste water, a field experiment was carried out to determine the morphological characteristics of four scented and oil bearing species of high value floricultural crop of roses under raw sewage untreated and treated waste water in peri-urban area.

## MATERIALS AND METHODS

**Experimental site, soil analysis and experimental treatments:** The experiment was carried out at the Agronomy Research Area of University of Agriculture, Faisalabad (31°25' N, 73°09' E and altitude of 300m above mean sea level) Pakistan from 2<sup>nd</sup> January 2012 to 30<sup>th</sup> December, 2013. Soil of this experimental area is clay loam which collects sewage waste water from the students living hostels of University of Agriculture Faisalabad and canal water from main canal of the city. Before start of experiment, sixteen soil samples were randomly collected at the depth of 15 and 30 cm. Composite soil samples were analyzed according to the standard procedures (U.S. Silinity Laboratory Staff, 1954) and results are presented in Table 1.

There were two treatment factors of this experiment i.e. irrigation water (canal water, treated waste water and untreated waste water) and *Rosa* species (*R. centifolia*, *R. damascena*, *R. bourboniana* and *R. Gruss-an-Teplitz*).

**Water treatment and analysis:** In this experiment, untreated waste water 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) in three large plastic tanks of 1500 gallons water storage capacity, in three step process i.e. primary, secondary and tertiary treatment (Pescod, 1992). Physio-chemical properties of all irrigation water types were determined by standard methods of waste water 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, Pakistan.

**Rosa species and morphological characteristics:** Two years old cuttings of fragrant *Rosa* species were planted during first week of January 2012 and irrigated by canal water, treated and untreated waste water. Data regarding vegetative parameters (plant height, number of leaves per branch, leaf area) and floral characteristics (days to flower emergence, bud and flower diameter, number of flowers per plant, number of petals per flower) were studied.

**Experimental design and statistical analysis:** Experimental treatments were set according to randomized complete block design (RCBD) and there were 15 plants of every single species in each treatment with total of 180 plants in single replication which were repeated thrice. 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 (LSD) test at 5% level of probability (Steel *et al.*, 1997).

## RESULTS

Physio-chemical analysis of canal water, treated and untreated waste water was carried out (Table 2) before the experiment. Data of water analysis showed that EC of untreated waste water was above the standard limit values set by International Irrigation Water Quality Standards and National Environmental Quality Standards for municipal waste waters of Pakistan. Untreated waste water also contained higher BOD, COD, heavy metal (Cd, Pb, Co, Cu), Na and N level while treated waste water and canal water contained all physical and chemical values within permissible limits.

**Effect of treated and untreated wastewaters on vegetative growth parameters:**

**Plant height (cm):** The results showed that treated waste water produced taller rose plants compared to canal water and untreated waste water in 2012 where maximum plant height (148.10cm) was observed in *R. bourboniana* while minimum height was found in *R. damascena* (83.23cm) under untreated waste water. In 2013, *R. bourboniana* (137.20cm) under untreated waste water produced maximum height and minimum height was found in *R. centifolia* (66.80cm) under untreated waste water. It was observed that all *Rosa* species

**Table 1. Soil composition before experiment.**

Soil characteristics	Texture	pH	EC	OM (%)	N (%)	P (ppm)	K (ppm)	Pb (ppm)	Cd (ppm)	Ni (ppm)	Zn (ppm)	Cu (ppm)
00-15cm	Clay	8.20	2.54	1.12	0.041	10.50	194	3.16	0.04	0.36	5.28	3.04
16-30cm	loam soil	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)

**Table 2. Composition of canal water, treated and untreated waste water.**

Parameters	Canal Water	Treated Water	Untreated Water	IIWQS/NEQS**
EC ( $\mu$ S/L)	1.13	1.44	2.11	1.5 <sup>†</sup>
pH	7.42	7.58	8.31	6-9.2 <sup>†</sup>
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 <sup>†</sup>
COD (mg/L)	---	481	669	500 <sup>†</sup>
TDS (mg/L)	218	1281	1678	2500 <sup>†</sup>
SS (mg/L)	0.9	0.15	1.1	--
Total Solids (mg/L)	218	982	1372	--
TSS (mg/L)	24	63	194	400 <sup>†</sup>
Chlorides (mg/L)	138	290	436	1000 <sup>†</sup>
Cadmium (mg/L)	0.001	0.01	0.013	0.01 <sup>††</sup>
Nickel (mg/L)	0.10	0.08	0.12	0.2 <sup>††</sup>
Arsenic (mg/L)	ND	0.004	0.005	0.1 <sup>††</sup>
Zinc (mg/L)	0.18	2.62	3.48	5.0 <sup>††</sup>
Potassium (mg/L)	30.41	17.61	40.73	--
Lead (mg/L)	0.021	0.42	0.66	0.5 <sup>†</sup>
Iron (mg/L)	0.32	3.47	4.82	5.0 <sup>††</sup>
Cobalt (mg/L)	0.17	0.029	0.079	0.05 <sup>†</sup>
Copper (mg/L)	0.05	0.13	0.24	0.2 <sup>††</sup>
Chromium (mg/L)	0.04	0.067	0.093	0.1 <sup>††</sup>
Calcium (mg/L)	28.1	39.72	54.29	230 <sup>††</sup>
Sodium (mg/L)	36.47	178.23	252.77	230 <sup>††</sup>
Magnesium (mg/L)	30	47	63	100 <sup>††</sup>
Phosphorus (mg/L)	0.39	1.76	2.49	15 <sup>†</sup>
Total Nitrogen (mg/L)	4	5.72	8.0	5.0 <sup>†</sup>

\*\*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. Vegetative growth characteristics of *Rosa* species under different irrigation treatments.**

<i>Rosa</i> species	Plant height (cm)							
	2012				2013			
	CW	TW	UTW	Mean	CW	TW	UTW	Mean
R.B.	120.40±8.2 <sup>bc</sup>	148.10±6.4 <sup>a</sup>	135.10±9.8 <sup>ab</sup>	134.53 <sup>a</sup>	131.33±7.3 <sup>a</sup>	132.60±5.1 <sup>a</sup>	137.20±3.3 <sup>a</sup>	133.71 <sup>a</sup>
R.C.	93.63±6.8 <sup>efg</sup>	100.77±3.1 <sup>def</sup>	85.83±4.8 <sup>fg</sup>	93.41 <sup>c</sup>	76.17±2.9 <sup>ef</sup>	98.03±4.7 <sup>cd</sup>	66.80±1.9 <sup>f</sup>	80.33 <sup>d</sup>
G.T.	109.93±3.9 <sup>cd</sup>	113.50±2.8 <sup>cd</sup>	104.23±2.7 <sup>de</sup>	109.22 <sup>b</sup>	112.93±6.8 <sup>b</sup>	106.60±4.5 <sup>bc</sup>	103.93±4.9 <sup>bc</sup>	107.82 <sup>b</sup>
R.D.	100.77±4.7 <sup>def</sup>	114.13±2.3 <sup>cd</sup>	83.27±5.5 <sup>g</sup>	99.39 <sup>c</sup>	84.43±2.8 <sup>de</sup>	101.17±5.1 <sup>bc</sup>	81.37±4.1 <sup>e</sup>	88.99 <sup>c</sup>
Average	106.18 <sup>b</sup>	119.12 <sup>a</sup>	102.11 <sup>b</sup>		101.22 <sup>b</sup>	109.60 <sup>a</sup>	97.33 <sup>b</sup>	
Number of leaves per branch								
R.B.	28.95±1.0 <sup>e</sup>	33.66±0.9 <sup>abc</sup>	32.9±1.8 <sup>bcd</sup>	31.84 <sup>b</sup>	29.42±1.3 <sup>cde</sup>	29.14±1.1 <sup>cde</sup>	30.57±1.1 <sup>bcd</sup>	29.71 <sup>b</sup>
R.C.	29.00±1.1 <sup>e</sup>	29.81±1.4 <sup>de</sup>	29.14±1.2 <sup>e</sup>	29.32 <sup>c</sup>	26.41±1.1 <sup>e</sup>	27.28±0.9 <sup>e</sup>	27.19±1.1 <sup>e</sup>	26.96 <sup>c</sup>
G.T.	27.33±1.1 <sup>e</sup>	30.38±1.1 <sup>cde</sup>	29.04±0.9 <sup>e</sup>	28.92 <sup>c</sup>	27.9±0.9 <sup>de</sup>	32.19±1.6 <sup>abc</sup>	29.42±0.9 <sup>cde</sup>	29.84 <sup>c</sup>
R.D.	32.85±1.0 <sup>bcd</sup>	36.33±1.1 <sup>a</sup>	35.09±1.0 <sup>ab</sup>	34.76 <sup>a</sup>	33.19±1.0 <sup>ab</sup>	33.52±0.7 <sup>ab</sup>	33.95±0.7 <sup>a</sup>	33.55 <sup>a</sup>
Average	29.53 <sup>b</sup>	32.54 <sup>a</sup>	31.54 <sup>a</sup>		29.23 <sup>a</sup>	30.53 <sup>a</sup>	30.28 <sup>a</sup>	
Leaf area (cm <sup>2</sup> )								
R.B.	87.77±5.4 <sup>fg</sup>	83.42±4.3 <sup>g</sup>	92.2±5.2 <sup>efg</sup>	87.80 <sup>c</sup>	86.43±3.8 <sup>gh</sup>	89.33±4.6 <sup>fgh</sup>	83.26±5.2 <sup>h</sup>	86.34 <sup>c</sup>
R.C.	120.52±5.4 <sup>b</sup>	108.16±6.5 <sup>bcd</sup>	101.77±4.3 <sup>def</sup>	110.15 <sup>b</sup>	102.85±2.9 <sup>cde</sup>	115.65±4.7 <sup>abc</sup>	98.93±4.9 <sup>efg</sup>	105.81 <sup>b</sup>
G.T.	103.56±6.7 <sup>cde</sup>	101.93±3.9 <sup>def</sup>	107.61±4.4 <sup>bcd</sup>	104.37 <sup>b</sup>	108.21±3.8 <sup>b-e</sup>	101.49±3.7 <sup>def</sup>	105.68±4.4 <sup>cde</sup>	105.13 <sup>b</sup>
R.D.	135.63±4.3 <sup>a</sup>	117.42±6.8 <sup>bc</sup>	119.43±4.8 <sup>b</sup>	124.16 <sup>a</sup>	120.02±2.8 <sup>ab</sup>	125.22±6.3 <sup>a</sup>	114.8±5.4 <sup>abcd</sup>	120.01 <sup>a</sup>
Average	111.87 <sup>a</sup>	102.73 <sup>b</sup>	105.25 <sup>ab</sup>		104.37 <sup>ab</sup>	107.92 <sup>a</sup>	100.67 <sup>b</sup>	

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

responded positively to treated waste water whereas plant height was reduced in untreated waste water (Table 3).

**Number of leaves per branch:** Maximum leaves branch<sup>-1</sup> was recorded in *R. damascena* (36.33) produced under treated

waste water followed by same *Rosa* species in untreated waste water (35.09) in 2012 while minimum leaves were calculated in *R. Gruss-an-Teplitz* (27.33) under canal water treatment. During 2013, *R. damascena* (33.95) under untreated waste water produced maximum and *R. centifolia* (26.42) produced minimum number of leaves per branch under canal water treatment (Table 3).

**Leaf area (cm<sup>2</sup>):** Data showed that maximum leaf area was found in *R. damascena* (135.63cm<sup>2</sup>) under canal water while *R. bourboniana* under treated waste water treatment produced minimum value (83.42cm<sup>2</sup>) during 2012. Leaf area of *R. centifolia* and *R. Gruss-an-Teplitz* was in medium range than values of *R. damascena* and *R. bourboniana*. In 2013, *R. damascena* (125.22cm<sup>2</sup>) under treated waste water produced highest value while untreated waste water in *R. bourboniana* (83.26cm<sup>2</sup>) produced lowest value of leaf area.

**Effect of treated and untreated waste waters on floral growth characteristics:**

**Days to flower emergence:** The results of days to first flower

appearance are presented (Table 4) which showed that during *R. Gruss-an-Teplitz* in 2012 took 25.42 days to produce first flower under treated waste during 1<sup>st</sup> year of experiment. Maximum days (76.57) were taken by *R. damascena* in treated waste water treatment. During 2<sup>nd</sup> year, *R. Gruss-an-Teplitz* produced flower after 25.28 days under treated waste water that is lowest number of days to produce first flower whereas *R. damascena* (71.95) under canal water treatment took highest number of days to produce first flower during 2013 among counterparts.

**Flower bud diameter (mm):** The highest value of flower bud diameter was measured in *R. Gruss-an-Teplitz* (12.55mm) under untreated waste water while minimum value was recorded in *R. damascena* (9.37mm) under untreated waste water irrigation in 2012. Highest bud diameter values in *R. centifolia* and *R. bourboniana* were 10.87mm and 10.23mm respectively in treated and untreated waste water treatment respectively. In 2013, minimum flower bud diameter was found for *R. damascena* (8.98mm) under untreated waste

**Table 4. Floral characteristics of *Rosa* species under different irrigation treatments.**

Rosa species	Days to flower emergence							
	2012				2013			
	CW	TW	UTW	Mean	CW	TW	UTW	Mean
R.B.	72.57±3.1 <sup>a</sup>	68.81±3.6 <sup>a</sup>	69.76±5.2 <sup>a</sup>	70.38 <sup>a</sup>	71.14±4.2 <sup>a</sup>	68.9±4.5 <sup>a</sup>	65.38±2.8 <sup>a</sup>	68.47 <sup>a</sup>
R.C	38.00±2.9 <sup>bc</sup>	42.52±5.3 <sup>b</sup>	44.85±3.3 <sup>b</sup>	41.79 <sup>b</sup>	46.09±2.8 <sup>b</sup>	40.52±3.1 <sup>b</sup>	46.19±1.6 <sup>b</sup>	44.27 <sup>b</sup>
G.T	27.09±3.6 <sup>cd</sup>	25.42±3.5 <sup>d</sup>	27.52±1.9 <sup>d</sup>	26.68 <sup>b</sup>	27.14±2.5 <sup>c</sup>	25.28±2.9 <sup>c</sup>	31.09±1.7 <sup>c</sup>	27.84 <sup>c</sup>
R.D	75.85±2.9 <sup>a</sup>	76.57±5.3 <sup>a</sup>	73.42±3.6 <sup>a</sup>	75.28 <sup>a</sup>	71.95±3.6 <sup>a</sup>	68.04±3.8 <sup>a</sup>	68.85±3.1 <sup>a</sup>	69.61 <sup>a</sup>
Average	53.38 <sup>a</sup>	53.33 <sup>a</sup>	53.89 <sup>a</sup>		54.08 <sup>a</sup>	52.14 <sup>a</sup>	51.42 <sup>a</sup>	
	Flower bud diameter (mm)							
R.B.	9.79±0.12 <sup>fg</sup>	9.92±0.11 <sup>ef</sup>	10.23±0.11 <sup>def</sup>	9.98 <sup>c</sup>	10.08±0.12 <sup>cd</sup>	9.74±0.09 <sup>de</sup>	10.35±0.14 <sup>c</sup>	10.06 <sup>b</sup>
R.C	10.22±0.10 <sup>def</sup>	10.87±0.16 <sup>c</sup>	10.27±0.13 <sup>de</sup>	10.45 <sup>b</sup>	9.4±0.19 <sup>ef</sup>	11.25±0.13 <sup>b</sup>	10.18±0.13 <sup>cd</sup>	10.28 <sup>b</sup>
G.T	11.79±0.13 <sup>b</sup>	11.85±0.15 <sup>b</sup>	12.55±0.26 <sup>a</sup>	12.06 <sup>a</sup>	11.65±0.15 <sup>b</sup>	12.35±0.16 <sup>a</sup>	12.5±0.24 <sup>a</sup>	12.17 <sup>a</sup>
R.D	10.09±0.10 <sup>def</sup>	10.39±0.16 <sup>d</sup>	9.37±0.12 <sup>g</sup>	9.95 <sup>c</sup>	9.58±0.12 <sup>e</sup>	9.72±0.17 <sup>de</sup>	8.98±0.18 <sup>f</sup>	9.43 <sup>c</sup>
Average	10.47 <sup>b</sup>	10.76 <sup>a</sup>	10.60 <sup>ab</sup>		10.18 <sup>c</sup>	10.76 <sup>a</sup>	10.50 <sup>b</sup>	
	Number of flowers per plant							
R.B.	86.33±5.6 <sup>e</sup>	90.47±4.8 <sup>c</sup>	81.93±5.1 <sup>e</sup>	86.24 <sup>c</sup>	94.56±5.4 <sup>d</sup>	96.89±7.3 <sup>d</sup>	91.22±6.6 <sup>d</sup>	94.22 <sup>c</sup>
R.C	328.4±28.4 <sup>cd</sup>	367.27±25.9 <sup>c</sup>	296.8±18.1 <sup>d</sup>	330.82 <sup>b</sup>	342.44±15.5 <sup>b</sup>	368.56±19.6 <sup>b</sup>	283.56±8.9 <sup>c</sup>	331.52 <sup>b</sup>
G.T	473.73±25.5 <sup>b</sup>	535.67±20.8 <sup>a</sup>	456.07±17.4 <sup>b</sup>	488.49 <sup>a</sup>	508.56±18.9 <sup>a</sup>	561.67±15.1 <sup>a</sup>	523.89±16.0 <sup>a</sup>	531.37 <sup>a</sup>
R.D	87.0±6.4 <sup>e</sup>	97.2±6.2 <sup>e</sup>	84.67±7.1 <sup>e</sup>	89.62 <sup>c</sup>	88.11±8.2 <sup>d</sup>	97.56±8.6 <sup>d</sup>	83.56±5.1 <sup>d</sup>	89.74 <sup>c</sup>
Average	243.86 <sup>b</sup>	272.65 <sup>a</sup>	229.86 <sup>b</sup>		258.42 <sup>ab</sup>	281.17 <sup>a</sup>	245.56 <sup>b</sup>	
	Flower diameter (mm)							
R.B.	45.35±0.9 <sup>cd</sup>	45.10±0.8 <sup>d</sup>	45.97±1.2 <sup>cd</sup>	45.48 <sup>b</sup>	43.94±0.6 <sup>i</sup>	48.40±0.9 <sup>ef</sup>	44.53±0.9 <sup>hi</sup>	45.62 <sup>c</sup>
R.C	53.86±1.1 <sup>b</sup>	45.77±0.7 <sup>cd</sup>	41.38±0.9 <sup>e</sup>	47.01 <sup>b</sup>	51.56±0.9 <sup>c</sup>	46.61±0.5 <sup>fg</sup>	42.64±0.4 <sup>i</sup>	46.94 <sup>b</sup>
G.T	52.40±1.3 <sup>b</sup>	62.76±1.2 <sup>a</sup>	59.96±1.6 <sup>a</sup>	58.37 <sup>a</sup>	50.85±0.8 <sup>cd</sup>	58.48±0.5 <sup>a</sup>	55.47±0.9 <sup>b</sup>	54.93 <sup>a</sup>
R.D	48.39±1.1 <sup>c</sup>	44.45±0.6 <sup>d</sup>	44.22±0.6 <sup>de</sup>	45.69 <sup>b</sup>	49.40±0.6 <sup>de</sup>	46.63±0.5 <sup>fg</sup>	46.13±0.6 <sup>gh</sup>	47.39 <sup>b</sup>
Average	50.00 <sup>a</sup>	49.52 <sup>a</sup>	47.88 <sup>b</sup>		48.94 <sup>ab</sup>	49.27 <sup>a</sup>	47.95 <sup>b</sup>	
	Number of petals per flower							
R.B.	45.18±1.4 <sup>ab</sup>	45.76±1.3 <sup>ab</sup>	42.04±0.7 <sup>d</sup>	44.50 <sup>a</sup>	42.52±1.1 <sup>cd</sup>	45.66±0.7 <sup>a</sup>	44.47±1.1 <sup>ab</sup>	44.11 <sup>b</sup>
R.C	42.42±0.7 <sup>cd</sup>	45.19±1.2 <sup>ab</sup>	40.71±0.4 <sup>d</sup>	42.77 <sup>b</sup>	43.38±0.9 <sup>bcd</sup>	45.57±0.5 <sup>ab</sup>	41.47±0.6 <sup>d</sup>	43.47 <sup>b</sup>
G.T	44.90±0.7 <sup>abc</sup>	46.31±1.0 <sup>a</sup>	45.19±0.5 <sup>ab</sup>	45.28 <sup>a</sup>	44.85±0.3 <sup>ab</sup>	46.28±0.4 <sup>a</sup>	45.81±0.4 <sup>a</sup>	45.65 <sup>a</sup>
R.D	41.52±0.6 <sup>d</sup>	43.04±0.6 <sup>bcd</sup>	42.42±0.4 <sup>cd</sup>	42.33 <sup>b</sup>	41.41±0.5 <sup>d</sup>	42.09±0.8 <sup>d</sup>	42.0±0.9 <sup>d</sup>	41.83 <sup>c</sup>
Average	43.50 <sup>b</sup>	45.06 <sup>a</sup>	42.59 <sup>b</sup>		43.04 <sup>b</sup>	44.90 <sup>a</sup>	43.44 <sup>b</sup>	

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

water treatment whereas maximum value was recorded for *R. Gruss-an-Teplitz* (12.50mm) under untreated waste water (Table 4).

**Number of flowers plant<sup>-1</sup>:** The results revealed that *R. Gruss-an-Teplitz* (535.67) under treated waste water produced highest number of flowers plant<sup>-1</sup> in 2012 whereas *R. bourboniana* (81.93) under untreated waste water produced minimum number of flowers. During 1<sup>st</sup> year of the experiment, *R. centifolia* and *R. damascena* produced 367.27 and 97.20 flowers respectively under treated waste water treatment and these values were at top in respective species among all irrigation treatments. In 2013, *R. Gruss-an-Teplitz* (561.67) produced maximum and *R. damascena* (83.56) produced minimum number of flower plant<sup>-1</sup> year<sup>-1</sup> under treated waste water and untreated waste water treatment respectively. In *R. centifolia*, flower quantity was reduced from 367.27 to 296.80 from treated to untreated waste water respectively while in *R. bourboniana* (96.89) maximum flowers were produced under treated waste water (Table 4).

**Flower diameter (mm):** Data showed that highest value of flower diameter was recorded in *R. Gruss-an-Teplitz* (62.76mm) under treated waste water during 2012 while *R. centifolia* produced minimum value of flower diameter (41.38mm) under untreated waste water. During 2013, *R. Gruss-an-Teplitz* (58.48mm) under treated waste water produced maximum flower diameter whereas *R. centifolia* (42.64mm) in same irrigation treatment obtained minimum value (Table 4).

**Number of petals flower<sup>-1</sup>:** The highest value of flower petals was found in *R. Gruss-an-Teplitz* (46.31) followed by *R. bourboniana* (45.76) under treated waste water treatment during 2012 whereas minimum number of petals (40.71) was recorded in *R. centifolia* under untreated waste water treatment. In 2013, maximum petal numbers was produced in *R. Gruss-an-Teplitz* (46.28) under treated waste water treatment while minimum values were recorded in *R. damascena* (41.41) under treated waste water treatment (Table 4).

## DISCUSSION

Water used in this experiment was basic in nature as its pH was more than 7 and EC of untreated waste water was more than standard values set by international irrigation water quality standards (IIWQS) and national environmental quality standards (NEQS) for municipal waste waters of Pakistan. All other minerals and chemicals under treated waste water and canal water treatment were in permissible range. Untreated waste water contained higher concentration of some toxic heavy metals (i.e. Cd, Pb, Co, Cu) and for this reason its BOD and COD were high (Kakar *et al.*, 2011). Plants were silent sufferers, so their response against untreated waste water was reduced growth and lesser flower yield as compare to canal water and treated waste water treatments.

Fragrant *Rosa* species differed significantly for plant height in waste water irrigation. *R. bourboniana* seems to be most resistant species to pollutants in irrigation water as compare to others *Rosa* species and *R. centifolia* was least resistant. In all *Rosa* species, maximum height was recorded in canal water treatment which showed that waste water treatment has negative effect on plant height but in *R. bourboniana*, height was less affected under treated and untreated waste water. Effects of toxicity in irrigation water differ greatly from plant to plant but in some medicinal plants, toxicity in irrigation water does not alters/reduced the height (Bernstein *et al.*, 2009). These results verified the findings of Younis (2006) who also reported that *R. bourboniana* produced maximum height than other oil bearing *Rosa* species in Pakistan. Results of this experiment were also in line with the findings of Sridhar *et al.* (2005) as increased in toxicity in irrigation water plant height was reduced depending upon plant species. Andleeb *et al.* (2008) also verified results of this study and stated that increase in metal concentration (especially Cr) tends to decrease plant height in sunflower.

Singh and Agrawal (2010) and Sinha *et al.* (2007) observed significantly higher number of leaves in plants, irrigated by municipal waste water as compare to canal water irrigated plants. Results of present study also showed that there was more number of leaves under treated and untreated waste water treatment in all *Rosa* species. Findings of Aldesuquy (2014) supported the results of this study who argued that as load of heavy metals increases in irrigation water, leaf area in plants reduced but this character depends on plant type. Pandey and Tripathi (2011) also reported that concentration of heavy metals adversely affect the leaf area. Singh and Agrawal (2010) showed positive effects of waste water regarding leaf area and Rusan *et al.* (2008) reported that *R. indica* and *R. canina* showed highest leaf area in treated waste water treatment as compare to canal/fresh water.

It is the specific character of different *Rosa* species to produce flowers during different months of the year as *R. centifolia* and *R. Gruss-an-Teplitz* produced flowering during whole year while *R. bourboniana* and *R. damascena* produced flowers only in the months of March and April (Younis, 2006). In this study floral bud and flower diameter of *R. bourboniana* and *R. Gruss-an-Teplitz* were higher under treated and untreated waste water treatment as compare to canal water irrigation while this trend was opposite in case of *R. centifolia* and *R. damascena*. These results were comparable with the results of Rusan *et al.* (2008) and Marinho *et al.* (2013) who argued that due to availability of nutrients in treated and untreated waste water, flower buds and flower size of *Rosa* species responded positively and their diameter was higher as compare to flowers of fresh water treatment. These findings were contradictory to the results of Bernstein *et al.* (2009) who showed no effects of waste water on morphological attributes i.e. plant height, leaf area, flower bud diameter, flower size etc. in aromatic plant species. The

increase in flower numbers per plant of *R. Gruss-an-Teplitz* in this study under waste water treatments over canal water could also be credited to the presence of high organic matter in waste water that can improve soil structure and availability of nutrients (Brady and Weil, 2008). The production limitations of *Rosa* species other than *R. Gruss-an-Teplitz* under waste water treatments might be instigated by higher EC of polluted irrigation treatments. Similar effects of high EC waters have been mentioned by different authors for olive (Ben Ahmed *et al.*, 2008; Melgar *et al.*, 2008). These results were contradictory with the results of Friedman *et al.* (2007) who reported that waste water has no effect on flower number. Darvishi *et al.* (2010) found that there was an increment in number of flowers per plant by the application of domestically treated waste water.

In this study, there was not any remarkable difference among *Rosa* species for number of petals per flowers but some petals were produced in *R. centifolia* and *R. damascena* that were small in size and malformed in shape and were not considered as petals under treated and untreated waste water treatment. These findings were according to the results of Nirit *et al.* (2006) who found no overall effect on flower development and shape but these results were contradictory with the findings of Khan *et al.* (2011) who showed increment in rose flower petal numbers in waste water irrigation treatment.

**Conclusion:** From this study it was concluded that under treated waste water, all morphological attributes of *Rosa* species showed the maximum response and most of the characteristics were improved significantly. The increase in the floral characteristics was highly prominent in *R. Gruss-an-Teplitz* such as minimum days to produce flowers, maximum bud and flower diameter, number of flowers per plant and number of petals per flower whereas, plant height and number of leaves were maximum in *R. bourboniana* but leaf area recorded in *R. damascena* was highly increased. In contrast to treated waste water, untreated waste water, reduced the values of all species where most of the characteristics showed decreasing trend. The overall impact of high concentration of metals and other contaminants was more severe in *R. centifolia* whereas *R. Gruss-an-Teplitz* was least affected and best performed species under untreated waste water.

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## REFERENCES

Abu Qdais, H. and M. Al-Widyan. 2016. Evaluating composting and cocomposting kinetics of various agro-industrial wastes. *Int. J. Recycle Org. Waste Agri.* 5:273-280.

- 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. Blackie Glasgow and London. John Wiley & Sons. Inc. New York.
- Andleeb, F., M.A. Zia, M. Ashraf and Z.M. Khalid. 2008. Effect of chromium on growth attributes in sunflower (*Helianthus annuus* L.). *J. Environ. Sci.* 20:1475-1480.
- Anonymous. 2007. Water quality standards. Environmental Protection Agency. Government of Pakistan, Islamabad, Pakistan.
- Asgharipour, M.R. and H.R. Azizmoghaddam. 2012. Effects of raw and diluted municipal sewage effluent with micronutrient foliar sprays on the growth and nutrient concentration of foxtail millet in southeast Iran. *Saudi J. Biol. Sci.* 19:441-449.
- Ben Ahmed, C., B.B. Rouina and M. Boukhris. 2008. Changes in water relation, photosynthetic activity and proline accumulation in one year old tree (*Olea europaea* L. cv Chemlali) in response to NaCl salinity. *Acta Physiol. Plant.* 30:553-560.
- Bernstein, N., D. Chaimovitch and N. Dudai. 2009. Effect of irrigation with secondary treated effluent on essential oil, antioxidant activity and phenolic compounds in oregano and rosemary. *Agron. J.* 101:1-10.
- Brady, N.C. and R.R. Weil. 2008. The nature and properties of soils. 14<sup>th</sup> ed. Pearson Education Inc., New Jersey.
- 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.
- Eaton, A.D., L.S. Glescer, E.W. Rice and A.E. Greenberg. 2005. Standard methods for the examination of water and wastewater. 21<sup>st</sup> ed. American Public Health Association. Washington, USA.
- Farahat, E. and H.W. Linderholm. 2013. Effects of treated waste water irrigation on size-structure, biochemical products and mineral content of native medicinal shrubs. *Ecol. Eng.* 60:235-241.
- Friedman, H., N. Bernstein, M. Bruner, I. Rot, Z. Ben-Noon, A. Zuriel, R. Zuriel, S. Finkelstein, N. Umiel and A. Hagiladi. 2007. Application of secondary-treated effluents for cultivation of sunflower (*Helianthus annuus* L.) and celosia (*Celosia argentea* L.) as cut flowers. *Sci. Hortic.* 115:62-69.
- 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.
- Janko, A.M. and G. Alemu. 2014. Supply and marketing of flowers in Ethiopia. *Unique J. Agric. Eco. Rural Dev.* 1:9-24.

- Kakar, S.R., A. Wahid, R.B. Tareen, S.A. Kakar, M. Tariq and S.A. Kayani. 2011. Impact of municipal waste water of Quetta city on biomass, physiology and yield of canola (*Brassica napus* L.). Pak. J. Bot. 42:317-328.
- 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.
- Khan, M.A., S.S. Shaukat, A. Shahzad and W. Ahmed. 2011. Application of waste stabilization pond's effluent on cultivation of roses (*Rosa damascena* Mill). Pak. J. Bot. 43:1919-1923.
- 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 oleracea* L. var. Botrytis) and red cabbage (*Brassica oleracea* L. var. Rubra) grown on calcareous soil in Turkey. Agric. Water Manage. 95:716-724.
- Kurian, M. 2017. The water-energy-food nexus: trade-offs, thresholds and transdisciplinary approaches to sustainable development. Environ. Sci. Policy 68:97-106.
- Laurie, A. and V.H. Ries. 1950. Floriculture: Fundamentals and Practices, 2<sup>nd</sup> Ed. McGraw Hill Book Co., New York.
- Marinho, L.E.O., A.L. Tonetti, R. Stefanutti and B.C. Filho. 2013. Application of reclaimed wastewater in the irrigation of rosebushes. Water Air Soil Poll. 224:1669-1675.
- Melgar, J.C., J.P. Syversten and F. Garcia-Sanchez. 2008. Can elevated CO<sub>2</sub> improve salt tolerance in olive trees. J. Plant Physiol. 165:631-640.
- Murtaza, G., A. Ghafoor, M. Qadir, G. Owens, M.A. Aziz, M.H. Zia and Saifullah. 2010. Disposal and use of sewage on agricultural lands in Pakistan: A review. Pedosphere 20:23-34.
- Nasir, M.H., R. Nadeem, K. Akhtar, M.A. Hanif and A.M. Khalid. 2007. Efficacy of modified distillation sludge of rose (*Rosa centifolia*) petals for lead (II) and zinc (II) removal from aqueous solutions. J. Hazard Mater. 147:1006-1014.
- 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.
- Pandey, P. and A.K. Tripathi. 2011. Effect of heavy metals on morphological and biochemical characteristics of *Albizia procera* (Roxb.) Benth. seedlings. Int. J. Environ. Sci. 5:1009-1018.
- Pescod, M.B. 1992. Wastewater treatment and use in agriculture. Irrig. Drain. 47, FAO, Rome.
- Riaz, A., M. Arshad, A. Younis, A. Raza and M. Hameed. 2008. Effect of different growing media on growth and flowering of Zinnia elegance Blue point. Pak. J. Bot. 40:1579-1585.
- Rusan, M.J.M., I. Bashabsheh and M. Safi. 2008. Reuse of treated wastewater for cut flowers production and impact on soil and plant quality parameters. Int. Conf. Const. Build. Technol. 6:63-78.
- Singh, A. and M. Agrawal. 2010. Effects of municipal waste water irrigation on availability of heavy metals and morpho-physiological characteristics of *Beta vulgaris* L. J. Environ. Biol. 31:727-736.
- Sinha, S., S. Mallick, R.K. Misra, S. Singh, A. Basant and A.K. Gupta. 2007. Uptake and translocation of metals in *Spinacia oleracea* L. grown on tannery sludge-amended and contaminated soils: Effect on lipid peroxidation, morpho-anatomical changes and antioxidants. Chemosphere 67:176-187.
- 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, 3<sup>rd</sup> Ed. McGraw Hill Book Co., New York.
- Talal, A., I. Ismail, J.M. Basahi, 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.
- U.S. Salinity Lab. Staff. 1954. Diagnosis and improvement of saline and alkali soils. USDA Hand Book No. 60. Washington. D.C., U.S.A.
- WHO. 1989. Health guidelines for the use of waste water in agriculture and aquaculture. WHO Technical Report Series 778. Geneva, Switzerland.
- Younis, A. 2006. Phytochemical analysis and potential for exploitation of heterosis for essential oil contents in *Rosa* species. Ph.D. diss. Uni. Agric. Faisalabad, Pakistan.