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Households waste water effects on medicinal plant (*Achillea santolina* L.) and its soil characteristics

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Key words: Growth characteristics, heavy metals uptake, medicinal plant, metabolites, treated waste water.

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Abstract

This investigation was done in botanical garden university of Balochistan Quetta, medicinal plants *Achillea santolina* L. were irrigated by four different types of water including municipal waste water, semi-treated wastewater, completely treated waste water and ground water (Tube well). Results of the study indicated that the continual usage of polluted water for irrigating purpose led to the enhancement of micro-nutrients as well as heavy metals in the soil. Our results also showed significant variation in the physiological, biochemical and growth characteristic of *Achillea santolina* L. with respect to different water treatments. Per plant biomass and total yield/per plant was found non- significantly higher in waste water plants as compared to the ground and treated water plants. Similarly rate of translocation and uptake of heavy metals were also found maximum in plants grown in waste water as compared to other waters. On the other hand the plants irrigated with wastewater produced least secondary metabolites and antioxidants, as compared to the ground and other treated waste water. Among the heavy metals, Zn and Mn showed highest uptake and between the plant parts (leaves and flowers) the maximum uptake was noted in leaves then flowers. The least production of metabolites and antioxidants might be reduce the remedial activities of *Achillea santolina* L. and increase the uptake of heavy metals contents moreover it reduce the tolerant efficiency of pants against the negative impact of heavy metals and thus not improve the yield and biomass potential in plants irrigated by waste water.

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Introduction

Pakistan is one of those states where shortage of ground water resources is increasing at an alarming rate in the arid and semi-arid areas, which has increased the usage of sewage water for farming and other associated activities (Leghari *et al.,* 2013). Even though the elements (nutrients) existing in the sewage water are reflected as useful for the cultivation of different crops, however this polluted water contain considerable quantities of lethal substances such as heavy metals and their extensive application to agricultural fields may enhance meaningfully the contents of these trace poisonous metals in topsoil and also in the cultivated crops (Rattan *et al.,* 2005). So the toxic trace metals go in the food chain process of human and animals, which cause well-being threats (Chandran *et al.,* 2012). Therefore it is necessary to control the quality of food and herbal medicines because the plants are the main pathway by which heavy metals go in to the diet as stated by Antonious and Kochhar, (2009). There is a straight association among biochemical features of waste water irrigated topsoil, toxic metals absorption and biological and morphological replies of vegetation. Preeti and Tripathi, (2011); Mangabeira *et al.,* (2001) reported that the accumulation of heavy metal in plants is recognized to develop important biochemical and physical changes in vegetables. Long-term utilization of polluted water move to increase soil by vital micro and macro-elements (Dassand Kaul, 1992; Kanan *et al.,* 2005).

The build-up of toxic metals in waste water irrigated plant tissue may cause decrease in biochemical and physio-morphological activities of plants, as result there is reduction in biomass and yield (Scoccianti, 2006; Lin *et al.,* 2005) as indicated that the heavy metals effects on vegetation slow down the growth processes of roots and shoots and also reduce the photosynthetic tissues activities. Significant negative relationship with yield and the concentration of heavy metals in root and shoot was reported by Lin *et al.,* (2005), who stated that the significant action of toxic metal is to increase the production of reactive oxygen species (ROS) and this enrichment of ROS typically injuries the cell components like nucleic acids and membrane. Karenlampi *et al.,* (2000) reported that the ROS in plant produced lipid peroxidation of membrane which is the reflection of heavy metals stress that cause the injury at cellular level.

A type of natural reactive oxygen species (hydrogen peroxide H_2O_2) produced in plant due to different environmental and developmental inducements and this is the new gesture molecule which play significant role in different biological methods like cells growth, expansion, closing of stomata and automatic cause the death of cell (Chao *et al.,* 2008; Drgzkiewicz *et al.,* 2004; Cho and Park, 2000)as specified that Cu, Cd and Hg augment the amount of H_2O_2 in plants. Similarly the amplified build-up of H_2O_2, typically linked through variations in the status of cellular redox, signals the vegetation cells in contradiction of ecological pressures (Foyer and Noctor, 2003) and might improve the plants antioxidant reply by Ca signing in the expression of glutathione transferees' gene (Rentel and Knight, 2004). In several investigations the association of plant pressure tolerance was found by the manufacture of bound and conjugated polyamines while the motivation of polyamine oxidation (Gill and Tuteja, 2010).

The discharge of domestic and industrial sewage in to the water is the main problematic which disturbs agriculture land and all types of vegetation (medicinal, fruit and food crops). So in semi-urban ecology the municipal discarded water is frequently used for the crop cultivation, because of its informal expediency and shortage of underground water. Irrigation with wastewater is identified as a significant contributor of heavy metals to the soil. Extreme build-up of heavy metals in cultivated soils by untreated waste water not only finish in soil impurity, it also has an impact on food purity and security. This study was conducted to assess the comparative effects of households waste waters on physio-morphological, biochemical and growth characteristics of medicinally important plant species; *Achillea santolina* L.

Materials and methods

The experiment was performed to investigate the house holds waste water effects on morphological, physiological characteristics and heavy metal up take of medicinal plant (*Achillea santolina* L.) and their soil characteristics. The experiment was conducted in the field of Botanical garden, University of Balochistan, Quetta. Seed and soil used for experiment were obtained from botanical garden. For the comparison, plants were also grown under optimal conditions. The Physio-chemical analysis of waste water, wastewater irrigated soil, tube well water and tube water irrigated soil were also performed.

Water treatment

Waste water used for irrigation was taken from drain, where domestic waste water of Satellite Town Quetta runoffs and this drain pass along the side of Botanical garden University of Balochistan, Quetta. For irrigation purposes following four water treatments strategies were designed;

GWI: Ground /tube well water irrigation.
WWI: Waste water irrigation (directly from drain)
STWWI: Semi treated wastewater irrigation (Semi treatment was made by passing wastewater through sand, silt and stones mixture).
TWWI: Treated waste water irrigation (water used after proper treatment for irrigation).

Measurement of growth conditions

Seeds of *Achillea santolina* L. were surface-sterilized with 0.001 M $HgCl_2$ solution for 3 min and washed thoroughly with several changes of sterile distilled water. Ten seeds were sown in each pot (40 pots) containing 20 kg of loam soil (each pot was 40 cm in diameter and 25 cm deep) at a 3 cm depth. After sowing seeds all the pots were irrigated with tube well/fresh water for only first week. After that, these pots containing medicinal plants were divided into four groups of 10 pots each.

The first group continued to be irrigated by fresh water/ground water (GW); the second irrigated with wastewater (WW), third with semi treated waste water (STWW) and fourth groups were irrigated by properly treated wastewater (TWW). Pots were maintained in a greenhouse under natural lighting with an 8 hours photoperiod and average 25°C/10°C day/night temperatures. Plant material samples were taken after flowering stage (75 days after sowing), to measure the growth, yield, other physiological parameters and certain secondary metabolites with antioxidant activity in response to irrigation with different water treatments. Number of leaf and shoots were determined by counting randomly selected 3 plants and then their average was calculated. Leaf area per plant (cm^2) was determined by multiplying length x width x 0.75 (Quarrie and Jones, 1979) and graph paper methods (Leghari and Zaidi, 2013).

The harvesting of the plants was carried out after 100 days and yield components along with heavy metals in the plants were measured.

Heavy metals uptake and translocation ratio in plant

Translocation and uptake ratio of heavy metals in the plant samples were determined at the time of final reaping through following formula given by Singh and Agrawal (2007).

Heavy metal Uptake ($\mu g\ plant^{-1}\ d^{-1}$) = $(M_2W_2 - M_1W_1) / (T_2 - T_1)$

Where M_1 and M_2 are metal contents ($\mu g\ g^{-1}$) in plant tissue and W_1 and W_2 is the plant biomass (g) at initial T_1 and T_2 samplings. Translocation ratio was measured through dividing heavy metals concentration in shoot by root.

Physio-biochemical characteristics analysis

Photosynthetic rate (Pr) and stomatal conductance (Sc)

For the measurement of photosynthetic rate (Pr) and stomatal conductance (Sc) the photo-synthetically active radiation ranging from 1000-1200 μ mol $m^{-2}\ s^{-1}$ portable photosynthetic system (LI-6200, LICOR, Inc., Lincoln, NE, USA) was used. Fully expanded leaves from three randomly selected plants were collected from each pots and used. Experiment was performed between 10:30 a.m. to 12:30 p.m. after 40 days germination (DAG).

Florescence determination

The chlorophyll fluorescence capacities (Initial fluorescence (If), maximum fluorescence (Mf), variable fluorescence (Vf = Mf - If) and florescence ratio (Fr = Vf / Mf) from the same leaf were also done during 10:30 a.m. to 12:30 p.m by using the moveable plant efficiency analyzer (MK 29414, 174 Hansatech Instrument Ltd, U.K).

Water use efficiency (WUE)

Water use efficiency from plant was also determined by the ratio between Photosynthetic and transpiration rate using the following formula;

Water use efficiency (WUE) = (Pr / Tr).

Where; Pr is for Photosynthetic rate (µ mol CO_2 m^{-2} s^{-1}) and Tr is for Transpiration rate (mol H_2O m^{-2} s^{-1}). For the approximation of photosynthetic pigments, lipid peroxidation and different metabolites, fresh leaves were manually sampled at 40 DAG. Total chlorophyll and carotenoid contents were extracted in 80% acetone and estimated according to the methods of Maclachlan and Zalik, (1963); Duxbury and Yentsch, (1956),respectively. Protein, proline and ascorbic acid contents were determined by the methods used by Lowry *et al.,* (1951); Keller and Schwager, (1977); Bates *et al.,* (1973), respectively.

The total phenol contents were measured with methods described byBray and Thorpe, (1954) and peroxidase activity and thiol contents were measured as per method given by Britton and Mehley, (1955); Fahey *et al.,* (1978), respectively. The per-oxidation was noted as melondialdehyde (MDA) concentration (Heath and Packer, 1968).

Heavy metal examination from plants, waters and soils

For the analysis of metals (Cr, Ni, Mn, Zn, Pb, Cu and Cd), 1 gm dried leaves and 1 gm dried flowers were individually digested by 10 ml of ternary acid (HNO_3, H_2SO_4 and $HCLO_4$ in 5:1:1 ratio) at 80°C until a transparent solution was obtained (Allen *et al.,* 1986). 50 ml from each water samples were digested in 10 ml of concentration HNO_3 at 80°C until the solution became transparent (APHA, 2005).

The contents of heavy metals in filtrate were examined by using atomic absorption spectrophotometer (Model 2380, Perkin 203 Elmer, Inc., Norwalk, CT, USA), which was calibrated as per blank and drift standards after five determinations. Heavy metal accumulation in the soil was investigated at the end of the experimental period. Available heavy metals (Cr, Ni, Mn, Zn, Pb, Cu and Cd) in the soil were determined by extracting the soil with 0.01 M di-ethylene-triamine-penta acetic acid (DTPA) as described by Lindsay and Norvell, (1978). Heavy metal analysis from plant, water and soil were performed in the laboratory of PCSIR Balochistan, Pakistan through atomic absorption spectrophotometer.

Statistical analysis

Statistical analysis was done using SPSS program version 16. Significance of differences in measured parameters between ground and wastewater irrigated pots were assessed by conducting one way analysis of variance (ANOVA) followed by Duncan's multiple range test at 5% level.

Results and discussion

Experimental data about morphological and physiological characteristics of *Achillea santolina* L. grown in different waste water are shown in Table 1 and 2. Table 3 and 4 show the results of photosynthetic contents and metabolites level of *Achillea santolina* L. respectively. Heavy metals uptakes consequences in investigated plants, waters and soils are illustrated in Table 5-7, respectively.

Morphological characteristics of Achillea santolina L. plants grown in different water treatments

Growth characteristics (no of leaves per plant, leaf area, total yield, root and shoot length, root and shoot biomass) of *Achillea santolina* L. exhibited variation in different water treatments. Number of leaves

(47.7), no of shoots (5.0) and shoot length (12.0 cm) of plants were significantly higher in wastewater irrigated pots plants as compared to the respective STWWI and TWWI plant. However least number of leaves and shoot along with shoot length were found in ground watered plant (40.0, 3.33 and 9.47), respectively (Table 1).

Table 1. Growth characteristics of *Achillea santolina* L. plants grown in different water treatments.

Morphological parameters	Water Treatment			
	GWI	WWI	STWWI	TWWI
No. of leaves/Plant	40.0 ±3.61^{b}	47.7 ±2.5^{a}	45.0 ±1.7^{a}	42.3 ±2.3^{b}
No. of shoots	3.33 ±0.58^{b}	5.00 ±1.0^{a}	4.30 ±0.6^{ab}	4.33 ±0.6^{ab}
Shoot length (cm)	9.47 ±2.20^{b}	12.0 ±1.8^{a}	10.5 ±1.8^{ab}	10.7 ±1.5^{ab}
Leaf area (cm^{2})	1.60 ±0.58^{a}	1.5 ±0.2^{a}	1.40 ±0.5^{a}	1.73 ±0.3^{a}
Root length	12.0 ±2.65^{a}	10.5 ±0.6^{a}	11.0 ±1.0^{a}	11.7 ±1.3^{a}
Total yield (g/Plant)	24.0 ±5.29^{a}	23.3 ±2.3^{a}	23.7 ±3.5^{a}	24.0 ±2.0^{a}
Shoot biomass (g/Plant)	0.04 ±0.01^{a}	0.04 ±0.01^{a}	0.04 ±0.01^{a}	0.04 ±0.01^{a}
Root biomass (g/Plant)	0.03 ±0.02^{a}	0.03 ±0.0^{a}	0.03 ±0.01^{a}	0.04 ±0.02^{a}

Where; ± = standard deviation and each value is the mean of three replicate.

Similar observation was also found by Singh and Agrawal, (2010) in *Beta vulgaris* L. Significant increase in shoot length of *H. binata* was also found by Paliwal *et al.,* (1998)with the treatment of increased percentages of sewage water. Wheat plants irrigated by treated and untreated textile effluents showed increment in shoot length as compared to the plants irrigated by distilled water (Kaushik *et al.,* 2005). In this study the leaf area was found higher in treated waste water (1.73 cm^{2}) and ground water (1.70 cm^{2}) irrigated plants as compared to the waste water plant (1.5 cm^{2}), which clearly suggests that treated wastewater reduced negative effects of heavy metals in plant tissue which led to favorable growth as compared to the pots receiving continuous waste water. Results showed that the wastewater irrigated plants have less root length (10.5 cm) with respect to the STWWI (11.0 cm) and TWWI (11.7) and maximum root length (12.0 cm) was recorded in ground water plants (Table 1).

Table 2. Physiological characteristics of *Achillea santolina* L. plant grown in different water treatments.

Physiological Parameters	Water Treatment			
	GWI	WWI	SWWI	TWWI
Photosynthetic rate (µmol CO_2 m^{2} S^{-1})	10.4±0.7^{b}	14.7±0.58^{a}	14.0 ±1.0^{a}	11.0 ±1.0^{b}
Stomatal conductance (mol m^{-2} S^{-1})	0.93±0.1^{b}	1.57±0.06^{a}	1.43 ±0.1^{a}	1.00 ±0.1^{b}
Transpiration rate (mol H_2O m^{2} S^{-1})	2.80±0.1^{b}	5.50±0.36^{a}	4.53 ±0.1^{ab}	03.2 ±0.1^{b}
Water use efficiency (µmol CO_2 m^{2} S^{-1}/ mol H_2O m^{2} S^{-1})	3.72±0.4^{a}	2.67±0.23^{a}	3.09 ±0.2^{a}	3.43 ±0.2^{a}
Maximum fluorescence (mv)	2097±5.8^{a}	1500±21.0^{b}	1803±25.2^{ab}	1997±5.8^{ab}
Variable fluorescence (mv)	1500±20^{a}	797±15.3^{b}	1002±17.6^{ab}	1402±7.6^{a}
Fluorescence ratio (Fr)	0.72±0.0^{a}	0.53±0.02^{b}	0.56 ±0.02^{b}	0.70±0.01^{a}
Initial fluorescence (mv)	502±7.2^{b}	701±3.61^{a}	602.0±3.5^{ab}	551±13.0^{b}

Where; ± = standard deviation and each value is the mean of three replicate.

The reductions in the root length in waste water plants may be due to the increase of nutrient concentrations at top level under wastewater irrigation as compared to the ground water irrigated ones. Result reported by Singh and Agrawal (2010) in *Beta vulgaris* L. are also in support of our results.

There was non-significant differences in the total yield, shoot and root biomass accumulation of plants grown in wastewater (23.3, 0.04 & 0.03 gm/plant dw), semi waste water (23.7, 0.04 & 0.03 gm/plant dw), treated waste water (24.0, 0.04 & 0.04 gm/plant dw) and ground water (24.0, 0.04 & 0.03 gm/plant dw), respectively (Table 1). Similar observation was also reported by Singh and Agrawal (2010). Derome and Lindroos, (1998) reported that heavy metals in soil may interfere with the nutrient uptake. Higher bioavailability of heavy metals in wastewater irrigated sites may have reduced the nutrient availability to the plants that may be the cause for not showing significant increments in biomass of these plants as compared to the plants grown at ground water irrigated pots. So the consequences of this research indicate that favorable physiological and growth responses are not translated into increments in the biomass accumulation and yield of plants, as the photosynthates are utilized in the formation of secondary metabolites to ameliorate the negative influence of heavy metals.

Table 3. Total chlorophyll and carotenoid contents in *Achillea santolina* L. grown in different water treatments.

Photosynthetic contents	Water Treatment			
	GWI	WWI	SWWI	TWWI
Carotenoid contents (mg/g dw)	0.13 ±0.01^{b}	0.26 ±0.01^{a}	0.18 ±0.02^{ab}	0.15 ±0.01^{b}
Total chlorophyll contents	0.61 ±0.01^{b}	0.74 ±0.01^{a}	0.69 ±0.01^{a}	0.65 ±0.01^{ab}

Where; ± = standard deviation and each value is the mean of three replicate.

Physiological characteristics of Achillea santolina L. plant grown in different water treatment

Stomatal conductance and photosynthetic rate ((1.57 &14.7) were found higher in waste water irrigated plant as compared to the ground water (0.93 &10.4). There was non-significant variation in Photosynthetic rate and Stomatal conductance between GWI and TWWI plants but there was significant differences between GWI and WWI plants (Table 2). The positive response of physiological characteristics of the *Achilea santolina* L. at waste water irrigated suggest that the concentrations of toxic heavy metals may not be up to the extant causing adverse effects on photosynthetic apparatus.

Table 4. Contents of selected metabolites in *Achillea santolina* L. grown in different water treatments.

Metabolites	Water treatments							
	GWI		WWI		SWWI		TWWI	
	Leaf	Flower	Leaf	Flower	Leaf	Flower	Leaf	Flower
Peroxidase activity (µm purpurogallin min^{-1} g^{-1}F.leaf)	22.3±0.5^{a}	20.5±0.5^{a}	14.3±1.2^{b}	12.8±1.0^{b}	16.5±0.5^{b}	14.5±0.5^{b}	20±0.3^{a}	18.5±0.4^{a}
Proline (mg g^{-1}F.leaf)	2.9±0.08^{a}	2.1±0.12^{a}	1.5±0.2^{b}	1.3±0.21^{b}	2.5±0.2^{b}	2.0±0.05^{b}	2.2±0.2^{a}	1.7±0.15^{a}
Phenol (mg g-1 F. leaf)	3.5±0.03^{a}	2.4±0.21^{a}	2.0±0.5^{b}	1.8±0.25^{b}	2.7±0.1^{b}	2.4±0.21^{b}	2.5±0.2^{b}	2.0±0.2^{b}
Thiol (µ mol g^{-1}F.leaf)	10.6±0.4^{a}	10.2±0.4^{a}	6.2±0.5^{b}	5.6±0.3^{b}	9.4±0.2^{a}	9.0±0.25^{a}	9.7±0.4^{a}	7.8±0.8^{ab}
Ascorbic acid (mg g^{-1} F. leaf)	0.5±0.04^{b}	0.4±0.04^{b}	0.9±0.01^{a}	0.8±0.01^{a}	0.8±0.02^{a}	0.7±0.01a	0.6±0.03^{b}	0.5±0.04^{b}
Lipid peroxidation (n mol ml^{-1} F. leaf)	0.7±0.1^{b}	0.6±0.02^{b}	1.2±0.02^{a}	1.0±0.03^{a}	0.9±0.04^{a}	0.7±0.02^{ab}	0.8±0.03^{a}	0.7±0.03^{ab}
Protein (mg g^{-1})	13.5±0.2^{a}	12.4±0.4^{a}	9.0±0.5^{b}	7.4±0.1^{b}	12.3±1.2^{a}	10.4±1.4^{a}	10.2±0.3^{a}	8.1±0.2^{ab}

Where; ± = standard deviation and each value is the mean of three replicate.

The significant increase in photosynthetic as well as growth rate of plants grown in wastewater pots as compared to ground water irrigated ones led to higher uptake and translocation of heavy metals in plants. Florescence ratio (Fr) represent the efficiency of photosystem II. There were significant variations in Fr of plants under wastewater and ground water irrigation pots. Fr in present study was 0.53 for WWI and 0.72 for GWI plants, which clearly showed the unstressed condition for the photosynthetic apparatus of the plants. Similar observation was also reported by Singh and Agrawal, (2010). They reported non-significant variations in florescence ratio (0.77 - 0.85) of the plants under wastewater and ground water irrigation sites. In the present study the low Fr in waste water plant may be due to high up take of heavy metals contents. Observation reported by Demming and Bjorkman (1987) are also in favors of our research results.

Table 5. Heavy metal (μg g^{-1}) up take by *Achillea santolina* L. grown in different water treatment.

Heavy metals	*Water treatments*							
	GWI		WWI		SWWI		TWWI	
	Leaf	Flower	Leaf	Flower	Leaf	Flower	Leaf	Flower
Cr	1.5±0.02^{b}	1.0±0.0^{b}	2.6±0.03^{a}	2.3±0.02^{a}	2.4±0.02^{a}	2.3 ±0.1^{a}	1.6±0.01^{b}	1.3±0.02^{b}
Ni	0.01±0.0^{a}	0.0±0.0^{a}	0.1±0.0^{a}	0.07±0.0^{a}	0.08±0.0^{a}	0.06±0.0^{a}	0.04±0.0^{a}	0.03±0.0^{a}
Mn	48.0±5.6^{b}	45.2±4.0^{b}	64±5.5^{a}	61.2±5.1^{a}	52.4±4.9^{b}	49.7±3.1^{b}	50.3±3.0^{b}	47.1±4.2^{b}
Zn	34.5±4.0^{b}	31.2±0.2^{b}	55±0.2^{a}	51.5±0.5^{a}	48.2±0.7^{a}	45.3±0.3^{a}	52.4±0.7^{a}	50.1±0.8^{a}
Pb	0.52±0.1^{b}	0.49±0.0^{b}	0.7±0.01^{a}	0.63±0.0^{a}	0.56±0.0^{b}	0.5±0.01^{b}	0.54±0.0^{b}	0.51±0.0^{b}
Cu	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Cd	0.31±0.0^{b}	0.19±0.0^{b}	0.8±0.01^{a}	0.76±0.0^{a}	0.8±0.02^{a}	0.75±0.02^{a}	0.51±0.0^{b}	0.3±0.0^{b}

Where; ± = standard deviation and each value is the mean of three replicate.

Results illustrated in Table 3 exhibited variation in photosynthetic parameters of *Achillea santolina*. Waste water irrigated plant showed higher level of total chlorophyll (0.74 mg g^{-1}dw) and carotenoid contents (0.26 mg g^{-1}dw), which followed by semi and completely treated waste water, while lowest total chlorophyll contents were recorded (0.61 & 0.13) in ground water plants. The higher level of total chlorophyll and carotenoid were also found by Singh and Agrawal (2010) in *Beta vulgaris* L. which was irrigated by waste water as compared to the tube well water. Carotenoid is a photosynthetic pigment which functions as non-enzymatic antioxidant protecting plants from oxidative stress by changing the physical properties of photosynthetic membranes with involvement of xanthophyll cycle (Gruszecki and Strzatka, 1991). An increase in carotenoid content is suggested as a defense strategy of the plants to combat metal stress (Sinha *et al.,* 2007).

Table 6. Heavy metal concentrations (μg ml^{-1}) in different water used for the irrigation.

Heavy metals	Water treatment			
	GWI	WWI	SWWI	TWWI
Cr	0.05 ±0.002^{b}	0.52 ±0.020^{a}	0.50 ±0.10^{a}	0.47 ±0.06^{b}
Ni	0.07 ±0.003^{b}	0.10 ±0.004^{a}	0.09 ±0.00^{a}	0.08 ±0.07^{ab}
Mn	0.10 ±0.003^{b}	0.61 ±0.050^{a}	0.60 ±0.07^{a}	0.41 ±0.04^{ab}
Zn	0.65 ±0.100^{b}	1.74 ±0.080^{a}	1.61 ±0.16^{a}	0.85 ±0.13^{b}
Pb	0.09 ±0.006^{b}	0.24 ±0.002^{a}	0.24 ±0.12^{a}	0.14 ±0.07^{b}
Cu	0.00 ±0.002	0.09 ±0.004^{b}	0.07 ±0.002^{b}	Nd
Cd	0.05 ±0.002^{b}	0.21 ±0.002^{a}	0.20 ±0.03^{a}	0.08 ±0.05^{b}

Where; ± = standard deviation and each value is the mean of three replicate.

Metals accumulation in Achillea santolina L.

Since the beginning of human life, medicinal plants are significant part of human intake and play a vital role in human health treatment. Different parts of medicinal plants such as leaves, roots and flowers are also being consumed for the treatment of different diseases. In this study the content of heavy metals in leaves and flowers of *Achillea santolina* L. are shown in Table 5. It is evident from the result that heavy metal contents in both parts of *Achillea santolina* L. irrigated by wastewater was (Cr; 2.6 & 2.3, Ni; 0.1 & 0.07, Mn; 64.4 & 61.2, Zn; 54.8 & 51.5, Pb; 0.71 & 0.63 and Cd; 0.81 & 0.76) respectively which were significantly higher as compared to GWI(Cr; 1.5 & 1.0, Ni; 0.01 & Nd, Mn; 48.0 &45.2, Zn; 34.5 & 31.2, Pb; 0.52 & 0.49 and Cd 0.31 & 0.19) and TWWI (Cr; 1.6 & 1.3, Ni; 0.04 & 0.03, Mn; 50.3 & 47.1, Zn; 52.4 & 50.01, Pb; 0.54 & 0.51 and Cd; 0.51 & 0.3) plants. The investigated heavy metals maintained the order of Mn > Zn > Cr > Cd > Pb > Ni in all irrigation water treatment (WW, STWW, TWW and GW) plants in their leaves and flowers. Similar results were also reported by Khan *et al.,* (2015) who revealed that leaves up take more heavy metals instead of flowers and Cu remain undetectable in both parts of the plant. It was reported previously that plant leaves more sink metals than that of flowers (Li *et al.,* 2009). Our findings reveal that plants irrigated with heavy metals present in waste water might have reduced the metabolites contents which led to decrease the remedial activities. Moreover the significant reduction of heavy metal accumulation in TWWI plant with respect to the WWI plant is clearly an indication that toxic metals uptake can be reduced significantly by the treatment of waste water for agricultural purposes.

Table 7. Heavy metal concentration (μg/g) in soil used for cultivation by different waste water.

Heavy metals	Waste Water treatment			
	GWI	WWI	SWWI	TWWI
Cr	2.31 ±0.21[b]	29.8 ±2.03[a]	24.4 ±1.10[a]	12.53 ±1.2[a]
Ni	1.20 ±0.02[b]	5.28 ±0.47[a]	05.3 ±0.81[a]	12.22 ±2.5[a]
Mn	159.6 ±23.9[b]	464.4 ±10.8[a]	407.3 ±8.15[a]	372.5 ±7.5[a]
Zn	14.2 ±2.85[b]	70.2 ±3.57[a]	57.1 ±3.82[a]	29.84 ±3.8[a]
Pb	5.8 ±0.76[b]	20.8 ±1.81[a]	17.1 ±1.31[a]	13.23 ±1.8[ab]
Cu	2.41 ±0.76[b]	12.1 ±1.02[a]	10.4 ±1.72[a]	7.24 ±0.7[ab]
Cd	1.6 ±0.23[b]	5.09 ±0.50[a]	4.23 ±1.02[a]	3.73 ±0.2[a]

Where; ± = standard deviation and each value is the mean of three replicate.

Metabolites contents in plant

Achilleas antolina L. grown in different water exhibited variation in their metabolites contents. Antioxidative enzyme, peroxidase activity were found higher in plants (leaves and flowers) grown in ground water (22.3 & 20.5), followed by TWW and SWW plants, while the lowest activity was recorded in wastewater irrigated plants (14.3 & 12.8), respectively (Table 4). Peroxidases play a significant role in defense against oxidative stress (Radotic *et al.,* 2000). The low peroxidase activity in wastewater plant might be due to high uptake of heavy metals which may lead to reduction of remedial activity of this plant. Our results contradict to the finding of Singh and Agrawal, (2007) who found increase in peroxidase activity under heavy metal stress, in palak (*Beta vulgaris* Var All green) grown at different application rates of sewage sludge. Singh and Agrawal, (2010) reported that the Peroxidase activity had positive and significant relationship with heavy metals. The positive relationship suggests that with increase in the heavy metal concentrations, there was increase in the oxidative modifications to cellular components of the plants (Moller *et al.*, 2007). The consequence of this study indicate that the antioxidative capacity of medicinal plants increased in ground water plants with positive effects on its growth is also used to induce tolerance against heavy metals. Proline content was found lower in plants grown in wastewater irrigated pots (1.5 & 1.3) in both parts of plant (leaves and flowers) with respect to the ground water irrigated (2.9 & 2.1) plants respectively. Similarly protein content were also found least in plants (leaves and flowers) grown in waste water (9.0

& 7.4) as compared to the treated waste water (10.2 & 8.1), mixed water (12.3 & 10.4)) and highest was found in ground water irrigated plants (13.5 & 12.4) (Table 4). Least production of proline found in wastewater irrigated plant might due to high level of heavy metals which may lead to cause reduction in medicinal activities of the plant because proline regulate the protein level, hydroxyl radicals and NAD/NADH ratio in plants. Many other researchers have also found and stated that possible positive roles of proline include stabilization of proteins (Anjum, 2000), scavenging of hydroxyl radicals (Smirnoff and Cumbes, 1989) and regulation of NAD/NADH ratio(Alia and Saradhi, 1993) which helps to enhance the tolerance level of plant against toxic metals and also increase bio-chemical activity of plant. Phenol and thiol contents were also found least in plants (leaves & flowers) irrigated by wastewater (2.0 &1.8) and (6.2 & 5.6), respectively as compared to the ground water irrigated plants which showed highest contents of phenol and thiol (3.5 & 2.4) and (10.6 & 10.2), respectively (Table 4). Higher level of phenol and thiol in ground water plants may detoxify the metals contents and may play significant part in remedial activity. Singh and Agrawal (2010) reported that thiols do not represent a single compound, but are sulphur containing polypeptides, which are known as phytochelatins. Cysteine, a -SH containing amino acid is a key constituent of phytochelatins and plays an important role in metal detoxification. Kneer and Zenk, (1992) reported that phytochelatins are involved in the detoxification of heavy metals by immobilizing the metal ions and facilitating their further transport to the vacuolar portion (Ortiz *et al.,* 1995). The observation reported by Tripathi and Tripathi, (1999) contradict to our results, who found increase in phenolic components in waste water irrigated plant *Albizia lebbek* due to heavy metals such as Ni, Cr and Hg. Similar observation was also reported by Singh and Agrawal, (2010) in *Beta vulgaris* L. Disparate other metabolites ascorbic acid was higher (0.9 & 0.8) in wastewater irrigated plants in both parts (leaves and flowers) as described in Table 4. Ascorbic acid, a natural antioxidant scavenges free radicals generated by heavy metals as reported by Halliwell and Gutteridge, (1993). The higher level of ascorbic acid in waste water plant might be due to enrichment of heavy metals. Singh and Agrawal, (2010) reported that the ascorbic acid content positively and significantly correlated with Cd, Pb, Zn and Ni concentrations. Sinha *et al.,* (2007) have also reported higher production of ascorbic acid in fenugreek plants grown in soil amended with tannery sludge to nullify the adverse effects of heavy metals. *Colocasia esculentum* and *Raphanus sativus* grown in wastewater irrigated area of Durgapur, West Bengal showed higher production of ascorbic acid under wastewater irrigation (Gupta *et al.,* 2009). Melon-di-aldehyde (MDA) concentration is an indicator of lipid peroxidation. Plantsgrown in wastewater irrigated pots showed highest contents in both part of parts; leaves and flower (1.2 &1.0) which followed by the plant grown in STWW and TWW irrigated plants, while the least concentration was found in those plant grown in ground water (0.7 & 0.6) irrigated pots (Table 4). The high level of lipid peroxidation in wastewater plant may be due to the enrichment of heave metals contents in these plants. Moreover leaves showed more contents of lipid peroxidation as compared to the flowers. Enhancement in MDA concentrations in leaves of *Bruguiera gymnorrhiza* and *Kandelia candel* were also reported (Zhang *et al.,* 2007). Heavy metals are known to induce generation of reactive oxygen species (ROS) and free radicals, which can cause more lipid peroxidation in cell membrane leading to increased permeability and oxidative stress to the plants (Nada *et al.,* 2007). Similar results were also reported by Drgzkiewicz *et al.*, (2004), they specified that the toxic metal play important role to upturn the manufacture of ROS and this enhancement characteristically damages the cell mechanisms such as nucleic acids and membrane. Karenlampi *et al.,* (2000) also reported that the ROS in plant produced lipid peroxidation of membrane which is the reflection of heavy metals stress that cause the injury at cellular level.

Metals accumulation in waters and soils

The consequences of heavy metals content in water and soil used for irrigation are shown in Table 6 and 7. Waste water and wastewater irrigated soils showed elevated level of all metals when compared with ground/tube well water and ground water irrigated soil. All the investigated metals contents in waste water (Zn; 1.74, Mn; 0.61, Cr; 0.52, Pb; 0.24, Cd; 0.20 and Ni; 0.10) were observed maximum which followed by semi waste water and treated waste water respectively, whereas the concentration of these elements were found quite low in ground water. The concentration of Cu was not detected in ground and treated wastewater (Table 6). Similarly The heavy metals concentration in waste water irrigated soil was recorded (Mn; 464.4, Zn; 70.2, Cr; 29.8, 20.8, Cu; 12.1, Ni; 5.28 and Cd; 5.09) greater than that of SWWI and TWWI soils, while the lowest content was in ground water irrigated soil, respectively as described in Table 7.

The concentration of heavy metals in waste water and waste water irrigated soil was also reported by other researchers (Khan *et al.,* 2015). They found higher level of investigated metals in waste water and wastewater irrigated soil as compared to tube well water. Present consequences also indicate that by the use of treated waste water for agriculture activities, the soil can be significantly protected from heavy metals contamination. Toxic metals in soil may restrict the nutrient uptake (Derome and Lindroos, 1998). The greater bio-obtainability of toxic metals (heavy metals) in contaminated water irrigated soil might decrease the essential elements accessibility to the plants that might be the reason for not indicating significant increase in biomass and total yield of these plants with respect to the plants grown with ground water irrigated soils. So the promising physical and growing replies in wastewater irrigation are not interpreted into increases in the biomass buildup and total yield of plants as the photosynthates are used in the creation of subordinate metabolites to improve the bad effects of toxic metals.

Conclusion

Investigation of medicinal plant *Achillea santolina* L. grown in waste water irrigated pots showed excessive buildup of heavy metals. Consequences of waste water irrigation indicate that enrichment of heavy metal in wastewater plants may cause the reduction of metabolites, which may lead to the decrease of remedial activity of the medicinal plant. Present study also reveals that by the use of treated waste water application for agriculture activities, the soil can be significantly protected from heavy metal contamination. Study also concluded that promising morpho-physiological responses of waste water irrigated plants are not decoded into the significant enrichment of biomass and yield of these plants. This study thus suggests that the higher availability of heavy metals contents and lower concentration of metabolites in waste water irrigated medicinal plants restricts the remedial activity of the plant.

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