Environ Monit Assess (2019) 191: 107
https://doi.org/10.1007/s10661-019-7228-3

Waste water irrigation in the regulation of soil properties, growth determinants, and heavy metal accumulation in different *Brassica* species

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Received: 20 December 2017 / Accepted: 10 January 2019 / Published online: 28 January 2019

Abstract To evaluate the impact of waste water (WW) irrigation, four *Brassica* species, namely *B. campestris*, *B. juncea*, *B. napus*, and *B. nigra*, were grown for 2 years in the agricultural field. First-year experiment (2014–2015) was conducted with the comparative effect of WW and ground water (GW) under a uniform dose of NPK ($N_{80}P_{45}K_{45}$, kg ha^{-1}). WW irrigation proved efficacious over GW to increase growth, physiological, and yield parameters. Increase in all parameters was due to the use of WW which leads to the improvement in the physico-chemical properties of soil as compared to resulted soil from GW application. Second-year experiment (2015–2016) therefore deals with WW irrigation only but under interaction with two levels of NPK fertilizers ($N_{80}P_{45}K_{45}$ and $N_{60}P_{30}K_{30}$, kg ha^{-1}). Results of this year revealed that maximum enhancement in growth, physiological, and yield parameters was observed at WW × $N_{60}P_{30}K_{30}$ and the input of WW × $N_{80}P_{45}K_{45}$ was not of benefit. WW × $N_{60}P_{30}K_{30}$ treatment was beneficial also because, at this treatment level, the accumulation of Cr, Cu, Pb, Ni, and Cd in leaf and seed was comparatively lesser in amount than that of WW × $N_{80}P_{45}K_{45}$. The study concluded even though the use of WW was applicable to save freshwater, enhance soil nutrient status, and make N, P, and K balance at their lower inputs, WW irrigation caused accumulation of heavy metals in all *Brassica* crops far above the safe limits during a quite longer irrigation time (70 days and 105 days after sowing (DAS)). However, WW was safe to use only up to 35 DAS. Therefore, the study suggested that there should be regular monitoring of heavy metal concentrations in irrigation water as well as in various crop vegetables.

Keywords Heavy metals · Fertilizers · Rapeseed-mustard species · Contamination

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Introduction

Exploded population growth rate, high industrialization and urbanization, shifts in food production practices, increased living standards, and poor water use practices are interacting factors that influence all freshwater-related issues such as freshwater crisis, food security, and safe drinking water declination (WHO 2009; Wyman 2013). It has been estimated that an increase in water scarcity level will be more than 40% in the next 50 years which will affect the world's population in terms of demand and supply of food and water. The

United Nations has estimated that more than 1.8 billion people, by the end of 2025, will suffer from high competition for water use, particularly in water-scarce regions of urban and peri-urban areas in the world. Contrary to this, the volume of waste water (WW) is increasing day-by-day with their disposal issue. The crisis in the availability of the freshwater for irrigation is yet influenced in more than 25 countries including India, encouraging farmers to adopt the WW use in agricultural fields (UNEP 2008). As a matter of fact, WW use in agricultural practice is making it not only an important but also a meaningful approach in order to cope the trouble of water crisis in arid and semi-arid regions (Feigin et al. 1984; FAO 2010) of Asian especially Indian agriculture which is 81% freshwater user (WRI 2007). Furthermore, the input of WW has also been reported as the source of useful and important nutrient elements such as N, P, and K in addition to Na, Ca, Mg, S, Cl, Cu, Fe, B, Zn, salts, pH, organic matter, and microbial activity that are required for plants. Irrigation with WW adds components into soil which support soil health and plant growth and consequently permits a higher yield of various ranges of field crops including vegetables as compared to clean water irrigation (Singh et al. 2012; Chalkoo et al. 2014; Iqbal et al. 2015, 2017; Sahay et al. 2017). Waste water irrigation is now quite common worldwide where it has been used as a source of irrigation water and crop nutrients. But, safe practices and precaution should be taken while using it either treated or untreated because WW is also the source of heavy metals which may cause injuries to plants and the whole ecosystem by their accumulation in food chain and food web in their own ways, if applied in higher concentration for long-term effect (FAO 2010; Naaz and Pandey 2010; Gall et al. 2015; Ma et al. 2015); however, the toxicity of any contaminated water depends upon its quality components and type of sources.

The rapeseed-mustard group jointly includes *Brassica* species, such as *B. juncea*, *B. campestris*, *B. napus*, and *B. nigra*. It is the third most important oilseed crop in the world after palm (*Elaeis guineensis* Jacq.) and soybean (*Glycine max*) oil. In India, rapeseed-mustard holds the second rank among the seven major sources of edible oil after groundnut and shares 28.6% of India's oilseed economy. These crops are also the source of the condiment and leafy vegetable in the daily Indian diets (Vaughan and Hemingway 1959). The global production of rapeseed-mustard is around 38–42 million tons (mt), while India produces around 6.7 mt of rapeseed-mustard next to China (11–12 mt) and EU (10–13 mt) with a significant contribution in the world's rapeseed-mustard industry. Thus, India that is one of the largest rapeseed-mustard–growing countries maintained a premier position in terms of 20.23% of the area and a second position in 11.7% of production shares at the global level (USDA 2012). Other countries, i.e., China, Canada, Japan, and Germany, also grow them at major scale. Among the states of India, Uttar Pradesh was placed at the second position after Rajasthan in the contribution of 13.1% of the total area and 13.4% of total rapeseed-mustard production (source: Agricultural Statistics at a glance 2010; website: http://www.dacnet.nic.in). Besides, these crops have been considered for the good ability to grow under diverse agro-ecological situations such as at relatively low temperatures and at highly polluted soils or wastelands (Angelova and Ivanova 2009), without affecting the nutritive status of plants in terms of biomass and yields. It has been proposed that *Brassica* species are more able to accumulate heavy metals from the contaminated soil than those grown in uncontaminated soil. Thus, rapeseed-mustard species have been diversified as the domestic and industrial oil crops.

It is well known that nutrient supply is one of the key factors in augmenting or limiting the growth, development, and crop productivity (Streeter and Barta 1984; Mengel and Kirkby 1987; Marschner 2002). Thus, the productivity of any crop depends on the application of micro- and macroelements after irrigation. Although nitrogen (N), phosphorus (P), and potassium (K) and other chemical nutrient-based synthetic fertilizers have been applied more frequently into soil to achieve high crop yield (Food and Agriculture Organization (FAO) 2006, 2011), the excessive use of them has also been reported as the source of heavy metals (Curtis and Smith 2002; Atafar et al. 2010; Sahay et al. 2017). Interestingly, WW application has reduced the excessive use of synthetic fertilizers at great extent (Iqbal et al. 2015, 2017; Sahay et al. 2017). In the context to use two sources of nutrients, it is important to maintain appropriate/optimal levels of inorganic nutrients with available natural organic nutrients in WW, because surplus or less than the desired amount of supplied nutrients may have a great loss in crop yield along with degraded environmental quality.

The present study aimed to (i) characterize the physical and chemical properties of Aligarh City's waste water and its effect on soil properties; (ii) assess the effectivity of WW irrigation along with different NPK doses on growth, physiological activities, and yield response of the four *Brassica* species; and (iii) evaluate the concentration of various heavy metals in edible leaf and seed parts of rapeseed-mustard species. The level of heavy metals was compared with established safe limits to assess the phytotoxic health hazards due to WW irrigation.

Materials and methods

Study site description

The 2-year experiments were conducted at the research agricultural farm of the Department of Botany, Aligarh Muslim University, Aligarh. Aligarh District of Uttar Pradesh has an area of 3747 km^2 and is located at 27.88° N latitude, 78.08° E longitude, with an elevation of 178.45 m above sea level. The climate of the experimental site was semi-arid and subtropical with severe hot dry summer and intense cold winter seasons. The meteorological data recorded at the study area showed that the temperature ranged from 20 to 25 °C in winter and from 46 to 47.5 °C in summer with around 600–650 mm annual rainfall and 42–90% relative humidity. The average monthly humidity along with maximum and minimum temperatures during morning and evening of experimental period (October–March 2014–2015, 2015–2016) at the study area is presented in Fig. 1. Aligarh District is characterized with different soil types including sandy, loamy, sandy loam, and clayey loam; however, the soil at the experimental site was sandy loam.

Plant material and growth conditions

Field experiments were conducted in rabi mustard crop season (October–March) for 2 years (2014 and 2015) adopting four rapeseed-mustard species, namely *B. campestris* cv. Pusa Gold, *B. juncea* cv. Pusa Bold, *B. napus* cv. GSL1, and *B. nigra* cv. IC247. These crops are also known by their common name as Indian mustard, yellow mustard, gobhi sarson, and black mustard, respectively. The authentic newer seeds of each species were collected from the National Research Centre on Plant Biotechnology (NRCPB) of the Indian Agriculture Research Institute (IARI), New Delhi, India. In the laboratory, seeds were put into a pot filled with 0.5 l of water, and the seed materials were stirred slowly so that undeveloped or damaged seeds will float on the water surface. These were removed, and the seeds were kept healthy for use in disinfection treatment. The seeds were surface disinfected by dipping them with 5% aqueous solution of sodium hypochlorite (NaOCl) followed by repeated washings with double-distilled water (DDW) and then dried in shade before sowing (Sauer and Burroughs 1986). Seeds were sown under standard day-night conditions having a 16-h/8-h day/night cycle at photosynthetically active radiation (PAR) (> 950 mmol m^{-2} s^{-1}), temperature (~23 °C), relative humidity (~75%), and rainfall (30–40 mm).

Treatment details

Two field experiments were set in complete factorial design with 5 m × 2 m dimension plots, arranged with three replicates of each experimental crop. The treatment setup along with ground water (GW) and WW is schematically represented in Table 1. Before sowing, the plots were plowed manually up to a depth of 15–25 cm to make the soil well pulverized and to ensure maximum soil aeration and complete removal of weeds. Standard agro-techniques, recommended for the cultivation of rapeseed-mustard, were employed for preparing a well-leveled and weed-free field with the required number of experimental plots. The plots were provided with supplementary water to ensure proper soil moisture at the subsurface. This was done to avoid seed germination failure. During the first-year experiment, crops were irrigated with GW and WW along with the recommended uniform dose of N, P, and K (80:45:45 kg ha^{-1}) to obtain the suitable irrigation water. Based on the first-year experimental results, the second-year experiment was conducted with WW only along with two different, recommended and less than the recommended (80:45:45 kg ha^{-1} and 60:30:30 kg ha^{-1}) doses of NPK fertilizer, respectively, to test the feasibility of the research work using WW for fertilizer nutrients. Fertilizer NPK was applied into soil 1 day before sowing in the form of commercial grade urea, single superphosphate (SSP), and muriate of potash (KCl), respectively. Seeds (five to ten per hill) were sown in rows by the dibbling method. The date of sowing for both-year experiments was 20 October 2014 and 2015. The rows were kept at the distance of about 20 cm from one another. The mature crop was harvested on 20 March 2015 and 2016. The experimental plot received three irrigations up to crop maturation. The first irrigation was provided when vegetative seedlings had reached an age (A) of 20 days after germination. The

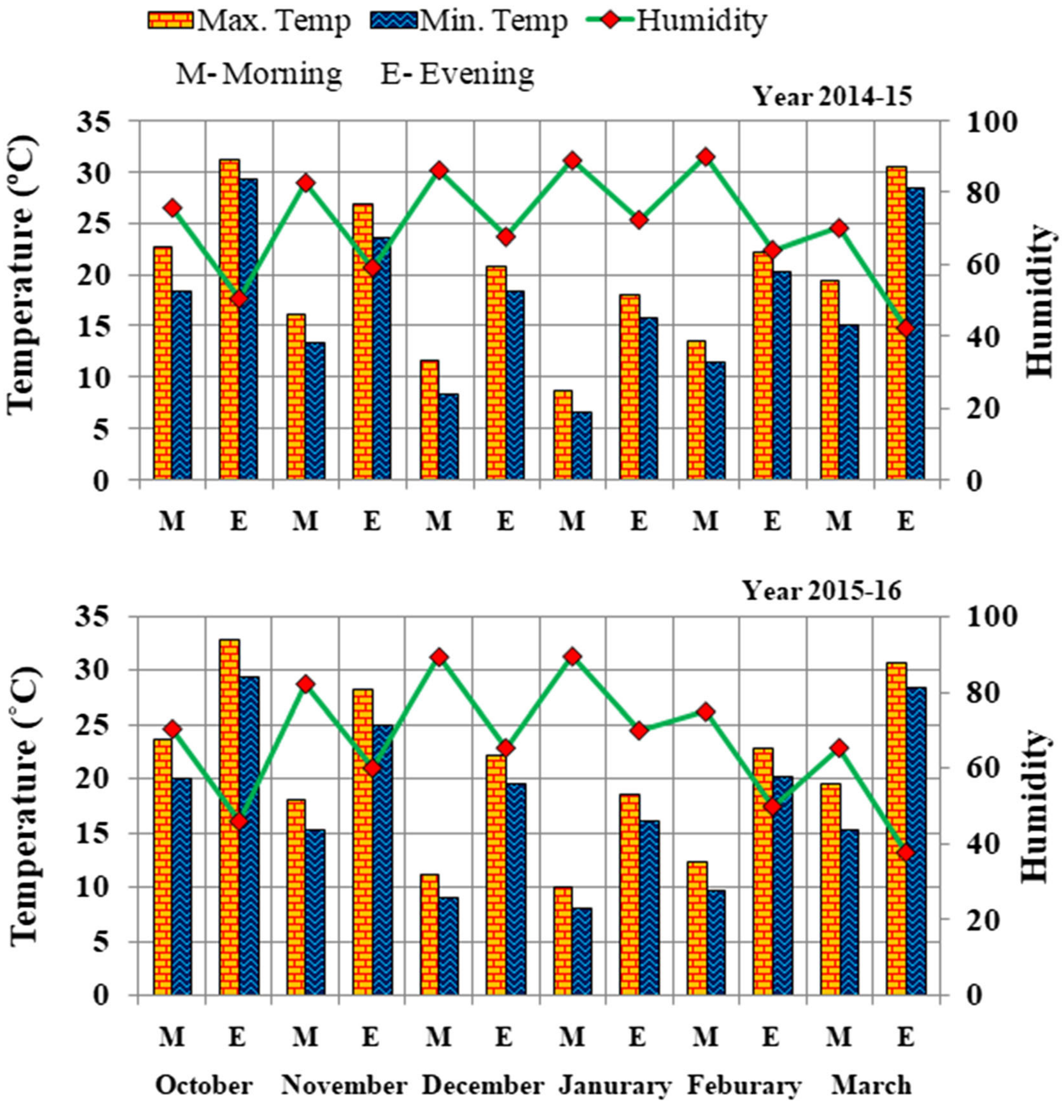

Fig. 1 Meteorological data showing the monthly mean temperature (°C) and humidity (%) range of the experimental period (October–March 2014–2015 and 2015–2016) at the Agriculture Research Farm, Aligarh Muslim University, Aligarh, India. The data was obtained from the Remote Sensing and GIS Applications Centre, Department of Physics, A.M. University, Aligarh

second irrigation was scheduled at the gap of 20 days from the first irrigation time as plants began to start growing faster. The third and last irrigation was done at the age of 70–80 days after germination. The weeding of the experimental plot during the whole cropping season was done manually to avoid the growth productivity. Further, the aphid attacks were managed effectively by applying pesticides at the start of the reproductive stage, when flowering of the plants began, and subsequently, when the formation of pods (siliquae) was taking place. As such, the crops are more susceptible to pests.

Sampling and analysis of ground water and waste water quality components

Aligarh District of Uttar Pradesh State is basically famous for lock manufacturing industries. Besides, in this district area, the agro-based industrial units based on the manufacturing of edible oil, dairy, decoration articles, and bakery products are also growing. These industries release a huge volume of waste water which mixed together with household and municipal units of waste water. The mixture of waste water flows through the drainage system and gets discharged into numbers of big

Table 1 Scheme of the treatments given in randomized complete block design during the 2-year experiments (2014–2015 and 2015–2016)

Brassica species	Year 2014–2015		Year 2015–2016	
	$N_{80}P_{45}K_{45}$ (applied uniformly)		WW+ $N_{60}P_{30}K_{30}$	WW+ $N_{80}P_{45}K_{45}$
	GW	WW		
B. campestris	+	+	+	+
B. juncea	+	+	+	+
B. napus	+	+	+	+
B. nigra	+	+	+	+

Subscript values denote the quantity of fertilizers in kilograms per hectare

GW ground water, *WW* waste water

drains which run outside the city. From these drains, local farmers pump this waste water for mustard and other crop irrigation in agricultural lands without being aware of any of its adverse effect on soil and plants. In the present study, WW for analysis was collected in 2-l plastic bottles from these drains. For watering of experimental plots, it was collected in 100-l jerry canes at weekly intervals. The samples of WW were analyzed for different physical and chemical properties as per the standard procedure of the American Public Health Association (APHA) (1985) and compared with GW and permissible limits set by Indian Standards Institution (ISI) (1974, 1983) (ISI Standards No. 2490, 10500) and Ayers and Wescot (1994) to ascertain the quality of WW components (Table 2). Experimental crops were irrigated with WW as well as GW, and the frequency of WW application was maintained at the alternate interval of WW application. It was scheduled till maturity. Prior to application of WW in experimental plots, it was screened and passed through filtration media to remove all large suspended objects then allowed to irrigate the crops.

Sampling and analysis of soil for physico-chemical properties

The soil samples from experimental plots were collected before and after application of waste water. The soil samples were sampled randomly from about 15 cm depth. In the laboratory, the soil samples were ground with the help of a mortar and pestle and then passed through a 2-mm sieve. The fine powder form of soil samples was oven-dried at 105 °C prior to use for the analysis of various physico-chemical characteristics and heavy metals. Soil characteristics of the experimental field samples before sowing (or application of GW and WW) during 2 years are presented in Table 3. However, the influence of GW and WW irrigation on some soil physico-chemical properties was also analyzed just after their application during the 2-year experiments which are presented in Table 4. Enhanced soil properties motivated us to select WW for irrigating the field during the second year (2014–2015) for further evaluation of its next feasibility as fertilizers on crops' growth and yield along with heavy metal contents. The detailed methodology for various soil parameters is given elsewhere (Sahay et al. 2015).

Measurements of agronomic variables

The measurements of all plant growth and physiological variables were carried out at the age of 35 days after sowing (DAS), 70 DAS, and 105 DAS which was named as the vegetative stage, flowering initiation stage, and siliquae development stage, respectively, while the yield variables were measured at the time of the crop harvest at maturity. Three plants of each *Brassica* species were dug out carefully from plots, washed, and soaked on a blotting sheet to record the fresh weight of shoot and root, separately using a digital weighing balance. Dry weight of shoot and root was determined after drying the samples in an oven at 80 °C. Length of the plants was recorded by using a meter scale. Leaf number was measured manually by counting the leaf per plant. Leaf area was measured by a LA211 leaf area meter (Systronics, Hyderabad, India).

Among the physiological and biochemical variables, nitrate reductase (NR) activity, carbonic anhydrase (CA) activity, total chlorophyll and carotenoid contents, and leaf N, P, and K contents were measured following the different method as described by Jaworski (1971), Dwivedi and Randhawa (1974), Hiscox and Israelstam (1979), Lindner (1944), and Fiske and Subba Row (1925). The detailed methodology employed for the determination of these variables has been provided earlier in our reports (Sahay et al. 2017).

The yield variables (such as the number of siliquae, seed number per siliqua, 1000 seed weight, siliqua length, seed yield, oil content, and oil yield) were

Table 2 Physico-chemical characteristics of ground water (GW) and waste water (WW) for the experiments in years 2014–2015 and 2015–2016 with the standard water quality showing the permissible toxic limit

Characteristics	2013–2014		2014–2015		Normal range[a,b]
	GW	WW	GW	WW	
pH	6.9 ± 0.3	8.3 ± 0.4	7.1±0.4	8.1 ± 0.7	6.5–8.5[a]
Electrical conductivity (EC) (μmhos cm^{-1})	711 ± 35.5	840 ± 42.0	723 ± 50.6	855 ± 68.4	250–3000[a]
Total solids (TS)	902 ± 45.1	1209 ± 60.4	907 ± 63.4	1244 ± 98.5	–
Total dissolved solids (TDS)	525 ± 26.2	632 ± 31.6	537 ± 37.5	657 ± 52.6	< 2000[a]
Total suspended solids (TSS)	420 ± 21.0	675 ± 33.7	425.5 ± 29.7	682 ± 53.5	100[b]
Dissolved oxygen (DO)	6.70 ± 0.3	2.22 ± 0.1	7.05 ± 0.4	2.26 ± 0.2	100[b]
Biological oxygen demand (BOD)	15.99 ± 0.8	160.75 ± 8.0	16.36 ± 1.1	164.54 ± 12.6	–
Chemical oxygen demand (COD)	35.20 ± 1.7	119.19 ± 5.9	38.24 ± 2.7	123.96 ± 9.7	–
Hardness	110.0 ± 5.5	320.0 ± 16.0	113.5 ± 7.4	325.5 ± 26.1	–
Magnesium (Mg^{++})	17.48 ± 0.8	128.17 ± 6.4	17.41 ± 1.1	125.69 ± 10.5	< 61[a]
Calcium (Ca^{++})	23.91 ± 1.1	41.48 ± 2.0	23.29 ± 1.3	42.07 ± 3.6	< 400[a]
Potassium (K^+)	6.08 ± 0.3	16.67 ± 0.8	6.80 ± 0.4	17.90 ± 1.4	< 2.0[a]
Sodium (Na^+)	16.36 ± 0.8	46.67 ± 2.3	16.87 ± 1.1	48.97 ± 9.3	< 460[a]
Bicarbonate (HCO_3^-)	61.00 ± 3.0	86.00 ± 4.3	60.5 ± 4.2	90.00 ± 7.2	< 610
Carbonate (CO_3^{--})	33.20 ± 1.6	118.24 ± 5.9	40.00 ± 2.8	119.76 ± 9.5	–
Chloride (Cl^-)	59.73 ± 2.9	113.10 ± 5.6	62.60 ± 4.3	116.76 ± 9.3	< 350[a]
Phosphate (PO_4^-)	0.37 ± 0.01	1.04 ± 0.05	0.26 ± 0.02	1.09 ± 0.1	< 2.0[a]
Sulfates (SO_4^{--})	35.28 ± 1.7	46.52 ± 2.3	57.57 ± 4.0	64.82 ± 5.1	–
Nitrate N (NO_3-N)	0.74 ± 0.03	1.20 ± 0.06	0.75 ± 0.05	1.21 ± 0.1	< 10.0[a]
Ammonium N (NH_3-N)	1.13 ± 0.05	5.21 ± 0.26	1.21 ± 0.08	5.31 ± 0.4	5.0[a]
Chromium (Cr)	0.009 ± 0.00	0.021 ± 0.001	0.011 ± 0.00	0.068 ± 0.001	0.05[b]
Copper (Cu)	0.090 ± 0.003	0.263 ± 0.013	0.105 ± 0.007	0.229 ± 0.018	0.05–1.5[b]
Nickel (Ni)	0.045 ± 0.001	0.375 ± 0.018	0.062 ± 0.001	0.418 ± 0.033	< 0.01[b]
Lead (Pb)	0.019 ± 0.00	0.038 ± 0.001	0.012 ± 0.00	0.044 ± 0.003	0.10[b]
Cadmium (Cd)	0.005 ± 0.00	0.008 ± 0.00	0.003 ± 0.00	0.013 ± 0.001	0.01[b]

All determinations are in milligrams per liter or as specified except for pH. All the values are the mean of three replicates ± SE. Dash (–) means no standard developed

[a] Food and Agriculture Organization (FAO) (2006, 2011) and Ayers and Wescot (1994)

[b] Indian Standards Institution (ISI) (1974, 1983) (ISI Standards No. 2490, 10500)

measured after harvesting the crops on 20 March of the years 2015 and 2016. For extraction of oil, seeds were crushed into a fine meal and allowed into a Soxhlet extractor filled with petroleum ether for 6–7 h. The extracted oil was used to determine the oil yield (seed yield × oil content) and oil content, as per the formula given elsewhere (Sahay et al. 2015, p. 8).

Heavy metal analysis

The concentrations of heavy metals such as chromium (Cr), Cu, nickel (Ni), lead (Pb), and cadmium (Cd) in soil, waters (GW and WW), and plant parts (leaves/seeds) were analyzed by employing a procedure described by Baker and Amacher (1982), Clesceri et al. (1989), and Allen et al. (1986), respectively. The heavy metal contents in plant samples were analyzed during the second-year experiment only.

The soil samples were ground in a stainless blinder and passed through the 2-mm sieves then kept in an oven for further analysis. Two-gram fine-powdered form soil samples were digested with a mixture of HF-HNO_3-$HClO_4$-H_2SO_4.

Table 3 Physico-chemical characteristics of the field soils before sowing used for the experiments in the years 2014–2015 and 2015–2016

Soil characteristics	Soil depth (0–15 cm)	
	Year 2013–2014	Year 2014–2015
Texture	Sandy loam	Sandy loam
Color	Light brownish	Light brownish
CEC (mEq 100 g^{-1} soil)	3.17 ± 0.22	2.92 ± 0.23
pH	7.1 ± 0.47	7.8 ± 0.62
Organic carbon (%)	0.821 ± 0.049	0.758 ± 0.06
EC (μmhos cm^{-1})	303.0 ± 24.2	295.0 ± 23.7
NO_3-N (g kg^{-1} soil)	0.311 ± 0.021	0.292 ± 0.02
Phosphorus (g kg^{-1} soil)	0.131 ± 0.007	0.136 ± 0.01
Potassium	25.00 ± 1.75	21.00 ± 1.68
Magnesium	31.68 ± 2.21	31.42 ± 2.51
Calcium	23.11 ± 1.84	19.31 ± 1.54
Sodium	13.66 ± 1.22	12.02 ± 0.96
Bicarbonate	22.81 ± 0.896	19.33 ± 1.54
Carbonate	95.89 ± 7.61	78.29 ± 6.26
Sulfate	18.61 ± 1.11	17.66 ± 1.41
Chloride	35.21 ± 1.76	28.22 ± 2.25
Chromium (mg kg^{-1} soil)	0.042 ± 0.002	0.031 ± 0.002
Copper (mg kg^{-1} soil)	17.23 ± 1.20	14.23 ± 1.20
Nickel (mg kg^{-1} soil)	11.31 ± 0.678	10.31 ± 0.678
Lead (mg kg^{-1} soil)	0.063 ± 0.003	0.050 ± 0.003
Cadmium (mg kg^{-1} soil)	0.009 ± 0.0007	0.011 ± 0.0007

All determinations are in milligrams per liter (1:5 soil:water extract), except for pH or as specified. All the values are the mean of three replicates ± SE

Samples of GW and WW were filtered, and 25 ml was taken in a glass beaker and kept for slow digestion with HCl-HF-HNO_3-$HClO_4$-H_2SO_4. $KMnO_4$ was added to eliminate the interference of sulfides, etc., in water samples.

The plant samples were collected and washed first with running tap water and then by distilled water to remove extraneous matter, and then the samples were oven-dried at a temperature of ~70 °C for 24 h. The dried samples were ground, passed through a 1-mm sieve, and further proceeded for analysis of heavy metal concentration through acid digestion. One-gram dried leaf powder and seed samples were digested with 10 ml of HNO_3, H_2SO_4, and $HClO_4$ mixture in a 5:1:1 ratio.

In all the three types of samples, digestion was carried on a hot plate digester (LabTech Graphite-EHD36S) at the temperature 80–150 °C. During heating, samples turned into black color along with reddish-orange smoke. Samples were cool down at ambient temperature, and two to three drops of concentrated HNO_3 and 30% H_2O_2 was added. The procedure of heating and cooling repeated three to four times, and digestion continued until a solution will not turn to transparent with white smoke that is an indication of complete digestion of samples. The blank sample containing only acids was also run parallel to these samples. The cooled samples were filtered, and volume was maintained by adding DDW. Afterwards, sample solutions were analyzed at different wavelengths for different heavy metal concentrations using an atomic absorption spectrophotometer (AAS) (SensAA GBC Avanta Var. 2.02). A series of *stock standard solutions* was used to prepare *working standard solutions* of Cr, Cu, Ni, Pb, and Cd, and then a magnitude of each metal was measured by their calibration curve which plotted between concentrations of standards versus absorbance. The concentration was calculated against the graph.

Statistical analysis

The results (mean ± SE) of three replicates ($n = 3$) were subjected to two-way analysis of variance (ANOVA) (Table 5), to analyze the statistical significance following the method given by Gomez and Gomez (1984). F test was applied to determine the least significant difference (LSD) at the level of probability ($p \leq 0.05$). All statistical analyses were performed using MS Excel and SPSS 14.0. The graphs were plotted using Origin 6.1.

Results and discussion

Water fertilization at different growth stages of the four species of *Brassica* was the considered objective to know the feasibility of WW irrigation based on the soil properties and plant growth responses. Results in the light of physico-chemical characteristics of irrigation water (Table 2) and soil (Tables 3 and 4) are described which showed WW irrigation has an improvement in soil nutrient status which, in turn, leads to increase in growth, physiological, and yield (Tables 6, 7, and 8; Figs. 2, 3, and 4, respectively) attributes with permissible metal content in leaves and seeds (Figs. 5 and 6, respectively). The results of WW quality components and their modulating effects during the 2-year experiments on the four *Brassica* species are described below.

Table 4 Effect of ground water (GW) and waste water (WW) irrigation on the relevant field soil properties with their phytotoxically excessive levels of five heavy metals in soil (PPM DW) (source: Pendias and Pendias 1992) for the experiments in the years 2015 and 2016

Treatments	Physico-chemical characteristics						Heavy metals				
	pH	EC	Organic carbon	NO_3-N	P	K	Cr	Cu	Pb	Ni	Cd
First year (2014–2015)											
Soil + GW	7.5 ± 0.45	348 ± 24.3	0.893 ± 0.03	0.333 ± 0.01	0.149 ± 0.05	26.3 ± 1.84	0.126 ± 0.002	18.09 ± 0.54	0.073 ± 0.00	12.09 ± 0.72	0.011 ± 0.00
Soil + WW	7.9 ± 0.55	372 ± 22.3	1.22 ± 0.05	0.352 ± 0.02	0.162 ± 0.08	29.0 ± 2.32	0.336 ± 0.01	20.79 ± 1.03	0.592 ± 0.23	15.08 ± 0.75	0.062 ± 0.001
Second year (2015–2016)											
Soil + GW	7.6 ± 0.31	339 ± 29.3	0.851 ± 0.11	0.320 ± 0.03	0.131 ± 0.03	21.9 ± 1.24	0.116 ± 0.001	17.77 ± 0.40	0.060 ± 0.002	11.84 ± 0.61	0.008 ± 0.00
Soil + WW	7.8 ± 0.24	389 ± 31.3	1.45 ± 0.09	0.372 ± 0.02	0.188 ± 0.10	41.0 ± 2.00	0.326 ± 0.01	22.79 ± 0.99	0.564 ± 0.19	16.02 ± 0.55	0.073 ± 0.001
Permissible limits	–	–	–	–	–	–	75–100[a–c]	100[a–d]	100[a–d]	100[a–d]	3–8[a–d]

[a] El-Bassam and Tietjen (1977)

[b] Linzon (1978)

[c] Pendias (1979)

[d] Kloke (1979)

Effect of waste water on relevant physico-chemical characteristics of soil

Overall, all the analyzed physico-chemical quality parameters showed much higher nutrient values for WW than for GW (Table 2). The physico-chemical analysis of Aligarh City's WW during both the experimental years has revealed that its alkaline nature (pH 8.1–8.3), electrical conductivity (EC, 840–855 μmhos cm^{-1}), total dissolved solids (TDS, 632–657), and ions of chloride (Cl^-, 113.10–116.76), calcium (Ca^{++}, 41.48–42.07), magnesium (Mg^{++}, 125.69–128.17), sodium (Na^+, 46.67–48.97), potassium (K^+, 16.67–17.90), and sulfate (SO_4^{--}, 46.52–64.82) (mg l^{-1}) were within the permissible limits of irrigation water quality except for the potassium set by Food and Agriculture Organization (FAO) (2006, 2011), Ayers and Wescot (1994), and Indian Standards Institution (ISI) (1974, 1983) (ISI Standards No. 2490, 10500). The content of Cl^- ion was also low and thus could not make WW to cause toxicity. The excessive richness of phosphorus (PO_4^{---}) and nitrate-nitrogen (NO_3^{--}-N) in WW may cause eutrophication, if diverted into the water body. In the present study, the contents (1.04–1.09 mg l^{-1} and 1.20–1.21 mg l^{-1}, respectively) in WW were not in excess and their presence in WW makes it an excellent source to supplement them which leads to a lower need of inorganic fertilizers and less degradation of environment (Singh et al. 2012).

Nutrient elements in WW were also more when compared to those in control soil except for the potassium which was due to Aligarh's soil known for being rich in potassium. The effect of GW and WW application on relevant physico-chemical parameters of soil is given in Table 4. The characteristics of control soil (pre-sowing soil) have been found to change positively more on irrigation with WW- than GW-irrigated soil. There was no drastic change which occurred in soil texture with the application of waste water. Further, WW had a significant effect on soil pH where the pH level of the control soil was found to be 7.1 (Table 3) while the pH level of the WW-irrigated soil was found to be slightly higher from that of the GW-irrigated soil which ranges from 7.5–7.6 to 7.8–7.9 (Table 4) which is said to be the most desirable and suitable of nutrients available in agricultural soil. A pH value at the 6.0–8.2 range provides bacterial activity predominantly and is favorably considered for the plant nutrient uptake and maximum yield of crops. The EC of control soil (295 μmhos cm^{-1} and 303 μmhos cm^{-1}) was also influenced positively, where that of WW increased up

Table 5 Model of the analysis of variance (ANOVA) experimental design and randomized complete block during the experimental years 2014–2015 and 2015–2016

Source of variation	*df*	SS	MSS		*F* (variance) value	Significant value ($p < 0.05$)
Waters/treatments	$(n-1)=1$	SS_{water}	MS_{water}	$=\frac{SS_{waters}}{n-1}$	$F1=\frac{MS_{water}}{MS_{interaction}}$	If $F1 > F_{0.05}$
Species	$(k-1)=3$	$SS_{treatment}$	$MS_{treatment}$	$=\frac{SS_{interactions}}{(n-1)\ (k-1)}$	$F2=\frac{MS_{treatment}}{MS_{interaction}}$	If $F2 > F_{0.05}$
Interactions	$(n-1)\times(k-1)=3$	$SS_{interaction}$	$MS_{interaction}$			
Error	14					
Total	23	SS_{total}	$(nk-1)=(N-1)$			

F1 is the variance of waters with *df* at $(n-1)$ vs $(n-1)(k-1)$. F2 is the variance of treatments with *df* at $(n-1)$ vs $(n-1)(k-1)$

df degree of freedom, *SS* sum of square, *MSS* mean sum of square

to 372 μmhos cm^{-1} and 389 μmhos cm^{-1} which was more as compared to GW application (339 μmhos cm^{-1} and 348 μmhos cm^{-1}) during both the years, respectively (Tables 3 and 4). However, the enhancement in EC indicates the tendency of WW remains good below the range from 840 to 855 μmhos cm^{-1} (Table 2). The organic carbon of control soil (0.821% and 0.758%) (Table 3) on WW irrigation increased up to 1.22% and 1.45% which is higher than GW soils, i.e., 0.839% and 0.821% for the 2-year experiments, respectively (Table 4). This indicates that the use of WW helps to enhance the fertility status of soil of rabi crops. As such, the organic carbon in the WW-mixed soils was observed to be significantly different from that in the GW-irrigated soils, as presented in Table 4. The quality of using WW in improving the soil fertility was also proved by the recorded significantly higher amount of nitrogen (as NO_3^{-}-N), phosphorus, and potassium in the WW-applied soil over the GW-applied soil and control soil (Table 4). Among the three nutrients, plants respond quickly to the application of nitrogen, thereby encouraging the vegetative growth. The increase in nitrogen is due to the use of WW which contains the higher amount of NO_3^{-}-N than GW (Table 2). The soil analysis data across the water treatments showed that the microelements, viz., Cr, Cu, Pb, Ni, and Cd, were found slightly higher in soil applied with GW and WW in respect to the control soil (Table 4). The extractable metals in WW and WW-irrigated soil were observed within the permissible toxic limit of Indian Standards Institution (ISI) (1974, 1983) (ISI Standards No. 2490, 10500) and Pendias and Pendias (1992), respectively. Overall, WW has increased the soil pH, EC, NO_3^{-}-N, P, K, and organic carbon including heavy metals positively, and the findings were in conformity with an earlier study reported by Kaushik et al. (2005).

Plant growth, physiological, and yield characteristics

The results of the first-year experiment on the comparative study of the effect of GW and WW under a uniform dose of recommended NPK, i.e., 80 kg ha^{-1}, 45 kg ha^{-1}, and 45 kg ha^{-1}, respectively, have revealed that WW was superior over GW as WW increased all growth, physiological, and yield parameters (Tables 6 and 7; Fig. 2). An increase of 19.25%, 27.09%, and 27.02% shoot fresh weight; 16.98%, 16.29%, and 21.35% root fresh weight; 17.92%, 17.73%, and 16.94% shoot dry weight; 21.88%, 19.70%, and 22.90% root dry weight; 8.65%, 17.27%, and 2.66% leaf area; and 18.58%, 18.74%, and 28.83% leaf number at 35 DAS, 70 DAS, and 105 DAS, respectively, was recorded with all the WW-subjected *Brassica* plants, approving its better efficiency than GW (Table 6). The role of WW application was again proved efficacious as it increased 12.70%, 10.50%, and 13.01% NR activity; 12.33%, 11.76%, and 9.51% CA activity; 12.33%, 13.47%, and 2.02% chlorophyll content; 8.73%, 13.07%, and 13.45% leaf N content; 9.85%, 11.33%, and 9.98% leaf P content; and 1.12%, 11.62%, and 13.63% leaf K content over GW application at all three growth stages, respectively (Table 7). The irrigation of *Brassica* crops with WW proved to be more efficient when seed and oil yield was found to be enhanced by 3.1% and 4.22% over GW irrigation, respectively (Fig. 2). The overall performance of WW during the first-year experiment indicated that WW may be a good source of irrigation water and it may help to reduce the load of more use of freshwater or ground water to irrigate the various crops including rapeseed-mustard in the agricultural field. Although WW has approved itself as the source of irrigation water, it was presumed that an increase in growth,

Table 6 Effect of ground water (GW) and waste water (WW) irrigation under the uniform dose of NPK (80:45:45 kg ha^{-1}) on the growth parameters of field-grown rapeseed-mustard species at the age of 35 days after sowing (DAS), 70 DAS, and 105 DAS for the year 2014–2015

Age (DAS)	Treatments	Fresh weight (g)				Dry weight (g)				Leaf characteristics			
		Shoot		Root		Shoot		Root		Leaf area		Leaf number	
		GW	WW	GW	WW	GW	WW	GW	WW	GW	WW	GW	WW
35	*B. campestris*	5.22	6.32	0.334	0.394	0.671	0.782	0.045	0.057	35.75	40.67	7.72	8.43
	B. juncea	9.34	11.22	0.457	0.537	0.843	1.01	0.065	0.077	43.49	55.58	6.86	7.24
	B. napus	7.24	8.33	0.442	0.486	0.753	0.871	0.061	0.069	38.38	43.73	5.61	6.23
	B. nigra	3.33	4.10	0.380	0.470	0.556	0.666	0.040	0.054	31.84	37.26	7.95	8.67
	Mean	*6.28*	*7.49*	*0.403*	*0.471*	*0.705*	*0.832*	*0.052*	*0.064*	*37.36*	*44.31*	*7.03*	*7.64*
70	*B. campestris*	16.25	21.53	1.34	1.61	2.08	2.54	0.376	0.470	43.00	48.87	14.48	17.10
	B. juncea	19.85	25.37	1.82	2.16	4.27	5.04	0.535	0.638	58.95	71.58	16.76	20.42
	B. napus	35.83	41.43	3.23	3.61	4.70	5.49	0.689	0.805	71.37	84.09	14.95	16.47
	B. nigra	22.23	31.34	1.65	1.97	3.55	4.12	0.460	0.553	61.44	74.22	12.62	14.98
	Mean	*23.54*	*29.91*	*2.01*	*2.33*	*3.65*	*4.29*	*0.515*	*0.616*	*58.69*	*69.69*	*14.70*	*17.24*
105	*B. campestris*	30.45	39.87	2.62	3.23	7.52	8.16	0.819	1.00	49.46	62.00	45.38	59.29
	B. juncea	49.71	68.41	4.44	5.44	10.02	12.67	2.41	2.88	99.36	132.10	62.95	83.90
	B. napus	60.46	73.56	5.99	7.12	11.28	13.17	2.63	3.23	58.09	68.68	36.95	42.42
	B. nigra	50.38	60.78	4.42	5.41	12.72	14.58	1.83	2.34	46.14	63.24	52.81	65.19
	Mean	*47.75*	*60.65*	*4.36*	*5.30*	*10.38*	*12.14*	*1.92*	*2.36*	*63.26*	*81.50*	*49.52*	*62.70*
LSD (5%)	35	0.394		0.016		0.013		0.005		1.66		0.488	
	70	0.555		0.075		0.052		0.013		2.01		0.704	
	105	1.16		0.102		0.313		0.322		1.93		1.63	

Interaction (T × S) mean values are shown in italics

Table 7 Effect of ground water (GW) and waste water (WW) irrigation under the uniform dose of NPK (80:45:45 kg ha^{-1}) on the physiological parameters of field-grown rapeseed-mustard species at the age of 35 days after sowing (DAS), 70 DAS, and 105 DAS for the year 2014–2015

Age (DAS)	Treatments	Enzyme activity				Total Chlorophyll		Macro-nutrients					
		NR		CA				N		P		K	
		GW	WW	GW	WW	GW	WW	GW	WW	GW	WW	GW	WW
35	*B. campestris*	242.67	280.24	2.22	2.56	0.962	1.07	2.53	2.82	0.390	0.431	2.46	2.79
	B. juncea	296.17	341.07	3.45	3.91	1.12	1.22	3.54	3.89	0.437	0.484	3.26	3.67
	B. napus	280.88	312.69	3.21	3.45	1.16	1.35	3.18	3.37	0.420	0.454	3.00	3.25
	B. nigra	247.77	269.11	2.71	3.10	0.942	1.06	3.00	3.24	0.357	0.393	2.32	2.57
	Mean	*266.87*	*300.77*	*2.89*	*3.25*	*1.04*	*1.17*	*3.06*	*3.33*	*0.401*	*0.440*	*2.76*	*3.07*
70	*B. campestris*	345.05	406.25	3.20	3.59	1.58	1.81	3.38	3.87	0.522	0.582	3.47	3.91
	B. juncea	466.19	434.73	4.69	5.36	1.93	2.22	4.35	4.97	0.583	0.656	4.38	4.97
	B. napus	388.84	473.91	4.39	4.83	1.79	2.02	4.03	4.51	0.559	0.612	4.02	4.39
	B. nigra	357.12	406.18	3.70	4.08	1.75	1.95	3.84	4.29	0.479	0.536	3.36	3.73
	Mean	*389.3*	*430.26*	*3.99*	*4.46*	*1.76*	*2.00*	*3.90*	*4.41*	*0.535*	*0.596*	*3.80*	*4.25*
105	*B. campestris*	327.76	376.67	2.96	3.22	1.23	1.44	3.04	3.32	0.475	0.521	3.02	3.47
	B. juncea	383.05	439.71	4.07	4.55	1.39	1.85	3.97	4.35	0.543	0.603	3.84	4.47
	B. napus	360.29	402.67	3.88	4.22	1.50	1.70	3.59	3.95	0.514	0.556	3.49	3.88
	B. nigra	323.71	357.29	3.18	3.44	1.27	1.49	3.15	3.98	0.440	0.489	2.70	3.01
	Mean	*348.70*	*394.08*	*3.52*	*3.85*	*1.34*	*1.62*	*3.43*	*3.90*	*0.493*	*0.542*	*3.26*	*3.70*
LSD (5%)	35	11.04		0.024		0.012		0.033		0.007		0.150	
	70	13.11		0.035		0.012		0.025		0.012		0.078	
	105	8.81		0.044		0.011		0.032		0.007		0.116	

Interaction (T × S) mean values are shown in italics

Table 8 Effect of waste water (WW) irrigation along with 60:30:30 kg of NPK ha^{-1} and 80:45:45 kg of NPK ha^{-1} on yield and quality parameters of the four rapeseed-mustard species at harvest for the year 2015–2016

Treatments	Yield characteristics						
	Number of siliquae	Siliqua length (cm)	Number of seeds	1000 seed weight (g)	Seed yield (kg ha^{-1})	Oil content (%)	Oil yield (kg ha^{-1})
WW + $N_{60}P_{30}K_{30}$							
B. campestris	75.80 ± 2.45	8.5 ± 0.20	29.75 ± 0.94	3.9 ± 0.04	555 ± 44.35	43.39 ± 0.20	240.20 ± 9.76
B. juncea	125.86 ± 1.85	10.33 ± 0.26	18.5 ± 0.76	4.79 ± 0.03	729 ± 65.76	42.15 ± 0.26	307.42 ± 21.05
B. napus	227.00 ± 1.01	11.41 ± 0.09	20.13 ± 0.13	3.71 ± 0.11	801 ± 64.33	44.41 ± 0.78	355.13 ± 17.67
B. nigra	403.33 ± 2.02	2.91 ± 0.12	9.33 ± 0.66	2.11 ± 0.03	415 ± 29.05	41.19 ± 0.10	170.12 ± 10.12
WW + $N_{80}P_{45}K_{45}$							
B. campestris	71.33 ± 0.66	8.23 ± 0.23	28.56 ± 0.44	3.73 ± 0.05	514 ± 41.12	42.71 ± 0.29	219.23 ± 14.15
B. juncea	120.00 ± 4.72	9.88 ± 0.23	17.33 ± 1.76	4.55 ± 0.05	669 ± 40.14	41.72 ± 0.23	279.82 ± 15.99
B. napus	215.00 ± 6.11	10.88 ± 0.06	19.00 ± 2.30	3.32 ± 0.21	722 ± 50.54	43.25 ± 0.54	312.51 ± 15.37
B. nigra	392.00 ± 4.93	2.7 ± 0.057	8.67 ± 0.66	1.96 ± 0.04	382 ± 26.74	40.76 ± 0.09	155.46 ± 9.30
$LSD_{0.05}$							
T	5.94	0.308	N.S	N.S	10.14	0.480	8.30
S	8.40	0.435	2.65	2.65	15.17	0.678	11.75
T × S	N.S	N.S	N.S	N.S	24.21	N.S	N.S

physiological, and yield parameters may be due to essential elements enriched in WW playing a significant role together with soil. This was checked and proved during the second-year experiment as it was found that WW supplementation into control soil enhances its fertility status by affecting some relevant physical and chemical characteristics of soil positively (Table 4). The application of WW to maintain the soil physical and chemical health has also been reported (Kiziloglu et al. 2008; Singh et al. 2012).

During the second-year experiment, the effect of WW under the two recommended and less than the recommended NPK fertilizer treatments was observed to determine the feasibility of WW for inorganic fertilizers. The use of WW had favorably influenced the crop growth and productivity. As such, ANOVA results showed that the growth, physiological, and yield parameters of all the four *Brassica* crops irrigated by WW coupled with $N_{60}P_{30}K_{30}$ (less than the recommended dose) were found to be better than those by WW coupled with $N_{80}P_{45}K_{45}$ (recommended dose). However, *Brassica* species and their interaction with fertilizers (treatments × species) responded differently at a 5% level of significance at each growth age as presented in Figs. 3, 4, and 5.

In Fig. 3, between the two NPK doses supplemented with WW, $N_{60}P_{30}K_{30}$ showed an increase of 12.08%, 10.94%, and 5.42% shoot fresh weight; 10.20%, 9.12%, and 15.76% root fresh weight; 11.35%, 9.29%, and 5.82% shoot dry weight; 9.22%, 5.82%, and 5.85% shoot length; and 5.69%, 8.39%, and 6.92% leaf area over $N_{80}P_{45}K_{45}$ which proved to be excessive at 35 DAS, 70 DAS, and 105 DAS, respectively. In case of root dry weight and leaf number, the ANOVA test also showed that both the treatments did not significantly differ at the first and second ages of plant; however, at the third age, both the treatments were recorded to have significantly different values where WW with N_{60}-containing treatments enhanced the increase of root dry weight (10.30%) and lead number (7.73%) over WW with N_{80}-containing fertilizer treatment. Root length was found to be not significant at 35 DAS, while at the last two ages of the plant (i.e., 70 DAS and 105 DAS), an improvement of 12.86% and 10.01% was recorded over the latter treatment by the former treatment of WW + NPK.

All the four *Brassica* species were found to be different to one another at each stage of their ages and revealed significant differences ($p < 0.05$). The significantly higher growth production was recorded due to the application of WW over high fertilizer use. As such, WW contains a large amount of nutrients which proved to be applicable in making N, P, and K fertilization optimum or sufficient at

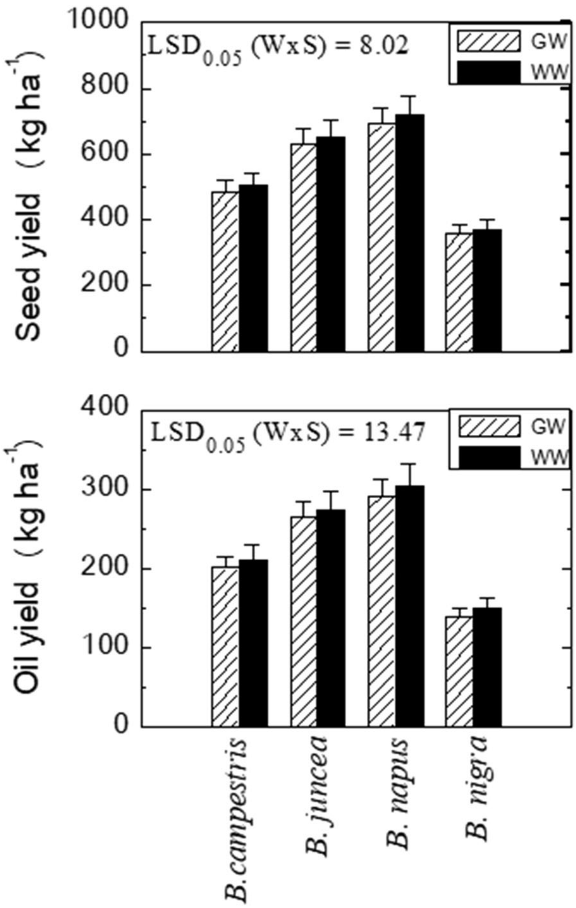

Fig. 2 Bar ± SE showing the comparative effect of ground water (GW) and waste water (WW) under uniform NPK dose (80:45:45 kg ha^{-1}) on seed yield and oil yield of the four rapeseed-mustard species at harvesting for the year 2014–2015

their lower dose, instead of higher dose. Therefore, WW could be used to reduce fertilizer application load as also evident by the results reported by Singh et al. (2012), Chalkoo et al. (2014), and Iqbal et al. (2015, 2017).

The effects of irrigation water and fertilizer with respect to plant age on the response of physiological parameters, viz., NR activity, CA activity, total chlorophyll, carotenoid content, nitrogen content, phosphorus content, and potassium content during the second year (2014–2015), are presented in Fig. 4. As shown in the figure, the use of waste water proved to be more beneficial along with $N_{60}P_{30}K_{30}$ rather than WW × $N_{80}P_{45}K_{45}$ in providing the suitable nutrients required for appropriate physiological processes of growth. As such, WW × $N_{80}P_{45}K_{45}$ showed an inhibited effect on physiological parameters which may be because of unbalanced management of WW nutrients with fertilizers. This indicates that surplus supplementation of nutrient affects plant growth negatively through a disturbance in optimum requirement of nutrient elements for various morpho-physiological developments and nutrient uptake processes from soil (Donahue et al. 1977). Further, it also gives alertness to focus on water productivity rather than yield enhancement, because the proper use of WW can reduce pollution, water disease, and fertilizers which, in turn, lead to significant crop productivity. The treatment WW × $N_{60}P_{30}K_{30}$ gave 4.49%, 6.27%, and 3.91% more values of NR activity; 5.24%, 3.21%, and 3.86% of CA activity; 5.83%, 5.58%, and 6.28% of total chlorophyll; 4.31%, 8.04%, and 3.69% of carotenoid content; 5.21%, 5.65%, and 5.48% of leaf nitrogen content; 15.97%, 4.31%, and 7.41% of leaf phosphorus content; and 6.28%, 4.25%, and 5.26% of leaf potassium content over WW × $N_{60}P_{30}K_{30}$ at the plant's age of 35 days, 70 days, and 105 days, respectively.

The final manifestation of growth is crop yield. The yield of rabi *Brassica* crops was significantly influenced by WW irrigation through the two different NPK treatments. The yield and yield characteristics of crops treated with WW coupled with $N_{60}P_{30}K_{30}$ were found to be better as compared to the application of WW with $N_{80}P_{45}K_{45}$. The ANOVA results revealed a significant variation in the crop yield due to the WW × fertilizer treatments into the soil (Table 8). The observations of yield characteristics at two WW treatment levels were found to be on similar pattern as observed in growth and physiological parameters. Hence, the maximum yield production was recorded with WW at low fertilizer level ($N_{60}P_{30}K_{30}$) and thus marked as appropriate/optimum treatment. However, the use of WW makes the higher fertilizer level ($N_{80}P_{45}K_{45}$) a luxury. As such, the result of two-way ANOVA test showed that WW × $N_{60}P_{30}K_{30}$ increased the seed yield by 7.00% in comparison with the WW × $N_{80}P_{45}K_{45}$. The other yield parameters such as the number of siliqua per plant, seeds per siliqua, 1000 seed weight, biological yield, oil content, and oil yield were increased by 4.21%, 5.60%, 4.54%, 6.78%, 2.90%, 1.59%, and 8.03%, respectively, under the optimum fertilizer treatment. The treatment $N_{80}P_{45}K_{45}$ proved to be excessive as it decreased the seed yield and other yield parameters significantly. This indicates the significance of WW in lowering fertilizer dose.

Among the crops, all the four species were significantly different to each yield parameter observed

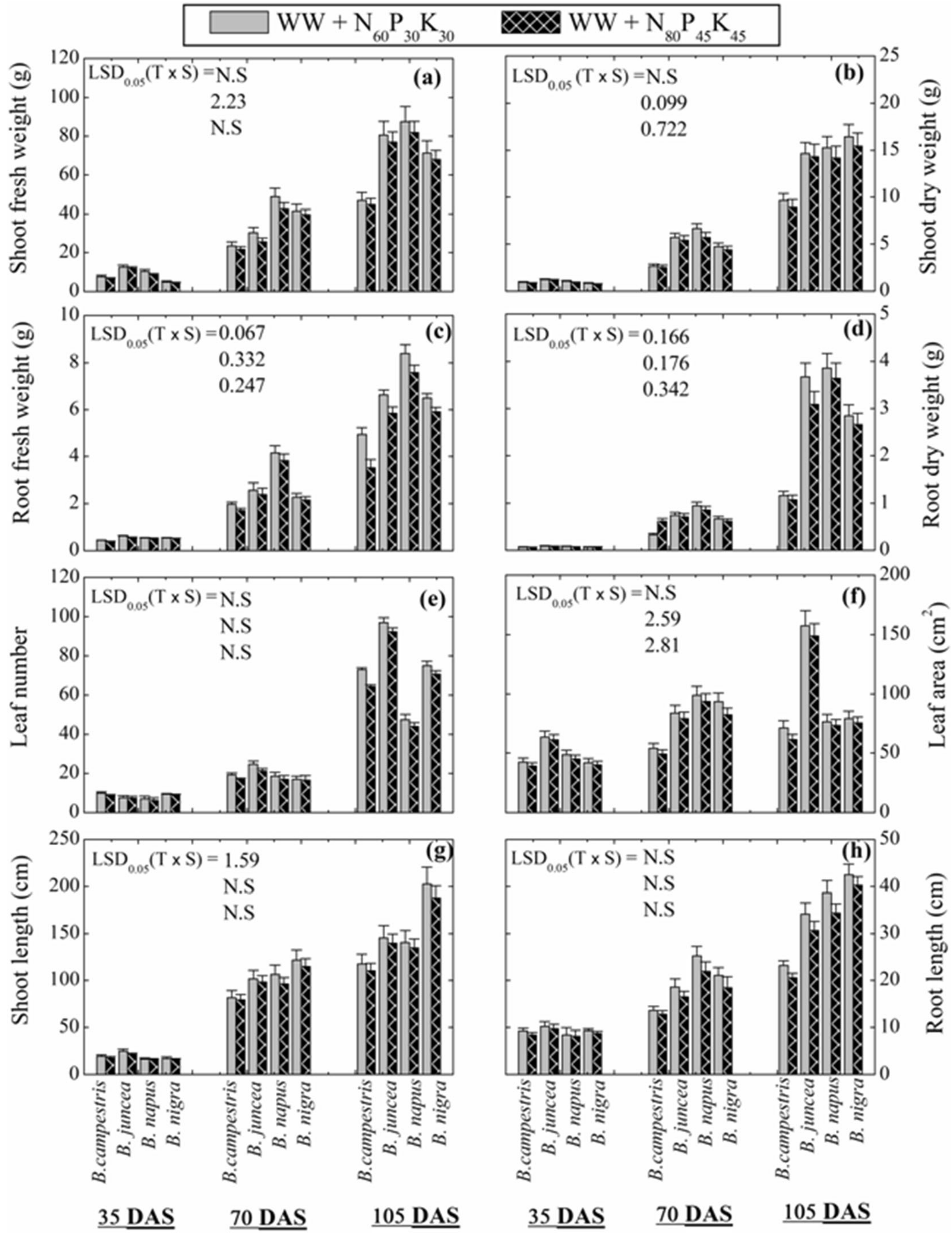

Fig. 3 Bar ± SE showing the effect of waste water along with 60:30:30 kg NPK ha^{-1} and 80:45:45 kg NPK ha^{-1} levels on the following growth parameters: **a** shoot fresh weight, **b** shoot dry weight, **c** root fresh weight, **d** root dry weight, **e** leaf number, **f** leaf area, **g** shoot length, and **h** root length of the four rapeseed-mustard species for the year 2015–2016. Values are presented as mean ± SE ($n = 3$). Least significant difference (LSD) test was determined to compare the significant mean difference at a p value < 0.05 for species (S), treatment (T), and their interaction (T × S)

at a 5% level of significance. As such, among them, the maximum number of siliquae was recorded for *B. nigra*, while *B. campestris* gave the maximum number of seeds, and siliqua length was more in

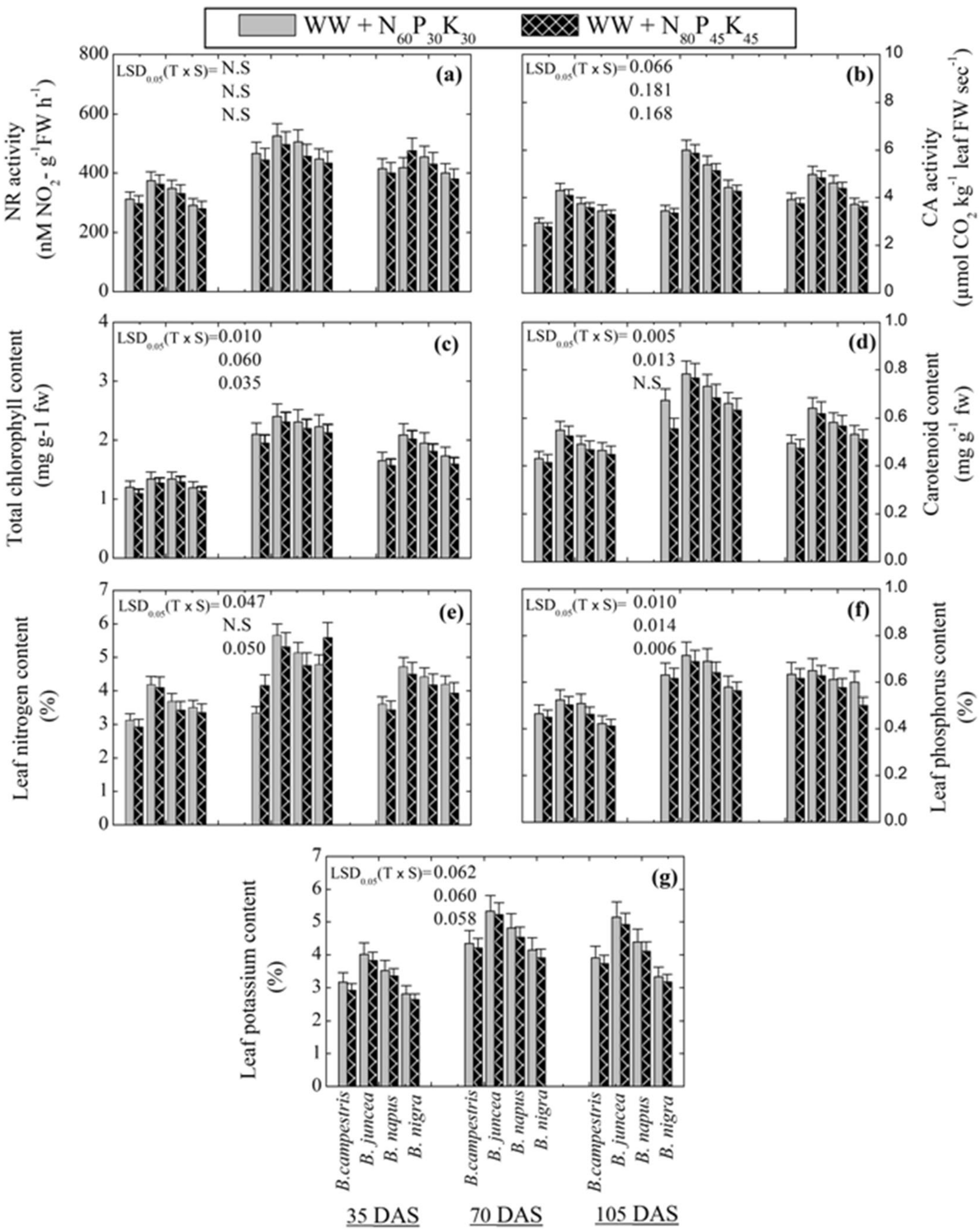

Fig. 4 Bar ± SE showing the effect of waste water along with 60:30:30 kg NPK ha^{-1} and 80:45:45 kg NPK ha^{-1} levels on the following physiological parameters: **a** nitrate reductase (NR) activity, **b** carbonic anhydrase (CA) activity, **c** total chlorophyll, **d** carotenoid content, **e** leaf nitrogen (N) content, **f** leaf phosphorus (P) content, and **g** leaf potassium (K) content of the four rapeseed-mustard species for the year 2015–2016. Values are presented as mean ± SE ($n = 3$). Least significant difference (LSD) test was determined to compare the significant mean difference at a p value < 0.05 for species (S), treatment (T), and their interaction (T × S)

B. napus. Further, *B. juncea* proved to be the best, giving maximum test weight (1000 seed weight) among the rest of three species. However, *B. napus* obtained the status of high seed yield, oil content, and oil yield, where *B. juncea* was closely followed by *B. napus*. The effect of fertilizer treatment,

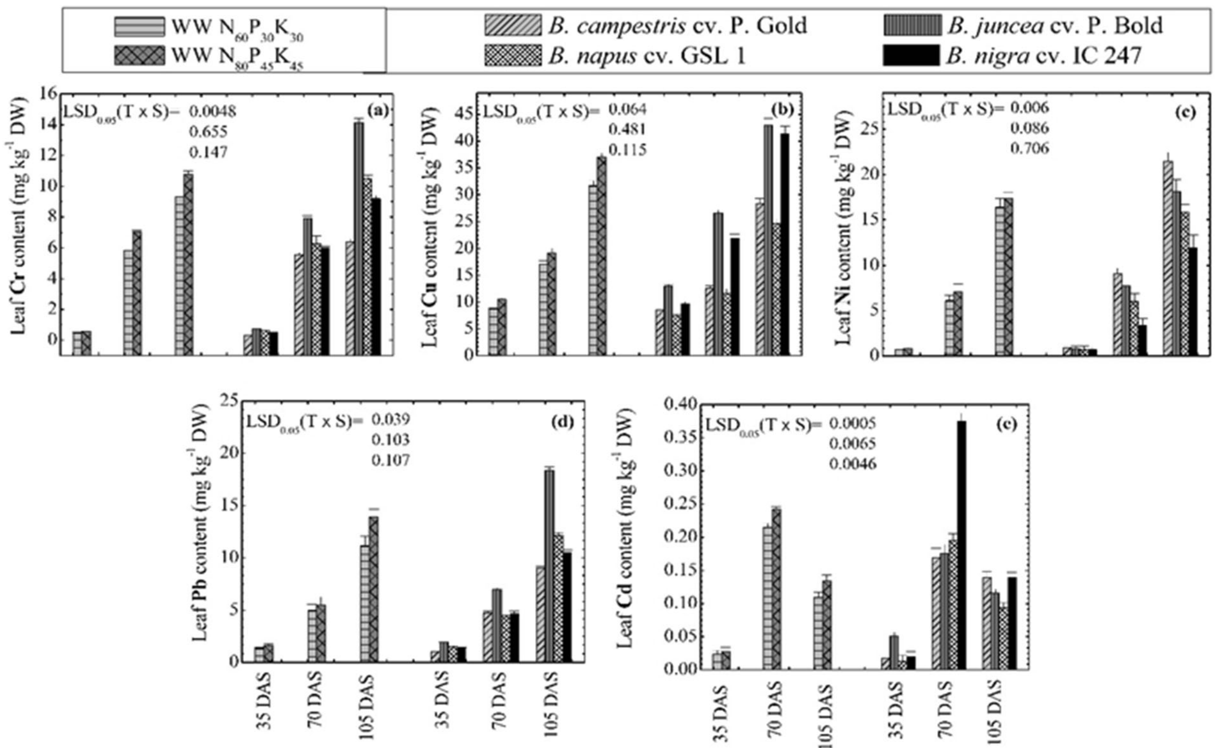

Fig. 5 Bar ± SE showing the effect of waste water along with 60:30:30 kg NPK ha^{-1} and 80:45:45 kg NPK ha^{-1} levels of content of the following metals on leaf: **a** chromium (Cr), **b** copper (Cu), **c** nickel (Ni), **d** lead (Pb), and **e** cadmium (Cd) of the four rapeseed-mustard species for the year 2015–2016. Values are presented as mean ± SE ($n = 3$). Least significant difference (LSD) test was determined to compare the significant mean difference at a p value < 0.05 for species (S), treatment (T), and their interaction (T × S)

Brassica species, and their interaction was nonsignificant for biological yield. The seed yield of yellow sarson (514 kg ha^{-1}), Indian sarson (669 kg ha^{-1}), gobhi sarson (722 kg ha^{-1}), and black mustard (382 kg ha^{-1}) obtained by WW × $N_{60}P_{30}K_{30}$ was found to be more than the seed yield of those obtained by WW × $N_{80}P_{45}K_{45}$. It was due to the appropriate management of WW nutrient level with the lesser amount of inorganic fertilizer in order to deliver plant requirement at their optimum. These differences in yields and yield parameters of the *Brassica* crops can be attributed to their inherent genetic variations and may presumably also arise, as pointed out by Gregory and Crowther (1928), from differences in the efficiency of absorption and utilization of the nutrient constituents of the soil. In fact, there are sufficient references where genotypes have been found to differ very considerably in their ability not only in the absorption but also in the subsequent distribution of nutrients in various parts (Vose 1963; Epstein and Jafferies 1964).

Thus, the second-year experimental data proved the role of WW application as fertilizer nutrients by increasing all growth, physiological, and yield parameters of $N_{60}P_{30}K_{30}$ treatment instead of $N_{80}P_{45}K_{45}$. It indicated that WW irrigation was efficient in providing supportable essential nutrients to crops at their optimum by lowering the fertilizer consumption. The utility of WW to improve soil nutrient levels, as well as crop growth, was also reported by Kiziloglu et al. (2008), Singh et al. (2012), Chalkoo et al. (2014), and Iqbal et al. (2015).

Influence of waste water on heavy metal accumulation

During the second-year experiment, the effect of WW also on heavy metal concentration across the two fertilizer treatments was noted for obtaining a clear picture of results which was expected whether any economy of fertilizer with the management of water would be achieved by using waste water at the cost of heavy metals. The level of heavy metals (Cr, Cu, Pb, Ni, and Cd) in the leaves and seeds of the four *Brassica* species

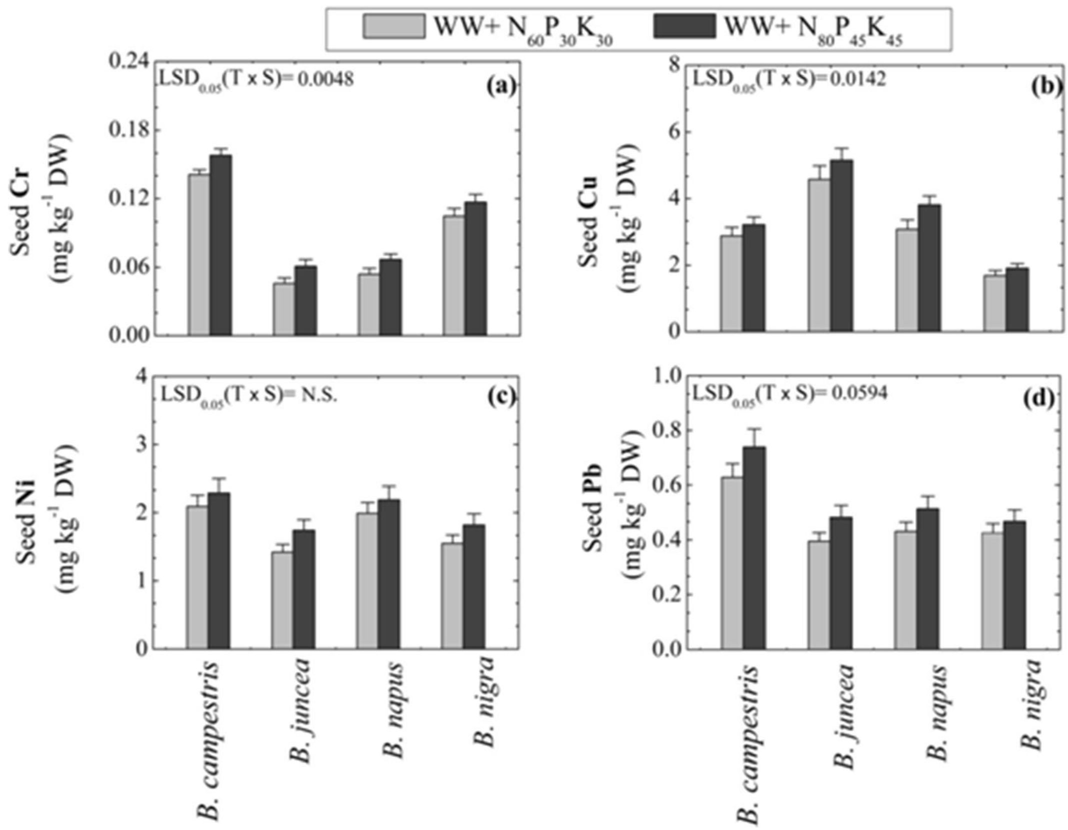

Fig. 6 Bar ± SE showing the effect of waste water along with 60:30:30 kg NPK ha^{-1} and 80:45:45 kg NPK ha^{-1} levels of content of the following metals on seed: **a** chromium (Cr), **b** copper (Cu), **c** nickel (Ni), and **d** lead (Pb) of the four rapeseed-mustard species for the year 2015–2016. Values are presented as mean ± SE ($n = 3$). Least significant difference (LSD) test was determined to compare the significant mean difference at a p value < 0.05 for species (S), treatment (T), and their interaction (T × S)

in WW-treated $N_{60}P_{30}K_{30}$ was significantly lower than that in WW-treated $N_{80}P_{45}K_{45}$ experimental plots (Figs. 4 and 5). It indicates that former fertilizer is, therefore, better because at this treatment level, significantly higher crop production with less metal accumulation was observed as compared to at the latter treatment. Therefore, WW × $N_{80}P_{45}K_{45}$ application proved to be toxic and wasteful which might be due to the level of nutrients in this fertilizer treatment together with the concentration of WW nutrients which become high and could not support to make balance together. It indicated that the use of excessive synthetic fertilizers may be sources of more heavy metal accumulation as also pointed out by Curtis and Smith (2002). It is well reported that the continuous use of WW over the year may deteriorate the quality of the soil which directly affects the nutritive element movement and their availability in soil's air and water. In the present study, however, no significant adverse change in soil quality was observed which may be due to improvement in the soil's physical and chemical status under WW applications (Table 4), and some heavy metals in WW and WW-irrigated soil were under the given toxic limit. Various diverse studies have also been shown that WW irrigation increases and improves the productivity of poor-fertility soil (Kiziloglu et al. 2007) as well as the concentrations of different nutrients involved in plant growth (Rezapour and Samadi 2011; Sacks and Bernstein 2011).

The ANOVA test revealed a significant variation in *Brassica* species to Cr, Cu, Pb, Ni, and Cd accumulation in the plant parts (leaf and seeds) at different ages. As such, accumulation of each metal was maximum in leaves than in seeds which is a common process in many various crops and also reported by Kloke et al. (1984), and further, this difference noted could be due to different cellular mechanisms of bioaccumulation of metals that may control their translocation and partitioning in the plant systems as suggested by Sinha et al. (2007). In addition, heavy metal uptake from medium and their accumulation also varies from plant species to species capacity (Alloway et al. 1990; Zurayk et al. 2001) which affects by several factors such as metal type, soil type, organic matter content in soil, pH of soil, redox potential, cation exchange capacity of soil, surface area and texture of soil particle, the presence and concentration of foreign ions, plant growth rate, and growth conditions (Salim et al. 1993). It was also noted that the concentration of each metal in the seeds was within the limits recommended by prevention of Food Adulteration Act 1954 (Awashthi 2000). In leaves, the concentration of heavy metals for all the four *Brassica* species was below the safe limits at 35 DAS, but not within the safe limits at 75 DAS and 105 DAS recommended by Awashthi (2000) (for Cr, 20 mg kg^{-1}; for Cu, 30 mg kg^{-1}; for Ni, 1.5 mg kg^{-1}; for Pb, 2.5 mg kg^{-1}; and for Cd, 1.5 mg kg^{-1}) and European Union (2001) standard (for Cu, 20 mg kg^{-1}, and for Cd, 0.2 mg kg^{-1}) for green leafy vegetables. This indicated that irrigation of WW for a short term may, thus, not pose any health harm for consumers using leaves of these four crops at market values (35 DAS). However, a practice of long-term reuse of WW (up to 70 DAS and 105 DAS) involved in the excessive accumulation of heavy metals and placed a risk to the urban population consumption due to affected food safety. The plants were still looking healthy and growing well in WW irrigation at a long term which was expected due to accumulating heavy metals to concentration which did not reached up to the recommended phytotoxic level (Cr, 5–30 mg kg^{-1}; Cu, 20–100 mg kg^{-1}; Pb, 30–300 mg kg^{-1}; Ni, 10–100 mg kg^{-1}; and Cd, 5–30 mg kg^{-1}; Pendias and Pendias 1992).

Considering different growth stages and crop growth responses, it is logical to conclude that the enhanced growth influenced the yield-attributing characteristics which were finally manifested in seed yield. In this context, mention may be made by Bunting and Drennan (1966) who emphasized that "the vegetative stage may have an important and direct effect on seed yield." As also evident in the present study, it was observed that the growth parameters like shoot fresh and dry weight increased with an increase in plant age up to the 105-DAS sampling, and the increase was comparatively more from flowering to fruiting stage, which is a common phenomenon in the growth of plants as it happens due to the increase in growth on sigmoid pattern where the growth is comparatively slower initially and faster during the lag phase (Salisbury and Ross 1992). It may further be added that the development of seeds is a death massage to older leaves as most of the mobile nutrients get translocated towards the developing organs (Bidwell 1979). It is based on the fact that nutrients attain their highest concentration in plants during the early stage of growth and exhibit a decline towards maturity. This decrease may be due to the exponential increase in growth (weight and volume) of plants, and as a result of which, "dilution with growth effect" occurs where even high quantities of nutrients appear to be less when expressed on the percent basis (Moorby and Besford 1983).

It may be pointed out that Cr, Cu, Ni, and Pb contents in leaf increased linearly (Fig. 5), which is contrary to N, P, and K contents in leaf (Table 7, Fig. 4). Metal concentrations may rise as leaves age simply due to the continuous passive metal transport into leaf tissues. Movement of metals into older leaves is a way that some plants have to eliminate some of their metal excess by leaf shedding. Among the heavy metals, Cd responded differently which may be due to the young leaves retaining more Cd than the older ones which were in agreement with Perronnet et al. (2003) who explained how heavy metal concentration vary with stage and plant organ.

Conclusions

Aligarh City's WW contained the high amount of organic matter, essential nutrients, and some heavy metals which were not toxic to plants as they were noted not beyond the permissible limits. The use of WW enhanced the physical and chemical status of soil as compared to GW application. The present two first- and second-year field experiments proved WW as a good source of irrigation water and plant nutrients, respectively. As such, application of WW in combination with less fertilizer ($N_{60}P_{30}K_{30}$) gave significantly higher crop growth, seed yield, and oil production rather than the use with high fertilizer dose ($N_{80}P_{45}K_{45}$). It was correlated to WW-supplied nutrients at the optimum requirement of plants at lower NPK levels and helped to save the 20 kg N, 15 kg P, and 15 kg K levels of fertilizers. Therefore, the use of WW in the cultivation of rapeseed-mustard could be beneficial, and it was concluded that WW irrigation can encounter the problems in Aligarh City and in other areas of India suffering from limited water sources, high-cost treatment of waste water, and the use of high-priced fertilizers. Although WW irrigation enhanced properties of soil and crop yield, its use has ensured food safety for seeds and only leaves produced at the age of 35 DAS with respect to heavy metal concentration. As a matter of fact, the indiscriminate use of WW for longer time (up to 70 DAS and 105 DAS) for the crop production resulted in heavy metals to reach a concentration which is not under admissible safe limits for human consumption as proposed by Awashthi (2000) and European Union (EC) (2001) for leafy green vegetables. Therefore, the short-term use of WW may have safe disposal and may contribute to filling the gap between water availability and water demand regardless of long-term use which will hold clinical health disorders in human beings and animals consuming these plants as leafy vegetables.

Acknowledgements The authors are grateful to the Chairman of the Department of Botany, A.M.U., Aligarh, for provide the agricultural research field and all the necessary facilities in the laboratory to carry out the research work. Thanks to Prof. Akhtar Inam and Prof. Arif Inam to draw the experimental design. Also, thanks to Dr. Meetu Gupta for the guidance during the writing of the manuscript.

Author contribution Seema Sahay and Saba Iqbal performed the experiment and the analysis and interpretation of the data. All authors discussed the results and contributed equally to final version of submitted manuscript.

Funding This work was financially supported by the University Grant Commission (UGC) funding agency to one of the authors (S. Sahay) in form of Junior Research Fellow and Senior Research Fellow of Rajiv Gandhi National fellowship (RGNF-JRF-SRF) and Post-Doctoral fellowship via award letter number F. No. 16/1274/SC (SA-III) and No. F./PDFSS-2015-17-UTT-12296, respectively.

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