Agric Res (October–December 2012) 1(4):379–391
DOI 10.1007/s40003-012-0044-4

FULL-LENGTH RESEARCH ARTICLE

Effects of Waste Water Irrigation on Physical and Biochemical Characteristics of Soil and Metal Partitioning in *Beta vulgaris* L.

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Received: 15 May 2012 / Accepted: 1 November 2012 / Published online: 25 November 2012

Abstract The present study deals with the assessment of changes in physical and biochemical characteristics of soil and metal partitioning in *Beta vulgaris* L. grown in farmer's fields irrigated with waste water in Dinapur and Lohta areas of Varanasi, India, during December to February, 2007–2008 and 2008–2009. Nutrient concentrations, organic carbon, microbial biomass, C, N, and P, enzymatic activities and heavy metal concentrations in soil and plant parts were estimated at waste water (DW_1, DW_2 and LW) and clean water-irrigated sites (DC and LC). Sites receiving waste water irrigation showed an increase in organic C by 36 and 64 % and in available phosphorus by 15 and, 21 % at DW_1 and DW_2 sites compared to DC and 88 and 29 % at LW compared to LC during the first year. Dehydrogenase and urease activities increased two to threefold at waste water-irrigated sites compared to the respective clean water-irrigated ones during both the years of study. Microbial biomass (C, N, and P) and concentrations of exchangeable cations (Na^+, K^+, and Ca^{+2}) also showed increments varying from two to threefold at waste water-irrigated sites. During both the years, total heavy metal concentration in soil was the highest for Mn followed by Zn, Pb, Ni, Cu, Cr, and Cd at Dinapur, whereas at Lohta the trend was Mn, Zn, Cr, Pb, Cu, and Cd. The accumulation of heavy metals in the plants was several-fold higher in roots and shoots at waste water-irrigated sites, and Cd, Pb, and Ni were above the safe limits in edible tissues. Lower metal concentrations were recorded at DW_1 site compared to DW_2 and LW sites. The study suggests that waste water irrigation led to beneficial changes in physico-chemical and biological properties of the soil, but increased the soil contamination of heavy metals. However, the intermittent use of clean water in such areas may not only reduce the metal contamination in the plants but will also maintain soil fertility.

Keywords Heavy metal · Microbial biomass · Nutrient concentration · Waste water

Introduction

Population growth, especially in the developing countries, has increased the demand for a huge quantity of water for domestic, municipal, and industrial sectors. With the increasing scarcity of freshwater resources that are available to agriculture, the use of urban waste water for irrigation is increasing, especially in the arid and semi-arid regions of the world. The use of untreated waste water is particularly intense in areas where there is poor access to other sources of irrigation water [20]. The use of waste water for irrigating agricultural soil has been shown to be associated with a number of potential beneficial changes such as an increase in organic carbon, available nitrogen, phosphorus, potassium, and magnesium contents in soil as compared to the clean ground water-irrigated soil [36]. Waste water is a valuable source of plant nutrients and organic matter needed for maintaining fertility and productivity levels of the soil [39]. Irrigation with waste water has been shown to result in increase in growth, yield, and plant constituents [2].

Waste water contains significant amounts of organic and inorganic nutrients. There is a potential for the nutrients

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present in recycled water to be used as a fertilizer source when the water is recycled for irrigation. Soil microorganisms show increased metabolic activities under sewage effluent irrigation [28, 37]. Organic carbon, total nitrogen, microbial biomass C and N and microbial activities increased with increase in the time duration of waste water irrigation [37]. Concentrations of total Mg, Hg, Mo, Ca, Cu, and Cr, and available concentrations of Pb, Cd, and Cu increase significantly in soils under waste water irrigation, and the concentrations remain below the hazardous levels [37].

Soil enzyme activity was used to test the biochemical status of the soil system in Brazilian Oxisol irrigated with treated sewage effluent [16]. Results showed rapid mineralization of dissolved organic matter and rapid nitrification from ammonia and organic nitrogen due to treated sewage effluent [16]. Soil water-soluble organic carbon, soil microbial biomass, and β-glucosidase and alkaline phosphatase activities increased under treated waste water irrigation in the Mediterranean island of Mallorca, Spain [1]. Liu and Haynes [24] have shown that microbial community increased under waste water irrigation.

In contrast, some harmful effects like inhibition of root and shoot growth, and reduction in yield due to the accumulations of heavy metals in plants grown at waste water-irrigated fields are also reported [43].

Heavy metal accumulation is one of the major drawbacks in using waste water for irrigation [44]. Therefore, the present study was undertaken to evaluate the effect of waste water irrigation on physical and biochemical characteristics of soil and heavy metal buildup in soil and plants under natural field conditions with different intensities (wholly/intermittently) of waste water use. It was hypothesized that intermittent use of clean water between waste water irrigation will not only reduce the heavy metal load but also enhance the soil microbial activities to increase the fertility of soil. The pollution index was calculated to show the level of contamination in soil at differently waste water-irrigated sites.

Materials and Methods

Study Area

The study was conducted at Dinapur and Lohta areas of Varanasi (25°18'N latitude and 83°01'E longitude and 76.19 m a.s.l.) situated in the eastern Gangetic plain of India having long practices of waste water irrigation (about 20 years). At Dinapur, the main source of waste water is a sewage treatment plant (DSTP) of 80 million liters per day (MLD) capacity installed in 1986. DSTP not only receives sewage but also effluents discharged from various industries such as fabric printing, batteries, and paint in the urban areas of Varanasi. The treated waste water from the main outlet is directly used by farmers for irrigation. At Lohta area, sewage and industrial effluents from more than a hundred local industries located upstream of the drain at Chandpur, Maruadih, Lahartara, and Lohta industrial estates and from treatment plant of a large diesel locomotive works, manufacturing diesel engines are discharged and directly used by farmers for irrigating the agricultural fields.

The soil of the study sites is classified as Inceptisol, and is pale brown in color and sandy loam in texture (58 % sand, 15 % silt, and 27 % clay).

Based upon the type of irrigation practices, three subsites were selected at Dinapur (DW_1, DW_2, and DC) area. The DW_1 site receives both clean (from bore well) as well as treated sewage water for irrigation, whereas the DW_2 site receives only treated waste water. At the DC site, only clean water from bore wells is used for irrigation. At Lohta area, two sites were selected (LW and LC). The LW site is irrigated by untreated and treated waste water, while LC site is irrigated by clean water from a bore well.

Field Preparation and Raising of Plants

Farmer's fields following different irrigation regimes in Dinapur and Lohta were marked and a whole plot of 6.5×4.5 m^2 was prepared at each site (DW_1, DW_2, DC, LW, and LC). The whole plot was then divided into six subplots of 1.5×1.5 m^2 having margins of 0.5 m. Genetically uniform seeds of palak (*B. vulgaris* L. var. All Green H1) procured from the Indian Institute of Vegetable Research, Varanasi, were sown 2 cm deep in five rows in each subplot. The irrigation was done either with the waste water at DW_1, DW_2, and LW sites and clean water at DC and LC at regular interval following the common schedule. The experiment was conducted from December 2007 to February 2008 and then repeated in the same months of 2008–2009.

Clean and waste water used for irrigation at Dinapur and Lohta showed variations in selected physico-chemical properties. In waste water, the values for pH, conductivity, total dissolved solid (TDS), and biological oxygen demand were 6.89, 0.78 ds m^{-1}, 596 μg ml^{-1}, 65.55 μg ml^{-1}1, respectively, at Dinapur and 7.15, 0.91 ds m^{-1} 691.6 μg ml^{-1}, 320.32 μg ml^{-1}, respectively, at Lohta site. Clean water did not show significant variations in different characteristics between Dinapur and Lohta, and the values for pH, conductivity (ds m^{-1}), and TDS (μg ml^{-1}) ranged from 7.90–8.00, 0.57–0.59, and 425–461.6, respectively. The concentrations of NO_3^-–N, NH_4^+–N, total P, N, Na^+, K^+, and Ca^{+2} (μg ml^{-1}), respectively, were 2.00, 11.7, 0.24, 5.4, 262.5, 135.4, and 248.5, in clean water and 5.30, 15.7, 9.7, 61.8, 281.5, 193.7, and 303.0 in waste water at Dinapur

and 2.57, 10.36, 0.30, 6.20, 291.25, 245.4, and 252.1 in clean water and 7.56, 17.0, 10.9, 75.4, 305.0, 273.7, and 363.5 in waste water at Lohta. The concentrations of Cd, Cu, Pb, Zn, Mn, Ni, and Cr were non-detectable in clean water at both the sites, whereas in waste water the concentrations of these metals (μg ml^{-1}) were 0.04, 0.053, 0.043, 0.117, 0.077, 0.020, and 0.050 at Dinapur and 0.037, 0.043, 0.063, 0.093, 0.110, 0.050, and 0.147 at Lohta, respectively.

Soil Sampling and Preparation

Monoliths of 10 × 10 × 15 cm^3 soil in triplicates were collected from different subplots separately at DW_1, DW_2, DC, LW, and LC sites. Fresh soil samples were used for estimation of microbial biomass and soil enzyme activities and the rest of the soil samples were air-dried at room temperature, crushed, and passed through a sieve of 2-mm-mesh size. The sieved samples were kept for analyzing various characteristics of the soil.

Plant Sampling

Plant samples were collected carefully without any disturbance to the root system at the time of maturity. Samples were then washed with the help of running tap water after keeping them on a 2.5-mm-mesh sieve to remove the soil particles adhering to the roots. The samples were separated into root and shoot parts and oven-dried separately at 80 °C until a constant weight was achieved. Plant samples were then powdered separately and stored at ambient temperature for further analysis of heavy metals. Samples collected from different plots were treated as replicates.

Soil Analysis

Soil pH was measured in suspension of 1:5 (soil: water w/v) using a glass electrode standardized with pH 4, 7, and 9.2 buffer tablets attached to an ion analyzer (Model E.A 940, Orion, USA). The organic carbon content was determined by using modified Walkley and Black's rapid titration method [7]. Nitrate nitrogen (NO_3^--N) and ammoniacal nitrogen (NH_4^+-N) were estimated by NO_3^--N and NH_4^+-N electrodes, respectively, with the help of the ion analyzer (Model E.A 940, Orion, USA).

The total nitrogen content was determined by following the Gerhardt-Kjeldahl technique through the Gerhardt automatic analyzer (Model KB8S, Kjeldatherm, Germany). Available phosphorus ($NaHCO_3$ extractable) was determined by the method given by [6]. Exchangeable cations such as Na, K, and Ca were extracted using ammonium acetate solution through repeated leaching technique [19] and contents were determined by atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA).

Digestion and Analysis for Heavy Metals in Soil and Plant Parts

The oven-dried and sieved samples of soil and plant parts (1 g), were digested by adding tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in a 5:1:1 ratio) at 80 °C until a transparent solution was obtained [6]. After cooling, the digested sample was filtered using Whatman no. 42 filter paper and the filtrate was finally maintained to 50 ml with distilled water. Concentrations of heavy metals in the filtrate of digested soil and plant samples were estimated by using atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA) fitted with a specific lamp of particular metal using appropriate drift blank.

Phytoavailable heavy metals in the soil samples were extracted by the method given by Quevauviller et al. [34]. A sieved soil sample of 10 g was shaken with 20 ml of 0.05 N EDTA solution (pH 7) for 1 h and then kept for 24 h before filtering. The concentrations of phytoavailable heavy metals in the filtrate were determined by using atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA).

Pollution Index

The pollution index (PI) was calculated by the following formula [36]:

$$PI = Ci/Si$$

Ci: Heavy metal content in a soil sample (mg kg^{-1}); *Si*: Permitted standard of the same metal (mg kg^{-1}).

When the *PI* values exceed 1.0, then soil is said to be contaminated by anthropogenic inputs and requires continuous environmental monitoring of the area.

Quality Control Analysis

Precision and accuracy of heavy metal analysis was assured through repeated analysis of samples against the National Institute of Standard and Technology, Standard Reference Material (SRM 1570) for all the heavy metals. The results were found within ± 2 % of the certified value. Quality control measures were taken to assess contamination and reliability of data. Blank and drift standards (Sisco Research Laboratories Pvt. Ltd., India) were run after five determinations to calibrate the instrument. The coefficients of variation of replicate analysis were determined for different determinations for precision of analysis and variations were found to be less than 10 %.

Enzymatic Activities

Dehydrogenase activity in the soil was measured by the method given by Tabatabai [46]. A soil sample of 5 g was taken into a culture tube and 2.5 ml of 1 % sterile triphenyltetrazolium chloride solution and 2.5 ml of 1 % sterile glucose were added. The mixture was incubated for 48 h at 28 °C. After incubation, 25 ml of methanol was added and the mixture was again incubated for 9 h at 28 °C. Its activity was determined by taking the absorbance of triphenyl formazan (TPF) at 485 nm using a UV–VIS spectrophotometer (Model 119, Systronics, India). Activity was calculated by the following formula.

$$\text{Dehydrogenase activity } \left(\mu\text{g TPF g}^{-1}\text{ hr}^{-1}\right) = \frac{O.D \times V}{W \times t}$$

where V = volume of the extract, W = weight of soil, t = incubation time.

Urease activity in the soil sample was estimated by following the method of Tabatabai [46]. A soil sample of 0.2 g taken in a culture tube was mixed with 0.1 ml of toluene and 2 ml of Tris buffer (pH 9). Then, 0.5 ml of urea solution was added and the mixture was incubated at 37 °C for 90 min. After incubation, 0.2 ml of 5 N NaOH solutions along with distilled water was added to dilute the sample to 10 ml. Activity of urease enzyme was measured by estimating the concentration of ammonium produced using an ammonium selective electrode connected to an ion analyzer (Model EA 940, Orion, USA).

$$\text{Urease activity } \left(\text{mmol NH}_3\text{ g}^{-1}\text{h}^{-1}\right) = \frac{\text{Concentration of ammonium ion}}{W \times t}$$

W = weight of soil, t = incubation time.

Microbial Biomass

Soil microbial biomass (C, N, and P) was analyzed on the field moist soil. The samples were stored for 7 to 10 days at room temperature (25–28 °C) to settle down respiration. Microbial biomass C was determined using the $CHCl_3$ fumigation extraction method of Vance et al. [47]. Biomass N and P was determined on the same field moist soil sample by following $CHCl_3$ fumigation extraction methods proposed by Brookes et al. [8] and Brookes et al. [9], respectively.

Statistical Analysis

The data of heavy metal concentrations in the soil and in plant parts at different sites were subjected to ANOVA followed by Duncan's test for assessing the significance of differences. All the statistical tests were performed using SPSS software (SPSS Inc., version 12).

Table 1 Physico-chemical characteristics of the soil samples at different experimental sites during 2007–2008 and 2008–2009 (mean ± 1 SE)

Characteristics	2007–2008					2008–2009				
	DW_1	DW_2	DC	LW	LC	DW_1	DW_2	DC	LW	LC
pH	$7.17^{b} \pm 0.02$	$7.16^{b} \pm 0.02$	$8.16^{a} \pm 0.02$	$7.33^{b} \pm 0.21$	$8.23^{a} \pm 0.03$	$8.12^{b} \pm 0.02$	$8.30^{b} \pm 0.01$	$8.90^{a} \pm 0.03$	$8.45^{b} \pm 0.07$	$8.99^{a} \pm 0.04$
Conductivity (ds m^{-1})	$0.13^{c} \pm 0.01$	$0.16^{b} \pm 0.01$	$0.11^{d} \pm 0.01$	$0.19^{a} \pm 0.01$	$0.12^{d} \pm 0.01$	$0.22^{b} \pm 0.03$	$0.25^{a} \pm 0.04$	$0.16^{c} \pm 0.02$	$0.27^{a} \pm 0.05$	$0.15^{c} \pm 0.02$
Organic C (%)	$1.52^{c} \pm 0.02$	$1.84^{b} \pm 0.01$	$1.12^{e} \pm 0.02$	$2.43^{a} \pm 0.04$	$1.29^{d} \pm 0.01$	$2.80^{b} \pm 0.04$	$3.23^{a} \pm 0.01$	$2.00^{c} \pm 0.02$	$3.12^{a} \pm 0.05$	$2.14^{c} \pm 0.09$
Total N (%)	$0.22^{a} \pm 0.01$	$0.24^{a} \pm 0.01$	$0.090^{b} \pm 0.01$	$0.23^{a} \pm 0.01$	$0.07^{b} \pm 0.01$	$0.12^{b} \pm .002$	$0.18^{ab} \pm .001$	$0.10^{c} \pm 0.002$	$0.19^{a} \pm 0.001$	$0.09^{c} \pm 0.001$
NO_3^-–N(μg g^{-1})	$15.17^{b} \pm 0.73$	$15.70^{b} \pm 0.43$	$14.10^{c} \pm 0.67$	$17.50^{a} \pm 0.76$	$13.10^{c} \pm 0.46$	$11.12^{a} \pm 0.04$	$12.10^{a} \pm 0.15$	$9.60^{b} \pm 0.90$	$12.67^{a} \pm 0.06$	$10.34^{b} \pm 0.07$
NH_4^+–N(μg g^{-1})	$4.51^{c} \pm 0.25$	$4.50^{c} \pm 0.25$	$3.11^{d} \pm 0.05$	$6.95^{a} \pm 0.02$	$5.73^{b} \pm 0.37$	$3.00^{c} \pm 0.03$	$2.98^{c} \pm 0.05$	$1.98^{d} \pm 0.03$	$4.78^{a} \pm 0.07$	$3.98^{b} \pm 0.15$
Available P(μg g^{-1})	$12.0^{b} \pm 0.21$	$12.63^{b} \pm 0.32$	$10.40^{c} \pm 0.41$	$17.60^{a} \pm 0.43$	$13.60^{b} \pm 0.46$	$10.45^{a} \pm 0.42$	$11.67^{a} \pm 0.12$	$8.45^{b} \pm 0.35$	$12.37^{a} \pm 0.56$	$10.03^{b} \pm 0.45$
Ex. Na(μg g^{-1})	$135.00^{b} \pm 2.89$	$146.33^{b} \pm 3.18$	$85.11^{c} \pm 2.79$	$225.00^{a} \pm 8.86$	$90.95^{c} \pm 5.63$	$167.7^{c} \pm 2.34$	$245.9^{b} \pm 1.62$	$89.90^{d} \pm 2.18$	$278.9^{a} \pm 3.89$	$105.0^{d} \pm 1.08$
Ex. K(μg g^{-1})	$143.00^{c} \pm 3.8$	$159.91^{b} \pm 0.75$	$93.49^{d} \pm 0.94$	$250.0^{a} \pm 8.66$	$100.90^{d} \pm 2.99$	$170.24^{b} \pm 1.06$	$178.34^{b} \pm 6.78$	$99.78^{c} \pm 4.67$	$300.12^{a} \pm 4.00$	$110.0^{c} \pm 2.26$
Ex. Ca(μg g^{-1})	$433.87^{e} \pm 6.7$	$491.70^{b} \pm 6.65$	$105.73^{e} \pm 3.54$	$653.1^{a} \pm 4.04$	$215.25^{d} \pm 8.17$	$600.05^{b} \pm 2.89$	$626.65^{b} \pm 10.79$	$118.56^{d} \pm 5.17$	$782.34^{a} \pm 3.45$	$236.89^{c} \pm 7.67$

DW_1 treated waste water + clean water, *DW_2* treated waste water, *DC* clean water, *LW* untreated waste water, *LC* clean water

Different letters in each row show significant difference at $p \leq 0.05$

Results

Physico-chemical Characteristics of Soil

During the first year (2007–2008), soil from clean water-irrigated sites, i.e., DC and LC, showed significantly ($p \leq 0.05$) higher pH values as compared to the waste water-irrigated sites, i.e., DW_1, DW_2, and LW (Table 1). Conductivity (ds m^{-1}) of soil ranged from 0.136 to 0.196 at waste water-irrigated sites and 0.113–0.123 at clean water-irrigated sites (Table 1). During the second year (2008–2009), clean water-irrigated sites also showed significantly higher pH ($p \leq 0.05$) and significantly lower conductivity ($p \leq 0.05$) compared to the waste water-irrigated sites at Dinapur as well as Lohta sites (Table 1).

Organic C ($p \leq 0.05$) total N ($p \leq 0.05$) and available P ($p \leq 0.05$) contents were significantly higher at waste water-irrigated sites (DW_1, DW_2, and LW sites) as compared to the clean water-irrigated sites (DC and LC) during both years. Organic C content (%) in the soil was 1.52, 1.84, 1.12, 2.43, and 1.29 at the DW_1, DW_2, DC, LW, and LC sites, respectively, during the first year of sampling (Table 1). Total N and available P content were also higher in waste water (DW_1, DW_2, and LW sites) irrigated sites as compared to respective clean water-irrigated sites (DC, LC). During the second year also, total N, organic C, and available P increased significantly ($p \leq 0.05$) by 20, 40, and 24 % at DW_1, 80, 62, and 38 % at DW_2 and 111, 45, and 23 % at LW sites, respectively, as compared to their respective clean water-irrigated sites (Table 1).

During both of the years, NO_3^--N and NH_4^+-N concentrations were significantly ($p \leq 0.05$) higher at DW_1, DW_2, and LW sites as compared to respective sites receiving clean water for irrigation (Table 1). NO_3^--N concentrations increased by 16, 26, and 23 % and NH_4^+-N increased by 52, 51, and 20 % at DW_1, DW_2, and LW sites, respectively, as compared to the respective clean water-irrigated sites during 2008–2009 (Table 1).

Soil Enzyme Activities

Dehydrogenase and urease activities in the soil were significantly higher ($p \leq 0.05$) at waste water-irrigated sites (DW_1, DW_2, and LW) as compared to the clean water-irrigated sites (DC and LC) in each year (Table 2). During 2007–2008, the average value of dehydrogenase activity was 34.67, 39.00, 22.67, 37.87, and 21.47 μM TPF g^{-1} h^{-1} and urease was 2.37, 3.09, 1.21, 2.83, 1.14 m mol NH_3 $g^{-1}h^{-1}$ at DW_1, DW_2, DC, LW, and LC sites, respectively (Table 2). The trends of variations in enzyme activities between clean and waste water-irrigated sites were also similar for 2008–2009 (Table 2).

Table 2 Enzymatic activity and microbial biomass of the soil samples at different experimental sites during 2007–2008 and 2008–2009 (mean ± 1 SE)

Characteristics	2007–2008					2008–2009				
	DW_1	DW_2	DC	LW	LC	DW_1	DW_2	DC	LW	LC
Dehydrogenase activity (μg TPF g^{-1} h^{-1})	$34.67^a \pm 3.71$	$39.00^a \pm 4.58$	$22.67^b \pm 0.67$	$37.86^a \pm 3.52$	$21.46^b \pm 0.29$	$42.0^a \pm 0.93$	$44.33^a \pm 2.60$	$23.00^b \pm 0.57$	$43.33^a \pm 2.02$	$32.00^b \pm 3.60$
Urease activity(mmol NH_3 g^{-1} h^{-1})	$2.37^b \pm 0.01$	$3.09^a \pm 0.33$	$1.21^c \pm 0.02$	$2.83^{ab} \pm 0.34$	$1.14^c \pm 0.03$	$3.52^b \pm 0.02$	$4.83^a \pm 0.31$	$1.58^c \pm 0.33$	$4.60^a \pm 0.10$	$1.18^c \pm 0.17$
Microbial biomass 'C' (μg g^{-1})	$377.33^a \pm 13.2$	$402.67^a \pm 11.05$	$193.33^b \pm 52.06$	$423.33^a \pm 17.63$	$216.00^b \pm 19.21$	$392.33^b \pm 6.69$	$407.0^b \pm 8.02$	$199.00^c \pm 50.63$	$512.67^a \pm 0.33$	$213.00^c \pm 18.78$
Microbial biomass 'N' (μg g^{-1})	$45.33^b \pm 2.03$	$49.30^{ab} \pm 2.33$	$22.30^c \pm 1.45$	$53.67^a \pm 2.90$	$24.33^c \pm 2.60$	$51.30^b \pm 1.86$	$52.00^b \pm 1.73$	$24.67^c \pm 2.18$	$59.00^a \pm 0.58$	$22.00^c \pm 1.73$
Microbial biomass 'P' (μg g^{-1})	$27.30^a \pm 1.67$	$29.00^a \pm 0.58$	$17.33^b \pm 1.20$	$29.67^a \pm 0.33$	$18.30^b \pm 2.30$	$31.00^b \pm 1.53$	$39.33^a \pm 0.33$	$18.33^c \pm 1.20$	$38.67^a \pm 1.85$	$18.30^c \pm 1.76$

DW_i treated waste water + clean water, *DW_2* treated waste water, *DC* Clean water, *LW* untreated waste water, *LC* clean water

Different letters in each row show significant difference at $p \leq 0.05$

Microbial Biomass C, N, and P

Microbial biomass C, N, and P was significantly higher ($p \leq 0.05$) for waste water-irrigated DW_1, DW_2, and LW sites as compared to respective clean water-irrigated DC and LC sites (Table 2). Microbial biomass C in soil samples during 2007–2008 was 377.33, 402.67, 423.30, 193.33, and 216.00 μg g^{-1} at DW_1, DW_2, LW, DC, and LC sites, respectively. The values of microbial biomass N were significantly ($p \leq 0.05$) higher than the values of microbial biomass P at all the sites (Table 2). Values of microbial biomass C, N, and P were higher at waste water-irrigated sites during the second year compared to the first year of sampling (Table 2).

Exchangeable Na, K, Ca, and Heavy Metal Concentrations

During 2007–2008, exchangeable Na and K concentrations (μg g^{-1}) were about twofold higher and of Ca about fourfold higher at all the waste water-irrigated sites as compared to the clean water-irrigated sites (Table 1). During 2008–2009, increments in concentrations were about 24, 68, 6, 24, and 15 % for Na, 19, 12, 7, 20, and 9 % for K, 55, 33, 12, 20, and 10 % for Ca at DW_1, DW_2, DC, LW, and LC sites, respectively, as compared to respective cations recorded during 2007–2008 (Table 1).

At all the sites, phytoavailability of Mn in the soil was highest followed Cu, Pb, Cr, Cd, Zn, and Ni. Waste water-irrigated sites showed significantly higher ($p \leq 0.05$) values of phytoavailable heavy metals as compared to the clean water-irrigated sites during both years of sampling (Table 3).

During both the years, total heavy metal concentration in the soil was the highest for Mn followed by Zn, Pb, Ni, Cu, Cr, and Cd at Dinapur, whereas at Lohta it was the highest for Mn followed by Zn, Cr, Pb, Cu, Ni, and Cd (Fig. 1a, b). Samples collected during the second year showed that among all the heavy metals, Pb, Mn, Ni, and Cr concentrations were the highest at the LW site as compared to the waste water-irrigated sites of Dinapur. Among all the waste water-irrigated sites, DW_1 site showed lower concentrations of all the metals compared to DW_2 and LW sites (Fig. 1a, b). The pollution index value was less than 1 for all the metals except for Cd at waste water-irrigated site LW during 2007–2008 (Table 4).

Heavy Metal Concentrations in Root and Shoot Parts of Plant

Heavy metal concentrations in root and shoot portions were significantly higher ($p \leq 0.05$) at waste water-irrigated sites as compared to clean water-irrigated ones in both

Table 3 Phytoavailable heavy metal concentrations (μg g^{-1} dw) in the of soil samples at the time of initial sampling at different experimental sites during 2007–2008 and 2008–2009 (mean ± 1 SE)

Heavy metals	2007–2008					2008–2009				
	DW_1	DW_2	DC	LW	LC	DW_1	DW_2	DC	LW	LC
Cd	$3.08^{a} \pm 0.03$	$3.00^{a} \pm 0.07$	$0.50^{b} \pm 0.02$	$2.55^{a} \pm 0.15$	$0.40^{b} \pm 0.10$	$4.00^{a} \pm 0.05$	$4.12^{a} \pm 0.06$	$0.56^{b} \pm 0.06$	$3.55^{a} \pm 0.03$	$0.48^{b} \pm 0.10$
Cu	$11.20^{a} \pm 0.80$	$10.07^{a} \pm 0.95$	$3.35^{b} \pm 0.08$	$10.36^{a} \pm 1.21$	$3.82^{b} \pm 0.03$	$11.77^{a} \pm 1.20$	$11.00^{a} \pm 1.00$	$4.00^{b} \pm 1.12$	$11.45^{a} \pm 0.98$	$4.16^{b} \pm 1.22$
Pb	$4.58^{b} \pm 0.14$	$7.55^{a} \pm 0.12$	$0.68^{c} \pm 0.14$	$8.17^{a} \pm 0.09$	$0.53^{c} \pm 0.02$	$5.00^{b} \pm 0.20$	$6.88^{a} \pm 0.32$	$0.59^{c} \pm 0.09$	$7.89^{a} \pm 0.08$	$0.49^{c} \pm 0.04$
Zn	$1.84^{a} \pm 0.20$	$2.11^{a} \pm 0.34$	$0.36^{b} \pm 0.17$	$1.91^{a} \pm 0.04$	$0.76^{b} \pm 0.04$	$1.84^{a} \pm 0.34$	$2.11^{a} \pm 0.56$	$0.36^{b} \pm 0.30$	$1.91^{a} \pm 0.06$	$0.76^{b} \pm 0.03$
Mn	$174.4^{a} \pm 2.89$	$189.8^{a} \pm 8.33$	$64.95^{c} \pm 6.55$	$151.82^{b} \pm 3.92$	$57.23^{c} \pm 9.52$	$187.3^{a} \pm 2.89$	$190.83^{a} \pm 9.00$	$64.95^{c} \pm 5.78$	$151.8^{b} \pm 4.00$	$57.23^{c} \pm 8.72$
Ni	$1.54^{b} \pm 0.02$	$2.33^{a} \pm 0.34$	$0.23^{c} \pm 0.02$	$2.42^{a} \pm 0.06$	$0.21^{c} \pm 0.02$	$1.67^{b} \pm 0.12$	$1.93^{a} \pm 0.50$	$0.45^{c} \pm 0.06$	$2.56^{a} \pm 0.06$	$0.35^{c} \pm 0.04$
Cr	$4.47^{b} \pm 0.07$	$6.19^{b} \pm 0.01$	$0.02^{c} \pm 0.005$	$8.17^{a} \pm 0.09$	$0.03^{c} \pm 0.01$	$5.00^{b} \pm 0.07$	$7.92^{b} \pm 0.01$	$0.06^{c} \pm 0.005$	$9.00^{a} \pm 0.09$	$0.05^{c} \pm 0.001$

DW_1 treated waste water + clean water, *DW_2* treated waste water, *DC* clean water, *LW* untreated waste water, *LC* clean water, *DW* dry weight

Different letters in each row of each year show significant difference at $p \leq 0.05$

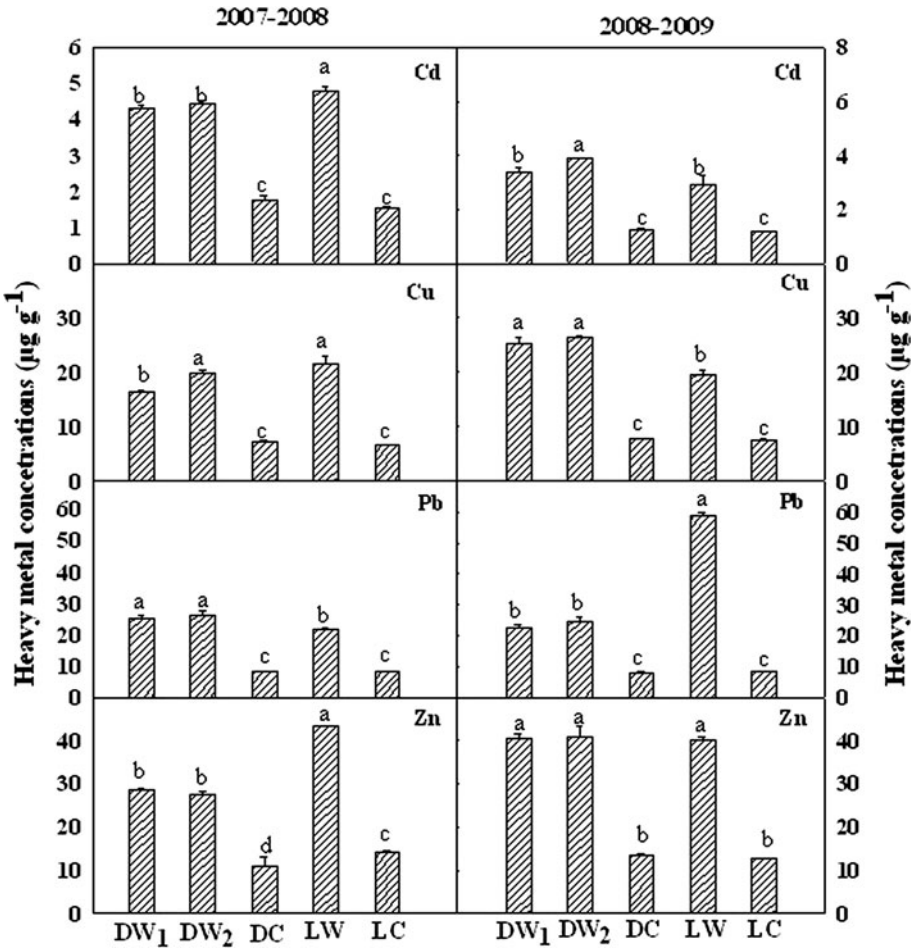

Fig. 1 **a** Concentrations of Cd, Cu, Pb, and Zn (μg g^{-1} dw) of the soil samples at different experimental sites during 2007–2008 and 2008–2009. Values are mean ± 1 SE. *Bars with different letters* in each group show significant difference at $p < 0.05$. **b** Concentrations of Mn, Ni, and Cr (μg g^{-1} dw) of the soil samples at different experimental sites during 2007–2008 and 2008–2009. Values are mean ± 1 SE. *Bars with different letters* in each group show significant difference at $p < 0.05$

years, but were higher in 2008–2009 compared to 2007–2008 (Figs. 2, 3). At both the samplings, heavy metal concentrations were higher in shoot compared to the root portion during both the samplings (Figs. 2, 3). Among all the heavy metals, Zn concentration was the highest and Cd concentration was the lowest in root part at all the sites. In shoot portion, Mn concentration was the highest and Cd was the lowest (Figs. 2, 3). During 2008–2009 at DW_1, DW_2, DC, LW, and LC sites, Cd concentrations (μg g^{-1} dw) in edible portion ranged from 4.75 to 6.16, Cu from 20.28 to 25.10, Pb from 25.50 to 30.33, Zn from 53.02 to 56.73, Ni from 21.32 to 33.28, and Cr from 6.03 to 8.59 (Fig. 3). In the edible portion of palak (shoot), the concentrations of Cd, Pb, and Ni were higher than the Indian permissible limits (Awashthi 2000), and permissible value in food provided by FAO/WHO (Codex Alimentarius, 2007) at all the waste water-irrigated sites.

Discussion

The soil of both the sites irrigated by waste water in both years had significantly lower pH and higher conductivity than the soil of clean water-irrigated sites. A reduction in soil pH due to waste water irrigation compared to irrigation by potable water has been reported [29]. Higher concentrations of ammonium ions in waste water may lead to a higher rate of nitrification releasing free hydrogen ions in the soil, thus lowering the soil pH [18, 48]. Higher accumulation of total dissolved solids in the soil due to continuous use of waste water may enhance the conductivity of soil at waste water-irrigated sites. Mohammad and Mazahreh [29] have also recorded higher conductivity in waste water-irrigated than clean water-irrigated soil.

Similar to the present study, Onweremadu [32] also showed increments in organic matter, available P and total N, at waste water-irrigated soil as compared to the clean water-irrigated soil of Imo State University, Owerri, Nigeria. Similarly, Masto et al. [27] have recorded 18.2 and 240.67 % increments in total N and available P, respectively, in the soil irrigated by sewage water from a sewage treatment plant at IARI farm, New Delhi. The range of organic C (1.52–2.43 %) recorded during the present study was higher than the range (1.68–1.78 %) reported by Yadav et al. [50] in soil irrigated by sewage water from urban estate of Kurukshetra, Haryana. Friedel et al. [13]

Fig. 1 continued

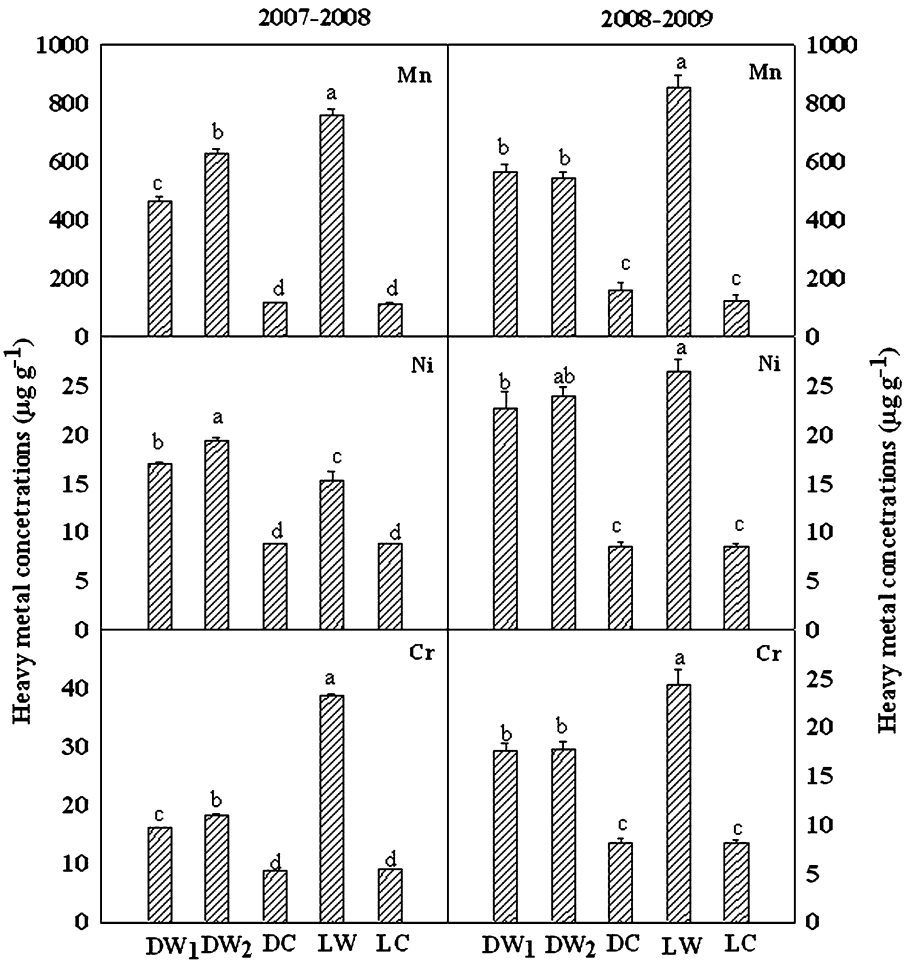

Table 4 Pollution index at different experimental sites during 2007–2008 and 2008–2009

Heavy metals	2007–2008			2008–2009		
	DW_1	DW_2	LW	DW_1	DW_2	LW
Cd	0.95	0.98	1.06	0.74	0.86	0.65
Cu	0.08	0.09	0.11	0.12	0.13	0.09
Pb	0.10	0.10	0.08	0.18	0.19	0.46
Zn	0.06	0.07	0.09	0.09	0.10	0.08
Mn	n/a	n/a	n/a	n/a	n/a	n/a
Ni	0.15	0.17	0.14	0.20	0.21	0.23
Cr	n/a	n/a	n/a	n/a	n/a	n/a

DW_1 treated waste water + clean water, *DW_2* treated waste water, *DC* clean water, *LW* untreated waste water, *LC* clean water, *n/a* not available (permissible limits for Mn and Cr in soil are not available)

have also reported 2.5-fold increments in organic carbon content of soil irrigated by untreated sewage effluents from Mexico City. The Lohta site, where untreated waste water was used for irrigation, showed higher concentrations of exchangeable cations compared to Dinapur, having the use of treated waste water for irrigation. Garg and Kaushik [15] have shown higher concentrations of exchangeable Ca and K in untreated textile mill waste water as compared to the treated ones. Waste water irrigation was found to act as a supplement to soil fertility by adding organic matter and mobile compounds of nutrients [21, 40]. Soil irrigated with waste water showed increments in total phosphorus, nitrogen, sodium, potassium, and iron concentrations [30].

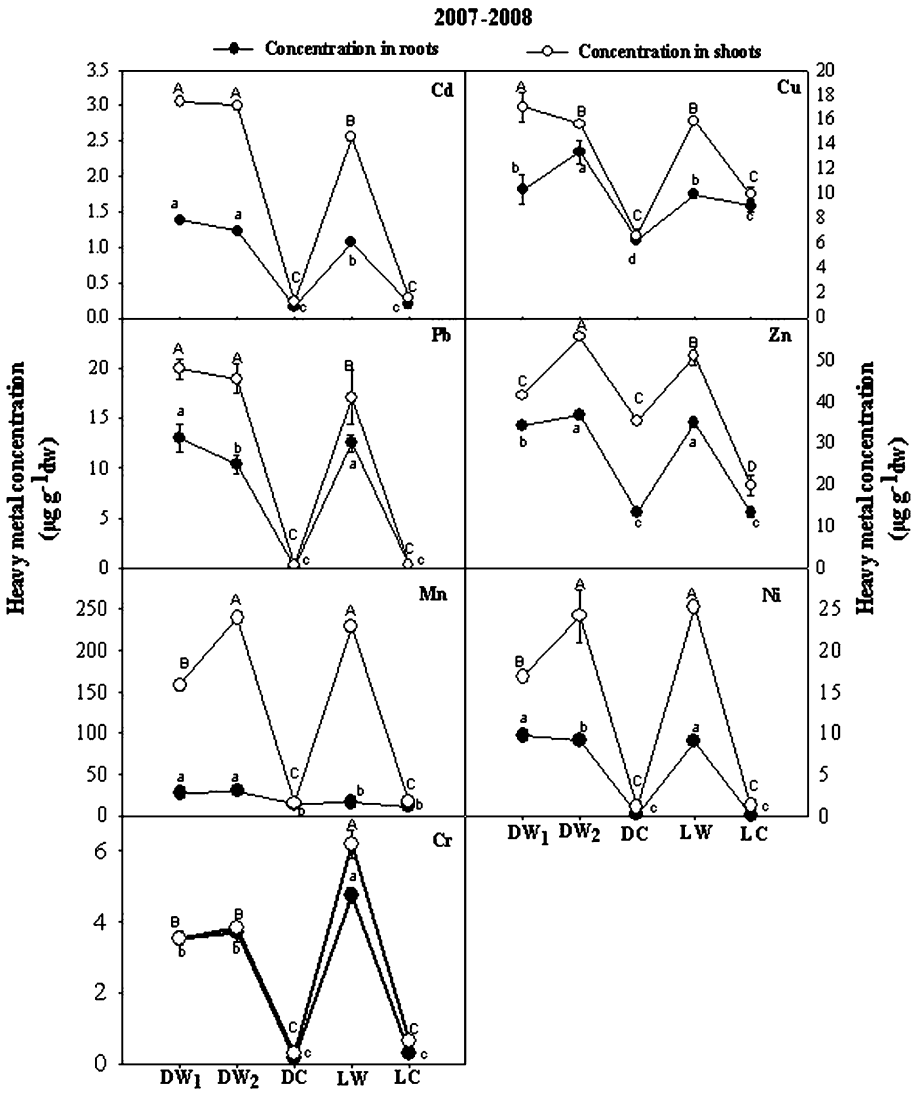

Fig. 2 Metal concentration (μg g^{-1} dw) in shoots and roots of plants grown at different experimental sites during 2007–2008. *Curved line with different capital letters* in each group show significant difference in shoot and *curved line with different small letters* show significant difference in root part of plant at $p \leq 0.05$

Microbial biomass is a more sensitive indicator of changing soil condition than the total organic matter. Microbial biomass C, N, and P were higher at waste water-irrigated compared to the clean water-irrigated sites. Soil irrigated by municipal waste water showed enhanced microbial activity and their biomass [24, 37]. The study of the long-term effect of waste water irrigation on selected microbiological and biochemical characteristics of the soil showed that microbial biomass and activity of dehydrogenase enzyme in soil increased due to waste water irrigation [17]. Higher microbial activity and organic C content in treated waste water-irrigated field located in the central coastal region of Israel was also reported [12].

An increase in microbial activity due to waste water irrigation led to the increments in the activities of dehydrogenase and urease enzymes during the present study. Low (60–75 mm waste water per treatment) and high dose (120–150 mm waste water per treatment) of waste water treatments increased the dehydrogenase activity by 44 and 27 %, respectively, in the soil from Lubin, Poland [10]. Dehydrogenase is an intracellular enzyme involved in microbial O_2 metabolism. Activity of this enzyme depends upon the metabolic state of the soil biota and thus is a good indicator of soil microbial activity [14]. Urease enzyme is involved in the hydrolysis of C–N bonds of amide and urea. Due to the higher amount of organic N incorporated in the soil through waste water irrigation, urease synthesis may have increased. Although the long-term use of waste water also led to an increase in heavy metal concentrations in the soil, the levels were not up to the extent that may have caused any negative effects on the measured enzymatic activities. Madejon et al. [25] have also reported that the addition of organic materials through municipal solid waste at doses of 50,000 kg ha^{-1} did not negatively affect the dehydrogenase and urease activities of the soil even at higher availability of heavy metals. Chatzakis et al. [11] have shown that activities of dehydrogenase enzyme were enhanced by irrigating the soil with secondary treated

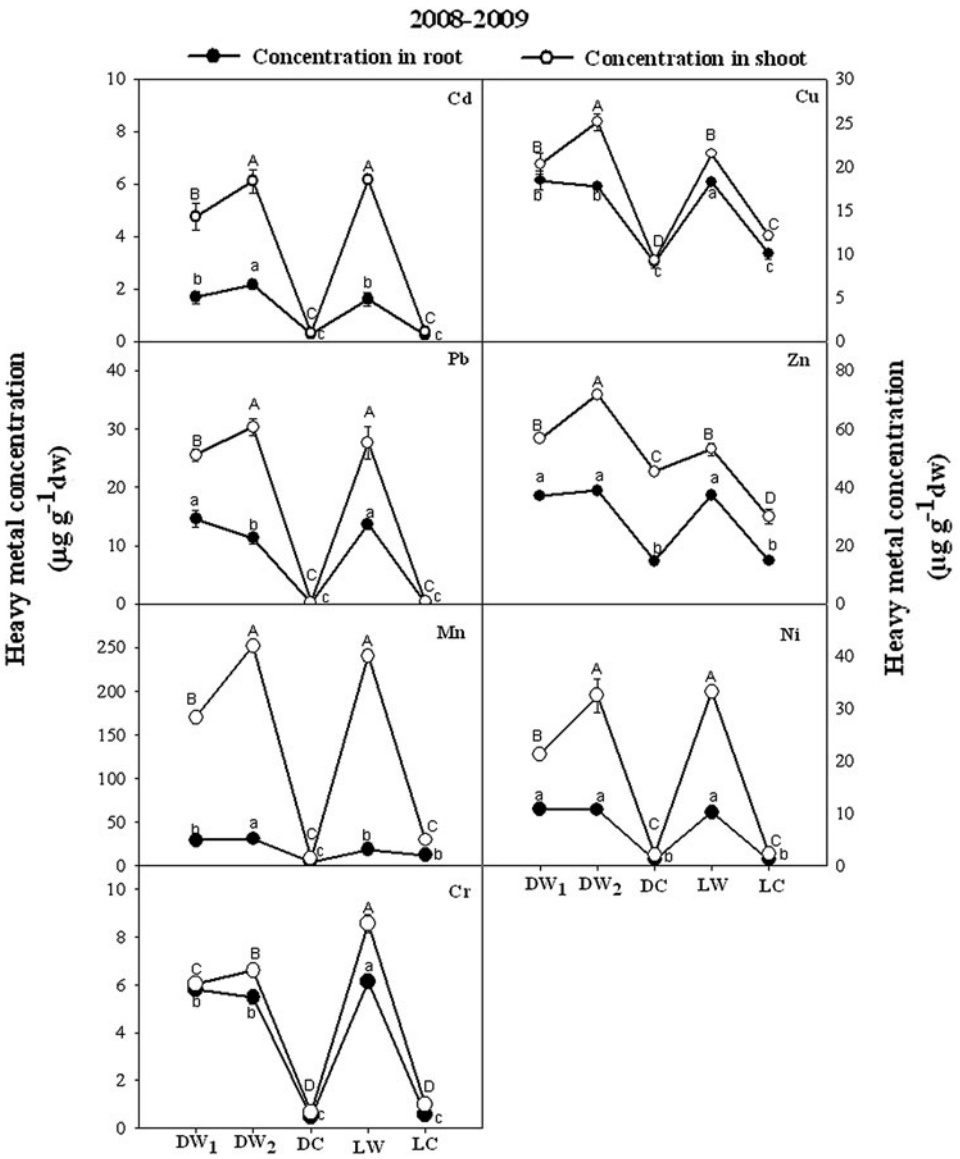

Fig. 3 Metal concentration (μg g^{-1} dw) in shoots and roots of plants grown at different experimental sites during 2008–2009. *Curved line with different capital letters* in each group show significant difference in shoot and *curved line with different small letters* show significant difference in root part of plant at $p \leq 0.05$

waste water effluent as compared to the fresh water irrigated one.

Low pH of waste water-irrigated soil increased the metal availability since the hydrogen ions have a greater affinity for competing with metal ions and releasing them from the soil solution for uptake. Total and phytoavailable heavy metal concentrations were significantly high in the soil at waste water-irrigated sites for all the metals as compared to the clean water-irrigated sites. Availability of heavy metals to plants depends upon solubility rate and various physico-chemical properties of the soil, like pH, organic carbon, cation exchange capacity, and soil texture [49]. The waste waters at both the sites had higher concentrations of heavy metals, while no heavy metals were detected in clean water.

In the present study, Mn concentration was 546.65 μg g^{-1} and Cd was 5.62 μg g^{-1} in the soil of Dinapur (DW_1 site). These values were higher than for the Mn (156.96 μg g^{-1}) and Cd (2.80 μg g^{-1}) reported by Sharma et al. [41] during 2006–2007 at the same site, but more or less similar to the values reported by Singh et al. [43] during 2008-2009. Cu (16.43 μg g^{-1}), Pb (25.33 μg g^{-1}), Zn (28.45 μg g^{-1}), and Cr (16.00 μg g^{-1}) concentrations at the DW_1 site in the present study were lower than the reported for Cu (74.48 μg g^{-1}), Pb (133.86 μg g^{-1}), Zn (132.91 μg g^{-1}) and Cr (54.87 μg g^{-1}) by Singh et al. [43] at the same site. Lower concentrations of heavy metals (Cd, Cu, Mn, Ni, and Cr) in the soil at DW_1 site may be ascribed to the intermittent use of clean water for irrigation, which has caused lower accumulation of heavy metals in the soil compared to the DW_2 site, where use of treated and untreated waste water for irrigation is a regular practice. The Lohta site contained the highest concentrations of most of the estimated heavy metals in the soil, due to the fact that many of the industries in this area are associated with significant heavy metal discharge

and irrigation water is drawn directly from industrial effluents and waste water containing channels [3]. The waste water from Lohta had higher concentrations of heavy metals compared to Dinapur.

Pollution index calculation suggests that heavy metal concentrations in soil at waste water-irrigated sites DW_1, DW_2, and LW were mostly within the permissible limit, except for Cd during 2007–2008. The pollution index for Cd was the highest among all the heavy metal at all the sites. As agriculture is continuously practiced in the area throughout the year, soil buildup of heavy metals is minimized due to absorption by plants and leaching to lower horizons.

Heavy metal concentrations in root and shoot portions of palak plants were significantly higher at waste water-irrigated sites as compared to the clean water-irrigated ones. Similar to the present study, many-fold increments in the concentrations of Zn, Cu, Mn, Pb, Cd, and Ni in roots and leaves of cauliflower, Indian spinach, and carrot plants grown under municipal sewage water irrigated soils were reported by Malla and Totawat [26]. Irrigation by effluent water from the Esfahan treatment plant increased the concentrations of Fe, Cu, and Zn in the roots of wheat and Mn and Zn in grains of wheat as compared to those irrigated with well water [35]. A field experiment was conducted to investigate the extent of translocation of heavy metals to tomato (*Solanum lycopersicon* L. cvs. "GS12" and "RS589956") fruit produced in an open field near Abu-Nusiar Waste Water Treatment Plant, Amman, Jordan. Tomato fruits showed elevated concentrations of Fe, Cu, Ni, Mn, and Zn under waste water irrigation [4, 5]. Kalavrouziotis et al. [22] have evaluated effects of municipal reclaimed waste water on the macro- and micro-element status of the soil and of *Brassica oleracea* var. Italica and Gemmifera. Use of treated municipal waste water for irrigation led to increases in the heavy metal load for both the varieties. Mojri and Amirossadt [31] have also observed high concentrations of Cd, Mn, and Ni in *Zea mays* grown in urban waste water-irrigated fields compared to clean water-irrigated ones.

Palak plants growing at all sites showed a higher accumulation of heavy metals in shoot than root. Sinha et al. [45] have also reported lower concentrations of heavy metals in the root as compared to the shoot in palak plants grown at sites near Gomti River, Lucknow. Heavy metals tend to remain in root tissue in most of the horticultural crops [23, 33], but palak did not show a similar trend. This may be ascribed to several-fold larger above-ground biomass of palak than the below-ground and also to high relative growth rate of this plant. Heavy metals like Zn, Cu, Fe, Mn, Ni, etc., were reported to accumulate more in shoots of leafy vegetables, especially spinach as compared to the grain-yielding plants [38]. In between DW_1 and DW_2 sites, DW_1 showed lower concentrations of the heavy metals in the edible portion of palak, which may be due to intermittent use of clean water for irrigation at the site. So our hypothesis that intermittent use of clean water with waste water will not only increase the yield but also maintain soil fertility has proven correct.

Conclusions

The results of both the years of study clearly showed that physico-chemical and biological properties of the soil were modified under long-term uses of waste water irrigation in Dinapur and Lohta areas. Soil pH decreased, whereas organic C, total N, available P, and exchangeable cations increased under the waste water-irrigation regime. Heavy metal concentrations in the soil increased several fold at waste water-irrigated sites as compared to the clean water-irrigated ones. Higher values of microbial biomass C, N, and P and higher enzymatic activities at waste water-irrigated sites maintained higher soil fertility level compared to clean water-irrigated sites. Pollution index values showed that the concentrations of heavy metals in soil at waste water-irrigated sites were mostly not at the levels that cause metal pollution, but Cd has the potential to cause adverse effects in view of higher index. The present 2-year study conducted in natural field conditions having long-term practices of using waste water irrigation suggests that heavy metal contamination of the food chain with Cd, Pb, and Ni is a major issue in the area, having risks to human health. Among different irrigation patterns, the intermittent use of clean water with waste water reduced the metal concentrations in soil and plants. Therefore, this kind of irrigation pattern enhanced the potential for long-term use of waste water for irrigation by reducing the translocation of metals in plants from soil, which will maintain food safety and reduce the vulnerability to human health.

Acknowledgments Anita Singh is thankful to the Center for Advanced Study in Botany, Banaras Hindu University, Varanasi, for Senior Research Fellowship during the work.

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