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Treated municipal wastewater for irrigation: effect on turnip (*Brassica rapa*)

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ABSTRACT

A study was conducted to explore the possibility of using treated municipal wastewater to grow turnip (*Brassica rapa*). Two aspects namely (1) effect on plant growth and (2) accumulation of Cd, Cr, Ni, Fe Cu, Mn and Zn in leaves and roots of the plant have been presented in this paper. The heavy metal concentration of wastewater used for irrigation was within the limits however, the concentration in the plant parts showed a significant rise due to continuous use. The levels of all the heavy metals except Mn in the edible plant parts (leaves and roots) were estimated to be more than the toxic limits given by Pendias and Pendias. The concentration of heavy metals was at excessive levels in 40 and 55 days after sowing (DAS), while at 70 DAS, metal concentration was low. Concentration of heavy metals in plants was found in the order of Fe > Zn > Ni > Mn > Cr > Cu > Cd.

Keywords: Wastewater irrigation; Heavy metals; Turnip

1. Introduction

Agriculture is the greatest user of water all over the world. The water consumption for crop irrigation amounts to 70% and in some cases 90% of the world water requirements [1]. In view of the irrigation water shortage faced in many countries, wastewater reuse constituents an alternative source of irrigation water [2]. The application of treated wastewater for agricultural purposes has been evaluated as the most convenient recycling option for environmental and economic reasons. In many countries, huge quantities of reclaimed water are produced from the wastewater treatment processes. This wastewater is not only a source of irrigation water, but it is also a carrier of significant quantities of macro and micro nutrients and organic matter. However, apart from these benefits, wastewater pollution by trace metals and toxic organic contaminants must also be taken into account. The irrigation water quality has been shown to affect soils, crops, food quality, safety [3] and the management of water [4]. Industrial and domestic effluents are either used or disposed off on land for irrigation purposes that create both opportunities and problems. The main concerns are the risk due to pathogens, heavy metals and other chemicals that may be present in the wastewater [5]. The treated municipal wastewater is basically a carrier of plant nutrients (N, P, K, S, etc.) and generally has low levels of heavy metals

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Table 1
Scheme of treatments applied in experiment

Fertilizer treatments (kg NKP ha^{-1})	Irrigation water treatments		Remarks (NKP kg/ha)*	T. No.	Treatments	T. No.	Treatments
	50% WW	100% WW					
N_0K_0 P_0	+	+	No fertilizer	T1	50%ww N_0K_0 P_0	T5	100%ww N_0K_0 P_0
$N_{50}K_{25}$ $P_{12.5}$	+	+	(50 + 25 + 12.5)	T2	50%ww $N_{50}K_{25}$ $P_{12.5}$	T6	100%ww $N_{50}K_{25}$ $P_{12.5}$
$N_{50}K_{25}$ P_{25}	+	+	(50 + 25+25)	T3	50%ww $N_{50}K_{25}$ P_{25}	T7	100%ww $N_{50}K_{25}$ P_{25}
$N_{50}K_{25}$ P_{50}	+	+	(50 + 25+50)	T4	50%ww $N_{50}K_{25}$ P_{50}	T8	100%ww $N_{50}K_{25}$ P_{50}

(Pb, Cd, Ni, Co, Cr, etc.) [6]. Heavy metals present in wastewater may be toxic to plants and disturb a wide range of biochemical and physiological processes, such as photosynthesis, pigment synthesis, protein metabolism and membrane integrity if taken up at excessive levels [7]. It was also found that the treated wastewater could act as a factor that intensified some of the interactions between heavy metals, as well as macro and micro elements in the soil and plants. These interactions could increase or decrease the level of the interacting heavy metals, depending on whether they were synergistic or antagonistic. Also, they could take place in the plant, in its various organs that is, roots, leaves and heads or sprouts, thereby, contributing to the spatial distribution of the heavy metals in the plant, an issue of great importance, as it is related to human health [8]. The toxicity due to heavy metals depends on the total concentration, as well as the form or species in which these are present in the soil, water or wastewater. The ability of plants to accumulate trace elements in their edible parts varies between plant species and among genotype within species. Thus there are genetic controls over the trace elements concentrations found in edible portions of higher plants [9]. Keeping these variations in mind, and due to the presence of nutrients mentioned above study was undertaken to investigate the effect of 34 ML/d wastewater (STP located at Noida) on the growth of turnip, as well as accumulation of heavy metals in turnip (*Brassica rapa*).

2. Materials and method

2.1. Preparation of pots

An experiment was conducted in pots using a statistical design of factorial randomised block design. Experiments were conducted in pots of 25 cm diameter during the rabi season of 2007–2008 in the month of September. The general purpose of the experiment was to study the effects of the treated municipal wastewater on growth, and heavy metal content in leaves and roots of turnip, with a view to examine the possibility of the treated municipal wastewater reuse, for the irrigation of vegetables.Ten seeds of turnip (*B. rapa*) were sown manually at equivalent distances in each pot. The pots were kept in open. Each pot was initially watered with 200 mL tap water till germination. Seeds germinated within 10 days. Afterwards thinning was done to maintain a single plant in each pot. Sampling for the study of growth was done after 40, 55 and 70 days after sowing (DAS) in each experiment, respectively. Turnip was thus grown under eight different conditions. Nine pots were maintained at each condition. There were two set of pots, one set was daily watered with 100 mL of treated sewage (100%). To the other set 100 mL treated wastewater mixed with tap water in the ratio 1:1 designated as 50% wastewater, with uniform basal dose of nitrogen (N_{50}) and potassium (K_{25}), and varying amount of phosphorus (P_0, $P_{12.5}$, P_{25} and P_{50}) in 50%, as well as 100% wastewater. The scheme of treatments of wastewater used for watering the pots and fertilizer doses is given in Table 1.

2.2. Plant analysis

Three replicates of plant samples were washed with distilled water, oven dried at 70 °C and crushed. The well ground samples were digested and analysed for Cd, Cr, Ni, Fe, Cu, Mn and Zn by atomic absorption spectrophotometer (Model GBC Avanta M). Each pot was analysed for root diameter, number of leaves, plant height, plant fresh weight, plant dry weight and metal accumulation. Out of which three pots were

Table 2
Characteristics of treated effluent and soil before sowing

Parameters	Wastewater[a] (range)	Parameters	Soil[b] (range)
pH	7.11–7.16	pH	7.92–8.09
TDS (mg/L)	1,122–1,138	Soil texture	Sandy loam
BOD (mg/L)	41–42	Total potassium (mg/kg)	50–80
COD(mg/L)	147–150	Total phosphorus (mg/kg)	200–245
DO(mg/L)	0	Total nitrogen (mg/kg)	145–224
Calcium (mg/L)	116–119	Organic carbon (%)	0.72–0.95
Magnesium (mg/L)	61–63	Cadmium (Cd) mg/kg	8.2–15.0
Sodium (mg/L)	330–333	Chromium (Cr) mg/kg	35–37
Potassium (mg/L)	23–24	Nickel (Ni) mg/kg	78–159
Phosphate (mg/L)	6.0–6.2	Iron (Fe) mg/kg	13,084–15,990
Nitrate Nitrogen (mg/L)	1.4–1.6	Copper (Cu) mg/kg	18–21
Ammonical Nitrogen(mg/L)	73–75	Manganese (Mn) mg/kg	31–34
Cadmium (Cd) mg/L	0–0.005	Zinc (Zn) mg/kg	72–184
Chromium (Cr) mg/L	0.003–0.031		
Nickel (Ni) mg/L	0.014–0.31		
Iron (Fe) mg/L	0.049–0.212		
Copper (Cu) mg/L	0.009–0.019		
Manganese (Mn) mg/L	0.005–0.054		
Zinc (Zn) mg/L	0.006–0.112		

[a]Results of analysis of triplicate sets samples collected every month for three months (nine samples).
[b]Results of analysis of triplicate samples collected before sowing.

randomly selected successively for analysis 40 and 55 DAS, respectively. Remaining three pots were analysed 70 days after sowing.

2.3. Water analysis

Treated effluent was characterised for physico-chemical parameters and heavy metals in accordance with the procedure laid down in standard method [10]. Results are given in Table 2. COD was determined by closed reflux method. BOD at three days and at 27 °C was analysed by BOD bottles equipped with pressure sensors and with inductive stirring system. A substrate prepared by mixing 150 mg each of glucose and glutamic acid [10], was used as check solution for standardisation. DO was analysed with Aqualytic OX 24 DO meter. Measurements were made in triplicate. Data presented is the average or the range of value. A spectrophotometer (DR/4000, Hach, USA) was used for measuring nitrate nitrogen, ammonical nitrogen and phosphorus. Hardness, alkalinity, chloride, calcium and magnesium were estimated by titration. Sodium and potassium were measured by flame photometer, while sulphate was determined by turbidity metric method. Samples collected for heavy metal analysis were immediately acidified at sampling point to $pH < 2.0$ by adding HNO_3 to prevent the precipitation of metals. Acidified samples (350 mL) were digested with HNO_3 and filtered. The filtrate was aspirated into Atomic Absorption Spectrophotometer (Model GBC Avanta M) for the analysis of Cd, Cr, Ni, Fe, Cu, Mn and Zn.

2.4. Soil analysis

Samples of soils were collected from the experimental pots prior to each experiment and also before the addition of NPK and analysed. Soil samples were air dried, ground and passed through 2 mm sieve and soil was analysed for pH, soil texture, total N, P, K and heavy metals (Cd, Cr, Ni, Fe, Cu, Mn and Zn). Measurements were made in triplicate. Soil texture was measured by using USDA soil textural triangle after determining the percentage of sand, silt and clay by hydrometry and sieve analysis [11]. Organic carbon and pH was determined by [12], total phosphorus by [13]. Total Kjeldhal nitrogen, [14] while Cd, Cr, Ni, Fe, Cu, Mn and Zn were digested with sulphuric acid procedure followed [14] and measured by means of Atomic Absorption Spectrophotometer (Model GBC Avanta M).

2.5. Statistical analysis

The data obtained were analysed statistically taking into consideration the variables in experiment

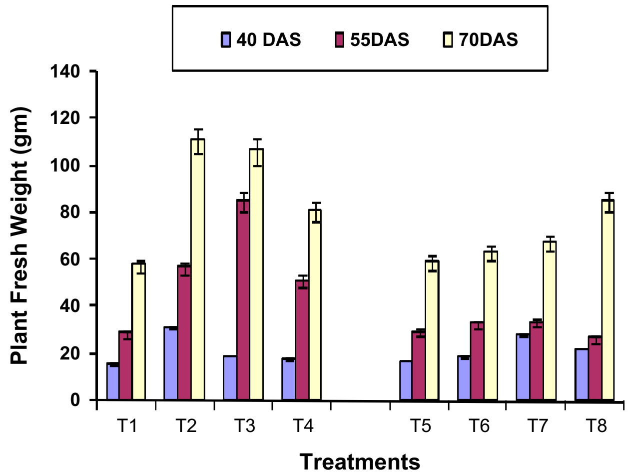

Fig. 1. Plant fresh weight.

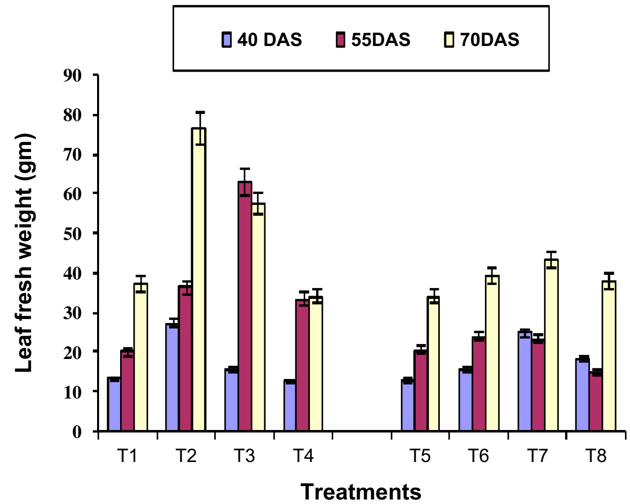

Fig. 3. Leaf fresh weight.

according to [15]. The "F" test was applied to assess the significance of data at 5% level of probability ($p \leqslant 0.05$). The error due to replication was also determined. The model of analysis of variance, Critical difference (CD) was also calculated to compare the mean value of various treatment.

3. Results and discussion

3.1. Growth of the plant

About 34 ML/d wastewater after dilution and lower phosphorus dose 50%ww $P_{12.5}$ proved beneficial in enhancing the growth as plant fresh weight (Fig. 1) and root fresh weight (Fig. 2) were maximum which was at par with 50%ww P_{25} indicating the usefulness of wastewater. While, higher P dose (P_{50}) with 50% wastewater was excessive as it decreased the plant growth. Growth of the plant measured was measured as plant fresh weight, root fresh weight, leaf fresh weight, plant height, leaf number and root diameter is shown through Figs. 1–6. Weights of the plants' roots and leaves increased with the increase in growth period and increase in the root fresh weight was more than that of leaves (Figs. 1–3). The root diameter also increased with the increase in growth (Fig. 6). Root diameter has not shown excessive effect of P_{50} alone while leaf fresh weight, another important growth parameter, was decreased with this dose. Plant height increased marginally (Fig. 4). Number of leaves, however, did not exhibit any trend. Shoots obviously grow first which then provide food to the roots. Fresh and

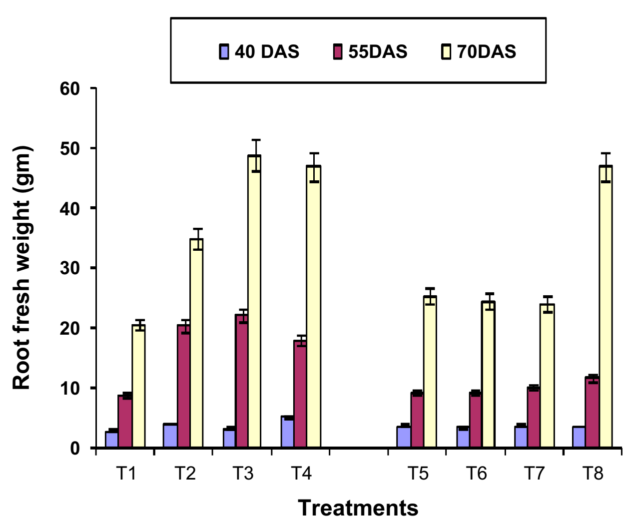

Fig. 2. Root fresh weight.

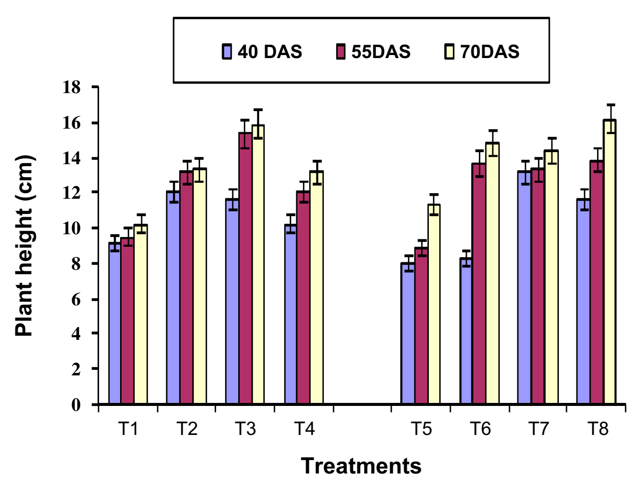

Fig. 4. Plant height.

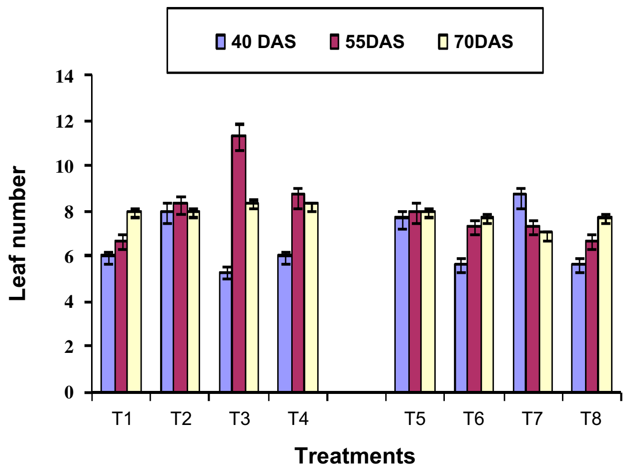

Fig. 5. Leaf number.

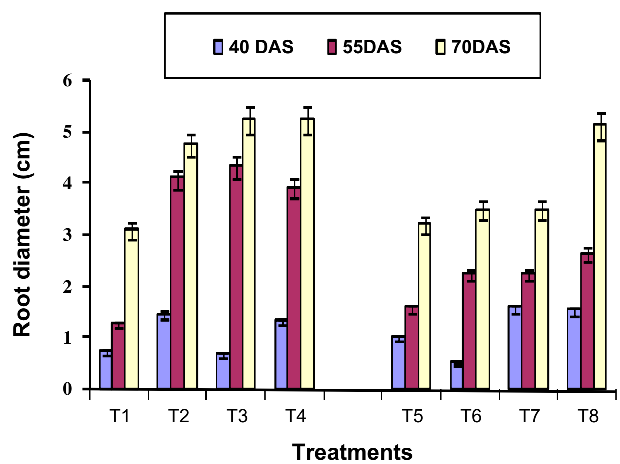

Fig. 6. Root diameter.

dry weight of plant roots and leaves and root diameter increased with the advancement of age up to 70 days. On the contrary, the increase in leaf number from 40 to 55 days followed by a decline in some cases was observed which may be due to the senescence in older leaves at last stage of growth. Among the various phosphorus levels P_{25} with 50% wastewater (T3 treatment) proved optimum for most of the growth characteristics shown in Figs. 1–6.

Irrigation with 100% wastewater proved less effective than 50% wastewater. Wastewater after dilution and lower phosphorus dose ($P_{12.5}$) proved beneficial in enhancing the growth as plant fresh weight and leaf fresh weight (Figs. 1 and 3). Root fresh weight, plant height and root diameter were found maximum in T3. While, higher P dose (P_{50}) was excessive as it decreased the plant growth. Root diameter has not shown excessive effect of P_{50} alone while leaf fresh weight, another important growth parameter in turnip, was decreased with this dose. It may be pointed out that root growth often increased if sufficient phosphorus is provided, relative to shoot growth [16] as P is an integral part of many important metabolites [17]. It also promotes ribulose-1, 5-biphosphate regeneration [18,19], ribulose-1, 5-bisphosphate carboxylase and adenosine tri-phosphate synthesis [20] and carbon dioxide assimilation [21], which was helpful in enhancing the photosynthetic process thereby enhanced the root and shoot growth as observed in this study. These results also corroborate the findings of Greenwood et al. [22] and Zheng-miao et al. [23] who worked on effect of P fertilizer and growth.

3.2. Heavy metal accumulation

The average concentration (along with minimum and maximum, obtained from triplicate) of heavy metals in leaves and roots of turnip on different days (viz. 40, 55 and 70 days) after sowing seeds is shown through Figs. 7–13. Range of heavy metal concentrations (mg/kg) in all the plants i.e. plants harvested on different days is tabulated in Table 3. Heavy metal

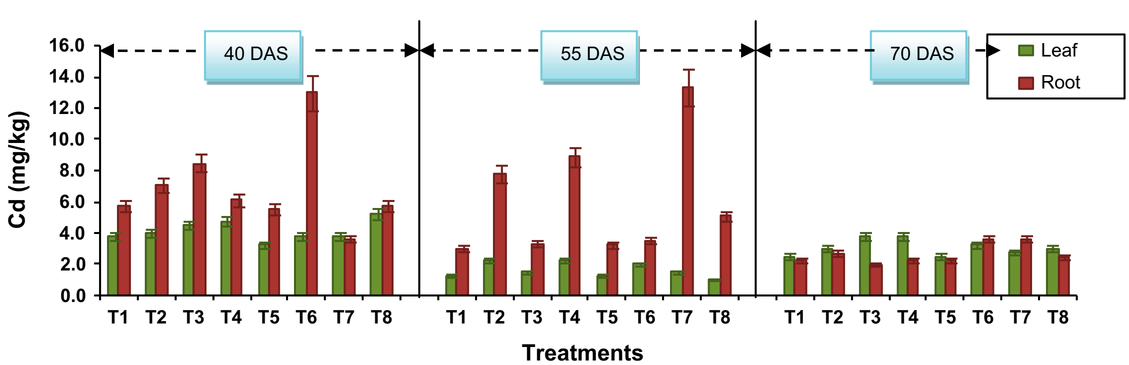

Fig. 7. Cadmium concentrations in leaf and root at different stages of growth.

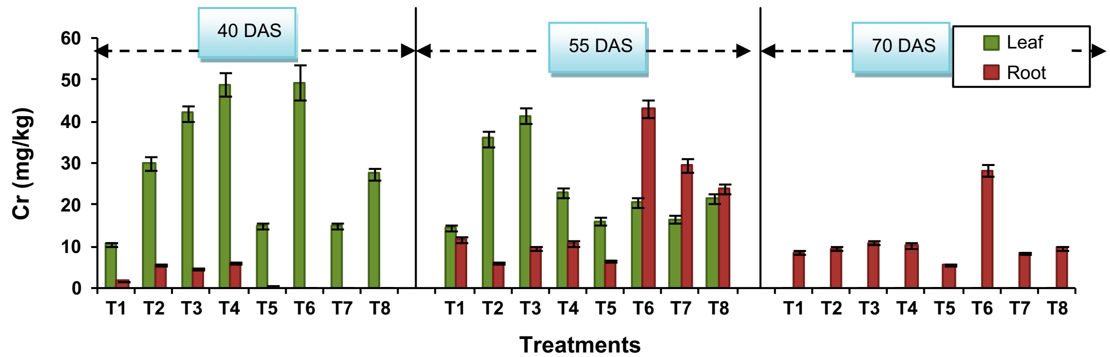

Fig. 8. Chromium concentration in leaf and root at different stages of growth.

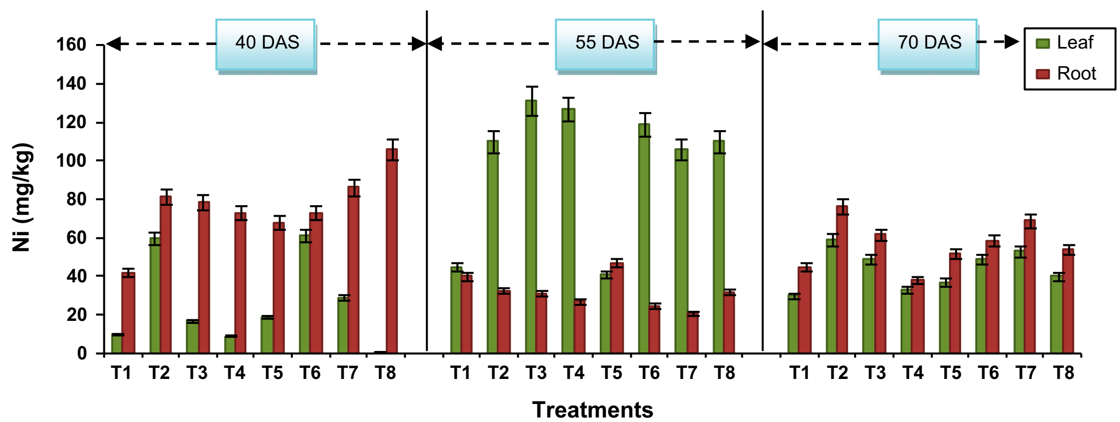

Fig. 9. Nickel concentration in leaf and root at different stages of growth.

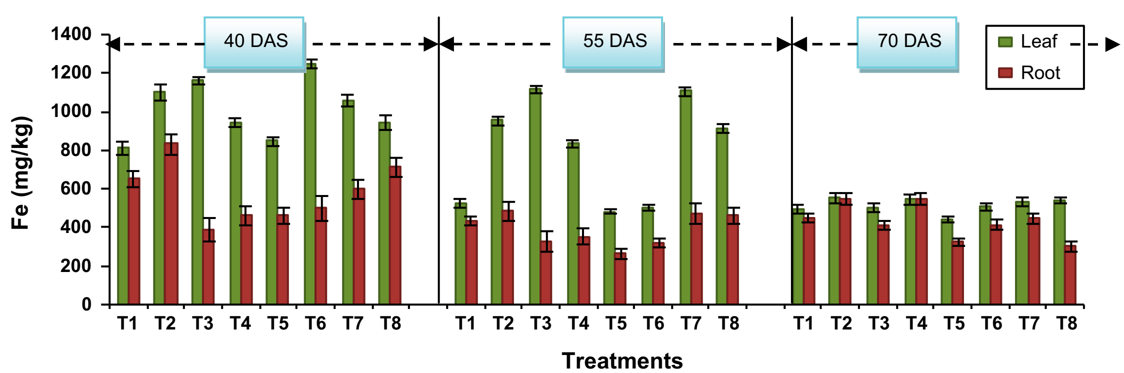

Fig. 10. Iron concentration in leaf and root at different stages of growth.

concentrations in the root and shoot tissues of plants were compared with the standards [24]. The level of Cd, Cr, and Ni in the edible parts i.e. leaves and roots were found to be more than the toxic limits. In case of leaf Cu and Zn was below the toxic limits. The application of wastewater generally led to changes in the physicochemical characteristics of soil and consequently heavy metal uptake by turnip. Heavy metal concentration was in the order of Fe > Zn > Ni > Mn > Cr > Cu > Cd. These results were more or less in agreement with the studies undertaken by Grytsyuk et al. [25], Zheng et al. [26], Arora et al. [27], and Khan et al. [3], while working on various vegetables including turnip. In our study Fe concentration was

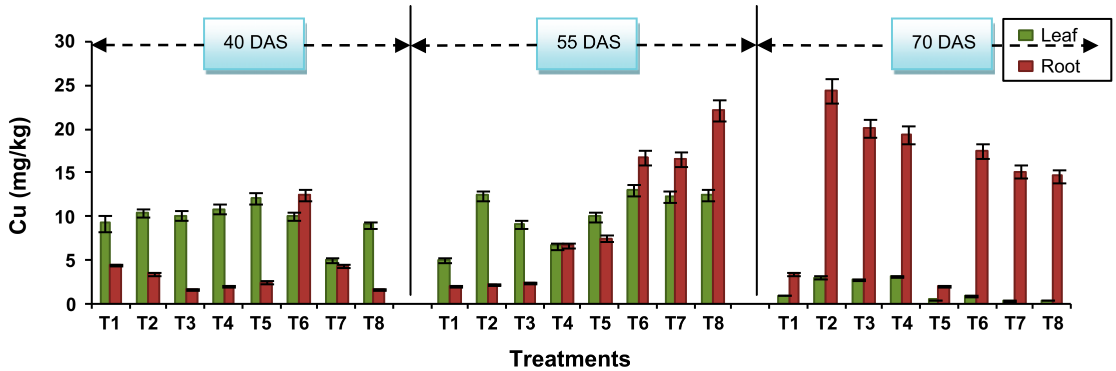

Fig. 11. Copper concentration in leaf and root at different stages of growth.

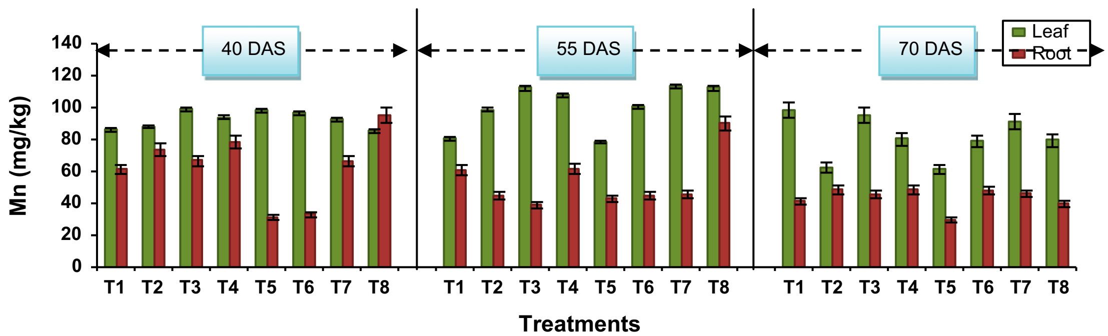

Fig. 12. Manganese concentration in leaf and root at different stages of growth.

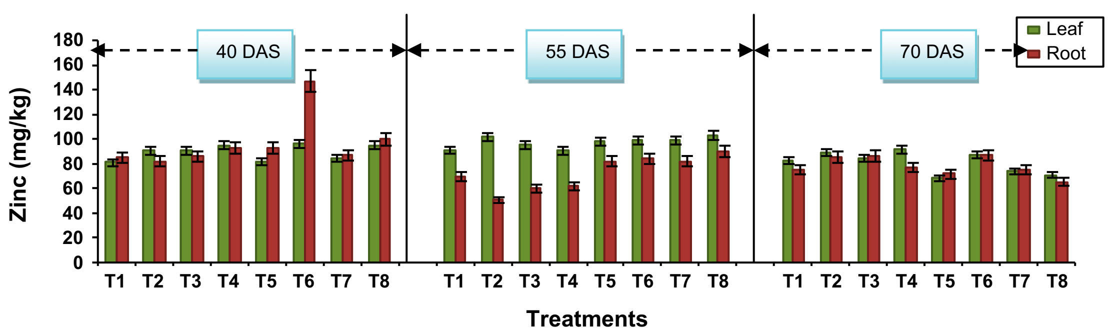

Fig. 13. Zinc concentration in leaf and root at different stages of growth.

maximum because of its high concentration in soil, as well as in wastewater compared to other elements. Zn was followed by Ni as sewage sludge may result in higher levels of Ni. In addition, it is also readily taken up by most plant species [28]. Among the seven trace elements comparatively lower concentration of Cu after Cd may be due to their greater dependability on solubility and soil pH. Most of the heavy metals decreased with growth in both organs. This decreasing trend can be ascribed to the exponential increase in growth and as a result of dilution with growth effect even higher quantities of elements appear to be less when expressed on per unit basis [29]. However, Ni in leaf and Cr, Cu in root were increased with growth which may be because of the selective properties of ion absorption in plants [17]. In addition, phyto-availability of metals also depends on the form of the metal ion and on the plant species which are tested. However, even if using the same species the uptake by plants does not necessarily correlate with

Table 3
CD at 5% at 40, 55 and 70 DAS of plant fresh weight, root fresh weight, leaf fresh weight and plant height

CD at 5%	Plant fresh weight			Root fresh weight			Leaf fresh weight			Plant height		
	40	55	70	40	55	70	40	55	70	40	55	70
Water	0.383	2.092	3.57	0.154	0.648	1.512	0.540	1.455	2.119	0.454	NS	0.579
Treatment	0.542	2.959	5.05	0.218	0.917	2.139	0.764	2.058	2.997	0.642	0.762	0.819
Interaction	0.766	4.184	7.14	0.308	1.297	3.025	1.081	2.911	4.239	0.907	1.077	1.158

the bio-available metal concentration in the soil or the total metal concentration. This is probably due to the genotype with inherent capability of different metal uptakes as pointed out above in the case of Ni, Cr and Cu. It may also be added that plants also differ in their transport of ions resulting in differences in concentration in plant parts [30] as observed in the present study also where the heavy metals fluctuated between the root and leaf depending upon the growth stage, availability of individual element and also their interactions. In many cases, heavy metal concentration was more in plants grown under 50%ww than the 100%ww in case of leaf and root. This was not surprising because metal uptake may differ in relation to external concentration and genotypes. It may also be pointed out that uptake is not linear in relation to increase in wastewater concentration in many cases which may be because metals are bound in the tissues causing saturation which is governed by the rate at which the metal is conducted away. Therefore, the uptake efficiency is more at low concentration which was observed in solution culture [31], as well as in soil [32] for Cd. It may be because of low metal concentration per absorption area giving low competition between the ions at the uptake sites while, the opposite occurs at high concentration (Table 5).

3.2.1. Cadmium

Cd concentration was increased with P doses in case of leaf only but it decreased in root. Its concentration generally decreased with increased in growth of the plant in case of root. While in leaf its concentration decreased with growth up to 55 days and increased towards harvest. Cd concentration was more in root then the leaf, which was also in agreement with the work of Demirezen and Aksoy, [35]. Among the phosphorus doses comparatively lower dose ($P_{12.5}$) at 40 and 70 DAS, gave higher Cd content while at 55 DAS, P_{25} accumulated more Cd. At 40 DAS, the interaction 100%ww$P_{12.5}N_{50}K_{25}$ showed maximum concentration which was different with others. At 55 DAS, 100%ww$P_{25}N_{50}K_{25}$ was statistically different with the other treatments and gave the maximum Cd content. Similarly at 70 DAS, also 100% ww$P_{12.5}N_{50}K_{25}$ accumulated more and it was at par with 100%ww$P_{25}N_{50}K_{25}$.In case of leaf, 50%ww enhanced the concentration throughout the growth. Among the phosphorus doses, P_{50} showed maximum Cd at 40 and 70 DAS while at 55 DAS, $P_{12.5}$ had more. Among interactions at 40 DAS, 100%ww$P_{50}N_{50}K_{25}$registered maximum concentration in leaf and this interaction was critically different with others. About 50% ww $P_{50}N_{50}K_{25}$ gave more Cd at 55 DAS, and it was at

Table 4
CD at 5% at 40, 55 and 70 DAS of leaf number and root diameter

CD at 5%	Leaf number			Root diameter		
	40	55	70	40	55	70
Water	0.383	2.092	3.57	0.050	0.134	0.184
Treatment	0.542	2.959	5.05	0.071	0.190	0.260
Interaction	0.766	4.184	7.14	0.102	0.268	0.368

CD: critical difference. DAS: Days after sowing.

Table 5
Heavy metal concentrations

Heavy metals	Range of heavy metal concentration in wastewater and soil sample		Range of heavy metals in plant part (mg/kg DW)		Range of excessive toxicant levels in plants (mg/kg DW) by Pendias and Pendias [24]	Permissible limits of Indian standards of heavy metals [3334]	
	Wastewater (mg/L)	Soil (mg/kg)	Leaf	Root		Water (mg/L)	Soil (mg/kg)
Cd	0–0.005	8.2–15.0	1.0–6.0	2.0–14.0	5–30	0.01	3–6
Cr	0.003–0.031	35–37	0–50	0–44	5–30	0.1	–
Ni	0.014–0.31	78–159	1–131	20–106	10–100	0.2	75–150
Fe	0.049–0.212	13,084–15,990	445–1,251	265–834	–	–	–
Cu	0.009–0.019	18–21	0–13	1.0–25.0	20–100	0.2	135–270
Mn	0.005–0.054	31–34	61–114	29–95	300–500	0.2	–
Zn	0.006–0.112	72–184	68–103	51–147	100–400	2.0	300–600

par with 50%ww $P_{12.5}N_{50}K_{25.}$ While at 70 DAS, also the same treatment accumulated maximum Cd was equaled by 50%ww $P_{25}N_{50}K_{25.}$In the present work, data indicated that the metal was largely retained in roots as compared by TF values which were <1. Significantly the level of Cd in root in the majority of the cases exceeded the toxic levels at 40 DAS, while at harvest stage Cd was surprisingly below the toxic level (Fig. 7). It was obviously due to the increased plant fresh weight at this stage, which was supposed to be responsible for its dilution although the total Cd in leaf was below the toxic levels at last stage of sampling as it was generally retained by the roots, instead of transporting it towards the shoot.

3.2.2. Chromium

In root, 50%ww recorded more Cr at early stage while at later two stages, 100%ww gave the maximum concentration (Table 4). Among the fertilizer doses higher concentration at 55 and 70 DAS, was given under lower concentration of P. At 40 DAS, the interaction, 100%ww$P_0N_0K_0$ accumulated more Cr content which was critically different with other treatments. At later stage another interaction 100% ww $P_{12.5}N_{50}K_{25}$ showed higher concentration and it also differed with other treatments, followed by 100%ww $P_{25}N_{50}K_{25,}$ 100%ww $P_{50}N_{50}K_{25}$ and 50%ww $P_0N_0K_{0.}$ While at last stage again like earlier stage, 100%ww $P_{12.5}N_{50}K_{25}$ gave the maximum value. It was followed by 50%ww $P_{25}N_{50}K_{25,}$ 50%ww $P_{50}N_{50}K_{25,}$ 100%ww $P_{50}N_{50}K_{25}$ and 50%ww $P_{12.5}N_{50}K_{25}$. In case of leaf also, the interactions 100%ww$P_{12.5}N_{50}K_{25}$ accumulated maximum Cr content as it was statistically similar with 50%ww $P_{50}N_{50}K_{25}$. While at 55 DAS, diluted wastewater (50%ww$P_{25}N_{50}K_{25}$) accumulated more. It was followed by 50%ww $P_{12.5}N_{50}K_{25}$ and 50%ww $P_{50}N_{50}K_{25.}$ Last treatment was at par with 100% ww$P_{50}N_{50}K_{25}$ which in turn was at par with 100%ww $P_{12.5}N_{50}K_{25}$. Surprisingly at 70 DAS, Cr was not detected in any of the treatments. At 40 and 55 days plant samples have toxic level of Cr while at 70 days Cr was not detected in leaf due to dilution after sufficient growth in plant. Its concentration was generally

Table 6
CD at 5% at 40, 55 and 70 DAS for Cd, Cr and Ni

CD at 5%	Cadmium (mg/kg)						Chromium (mg/kg)						Nickel (mg/kg)					
	Leaf			Root			Leaf			Root			Leaf			Root		
	40	55	70	40	55	70	40	55	70	40	55	70	40	55	70	40	55	70
Water	0.174	0.075	0.131	0.360	0.338	0.115	1.45	1.14	NS	0.184	0.903	0.564	1.56	4.53	1.96	3.28	1.37	2.53
Treatmnt	0.246	0.105	0.185	0.509	0.477	0.162	2.05	1.61	NS	0.261	1.278	0.798	2.20	6.41	2.78	4.64	1.93	3.58
Interaction	0.348	0.149	0.262	0.719	0.675	0.229	2.88	2.27	NS	0.369	1.807	1.129	3.11	9.06	3.93	6.57	2.73	5.06

CD: critical difference. DAS: Days after sowing.

more in leaf at 40 and 55 DAS, while at harvest stage more in root. Cr concentration generally decreased with the growth of plant in leaf. In root, 50%ww recorded more Cr at early stage, while at later two stages 100%ww gave the maximum concentration (Fig. 8). At early stage of growth, Cr was not detected probably it was not taken up at detection level by the root while at harvest it was not detected (T6, T7 and T8) in leaf, which may be due to differences in genotypes with inherent difference in metal uptake [30]. Alternatively it may be because of its release back from plant tissue as metal accumulation depends on both uptake into the tissue and leakage into the surrounding medium [36].

3.2.3. Nickel

Among the interactions 100%ww $P_{50}N_{50}K_{25}$ recorded more Ni as it was different with other interactions. It was followed by 100%ww $P_{25}N_{50}K_{25}$, 50% ww $P_{12.5}N_{50}K_{25}$, 50%ww$P_{25}N_{50}K_{25}$, 50%ww $P_{50}N_{50}K_{25}$ and 100%ww $P_{12.5}N_{50}K_{25}$. At 70 DAS, 50%ww $P_{12.5}N_{50}K_{25}$, gave maximum Ni content and was different with others. In case of leaf, 100%ww at 40 and 70 DAS, respectively, it gave the maximum values while at 55 DAS, 50%ww accumulated more. Among the phosphorus doses, $P_{12.5}$ at 40 and 70 DAS, and P_{25} at 55 DAS, showed maximum content. The interaction 100%ww $P_{12.5}N_{50}K_{25}$ gave the maximum Ni concentration at 40 DAS, and had similar effect with 50%ww $P_{12.5}N_{50}K_{25}$. While at 55 DAS, 50%ww $P_{25}N_{50}K_{25}$ accumulated more. It was equally effective with 50%ww $P_{50}N_{50}K_{25}$. At 70 DAS, 50%ww $P_{12.5}N_{50}K_{25}$ had more Ni content and was different with other interactions followed by 100%ww $P_{25}N_{50}K_{25}$ and 100%ww $P_{12.5}N_{50}K_{25}$. Last treatment was at par with 50%ww $P_{25}N_{50}K_{25}$. In case of root its concentration first decreased with growth and again increased at harvest, while in case of leaf first increase with growth up to 55 DAS, and then decreased at harvest. In case of root, 100%ww showed maximum concentration at 40 and 70 DAS, while at 55 DAS, 50%ww gave more value. Its concentration was more in root at 40 and 70 days, while at 55 days, it was more in leaf. It may be because of its easy mobility in plants therefore after initial growth up to 40 days, where its concentration was higher it was transported more towards the growing leaves at 55 days. However, at later stage due to senescence in some of the older leaves it was transported back towards the root where an elevated Ni concentration was observed (Fig. 9) Shacklette, [37] reported that the Ni content in vegetables ranges from 0.2 to 3.7 ppm (DW). In our study, it ranges from 1 to

Table 7
CD at 5% at 40, 55 and 70 DAS for Fe and Cu

CD at 5%	Iron (mg/kg)						Copper (mg/kg)					
	Leaf			Root			Leaf			Root		
	40	55	70	40	55	70	40	55	70	40	55	70
Water	17.57	36.43	14.52	14.69	16.98	19.05	0.416	0.453	0.090	0.402	0.483	0.746
Treatment	24.85	51.52	20.54	20.78	24.01	26.94	0.588	0.640	0.127	0.568	0.683	1.055
Interaction	35.14	72.85	29.04	29.39	33.96	38.10	0.831	0.905	0.180	0.804	0.965	1.492

CD: critical difference. DAS: Days after sowing.

131 mg/kg. Data obtained indicated that metal accumulated by turnip plants was largely retained in roots as indicated by TF values which were <1 at 40 and 70 days, while at 55 days TF value in general was >1 (Table 6).

3.2.4. Iron

Among the interactions, 50%ww $P_{12.5}N_{50}K_{25}$ accumulated more iron. It was critically different with other interactions. At later stage, 50%ww $P_{12.5}N_{50}K_{25}$ showed maximum Fe content and gave equal effect with 100%ww $P_{25}N_{50}K_{25}$ and 100%ww $P_{50}N_{50}K_{25}$. At last stage, 50%ww $P_{12.5}N_{50}K_{25}$ accumulated more Fe in root while in case of leaf at 40 DAS, 100%ww and 50%ww at 55 and 70 DAS, accumulated more iron. The interaction at 40 DAS, was maximum in 100%ww $P_{12.5}N_{50}K_{25}$ and also different with other interactions. At second stage, 50%ww $P_{25}N_{50}K_{25}$ accumulated more iron followed by 100%ww $P_{25}N_{50}K_{25}$, and 50%ww $P_{12.5}N_{50}K_{25}$. At 70 DAS, 50%ww $P_{12.5}N_{50}K_{25}$ gave the maximum value and was statistically similar with 50%ww $P_{50}N_{50}K_{25}$, 100%ww $P_{50}N_{50}K_{25}$, and 100% ww$P_{25}N_{50}K_{25}$ and the last treatment was also at par with 100%ww $P_{12.5}N_{50}K_{25}$.

In the present work all the samples contain Fe content more than 130 mg/kg. It was more in leaf than in root and in case of leaf, Fe content decreased with growth of the plant (Fig. 10). While in root it decreased up to 55 DAS, and increased at harvest. In roots 50%ww showed more Fe content at three stages. Among the phosphorus doses, $P_{12.5}$ at 40 and 70 DAS, while at 55 DAS, P_{50} gave higher iron content. The increase in Fe content under P fertilizers may be because of their roles in enhancing the root growth thereby the surface area for its greater absorption [38]. The TF values were generally >1 proving more concentration in leaf because about 75% of total leaf Fe is associated with chloroplast and up to 90% of Fe leaves occur with lipoprotein of chloroplast and mitochondria membrane. Its presence in larger amount in leaves was understandable [28].

3.2.5. Copper

Among the interactions, 100%ww $P_{12.5}N_{50}K_{25}$ giving higher concentration as it was statistically different with other treatments followed by 100%ww $P_{25}N_{50}K_{25}$, 50%ww $P_{12.5}N_{50}K_{25}$ and 100%ww $P_0N_0K_0$. At second stage, 100%ww $P_{50}N_{50}K_{25}$ recorded maximum Cu content followed by 100%ww $P_{12.5}N_{50}K_{25}$, which in turn was at par with 100%ww $P_{25}N_{50}K_{25}$. While at harvest, 50%ww $P_{12.5}N_{50}K_{25}$, accumulated more followed by 50%ww $P_{25}N_{50}K_{25}$, which was also statistically equal to 50%ww $P_{50}N_{50}K_{25}$, followed by 100%ww $P_{12.5}N_{50}K_{25}$, 100%ww $P_{25}N_{50}K_{25}$, and 100% ww $P_{50}N_{50}K_{25}$. In case of leaf at 40 and 70 DAS, 50% ww gave the maximum Cu while at second stage, 100%ww accumulated more. Out of P doses, $P_{12.5}$ accumulated maximum and among interactions, 100% ww $P_0N_0K_0$. At 55 DAS, 100%ww $P_{12.5}N_{50}K_{25}$ accumulated more and was equally effective with 100%ww $P_{50}N_{50}K_{25}$, 50%ww $P_{12.5}N_{50}K_{25}$ and 100%ww $P_{25}N_{50}K_{25}$. While at the last stage, 50%ww $P_{50}N_{50}K_{25}$ had more Cu and it was also similar to 50%ww $P_{12.5}N_{50}K_{25}$. In general, Cu was more in leaf at 40 and 55 days, while at 70 days it was more in root (Fig. 11). The highest concentrations of Cu in shoots are always in phase of intensive growth which was evident in our study. The distribution of Cu within plants is highly variable as within roots, Cu is associated mainly with cell wall and is largely immobile. Its normal concentration in plant tissue ranges from 5 to 20 ppm. It was found that the range of Cu varied from 0 to 25 mg/kg. Among the interactions T6 accumulated more Cu at 40 and T8 at 55 days, while at 70 days T2 accumulated more Cu. It may also be pointed out that phosphatic fertilization has also decreased the Cu concentration as Cu-P antagonism commonly occurs in root media [36] (Table 7).

Table 8
CD at 5% at 40, 55 and 70 DAS for Mn, and Zn

CD at 5%	Manganese (mg/kg)						Zinc (mg/kg)					
	Leaf			Root			Leaf			Root		
	40	55	70	40	55	70	40	55	70	40	55	70
Water	1.11	1.31	3.38	2.82	2.27	1.88	NS	2.726	3.48	4.105	2.953	3.347
Treatment	1.56	1.86	4.79	3.99	3.21	2.66	5.319	3.855	4.921	5.805	4.176	4.733
Interaction	2.21	2.63	6.77	5.65	4.54	3.77	NS	5.452	6.960	8.210	5.906	6.694

CD: critical difference. DAS: Days after sowing.

3.2.6. Manganese

At 40 DAS, among the wastewater and phosphorus doses 100%ww $P_{50}N_{50}K_{25}$ showed maximum Mn content followed by 50%ww $P_{50}N_{50}K_{25}$ and it was at par with 50%ww $P_{12.5}N_{50}K_{25}$. While at 55 DAS, 100% ww $P_{50}N_{50}K_{25}$ accumulated more and followed by 50% ww $P_{50}N_{50}K_{25}$ and the last treatment was at par with 50%ww $P_0N_0K_0$. At 70 DAS, 50%ww $P_{50}N_{50}K_{25}$ accumulated higher Mn content and it was equally effective with 50%ww $P_{12.5}N_{50}K_{25}$, 100%ww $P_{12.5}N_{50}K_{25}$, 100% ww $P_{25}N_{50}K_{25}$ and 50%ww $P_{25}N_{50}K_{25}$. In case of leaf at 40 and 55 DAS, 100%wwgave higher Mn content in leaf while at 70 DAS, 50%ww accumulated maximum Mn. Among the phosphorus doses, P_{25} registered more than the other doses. Phosphatic fertilizers have about 40–2000 ppm Mn [36] which can be a good source of Mn. The interactive effect was maximum in 50%ww $P_{25}N_{50}K_{25}$ and was at par with 100%ww$P_0N_0K_0$. At 55 DAS, 100%ww $P_{25}N_{50}K_{25}$ accumulated more Mn and was statistically similar with 100%ww $P_{50}N_{50}K_{25}$ and 50%ww $P_{25}N_{50}K_{25}$ while at 70 DAS, 50%ww$P_0N_0K_0$ registered higher value although at par with 50% ww$P_{25}N_{50}K_{25}$.

Mn concentration in plant samples were found within lower range of toxic level. Its concentration was more in leaf than in root. Its concentration was generally decreased with growth. In root at 40 and 55 days T8 accumulated more manganese, while at 70 days T4 accumulated higher Mn content. Its normal concentration in plants typically ranges from 20 to 500 ppm. In our study the range of Mn varied from 29 to 113 mg/kg. In case of leaf Mn concentration from 15 to 20 ppm considered deficient while in the present study higher amount of Mn were observed in leafy part of turnip (Fig. 12). The TF values in general were > 1 in all experiments although it reverses in some cases. It may be because Mn concentration fluctuates greatly within the plant parts and within the vegetative period. It is not only an effect of plant characteristics but also the pool of available Mn which is controlled basically by soil properties.

3.2.7. Zinc

The concentration of Zn was more in leaf than root at second stage of sampling. Its concentration in leaf increased first and then it decreased with growth. While in root, it was generally decreased with growth. At 40 DAS, interaction 100%ww $P_{12.5}N_{50}K_{25}$ accumulated maximum Zn content and was different with other combinations. At later stage, 100%ww $P_{50}N_{50}K_{25}$ gave more Zn and was statistically different with the other treatments. At 70 DAS, 100%ww $P_{12.5}N_{50}K_{25}$ accumulated more and had equal effect with 50%ww $P_{25}N_{50}K_{25}$ and 50%ww $P_{12.5}N_{50}K_{25}$. In case of leaf, at 55 DAS, 100%ww $P_{50}N_{50}K_{25}$ gave higher value and it was equally effective with 50%ww $P_{12.5}N_{50}K_{25}$, 100%ww $P_{12.5}N_{50}K_{25}$, 100%ww$P_{25}N_{50}K_{25}$ and 100%ww$P_0N_0K_0$. At 70DAS, 50%ww$P_{50}N_{50}K_{25}$ accumulated more and was equally effective with 50%ww $P_{12.5}N_{50}K_{25}$ and 100%ww $P_{12.5}N_{50}K_{25}$. Haq et al. [9] reported that normal range of Zn in healthy vegetables is 20–100 mg/kg, while Pendias and Pendias [24] reported that toxic or excessive levels of Zn in plants are 100–400 mg/kg.

Comparing with normal and toxic range, all the treatments were found within sufficiency range except one that is, T6 treatment in root. Its normal concentration range is 25 to 150 ppm in plants and its deficiency is usually associated with concentration of less than 20 ppm and toxicities with 400 ppm or more and in the present work it was from 51 to 147 mg/kg (Fig. 13) (Table 8).

4. Conclusion

Analysis of the wastewater revealed its suitability for the irrigation as most of the values for the analysed parameters were within the permissible limits of FAO/WHO/Pescod. It was concluded that the irrigation with treated effluent increased the concentration of heavy metals in plant parts. It is believed that translocation of heavy metals from the soil to plant occurred due to the presence of these metals in treated effluent. But wastewater irrigation increased the growth parameters. Plant fresh weight, root fresh weight, leaf fresh weight and root diameter increased with increasing age of the plants. The best harvest stage of turnip was 70 days after sowing. 50%ww proved best over 100%ww therefore it can be used after dilution. The combination 50%ww $P_{12.5}$ proved beneficial in enhancing the growth which was at par with 50%wwP_{25} indicating usefulness of wastewater where 12.5 kg P/ha could be saved. While, the 100% wastewater treatments proved toxic, therefore this water should not be used directly until it diluted beyond the range of 50% wastewater. The levels of Cd, Cr and Ni in the edible plant parts (leaves and roots) were more than the toxic limits given by Pendias and Pendias [36]. In leaf Cd, Cr, Fe, Cu, Mn and Zn concentration was below the toxic level at harvest stage. Heavy metal concentration was found in the order of Fe > Zn > Ni > Mn > Cr > Cu > Cd. Therefore, it is not advisable to consume turnip cultivated under this water.

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