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Original Article

Morphological and anatomical responses of *Capsicum annuum* L. as influenced by industrial effluents

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ABSTRACT

This research was conducted at the Botanical Garden, Bauchi Road Main Campus, University of Jos, Plateau State, Nigeria from June to November 2022, to investigate the impact of industrial effluent on the growth and anatomical structures on chili pepper (*Capsicum annuum* L.). The experiment was laid out in a completely randomized design with five treatments and six replicates. The treatments comprised 0% (control), 5%, 10%, 15%, and 20% industrial effluent concentrations, applied as irrigation water. The growth parameters were collected and data evaluated. The post-harvest pot soils were analyzed for heavy metals. The plants showed a significant reduction in plant height, leaf length, leaf width, fruit weight, plant weight, root length, number of flowers, and fruits with increased effluent concentrations. The morphological response of the plants to the effluent concentrations contributed to the reduction in the anatomical characters studied. Stem tissues such as cortical layer, vessel number, pith thickness, collenchyma, and parenchyma cell thickness decreased significantly ($P < 0.05$) with increased effluent concentrations. The root characters also showed similar trend. The application of higher concentrations of the effluent caused increase in the heavy metal toxicity in the soils, resulting in the reduction in the growth and yield of *C. annuum* as well as the anatomical structures. It is therefore important to educate farmers on the toxic effects of detergent effluents on plants especially at a high concentration.

Keywords: Anatomical structures, concentrations, growth, stem tissues

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INTRODUCTION

Waste created by industrial activity is referred to as industrial effluents, and the types of effluents produced vary based on the human activities that generate them.[1] The production of these wastes is a necessary component of industrial activities, but sadly, our inability to foresee or predict the types and magnitude of unintended consequences of the unrestricted release of effluents in our environment, combined with the expansion of industrialization, have led to massive and destructive operations in our ecosystems.[2] Industrial operations are desirable, but there are issues with how to manage and dispose of the massive amounts of waste that are produced by various industries. Almost all industrial wastewater is released untreated into waterways or onto land in underdeveloped nations, however in areas where effluent treatment facilities are present, the plants are rarely used, due to the high expense of treating effluents.[3] In addition, even after treating industrial wastewater/effluents, it is still impossible to completely remove all undesirable properties before releasing the effluents into the environment, particularly into rivers, estuaries, lagoons, and the ocean. As a result, these waste waters contaminate the water supplies, which, in turn, affects the agricultural land.[4]

Due to a lack of clean water, urban cities have seen an increase in the irrigation of food crops, particularly leafy and fruit vegetables, using water that has been contaminated by discarded effluents.[5] Urban farmers have also advocated for the use of these wastewater/effluents because they believe they contain high nutrients that encourage rapid vegetative development, lowering the cost of fertilization in both organic and inorganic forms.[6] According to reports, Nigeria produces vegetables all year round due to the availability of industrial effluent for irrigation.[7] To meet the high demand for vegetables in Nigeria, Uaboi-Egbenni *et al*.[2] also stated that many urban farmers divert effluents to farmland to irrigate vegetable farms. However, it was stated that caution should be exercised when

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using these effluents for the irrigation of tender herbaceous vegetables.

Capsicum annuum L. commonly known as red pepper or chili pepper belongs to the family Solanaceae. It is a short-lived, evergreen perennial plant that is usually grown as an annual plant.[8] *C. annuum* is one of the most significant vegetables in the world, valued for its colorful, fleshy, and pod-like fruit. Chili peppers are the third most significant cultivated vegetable, and every household consumes them; thus, their significance cannot be overstated.[9] The remarkable cherished flavor and the desirability of carotenoid pigments in food coloring continually increase its demand by consumers in local and urban markets.[9] In addition to being utilized as food colouring, chili peppers are also employed in the manufacturing of extracts for the pharmaceutical and cosmetics industries, as well as fresh and processed vegetables, spices, dried forms, dye, and bred as ornamental plant.[10] Chili peppers are in high demand in Nigeria and needed all year long due to their many benefits. The cultivation of *C. annuum* requires irrigation, and as a result of the high demand, the use of industrial effluents as irrigation water has significantly grown. Several authors have discussed the impact of using these effluents as irrigation water on vegetable growth, nutrient content, and heavy metal concentration.[2,7,11] This research is, therefore, aimed at studying the morphological and structural alterations in *C. annuum* in response to irrigation using detergent effluent.

MATERIALS AND METHODS

Experimental Location

The field research was conducted at the Botanical Garden, Bauchi Road Main Campus, University of Jos, Plateau State, Nigeria (latitude 09°56’N and longitude 08°53’E and altitude 1159 m above sea level). The experiment was carried out between the months of June and November 2022, rainy season.

Sources of Seeds, Detergent Effluent, and Soil

The variety of chili pepper seeds (cayenne) used for this investigation was purchased from a local seed shop at the Jos Museum gate, Jos, and identification was done in the herbarium of the University of Jos. The detergent effluent used was collected from NASCO Group of Company, Jos. The soil was obtained from the nursery of the Federal College of Forestry, Jos.

Experimental Design and Seed Planting

The pot experiment was laid out in a completely randomized design with five treatments and six replicates. The effluent collected was serially diluted with borehole water to give representative concentrations of 5%, 10%, 15%, and 20% and then used as irrigation water. The fifth treatment was the control experiment (0% concentration) which was set aside and irrigated with normal water for the period of the research. Five healthy seeds were planted in each pot and six replicates of each concentration were made. The pots were labeled accordingly as control (0%), 5%, 10%, 15%, and 20%. The first round of irrigation with 30 mL of each of the prepared concentrations of the effluent was carried out in the 1st week after planting (WAP). Effluent irrigation of the peppers was also carried at 2WAPafter which normal water irrigation commenced until the 18WAP when the experiment was terminated. The experiment was conducted in a greenhouse to protect the plants from direct rainfall and plants were transported to the field immediately after 3 weeks.

Data Collection and Recording

At 4, 8, 12, 16, and 18 WAP, plant height, leaf length, number of leaves, number of branches, number of flowers, and number of fruits were recorded. After harvest (at 18 WAP), the root length and weight of the plants were determined.

Sample Preparation for Anatomical Studies

The plants were carefully uprooted to prevent damage to the root tips and rinsed thoroughly in distilled water to remove debris. For each of the concentrations, transverse sections of 5–10 µm thickness of the stem and root were obtained using a rotary microtome. The sections were stained in 10 % aqueous safranin solution, counter stained in lactophenol and mounted in 5% diluted glycerol.[12] Specimen slides were observed using light microscopy and photomicrographs were captured under magnification of ×400 and ×100 using olympus microscope with attached celestron digital camera.

The arrangements of cortical cells, vascular bundles, and epidermis were observed and measurement of characters was done using an ocular micrometer.

Soil Analyses

The soil samples collected from the various pots irrigated with the different effluent concentrations were preserved and subjected to chemical analysis. A total of seven metallic elements were determined using atomic absorption spectrophotometry.[13] These include, iron (Fe), lead (Pb), copper (Cu), manganese (Mn), cadmium (Cd), nickel (Ni), and chromium (Cr).

Statistical Analysis

The means of the quantitative morphological and anatomical parameters were subjected to a two-way analysis of variance and means separated by Duncan multiple range test at $P < 0.05$. All statistics were carried out with the use of the Statistical Package for the Social Sciences software version 16.

RESULTS AND DISCUSSION

Plant Height

The effect of the industrial effluent on the mean height of *C. annuum* is presented in Table 1. The growth of the plant

Table 1: Effect of different effluent concentrations on the morphological characters of *Capsicum annuum*.

	Percentage concentrations of the effluent				
Characters	**Control**	**5%**	**10%**	**15%**	**20%**
Plant height (cm)	28.17±4.22^a	26.50±2.57^b	25.03±1.24^b	18.03±1.56^c	16.00±0.40^c
Leaf length (cm)	3.45±0.08^a	3.15±0.12^a	1.96±0.05^b	1.34±0.08^c	1.73±0.08^{bc}
Number of leaves	43.50±11.20^c	46.33±5.28^b	49.00±7.75^b	71.33±24.41^a	24.50±1.29^c
Number of branches	12.67±1.51^d	14.67±1.21^c	15.00±1.55^b	18.00±2.19^a	8.20±0.08^e
Number of flowers	7.00±1.67^a	5.17±2.93^b	3.67±0.52^c	0.67±0.52^d	0.00±0.00^e
Root length (cm)	12.45±0.75^a	10.65±0.77^b	7.50±3.74^c	5.35±4.18^c	0.14±0.26^d
Weight of plants (g)	11.27±2.59^a	8.67±2.16^b	6.61±3.88^b	3.73±3.86^c	2.33±2.88^c
Number of fruits	0.67±0.82^a	0.50±0.84^a	0.17±0.41^b	-	-

Data represented as a mean ± deviation. Means followed by the same letter (s) in each row are not significantly different at 5% level of probability (Duncan multiple range test)

decreased with increase in the application of the effluent. At 18 WAP, the *C. annuum* with no effluent contamination (control) showed the highest performance on plant height and was significantly different ($P < 0.05$) from other treatments. The *C. annuum* planted with 20% effluent concentration showed the least performance on plant height but was however not significantly different from those planted with 15% effluent concentration [Table 1]. The continuous reduction in the growth parameters, especially at 20% effluent concentration with lowest growth and development have been reported by Mohammad *et al.*[14] to be a result of accumulation of toxic metals from the application of the effluent to the soil over the growing period as well as change in the soil environment which includes changes in soil pH and organic matter contents through different anthropogenic activities such as mining and effluent discharge.

Leaf Length

The effect of the industrial effluent on the mean leaf length of *C. annuum* is shown in Table 1. The mean length of the plant decreased with increased effluent concentration. At 18WAP, the mean length was highest in the control (0% effluent concentration) and was significantly different ($P < 0.05$) from the other effluent concentrations except 5% concentration. The reduction in the leaf length in plants grown in polluted areas has been reported by Folorunso *et al.*[11] and Stevovic *et al.*[15] and considered adaptive advantage that enables leaves to develop and function in habitats with strong variations of heavy metals. Further, the increase in the leaf length at 20% effluent concentration could also be adaptive measures employed by the same characters in the plant so as to withstand the high effluent concentration.

Number of Leaves

The effect of the effluent concentrations on the mean number of leaves was also revealed in Table 1. At 4–10 WAP, the average number of leaves was highest for the 0% effluent concentration; however, there was a rapid increase in the number of leaves of *C. annuum* with 15% effluent concentrations from 12 WAP. Further, for all the sampling dates, the mean number of leaves was least for 20% effluent concentration and was significantly different ($P < 0.05$) from the other effluent concentrations.

Number of Branches

The effect of the industrial effluent on the mean number of branches of *C. annuum* is also shown in Table 1. The different effluent concentrations resulted in significantly different ($P < 0.05$) mean number of branches at 18 WAP. For all the sampling dates, the 20% effluent concentration resulted in a significantly lower mean number of branches compared to the other effluent concentrations, while 15% effluent concentration had a significantly higher ($P < 0.05$) mean number of branches.

Number of Flowers

Table 1 also shows the effect of the different concentrations of effluent on mean number of flowers. There was a reduction in the mean number of flowers with increased effluent concentrations. At 18 WAP, the mean number of flowers was significantly higher ($P < 0.05$) for the 0% effluent concentration (control); however, 20% effluent concentration had the least mean number of flowers.

Root Length, Weight of *C. annuum*, Number, and Weight of Fruits

The mean root length decreased with increased effluent concentrations [Table 1]. The mean root length was significantly longer ($P < 0.05$) for 0% effluent concentration and was lowest at 20% effluent concentration. This agrees with Raju *et al*.[16] who also reported that effluent caused reduction in the length of roots of wheat and rice cultivars.

The mean weight of the *C. annuum* also decreased with increase in the effluent concentrations [Table 1]. At 18 WAP, the control (0% effluent concentration) resulted in a significantly higher ($P < 0.05$) mean weight of the plant when compared to the 5, 10, 15, and 20% concentrations of the effluent.

At 18 WAP, the mean number of fruits yielded decreased with an increase in effluent concentration. The average number of fruits recorded is 0.67, 0.50, and 0.17 for 0%, 5%, and 10% effluent concentrations, respectively. However, at 18 WAP, no fruit was recorded for 15 and 20% effluent concentrations. The continuous reduction in the time of fruit formation, number, and weight of fruits of the *C. annuum* is an indication that the industrial effluent is not favourable to the growth of the plant. This could be as a result of the presence of toxic metals in effluents capable of affecting the metabolism and other physiological process of the plants. This agrees with Srivastava[17] and Ramasubramanian[18] who reported changes in plant physiological processes in radish and onion growth as a result of pepper mill effluent.

Effect of Effluent Concentrations on the Stem Anatomical Structures *C. annuum*.

Table 2 shows the effect of the effluent concentrations on the stem anatomical characters. The stem thickness and cortical layer thickness were significantly higher at 0% effluent concentration. The number of xylem vessels in the stem of the *C. annuum* reduced with increase in the effluent concentration (5%, 10%, and 15%); however, the reduction in number of the vessel was not significantly different at ≤0.05 level of probability [Table 2]. The phloem cells also deteriorated with increased effluent concentration. Reduction in the number of vessels and deterioration of the structure of phloem cells have been reported to be one of the effects of irrigating with effluent water.[19]

The thickness of the stem decreased significantly with an increase in the effluent concentration. The thickness of both the collenchyma and parenchyma cells similarly decreased as the effluent concentration increase [Figure 1]. However, the reduction was more advance in 15% and 20% effluent concentration. This supports Vijayakumar and Udayasoorian[20] who implied that at lower effluent concentration, the plants can maintain the production of the collenchyma and parenchyma cells which have an adaptive mechanism to regulate ion concentrations from entering the stem. However, at a higher concentration, there was reduction in the number of collenchyma and parenchyma cells so as to prevent excess amount of effluent from entering the vessels and also reduced toxicity from the effluent to the aerial parts. This agrees with Folorunso *et al*.[11] and Ogunkunle *et al*.[19] who reported similar effect of brewery effluents on collenchyma and parenchyma thickness of *Talinum triangulare* and *Amaranthus hybridus*. The varied effluent concentrations appear not to have a significant effect on the pith diameter, except where the plant irrigated with 20% effluent concentration had significantly smallest pith diameter [Figure 1].

Effect of Effluent Concentrations on the Root Anatomical Structures *C. annuum*

The effects of the effluent concentrations on the root of *C. annuum* are presented in Table 2. The number of vessels in the root reduced significantly with increased effluent concentration. The reduction in the number of vessels with increasing effluent concentrations had been reported by Folorunso *et al.*,[11] Ghouse and Yunus,[21] and Khudar *et al.*[22] to reduce the flow of water, mineral nutrients, and assimilates. Further, there was a reduction in the vessel diameter with increase in the effluent concentrations [Figure 2]. The reduction in vessel diameter has been reported by Folorunso *et al.*[11] to also reduce the flow of assimilates to the plant parts, thereby leading to stunted growth.

The cortical diameter reduced significantly at 5%, 10%, and 15% effluent concentration; however, at 20% effluent

Table 2: Effect of different effluent concentrations on stem anatomical structures of *Capsicum annuum*.

Characters	Percentage concentration of the effluent				
	Control	5%	10%	15%	20%
Stem anatomical characters					
Number of vessels/row	7.00±1.41[a]	6.00±1.63[a]	4.00±0.82[a]	3.25±0.50[a]	5.75±2.36[a]
Stem thickness (μm)	91.50±5.58[a]	89.50±2.94[ab]	85.63±3.75[b]	82.38±3.49[b]	67.50±7.36[c]
Cortical layer thickness(μm)	12.38±2.14[a]	11.88±2.39[a]	8.63±1.11[ab]	8.50±2.97[ab]	5.63±2.98[b]
Collenchyma thickness(μm)	6.2±1.175[a]	5.75±2.22[a]	4.38±0.48[a]	4.25±2.26[a]	3.38±1.65[a]
Parenchyma thickness(μm)	6.25±2.02[a]	4.13±1.65[a]	4.75±1.85[a]	4.50±1.87[a]	3.25±0.96[a]
Pith diameter (μm)	41.00±4.26[a]	38.75±3.01[a]	40.25±6.12[a]	38.00±2.35[a]	32.88±2.18[b]
Root anatomical characters					
Number of vessels/row	21.70±17.07[a]	20.60±10.59[a]	18.80±12.46[ab]	15.60±8.57[b]	7.20±4.42[c]
Vessel length (μm)	4.17±0.50[a]	3.73±0.82[a]	3.15±0.65[a]	3.15±0.88[a]	4.28±0.98[a]
Vessel diameter (μm)	5.88±8.06[a]	3.27±0.85[a]	2.75±0.69[a]	2.60±1.13[a]	2.52±0.81[a]
Cortical diameter (μm)	18.83±3.51[a]	16.96±4.58[b]	13.10±1.98[bc]	10.88±2.49[c]	18.65±7.03[a]

Means followed by the same letter(s) in a row are not significantly different at 5% level of probability (Duncan multiple range test)

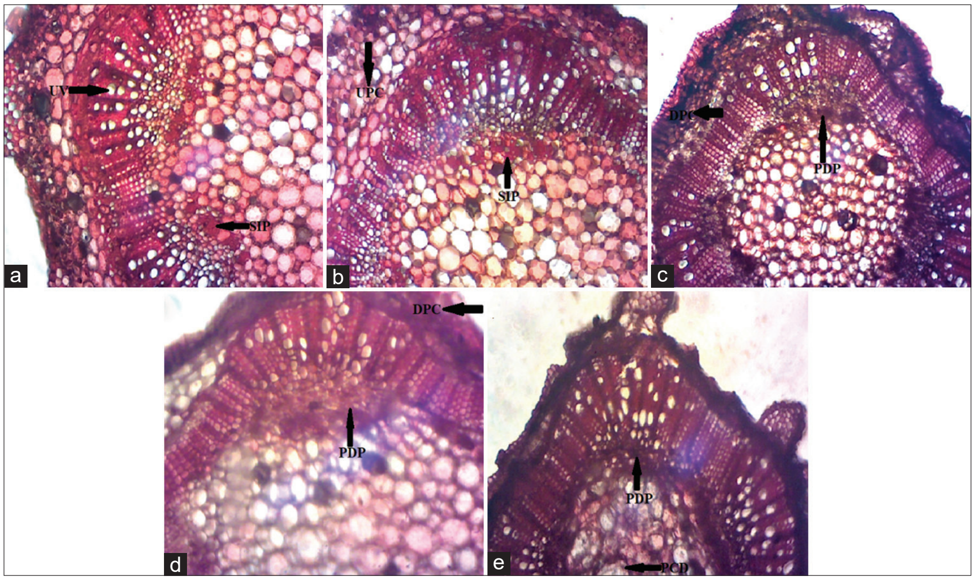

Figure 1: Transverse section of the stem of *Capsicum annuum* of (a) the control with undamaged vessels (UV) and structurally intact phloem (SIP), (b) 5% treatment with undamaged parenchyma cells (UPC) and SIP, (c) 10% treatment with damaged parenchyma cells (DPC) and pathological death of phloem (PDP), (d) 15% treatment with PDP and DPC, and (e) 20% treatment with PDP and destroyed pith cells (PCD) (×40)

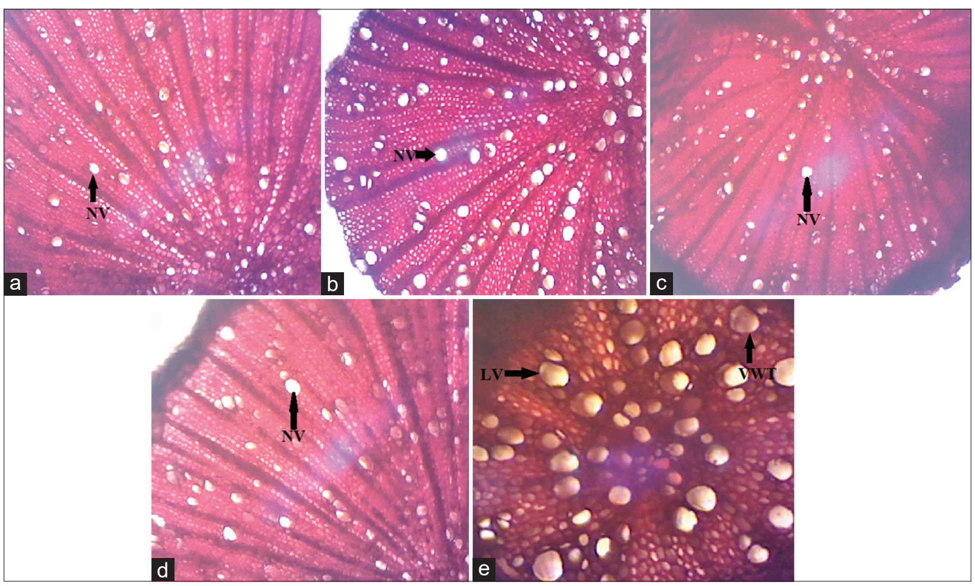

Figure 2: Transverse section of the root of *Capsicum annuum* of (a) control showing normal vessels (NV), (b) 5% treatment with numerous NV, (c) 10% treatment with NV (d) 15% treatment with NV, and (e) 20% with large vessels (LV) and vessel wall thickened (VWT) (×40)

concentration, there was an increase in the cortical diameter [Table 2]. The reduction in the cortical cells has been reported by Folorunso *et al.*,[11] Barcelo *et al.,*[23] and Kasim[24] to be the result of a decrease in elasticity of cell walls of the root. However, at a high concentration (20%), the effluent toxic effect resulted in an increase in the cortical cells of the root of the *C. annuum.* This could imply that there was an increase in the formation of cell wall thickening of root cortical cells as a result of the heavy metal content in the effluent. Multiple environmental stresses (high soil pH, high salt, and heavy metal content) have been reported to induce cell wall thickening of root cortical cells.[25,26]

Accumulation of Heavy Metals in the Experimental Soils Irrigated with Different Effluent Concentrations

Table 3 revealed that the soil pH was lower than the initial soil pH of 6.78 for all the different effluent concentrations. The

Table 3: Accumulation of heavy metals in the soil from effluent application

	Percentage concentration of effluent				
Heavy metals/ parameters	**Control**	**5%**	**10%**	**15%**	**20%**
Mn (mg/kg)	4.23	7.87	11.83	14.86	28.00
Cu (mg/kg)	6.93	7.76	16.93	12.19	40.00
Fe (mg/kg)	36.60	30.80	98.36	128.10	134.24
Cd (mg/kg)	0.033	0.10	0.37	0.004	2.07
Pb (mg/kg)	38.00	24.00	24.10	54.00	136.00
Ni (mg/kg)	1.17	3.70	7.45	22.10	24.66
Cr (mg/kg)	55.33	14.10	33.17	60.20	110.60
pH	6.76	5.82	5.98	5.00	4.44
$pHCaCl_2$	6.78	7.99	5.80	4.98	3.99
Temperature (°C)	25.00	26.00	26.67	27.97	27.97
Conductivity	1.23	0.06	1.98	2.00	2.13

Mn: Manganese, Cu: Copper, Fe: iron, Cr: Chromium, Pb: Lead, Cd: Cadmium, Ni: Nickel

soil pH was slightly acidic in the 0% effluent concentration, however as the concentration increased, soil pH decreased, becoming more acidic. Lower soil pH (acidic) has been reported to favor the availability, mobility, and redistribution of metals due to the solubility of the ions in an acidic environment.[27,28] This supports the presence of high levels of heavy metals at high effluent concentrations (15%, 20%) with low soil pH (4.44–5.00, respectively).

The accumulation of heavy metals in the contaminated soils varied with increasing effluent concentrations [Table 3]. The Mn content in the soil was highest (28.00 mg/kg) under the 20% effluent treatment, while the lowest amount of 4.23 mg/kg was in 0% treatment. Cu content in the experimental soil varied from 7.76 mg/kg to 40.00 mg/kg. The highest accumulation of Fe in the soil was 134.24 mg/kg under the 20% effluent concentration while the lowest (30.80 mg/kg) was in 5% effluent treatment. The highest level of Ni (24.66 mg/kg) was observed in 20% effluent concentration, while the control (0%) contributed the lowest level of Ni (1.17 mg/kg). The maximum level of Cd (2.07 mg/kg) was observed in 20% effluent treatment, while 15% effluent concentration contributed the lowest of Cd (0.004 mg/kg). The Cr content was highest (110.60 mg/kg) and lowest (14.10 mg/kg) in soil irrigated with 20% and 5% effluent concentration, respectively. The highest accumulation of Pb content in the soil was 136.00 mg/kg under the 20% effluent treatment, the lowest (24.00 mg/kg) was in 5% effluent treatment.

The maximum permissible limits of Mn, Cu, Fe, Cd, Pb, Ni, Cd, and Cr in soils recommended by WHO (1996) are 3.75 mg/kg, 36.00 mg/kg, 11.25 mg/kg, 0.80 mg/kg, 85.00 mg/kg, 35.00 mg/kg, and 100.00 mg/kg.[29] In the experimental soils, the concentrations of Ni, Cd, Cr, Cu, and Pb were observed to be below the maximum permissible limits except for the soil irrigated with 20% effluent concentration having very high heavy metal content [Table 3]. However, concentration of Mn and Fe in the soils exceeded the permissible limits. The presence of these heavy metals in high concentration has been reported to cause alterations in the germination processes as well as the growth of roots and stems.[14,30]

CONCLUSION

This research showed that the concentration of the heavy metals (Mn, Cu, Fe, Cd, Pb, Ni, and Cr) increased with an increase in effluent concentrations. The accumulation of these heavy metals in the experimental soils exceeded the critical permissible levels except for Ni and the effect was observed in the anatomical characters. The response of the anatomical characters to the heavy metals in the soil at different effluent concentrations resulted in the observed growth morphological characters of the *C. annuum*. The research also revealed that the growth of *C. annuum* was maintained at low concentration of the effluent; however, at high effluent concentration, the growth was affected.

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