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RESEARCH ARTICLE

Mixing tannery effluent had fertilizing effect on growth, nutrient accumulation, and photosynthetic capacity of some cucurbitaceous vegetables: A little help from foe

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Abstract

Tannery effluent contains a number of organic and inorganic elements as pollutants which reduce plant growth. To overcome shortage of water, use of diluted industrial wastewater such as tannery effluent can be a viable strategy for improving crop growth and yield. A pot experiment was conducted to determine the effects of tannery effluent and its various dilutions on physiological and biochemical characteristics of five cucurbitaceous vegetables. Tannery effluent was applied 0, 25, 50, 75 and 100% to 3-week-old plants of five cucurbitaceous vegetables (*Cucurbita maxima*, *Luffa cylindrica*, *Citrullus vulgaris*, *Cucumis melo*, and *Praecitrullus fistulosus*) for 4 weeks. Tannery effluent reduced the growth of all five cucrbitaceous vegetables. Diluted tannery effluent (25%) improved the growth of *Cucurbita maxima*, *Citrullus vulgaris*, and *Cucumis melo*. Moderately diluted (50%) did not affect the growth of *Citrullus vulgaris* and *Cucumis melo*. Toxic effects of tannery effluent were associated with high accumulation of heavy metals Cr, Cd, Mn, and Fe in leaves and roots. High accumulation of heavy metals in leaves reduced the accumulation of nutrients in leaves (N, P, K) and reduced photosynthetic pigments and photosynthetic rate. Changes in photosynthetic rates of all vegetable species due to tannery effluent were not associated with stomatal limitations (stomatal conductance, transpiration rate, internal CO_2). Toxic effects of tannery effluent on plants also include changes in N-metabolism (amino acid and protein). However, extent of these adverse effects of tannery effluent on vegetables was species specific. It is suggested that *Cucurbita maxima* can be grown by supplying 25% tannery effluent, whereas *Citrullus vulgaris* and *Cucumis melo* can be grown with moderately diluted (50%) tannery effluent.

Keywords Mixing tannery effluent · Photosynthetic rate · Quantum yield of PSII · Accumulation of nutrients · Cucurbitaceous vegetables · Cadmium

Introduction

Leather industry plays a vital role in the economy of Pakistan in terms of employment and business (Bareen and Tahira 2011). In Pakistan, there are more than 800 tanneries

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that include small tanning units, releasing a huge amount of effluent to the environment (Anonymous 2019). The discharged effluent from tanneries is either partially treated or untreated containing a number of pollutants (Lofrano et al. 2013; Asfaw et al. 2017; Rani et al. 2017). In tannery, various types of chemicals are used for tanning process, during which animal skin is converted into useful leather. These chemicals include salts of heavy metals and acids to remove meat, fats, and hair, thus producing large quantity of salts (TDS 13,000 to 21,000 mg/L) and toxic metals such as Cr (100–250 mg/L) (Afzal et al. 2014; Asfaw et al. 2017). Tannery effluent contains large amount of non-biodegradable organic compounds such as ammonium, aryl amides, aliphatic amides, sulfide, sulfonated cyclic aromatic compounds, fatty acids, diazo-compounds, etc. Treatment of tannery effluent required high cost of treatment or dilution before its discharge (Lofrano et al. 2013; Dixit et al. 2015). Conventional wastewater treatment did not remove organic compounds. Advance oxidation process by generating hydroxyl radicals for mineralization of pollutants in wastewater such as Fenton oxidation system can be used for wastewater treatment (Karthikeyan et al. 2011; Sekaran et al. 2013; Jo et al. 2017). However, most of the tanneries in developing countries including Pakistan do not have treatment plants and thus untreated effluent is released into open land, agricultural fields, or water body (Annonymous 2009; Afzal et al. 2014; Zereen et al. 2014). According to an estimate, about 80% of untreated tannery effluents are discharged into recipient water bodies or onto open land (Bareen and Tahira 2011; Afzal et al. 2014; Rekik et al. 2017). In view of water shortage, irrigation with wastewater became a common practice in agriculture in various countries including in Pakistan (Afzal et al. 2014). Due to irrigation with tannery wastewater, soil of agricultural fields is becoming contaminated with toxic pollutants. Plants growing on soil irrigated with wastewater had accumulated manifolds higher metal contents than in polluted soil, which indirectly affect the human health.

Several management strategies have been proposed to avoid toxic effects of wastewater on plants. Of these, one of the potential strategy is to use tannery effluent for irrigation by mixing with normal water (Tripathi and Tripathi 2011). The use of diluted effluent causes the lower accumulation of toxic elements in soil and do not largely affect the soil structure (Babyshakila and Usha 2009). In addition, several studies demonstrated the beneficial effects of diluted industrial effluent on different crop species (Yasmeen et al. 2014; Anwar et al. 2016; Asfaw et al. 2017; Khalid et al. 2017). However, use of wastewater for agriculture by inappropriate dilution causes adverse effects on growth and yield (Abbas et al. 2021). In addition, toxic chemicals in wastewater such as tannery effluent reached to the food chain as these metals are easily accumulated in edible parts of vegetables (Mapanda et al. 2005; Muchuweti et al. 2006). Accumulation of heavy metals in vegetative parts affects various physiological and biochemical processes such as accumulation of macronutrients (N, P, K, Ca^{2+}, Mg^{2+}, etc.), light reaction to generate ATP and NADPH, fixation of CO_2 in mesophyll cells, and N-metabolism (Athar et al. 2003; Athar and Mahmood 2003; Ali et al. 2009). By irrigating tannery effluent by mixing tap water, one can avoid the toxicity of salts of heavy metals particularly chromium. However, irrigation with tannery effluent to vegetables can cause serious health hazards to consumers. Thus, it is imperative to dilute tannery effluent and assess the growth and physiology of vegetables irrigated with tannery effluent.

The present study was aimed to assess whether or not mixing tap water to tannery effluent can reduce the phytotoxic effects on growth, mineral nutrient status, and photosynthetic capacity of some cucurbitaceous vegetables. Moreover, inter-specific variation for tolerance to leather tannery effluents was also assessed in some cucurbitaceous vegetables (pumpkin, sponge gourd, water melon, melon, and tenda).

Materials and methods

Seeds of five cucurbitaceous vegetables [(*Cucurbita maxima* L. (pumpkin), *Luffa cylindrica* Mill. (sponge gourd), *Citrullus vulgaris* Schrad (watermelon), *Cucumis melo L.* (melon), *Praecitrullus fistulosus* (stocks) Pangalo (tenda)] were purchased from the local market. Seeds of each cucurbitaceous species were disinfected with 5% sodium hypochlorite solution by immersing them for 10 min. The seeds were then washed twice with water. Seeds were germinated in 10-kg sand-filled plastic pots. The whole experimental work was carried out in naturally lit wire-net house. After 2 weeks of seed sowing, plants of each cucurbitaceous vegetable were thinned to four plants in each pot. The tannery effluent was collected from Khawaja Tanneries, Multan Pakistan. Khawaja Tanneries has wastewater treatment plant. The tannery effluent was collected before the treatment. Plants of each species were treated with varying concentrations of tannery effluent (0 as Hoagland's nutrient solution, 25, 50, 75%, and 100% leather tannery effluent) (Table 1). To avoid dehydration due to daily evapo-transpirational water loss, adequate amount of distilled water was applied. The experiment was conducted in randomized complete block design (RCBD). Five vegetables and five tannery effluent treatments were placed in block with five replicates. One can use a randomized complete block design (RCBD) to compare treatment means when there is an extraneous source of variability. In such cases, treatments are randomly assigned to experimental units within a block, with each treatment

Table 1 Physico-chemical characteristics of use tap water and tannery effluent and National Environmental Quality Standards (NEQS) limits from National Environmental Protection Agency (Afzal et al. 2014) for municipal waste water of Pakistan

Characteristics/parameters	Tap water	Tannery effluent	NEQS for water
Odor	Odorless	Very offensive	Odorless
Temperature	22 °C	28 °C	24
Color	Colorless	Black grey	grey
Turbidity	Very clear	Very turbid	clear
Total soluble solids (TSS)	45 mg/L	1950	400
Total dissolved solids (TDS)	180 mg/L	6540	2500
pH	6.8	8.4	6.0–7.5
Electrical conductivity (EC)	1.02 dS/m	16 dS/m	1.5 dS/m
Biological oxygen demand (BOD_5)	45 mg/L	340 mg/L	250 mg/L
Chemical oxygen demand (COD)	84 mg/L	930 mg/L	400 mg/L
Hardness	Nil	1624	500
Iron (Fe)	1.6 mg/L	3.93 mg/L	8.0 mg/L
Chromium (Cr)	Nil	3.20 mg/L	1.0
Zinc (Zn)	0.12 mg/L	0.39 mg/L	5.0 mg/L
Manganese (Mn)	0.03 mg/L	0.12 mg/L	1.5 mg/L
Cadmium (Cd)	Nil	0.096 mg/L	0.005 mg/L
Copper (Cu)	Nil	Nil	1.0 ug/g
Nickel (Ni)	Nil	Nil	1.0 ug/g
Cobalt (Co)	Nil	Nil	1.0 ug/g
Lead (Pb)	Nil	Nil	0.5 ug/g
Calcium (Ca)	26 meq/L	800 meq/L	200 meq/L
Magnesium (Mg)	0.03 meq/L	526 meq/L	150 meq/L
Sodium (Na)	32 meq/L	395 meq/L	250 meq/L
Chloride (Cl)	100 meq/L	2104 meq/L	1000 meq/L
Nitrogen (N)	0.24 meq/L	268 meq/L	0.25 meq/L
Potassium (K)	1.6 mmol/L	13.43 mmol/L	3.6–5.2 mmol/L

appearing exactly once in every block. Before each treatment all the pots were washed thoroughly with tap water to leach down the previous applied tannery effluents.

Biomass

Three weeks after tannery effluent treatment, plants of each cucurbitaceous species were uprooted and separated into shoots and roots. Fresh weights of shoots and roots of each cucurbitaceous species were recorded on digital balance. Plant parts were dried in oven at 80 °C for 4 to 6 days. Dry weights of plant parts were recorded. Before harvesting, the following parameters were measured.

Chlorophyll content as SPAD units

Photosynthetic pigments or total chlorophyll content were measured as SPAD measuring units. Photostosynthetic pigments were measured in fully developed leaves and avoid main veins of leaves. This was measured using SPAD meter (Minolta SPAD-502, Japan).

Quantum yield of PSII

Maximum quantum yield of PSII was measured on third mature leaf of each vegetable species to assess up to what extent structural stability and functional activity of PSII were affected by supplying plants with varying dilutions of tannery effluent. The leaves were dark adapted for 30 min before taking data using FlourPen FP 100 MX-LM (Photon Systems Instruments, Czech Republic).

Total soluble proteins and total free amino acids

Both these biochemical attributes were determined in the leaf extract of all vegetable species. Fresh leaves (100 mg) were ground in 5 mL of phosphate buffer and centrifuged at $6000 \times g$. Total soluble proteins were measured in the leaf extract. Leaf extract (100 µL) was mixed with 5 mL of Bradford's reagent following Bradford (1976). The absorbance of each sample was recorded at 595 nm. Total free amino acids were measured from the leaf extract. Leaf extract of each sample (1 mL) was taken in test tube. One milliliter of 10% cold pyridine was added in the test tube and mixed well in free environment to avoid inhaling any vapors of pyridine.

One milliliter of 2% ninhydrin solution was also added in the test tube. The mixture in test tubes was heated in water bath for 20–30 min following Hamilton and Van Slyke (1943). All samples were allowed to cool and diluted with distilled water to make up the final volume of 50 mL. The absorbance of the solution of each sample was recorded at 570 nm.

Gas exchange parameters

Measurements of gas exchange attributes were made on fully developed but youngest leaf usually 3rd or 4th leaf from the top to assess up to what extent tannery effluent affect gas exchange attributes of five vegetables. Gas exchange attributes were measured on intact leaves of all species using photosynthetic rate analyzer (LCA-4, ADC, UK). All photosynthetic attributes were measured when light intensity was almost constant and ranges from 800 to 900 µmol m^{-2} s^{-1} usually around at 11:30 a.m. to 1:30 p.m. The data were recorded for gas exchange attributes using specific instrumental adjustments. For example, leaf chamber with 11.25 cm^2 surface area was used. Gas flow rate in leaf chamber was fixed at 200 mL min^{-1}. Leaf chamber temperature during the measurements was 27.5 ± 2.75 °C and RH of the chamber ranged from 25.4 to 41.2%. Ambient CO_2 concentration was 406 µmol m^{-2} s^{-1}. Photosynthetic attributes measured were net CO_2 assimilation rate (A), transpiration rate (E), water use efficiency (A/E), sub-stomatal CO_2 concentration (C_i), ambient CO_2 (C_{an}) and C_i/C_{an}.

Mineral nutrients (macronutrients)

Dried plant leaf and roots were grounded into powder and 0.1 g leaf and root material was mixed with 1 mL of sulfuric acid digestion mixture in 10-mL flask. All samples were placed on hotplate and heated at 80 ℃ initially and then temperature was increased to 200 ℃. When samples in the flasks turned black, 0.5 mL of perchloric acid was added to decolorize it. The sample digests were diluted with distilled water up to 100 mL and filtered. The filtrate was used for the determination of various ions described below.

Sodium (Na^+) and potassium (K^+) cations in the digested samples of leaves were determined using flame photometer (Jenway PFP7, Essex, UK). For phosphorous (P), the digested leaves and root samples (4.5 mL) were mixed with Barton's reagent (0.5 mL) and absorbance were noted at 470 nm using a spectrophotometer (UV-1900 BMS). The concentration of P was calculated from the standard curve. Nitrogen (N) in the leaves and roots was determined by Kjeldahl method. The digested and diluted samples (25 mL) were taken in Kjeldahl's flask and mixed with 5 mL of 40% NaOH to neutralize acid in the digest and 50 mL distilled water. The mixture was distilled in Kjeldahl's apparatus. The distillate (30–35 mL) was collected in a 250-mL conical flask containing 5 mL boric acid indicator. Few drops of phenolphthalein as indicator were added in the collected distillate and was titrated using 0.01 N H_2SO_4 until light pink as end point appeared. A blank sample was also run as mentioned-above procedure. Nitrogen was then calculated by formula.

Micronutrients (Cr, Zn, Mn, Fe, Cd)

For the measurement of micronutrient, dry leaves and roots of all five cucurbitaceous vegetable species were digested in acid digestion mixture. However, the digested samples of leaves and roots of all five cucurbitaceous vegetables were diluted with deionized water and make the final volume up to 25 mL. Copper, zinc, nickel, iron, and chromium were estimated in the digested samples by atomic absorption spectrophotometer (Perkin Elmer, Analyst 300).

Statistical analysis

The data for various growth, biochemical, and physiological attributes obtained was subjected to two-way analysis of variance (ANOVA) using a statistical computer package COSTAT v. 6.5 (Cohort Software, Berkeley, USA). Since comparison of treatment means of different species at each tannery effluent concentration was not possible, the treatment means of each species were compared after carrying out one-way ANOVA followed by least significance difference (LSD) test (Snedecor and Cochran 1980).

Results

Addition of tannery effluent caused a significant decrease ($P < 0.001$) in shoot fresh and dry biomass of all five cucurbitaceous vegetables. However, increasing concentration of tannery effluent in water reduced the fresh and dry weights of all five vegetable species (Table 2; Fig. 1). Maximum reduction in fresh and dry weights was found at 100% tannery effluent treatment. All five cucurbitaceous species responded differentially to tannery effluent dilution treatments. The use of 25% tannery effluent caused a significant increase in fresh and dry weights of shoots of *Cucurbita maxima* and *Luffa cylindrica*. Moreover, maximal increasing effect of diluted tannery effluent on fresh and dry weights of shoot was found in *Cucumis melo* at 50% tannery effluent. In *Praecitrullus fistulosis*, shoot dry biomass remained unchanged at 25% tannery effluent treatment.

Root fresh and dry weights of all five cucurbitaceous species reduced due to increasing concentration of tannery effluent and all species differed significantly (Fig. 1). Root fresh and dry biomasses of all five vegetables either increased or remained same at 25% and 50% tannery effluent treatment.

Table 2 Mean squares from analysis of variance of data for shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), total free amino acids (TFAA), total soluble proteins (TSP), chlorophyll contents (SPAD values), quantum yield (QY), net assimilation rate (*A*), transpiration rate (*E*), internal carbon dioxide (Ci), internal carbon dioxide concentration/ambient carbon dioxide concentration ratio (Ci/Can), water use efficiency (WUE), leaf sodium, leaf potassium, potassium/sodium ratio in leaf, leaf nitrogen, root nitrogen, leaf phosphorous, root phosphorous, leaf manganese, root manganese, leaf zinc, root zinc, leaf iron, root iron, leaf chromium, root chromium, leaf cadmium, and root cadmium of five cucurbitaceous vegetables (*Cucurbita maxima*, *Luffa cylindrica*, *Citrullus vulgaris*, *Cucumis melo*, and *Praecitrullus fistulosus*), when different concentrations of tannery effluent in full strength Hoagland's nutrient solution were applied on 2-week-old plants for 4-week grown in sand culture

SOV	df	SFW	SDW	RFW	RDW	TFAA	TSP
Treatment (T)	4	16,736.8***	268.27***	160.68***	1.63***	49.26***	150.42
Species (S)	4	19,429.9***	116.96***	131.40***	1.88***	2.03***	33.67*
T×S	16	697.1***	7.74***	15.36***	0.20***	0.41***	6.38**
Error	100	4.64	0.18	0.43	0.002	0.145	1.065
SOV	df	SPAD values	QY	A	E	Ci	Ci/Can
Treatment (T)	4	3086.4	0.303***	541.72***	45.81***	42,254.04*	0.03***
Species (S)	4	102.31	0.001***	178.62***	7.30***	1498.13**	131.40***
T×S	16	93.68*	0.005***	15.35***	3.19***	3196.20**	15.36***
Error	100	2.60	3.012	0.45	0.13	208.26	0.43
SOV	df	WUE	Leaf Na	Leaf K	K/Na	Leaf N	Root N
Treatment (T)	4	11.11***	6.061***	512.68***	89.997***	16,736.8***	268.3***
Species (S)	4	55.88***	0.320***	15.16***	2.50***	19,429.9***	116.9***
T×S	16	1.88***	0.107***	0.38***	0.215***	697.1***	7.74***
Error	100	0.71	0.004	0.007	0.014	4.64	0.180
SOV	df	Leaf P	Root P	Leaf Mn	Root Mn	Leaf Zn	Root Zn
Treatment (T)	4	160.68***	1.63***	10,874.6***	11,923.89***	4315.535***	399.613***
Species (S)	4	131.40***	1.88***	50.38***	23.84***	82.88***	120.71***
T×S	16	15.36***	0.20***	1.68***	2.37***	7.92***	4.71***
Error	100	0.43	0.002	0.0442	0.052	0.658	0.195
SOV	df	Leaf Fe	Root Fe	Leaf Cr	Root Cr	Leaf Cd	Root Cd
Treatment (T)	4	7958.814***	14,630.2***	921.1***	2564.80***	75.916***	84.177***
Species (S)	4	96.06***	907.14***	7.08***	10.237***	0.322***	0.236***
T×S	16	19.86***	14.81***	0.62***	1.559***	0.051***	0.110***
Error	100	0.593	0.788	0.025	0.008	0.009	0.004

[*], **, *** = significant at 0.1, 0.01, and 0.001 levels

Na, sodium; *K*, potassium; *N*, nitrogen; *P*, phosphorous; *Mn*, manganese; *Zn*, zinc; *Fe*, iron; *Cr*, chromium; *Cd*, cadmium

Maximum reducing effect of tannery effluent was found at the 100% tannery effluent treatment. Maximum beneficial effect of diluting tannery effluent on root fresh and dry biomasses was found in *Citrullus vulgaris* and *Cucumis melo*.

Chlorophyll content measured as SPAD units reduced in all five cucurbitaceous species due to increasing fraction of tannery effluent in irrigation water, particularly at the highest tannery effluent treatment. All five cucurbitaceous species differed significantly in accumulation of photosynthetic pigments (Table 2; Fig. 1). Mixing 25% tannery effluent in irrigation water increased the photosynthetic pigments in all cucurbitaceous species except that in *Citrullus vulgaris* and *Praecitrullus fistulosis*. However, maximum reducing effect was found in *Cucurbita maxima* and *Luffa cylindrica*. Structural stability and functional activity of photosystem II (PSII) were measured as quantum yield of PSII and this was reduced in all five species when 75% and 100% tannery effluents was applied. Mixing tannery effluent 25% or 50% did not affect PSII activity or slightly affected. In *Cucumis melo*, quantum yield of PSII reduced at 100% tannery effluent treatment.

Mixing tannery effluent significantly decreased total soluble proteins and total free amino acids in all five cucurbitaceous species. All species significantly differed in accumulation of total soluble proteins and total free amino acids at varying concentrations of tannery effluent (Table 2; Fig. 1). Mixing 25% tannery effluent enhanced the total soluble proteins only in *Citrullus vulgaris*, *Cucumis melo*, and *Praecitrullus fistulosis*, whereas it caused a significant increase in total free amino acids in all species except that in *Citrullus vulgaris*. Application of 100% tannery effluent caused a maximal reduction in total

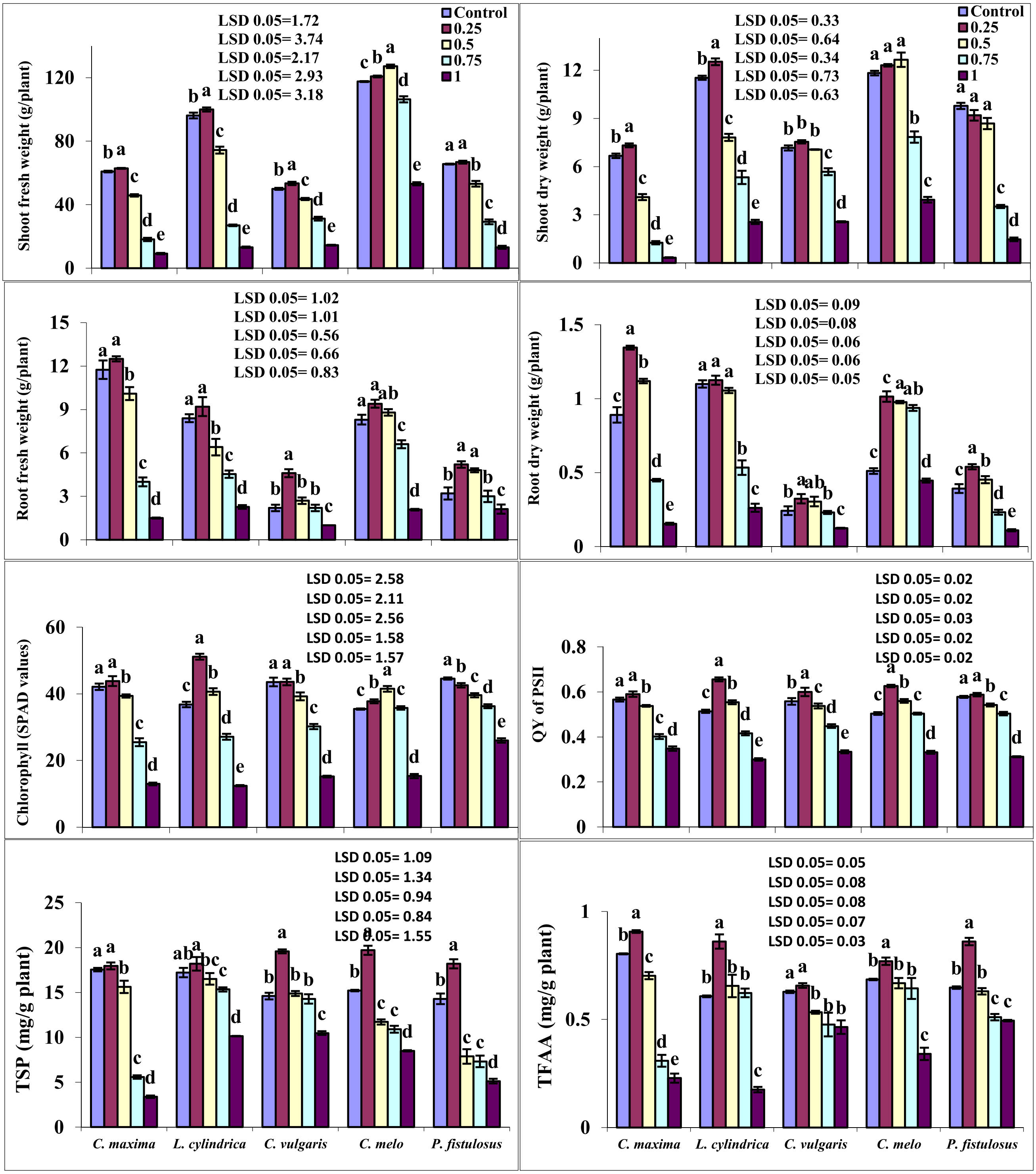

Fig. 1 Shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, chlorophyll (SPAD), quantum yield of PSII, total soluble proteins (TSP), and total free amino acids (TFAA) of five cucurbitaceous vegetables when 2-week-old plants grown in sand culture were applied varying concentration of tannery effluents in full strength Hoagland's nutrient solution for 4-week growth. Means were compared with LSD. LSDs of each species are given

soluble proteins and total free amino acids in *Cucurbita maxima*.

Photosynthetic rate measured as net CO_2 assimilation rate was reduced in all cucurbitaceous species only at 75% or at 100% tannery effluent treatment. Tannery effluent treatment at 25% or at 50% either significantly increased net CO_2 assimilation rate or did not affect this physiological attribute in all cucurbitaceous species (Table 2; Fig. 2). In *Cucumis melo* and *Praecitrullus fistulosis*, 50%, 75%, and 100% tannery effluent treatment caused a significant decrease in net assimilation rate higher concentration of tannery effluent or no mixing of water and 25% mixing of water had large quantities of heavy metals or remained above the threshold level that cause decrease in net CO_2 assimilation rate. Application of 25% and 50% tannery effluent did not change the internal CO_2 concentrations (C_i) in all species,

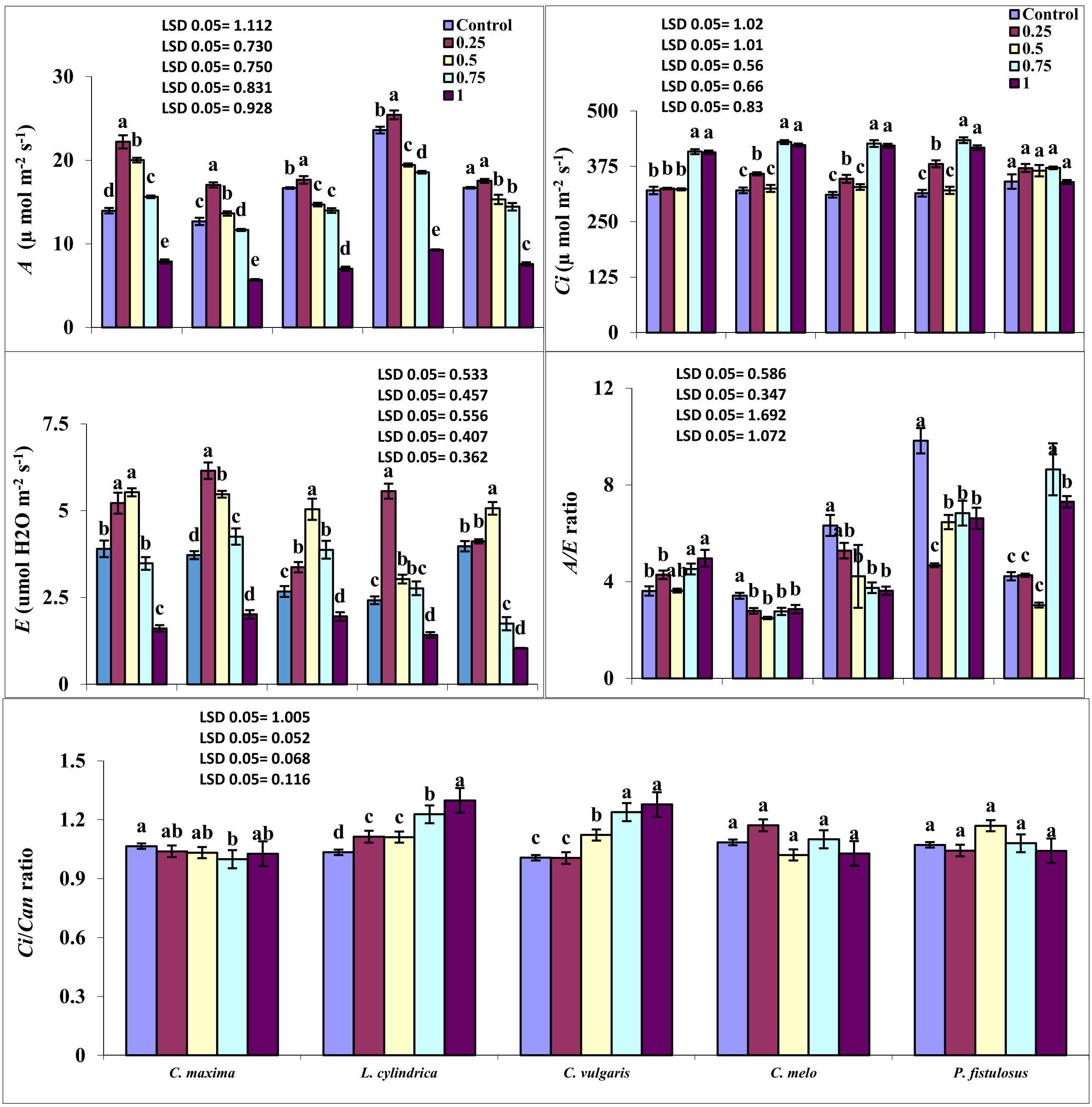

Fig. 2 Net assimilation rate (*A*), inter CO2 (Ci), transpiration rate (*E*), *A*/*E* ratio, and Ci/Can of five cucurbitaceous vegetables when 2-week-old plants grown in sand culture were applied varying concentration of tannery effluents in full strength Hoagland's nutrient solution for 4-week growth. Means were compared with LSD. LSDs of each species are given

whereas 75% and 100% caused a significant increase in C_i in all five species except that in *Praecitrullus fistulosis* where it remained same. This is due to lower CO_2 consumption rate in Calvin cycle due to tannery effluent that caused an increase in internal CO_2 concentrations (C_i). The effect of increasing tannery effluent on rate of transpiration (E) in all five species was significantly different. Application of 25%, 50%, and/or 75% tannery effluent caused a significant increase in E in all five species except that in *Praecitrullus fistulosis*. In addition, 100% tannery effluent treatment caused a significant reduction E in all five species. Water use efficiency (A/E) increased at 75% and 100% tannery effluent in *Cucurbita maxima* and *Praecitrullus fistulosis*, whereas it decreased in *Luffa cylindrica* and *Citrullus vulgaris*. However, in *Cucumis melo* the pattern of increase or decrease was not consistent with tannery effluent treatment. Ratio of ambient CO_2 to internal CO_2 (C_i/C_{an}) significantly increased in *Luffa cylindrica* and *Citrullus vulgaris* with tannery effluent treatment, whereas it remained unchanged in other cucurbitaceous species.

Accumulation of nitrogen in shoots and roots increased at 25% and 50% tannery effluent treatment except that in *Cucurbita maxima* where shoot nitrogen was maximal at 25% tannery effluent treatment. However, 75% and/or 100% tannery effluent treatment reduced the accumulation of nitrogen in the shoots and roots of all cucurbitaceous species. Accumulation of phosphorous in shoots and roots of all five cucurbitaceous species significantly decreased with increase in tannery effluent concentration. However, 25% tannery effluent treatment caused an increase in accumulation of phosphorous in shoots of *Praecitrullus fistulosis* and roots of *Cucurbita maxima* (Table 2; Fig. 3). Accumulation of Na^+ in shoots of all five species significantly increased with a concomitant decrease in accumulation of K^+ in shoots due to increasing concentration of tannery effluent. In addition, K^+/Na^+ ratio in shoots of all five species decreased with increase in concentration of tannery effluent. Change in accumulation of Na^+ and K^+ in shoots of all species was almost same (Table 2; Fig. 3).

Accumulation of iron (Fe^{2+}) in shoots and roots of all five species increased at 25% tannery effluent treatment. Application of 50% tannery effluent did not affect the accumulation of Fe^{2+} in shoots of all species, whereas 50% tannery effluent treatment reduced the accumulation of Fe^{2+} in roots of all five species. Irrigation with 75% or 100% tannery effluent caused a significant decline in accumulation of Fe^{2+} both in shoots and roots (Table 2; Fig. 4). In contrast, accumulation of manganese, zinc, cadmium, and chromium in shoots and roots of all five cucurbitaceous species consistently increased with consistent increase in concentration of tannery effluent in irrigation water. All cucurbitaceous species did not differ significantly in accumulation of all these micronutrients in shoots and roots (Table 2; Fig. 5).

Discussion

Tannery effluent is one of the environmental problems that limits plant vigor and productivity (Bareen and Tahira 2011). Due to shortage of good quality water, mixing tannery effluent with normal water could be viable strategy to overcome shortage of water. However, various studies on different crop species resulted in contradictory results regarding industrial effluent usage for irrigation. Thus, in this study, it was assessed up to what extent tannery effluent mixed with normal water which do not affect the growth of five potential cucurbitaceous vegetables using various morphological, biochemical, and physiological attributes.

In the present study, growth of all five cucurbitaceous vegetables either increased or remained same at the 25% tannery effluent treatment. These results are similar to some of earlier studies, in which it has been demonstrated that irrigation with sewage water or industrial wastewater improved the growth of vegetables and food crops (Yasmeen et al. 2014; Anwar et al. 2016; Khalid et al. 2017). While assessing effects of tannery effluent, Karunyal et al. (1994) demonstrated that water irrigation with diluted tannery effluent (25%) increased the growth of *Lycopersicon esculentum*, *Gossypium hirsutum*, *Vigna mungo*, and *Vigna unguiculata*. In this study, maximum growth enhancement due to 25% tannery effluent treatment was found in *Cucurbita maxima* and *Luffa cylindrica*. In addition, 25% and 50% tannery effluent treatment did not affect the growth of *Citrullus vulgaris* and *Cucumis melo*, whereas both 25% and 50% tannery effluent treatment reduced the growth of *Praecitrullus fistulosis*. These results indicated a genotypic variability against mixing tannery effluent treatment in all five cucurbitaceous vegetables, which is analogous to previous studies (Yasmeen et al. 2014; Zereen et al. 2014; Rekik et al. 2017). Growth enhancement due to diluted industrial effluent or sewage water can be explained as dilution or mixing effluent with normal water sufficiently lowered the levels of toxic elements and salts such as sulfates, phosphates, and chlorides of chromium, calcium, and magnesium, etc., which might have negligible effects on growth of plants (Rehman et al. 2009; Rekik et al. 2017). While assessing physico-chemical characteristics of 50% dilution of tannery effluent, Rekik et al. (2017) reported that 50% dilution considerably lowered the concentration of toxic elements (7–8 times lower than in effluent) and improved the COD and BOD characteristics.

Lesser dilution or higher concentration of tannery effluent reduced the growth of all five vegetables, particularly at 75% and 100% tannery effluent treatments. While working with different vegetables, Asfaw et al. (2017) found that 50% or more than 50% diluted tannery effluent caused reduction

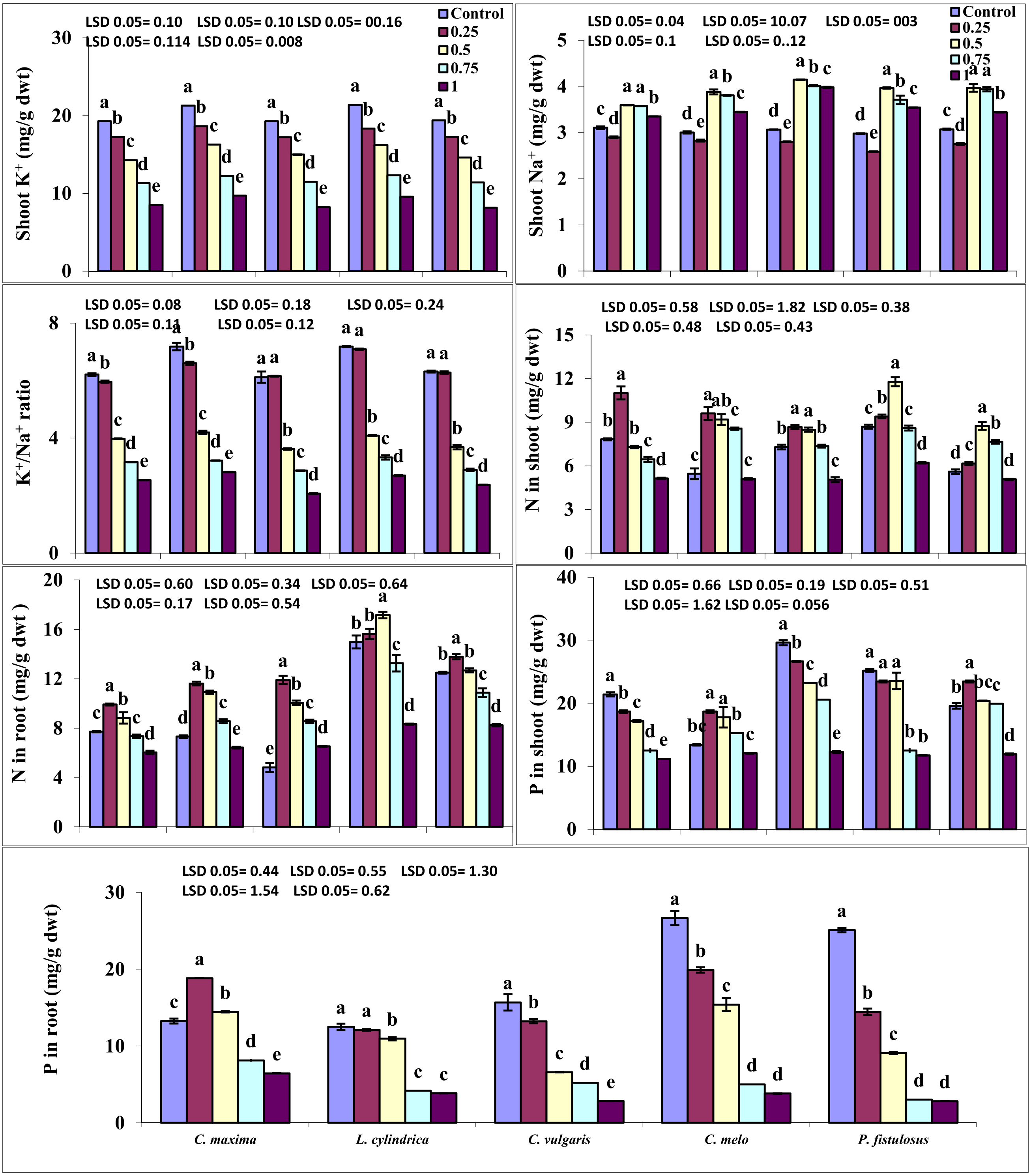

Fig. 3 Shoot potassium (K+), shoot sodium (Na+), potassium sodium ratio in shoot ((K+/Na+), shoot nitrogen (N), root nitrogen (N), shoot phosphorous (P), and root phosphorous (P) of five cucurbitaceous vegetables when 2-week-old plants grown in sand culture were applied varying concentration of tannery effluents in full strength Hoagland's nutrient solution for 4-week growth. Means were compared with LSD. LSDs of each species are given

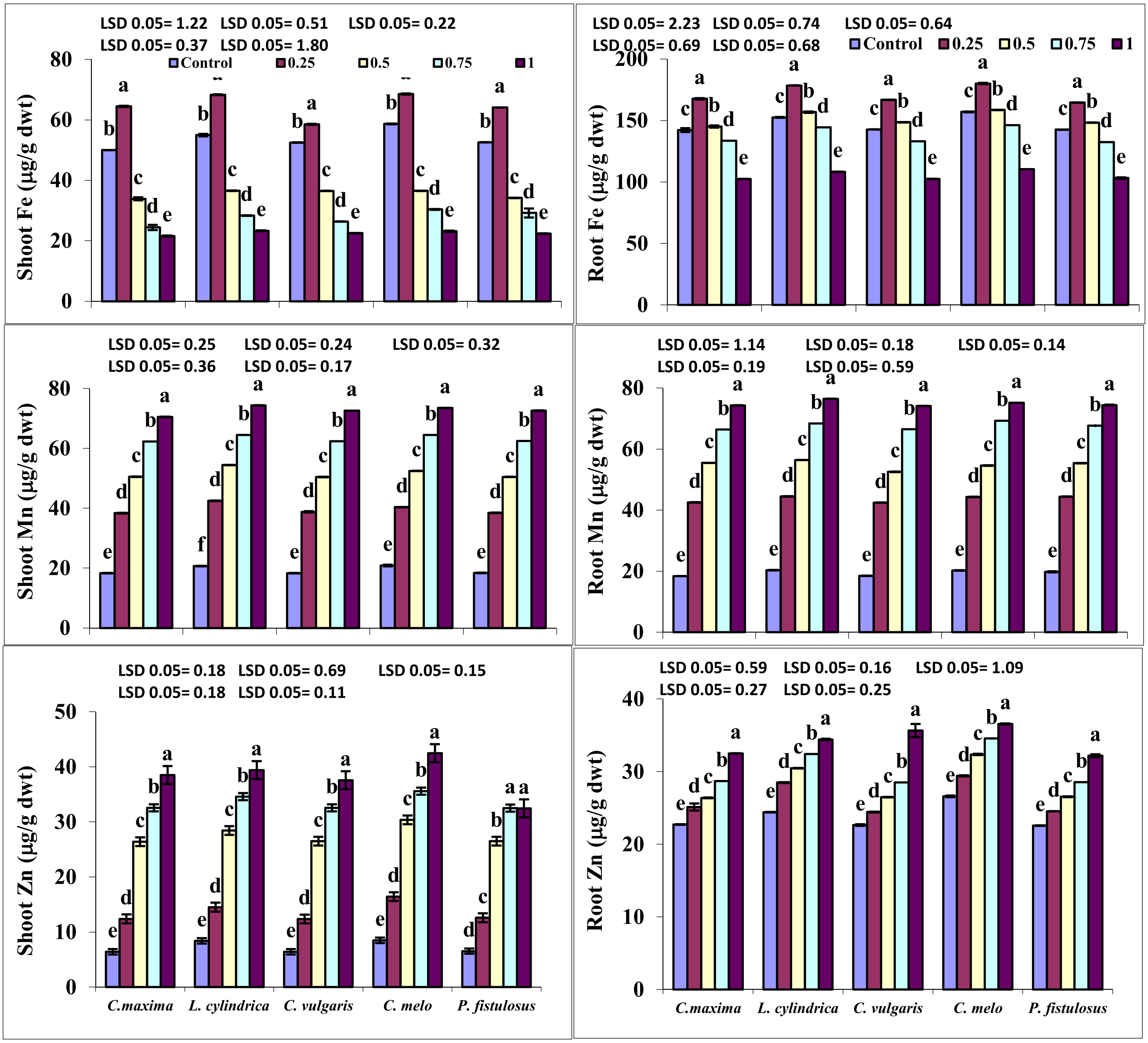

Fig. 4 Shoot iron (Fe), root iron (Fe), shoot manganese (Mn), root manganese (Mn), shoot zinc (Zn), and root zinc (Zn) of five cucurbitaceous vegetables when 2-week-old plants grown in sand culture were applied varying concentration of tannery effluents in full strength Hoagland's nutrient solution for 4-week growth. Means were compared with LSD. LSDs of each species are given

in growth. The extent of growth reduction is also species specific. Differential adverse effects of lesser diluted tannery effluent (50%) were also observed by Rastogi et al. (2007) and Pandey et al. (2019) who argued that every crop has its own self-tolerance limit and mechanism for coping different pollutants in industrial wastewater. Such growth reduction can be attributed to poor water uptake at the root level due to osmotic and toxic effects of high salt concentration in effluent (Rekik et al. 2017). In this study, used tannery effluent contains high amounts of nitrogen, potassium, sodium, calcium, magnesium, chromium, and cadmium; electrical conductivity (EC) was 16 dS/m (~ 150 mM NaCl salinity), whereas pH was 8.4 (Table 1). These attributes indicated that tannery effluent used in this study was highly saline containing heavy metals like chromium and cadmium, which is similar to earlier study (Balasubramanian et al. 1999). These results and earlier studies suggested that 50%, 75%, and 100% tannery effluent caused deleterious effects on growth of all five vegetables examined in this study due to having high EC and high toxic metals. However, diluted tannery effluent had lower amounts of salts and toxic heavy metals, thereby resulting in fertilization effect on plants, which depends on total dissolved salts, composition of salts in effluent, and type of species.

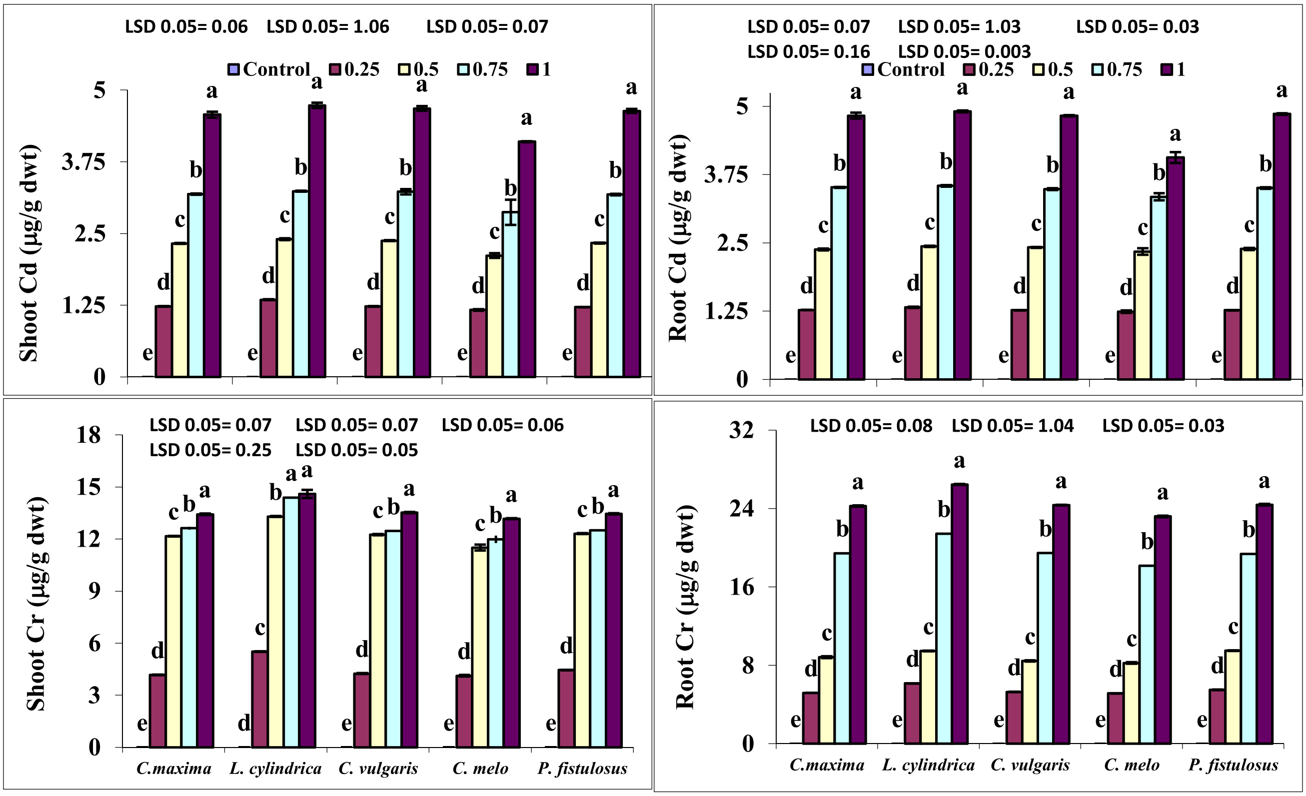

Fig. 5 Shoot cadmium (Cd), root cadmium (cd), shoot chromium (Cr), and root chromium (Cr) of five cucurbitaceous vegetables when 2-week-old plants grown in sand culture were applied varying concentration of tannery effluents in full strength Hoagland's nutrient solution for further 4-week growth. Means were compared with LSD. LSDs of each species are given

Uptake, transport, and accumulation of mineral nutrients though active or passive transport in plants is important physiological phenomenon. High amounts of heavy metals, high EC, or acidic/alkaline pH (as present in tannery effluent) in the growth medium can affect the uptake of both macro- and micro-nutrients. Irrigation with tannery effluent caused the high accumulation of heavy metals (Fe, Mn, Zn, Cd, Cr) and Na in roots and shoots, whereas it reduced the accumulation of N, P, and K. By drawing parallel between growth of all five cucurbitaceous vegetables and accumulation of nutrients in leaves and roots, it is clear that toxic effects of tannery effluent on plant growth were associated with lowering in NPK and high accumulation of heavy metals. Similar pattern of changes in nutrient accumulation has already been reported in *Phaseolus mungo* Roxb. (Nath et al. 2009), spinach (Maqbool et al. 2018), and *Brassica napus* (Zaheer et al. 2020). Diluting tannery effluent reversed the toxic effect and increased the accumulation of NPK and reduced the accumulation of heavy metals particularly, Cr, Cd, Zn, and Fe. It has been demonstrated that plants of different vegetables grown with various dilutions of tannery effluent treatment accumulated Cr in their root and leaves and exhibit some toxic symptoms such as chlorosis and necrosis of leaves. Although toxic symptoms were least observed in plants treated with higher dilutions of tannery effluent, plants still accumulate Cr in their roots and leaves. In view of this observation and UN's Sustainable Development Goal 2 (USDG 2, zero hunger), USDG 3 (good health and well-being), and USDG 12 (responsible consumption and production), accumulation of heavy metals in fruits and seeds needs to be assessed, particularly when this research will be replicated in field trials, because accumulation of Cr and Cd in fruits or seeds may enter in food chain and cause adverse effects on human health (Ahmed et al. 2022).

Over-accumulation of toxic elements in chloroplast causes the generation of reactive oxygen species (ROS) chloroplasts, thereby affecting plant pigment biosynthesis and thus photosynthetic activity. For example, greater accumulation of Cd reduced the chlorophyll by oxidative damage, reduced photosynthetic rate, and growth of all cucurbitaceous vegetables (Demirevska-Kepova et al. 2006). In this study, 25% effluent treatment had an increasing effect on photosynthetic pigments in *Luffa cylindrica* and *Cucumis melo*, whereas it did not affect the photosynthetic pigments of other cucurbitaceous vegetables. In contrast, 50%, 75%, and 100% tannery effluent reduced the photosynthetic pigments in all cucurbitaceous species, except that in *Cucumis melo* where 50% tannery effluent improved the photosynthetic pigments. Similar results of increase in photosynthetic pigments due to 25% and/or 50% tannery effluent treatment were found in *Phasoelus radiatus* (Verma and Verma 1995), *Cicer arietinum* L. (Narain et al. 2012), and in two *Vigna* species (Babyshakila and Usha 2009). In contrast, higher concentration of tannery effluent reduced the photosynthetic pigments. Correlation studies between photosynthetic pigments and accumulation of heavy metals in leaves indicated that higher accumulation of heavy metals cause the toxic effects on photosynthetic pigments. These results can be related to the findings of Zaheer et al. (2020) who found that tannery effluent treatment increased the accumulation of heavy metals (Cd, Cr), ROS, MDA, and reduced the Mg and Zn in the leaves of *Brassica napus*. They suggested that increased accumulation of Cr and Cd caused oxidative stress, reduced the Mg accumulation, and degrade the photosynthetic pigments. Similarly, in another study it has been reported that accumulation of heavy metals substituted Mg, Fe, or Zn as co-factor in chlorophyll biosynthesis enzymes, thereby reducing the biosynthesis of chlorophyll (Küpper et al. 1996, 1998). In addition, supplementation with Zn-lysine to tannery effluent–treated plants reduced the heavy metal accumulation, ROS, and MDA, while it enhanced chlorophyll in *Brassica napus* plants (Zaheer et al. 2020). These reports further supported our argument that diluted tannery effluent treatment corrected the mineral nutrient status and photosynthetic pigment biosynthesis in cucurbitaceous vegetables.

Conversion of solar energy into biochemical energy occurs by separation of charges at photosystem II (PSII) and transport of electron from PSII to PSI coupled with proton transport for ATP synthesis. This process supports the CO_2 fixation and plant photosynthetic capacity can partially be measured as PSII activity (Athar and Ashraf 2005; Kauser et al. 2006; Ogbaga and Athar 2019a; Ogbaga and Athar 2019b; Bano et al. 2021). Low concentration (25%) of tannery effluent application enhanced quantum yield of PSII activity in *Luffa cylindrica*, *Citrullus vulgaris*, and *Cucumis melo*. Application of 50% tannery effluent treatment did not affect the PSII activity of almost all five cucurbitaceous vegetables. However, 75% and 100% tannery effluent reduced the PSII activity. These results can be related to some analogous studies in which PSII activity reduced due to high accumulation of Cr, Cd, or reduced photosynthetic pigments under heavy metal stress conditions such as in canola (Farooq et al. 2018; Ayyaz et al. 2021), grapevine (Rhimi et al. 2016), and spinach (Maqbool et al. 2018).

Photosynthetic rate or CO_2 fixation is central to plant growth and this was measured as net CO_2 assimilation rate along with associated gas exchange attribute. In this study, 25% and/or 50% tannery effluent increased the photosynthetic rate and transpiration rate in most of the cucurbitaceous vegetables. In this study, high concentration of tannery effluent reduced the photosynthetic rate and transpiration rate. Changes in photosynthetic rate in plants are due to two main factors, i.e., stomatal limitations and metabolic limitations (Athar and Ashraf 2005; Roig-Oliver et al. 2021). In this study, changes in photosynthetic rate in cucurbitaceous vegetables are positively correlated with transpiration rate but not associated with internal CO_2. These results indicated that tannery effluent caused the stomatal closure, reduced transpiration rate, and reduced CO_2 fixation rate. However, internal CO_2 was high at the high concentration of tannery effluent in all five vegetable species, where photosynthetic rate was the lowest. These results indicated that tannery effluent caused some metabolic disturbances such as degradation of photosynthetic pigments, reduction in activity of rubisco, etc., in all five vegetables. These results are similar to Zaheer et al. (2020) who reported that tannery effluent reduced the photosynthetic rate in *Brassica napus*. They also found that reduction in photosynthetic rate was associated with stomatal as well as metabolic limitations.

Conclusion

In conclusion, increasing concentration of tannery effluent reduced the growth of all cucurbitaceous vegetables. Mixing 25% tannery effluent had beneficial effects on the growth of cucurbitaceous vegetables. These beneficial effects of 25% tannery effluent application were associated with lower accumulation of toxic elements, better photosynthetic pigments, higher photosynthetic rate, and nitrogen metabolism. Moreover, *Cucurbita maxima* and *Luffa cylindrica* had maximal beneficial effects of 25% tannery effluent. Thus, appropriate dilution of tannery effluent and selection of appropriate crop will provide safe and water use efficient strategy. Since daily intake of metal contaminated vegetables even with low quantity may increase the risk of human health, it is necessary to assess heavy metal accumulation in soil contaminated with tannery effluent or contaminated with diluted tannery effluent, transport rate of different heavy metals from root to shoot, portioning of heavy metals in different plant parts particularly edible parts of plants, and human health risk assessment in field trials before recommending this strategy as safe and water use efficient strategy.

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Author contribution Zafar Ullah Zafar and Habib-ur-Rehman Athar designed the experiment; Muhammad Zaheer, Muhammad Javed, and Muhammad Iqbal conducted the experiment; Hussan Bano, Misbah Amir, and Ayesha Khalid did analysis of tap water and tannery effluent; Muhammad Zaheer, Hamid Manzoor, Muhammad Kamran Qureshi, and Zafar Ullah Zafar did physiological analysis; Muhammad Zaheer and Zafar Ullah Zafar wrote first draft of the manuscript. Chukwuma C.

Ogbaga and Habib ur Rehman Athar edited the manuscript responded to the comments; all authors read and approved the final manuscript.

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Declarations

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Consent to participate Not applicable.

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Competing interests The authors declare no competing interests.

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