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Metal accumulation and its effects in relation to biochemical response of vegetables irrigated with metal contaminated water and wastewater

S. Nayek[a], S. Gupta[a,*], R.N. Saha[b]

[a] Department of Environmental Science, The University of Burdwan, Golapbag, Burdwan 713104, West Bengal, India
[b] Department of Chemistry, National Institute of Technology, Durgapur 713209, West Bengal, India

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ABSTRACT

Agricultural application of metal contaminated water resulted in elevated concentrations of metals in irrigated soil and vegetables. Metal enrichment in irrigated soil is in the sequence of Cr > Fe > Pb > Mn > Zn > Cu > Cd. High metal translocation was observed from soil to plants with varied accumulation pattern in different species. Fe, Mn, Cu, Zn, Cr showed higher translocation to the aerial parts, while Cd, Pb exhibits their restricted mobility and concentrated in roots and stems. Hyperaccumulation of metals in vegetative parts resulted significant decrease ($p < 0.05$) in total chlorophyll and soluble sugars, with elevated ($p < 0.05$) protein and proline content in cultivated vegetables. Oxidative stress due to high metal concentrations significantly induced ($p < 0.05$) the antioxidant-enzyme activity. Peroxidase (52–206%) and catalase (40–106%) activity was noticeably higher in all the examined species, while enhanced activity of ascorbate peroxidase (70–78%) was observed in pea and spinach.

1. Introduction

Contamination of agricultural field by heavy and trace metals due to irrigation with metal rich water and wastewater has become a major environmental concern due to translocation of metals into soil–plant system, and ultimately into the food chain. In developing countries like India, contaminated water and wastewater is being extensively used by local farmers for irrigation of crops and vegetables due to scarcity of underground irrigation water. Water contaminated with industrial discharge contains appreciable amounts of toxic metals and its long term application on agricultural lands contributes significantly to the build up of elevated concentration of toxic metals in irrigated soil and plants [1–5]. Sometimes natural abundance of metals also contributes significantly. Metals, being persistent in nature remains in soil for a long time are absorbed by plant roots and mobilise to the foliage and edible parts [6,7]. The metal accumulation in different plant parts depends on the availability and chemical form of metals in soil, their translocation potential, and type of plant species with their stage of maturity [4,7]. Excessive accumulation of metals in vegetables may pose serious threat to the local residence, who consumes crops/vegetables grown in contaminated areas. The bioconcentration of metals are often related with the phytotoxicity of plants, which depends on their binding affinity with different cellular entities. Hyperaccumulation of metals in plant tissue induced oxidative stress by generating reactive oxygen species, which can seriously disrupt normal biochemical and physiological functions of plants [8]. The plants possess unique resistant strategy with the help of various cellular components and antioxidant enzymes viz APX (ascorbate peroxidase), CAT (catalase) and POD (peroxidase) that reduces the oxidative stress by eliminating active oxygen species (AOS) from plant cell. Several investigations were carried out to assess the effects of different metals (singly or in combination) on various plants in relation to their biochemical response [1,2,6,8–11].

The field work was carried out at near by areas of Mangalpur industrial complex (Latitude 23°37′N and Longitude 87°08′E) in western part of the state West Bengal, India; consisting of a number of Sponge iron and Ferro alloy industries with integrated captive power plants. This area is an extension of 'Chotonagpur platue' possessing red laterite soil zone, and had coal mining activities in the past. The wastewaters discharged from these industries are diluted in a common open channel and terminated into an open cast pit pond (OCP) and contaminates its water. This contaminated OCP water is the main source of irrigation water in that region and extensively used by the local people for the cultivation of different vegetables in the surrounding areas. Sometimes these people also use industrial wastewater from wastewater channel directly to irrigate the cultivated vegetables. Earlier investigation [12] showed that the application of contaminated OCP water and wastewater for irrigation leads to enrichment of metals in agricultural soil as well as irrigated vegetables in those areas. But the previous investiga-

* Corresponding author. Tel.: +91 09433968712.
E-mail address: srimantagupta@yahoo.co.in (S. Gupta).

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tion was devoid of effects of metal contamination/stress in relation to the biochemical response of cultivated vegetables.

The present study deals with the assessment of irrigation suitability of wastewater and open cast pit-pond water for their applications in agricultural purpose. The investigation focuses on the metal accumulation in agricultural soil and vegetables irrigated with metal rich water, and the metal induced oxidative stress on biochemical constituents and antioxidant enzymes.

2. Materials and methods

2.1. Sample collection and preparation

The wastewater and OCP water samples are collected at regular intervals from open effluent channel and OCP in acid washed plastic containers (1 L). In laboratory, the wastewater samples were stored in a freeze and physicochemical analysis was performed within a week. Composite soil samples were collected from the 12 different sites of the wastewater irrigated field. Soil collection was done at growing crop season and also during harvesting of vegetables. Samples are collected from (0–10 cm depth) plant root zone and kept immediately into plastic bags. In laboratory conditions, soil samples were air dried, crushed and sieved through 2 mm mesh for further physicochemical analysis.

The most commonly cultivated garden vegetables were tomato (*Lycopersicum esculatum*; Family Solanaceae), pea (*Pysum sativum*; Family Fabaceae), beans (*Phaseolus vulgaris*; Family Fabaceae) and spinach (*Spinacia oleracea*; Family Chenopodiaceae). Vegetable samples were collected randomly from wastewater irrigated field. All the plant samples are collected at their harvestable maturity (tomato, pea and beans in fruiting stage, except spinach). Care was taken to collect the healthy uninfected vegetable species. Plant samples were thoroughly washed first with tap water followed by distilled water to get rid of soil/contaminants especially at root zone. Washed plant samples were refrigerated under suitable condition for biochemical analysis. For estimation of metal content in different plant parts, plant samples were divided into roots, stems and leaves and dried at 80 °C temperature for 24 h.

A control site was also selected in nearby areas where groundwater is used for irrigation. Uncontaminated soil samples were collected in similar way as described earlier and considered as control/background value and compared against contaminated soil to investigate the metal enrichment in wastewater irrigated fields. Vegetable samples from the control field were collected and the analysed for biochemical parameters (along with antioxidant enzymes) to assess the effect of metal stress on biochemical response of plants.

2.2. Physicochemical analysis of wastewater, soil and plant samples

The physicochemical analysis of wastewater and OCP water samples was performed as per Standard method, APHA [13]. For metal estimation, the wastewater samples (500 ml) were reduced to 50 ml, digested with 10 ml of conc. HNO_3 at 80 °C [13]. The solution was filtered through Whatman No 42 filter paper and diluted to 50 ml with distilled water.

Soil pH and electrical conductivity (EC) was measured by preparing soil suspension (1:5, w/v) with deionised water [14]. Organic matter (OM) content by Walkey and Black [15] method and cation exchange capacity (CEC) was determined by extracting major cations from soil samples (5 g) with 1 M CH_3COONH_4 buffer followed by titrimetric analysis [16]. For estimation of total metal content (Fe, Cr, Cd, Pb, Mn, Cu and Zn) 1 g of soil sample was digested with a mixture of concentrated HNO_3 and $HClO_4$ (4:1) [17]. The filtered solution was diluted to 50 ml with distilled water and was analysed by atomic absorption spectrophotometer (GBC, Avanta). Total Fe content was determined spectrophotometrically at 510 nm using digested extract. For determination of bioavailable metal fraction in wastewater irrigated soil, 5 g of soil (air dried) samples extracted with 50 ml of 1 M CH_3COONH_4, the extractant was filtered through Whatman 42 grade filter paper and clear solution were analysed by AAS, same as described earlier.

Metal concentrations in different parts of crop plant were analysed by microwave digestion method, 1 g of dried plant samples were treated with mixture (4:1) of concentrated HNO_3 and $HClO_4$ [17] for 20 min at 620 W and in closed Tephlon containers. The clear digestion mixture was analysed in AAS. The metal content was expressed as mg kg^{-1} plant dry matter (DM).

2.3. Estimation of biochemical parameters

Total chlorophyll content was measured according to Arnon [18] using 80% chilled acetone. Soluble sugar and protein content of foliar parts was determined following the procedure of Yemm and Willis [19] and Lowry et al. [20] respectively. Proline content in leaves was estimated according to Bates et al. [21]. The estimated biochemical parameters were expressed as $\mu g\, g^{-1}$ plant fresh matter (FM).

2.4. Antioxidant enzyme analysis

0.5 g of leaf tissues (without midribs or veins) were homogenized using 5 ml of phosphate buffer (0.1 M, pH 7.0). The homogenate was filtered and centrifuged in 15,000 rpm for 15 min. The supernatant was used for enzymatic analysis; all preparations were carried out less than 4 °C.

2.4.1. APX (EC 1.11.1.11)

The APX activity was measured according to Nakano and Asada [22] (absorbance coefficient 2.8 $mM^{-1}\, cm^{-1}$) as decrease in absorbance at 290 nm. The total mixture contained 50 mM phosphate buffer (pH 7.0), 0.5 mM sodium phosphate, 1 mM H_2O_2, 0.1 mM EDTA and 100 μl enzyme extract. The enzyme activity was expressed μmol of ascorbate oxidized $min^{-1}\, g^{-1}$ FM at 25 ± 2 °C.

2.4.2. CAT (EC 1.11.1.6)

CAT activity was determined [23] by measuring the decomposition of H_2O_2 at 240 mM (extinction coefficient of 0.036 $mM^{-1}\, cm^{-1}$). The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 100 μl enzyme extract. The activity was expressed in μmol of H_2O_2 reduced $min^{-1}\, g^{-1}$ FM at 25 ± 2 °C.

2.4.3. POD (EC 1.11.1.7)

POD activity was measured as the increase in absorbance at 470 mM due to guaiacol oxidation (extinction coefficient of 26.6 $mM^{-1}\, cm^{-1}$) [22]. The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, 0.05% guaiacol and 100 mM enzyme extract. The unit is expressed in μmol $min^{-1}\, g^{-1}$ FM.

2.5. Calculation of different indices/factors

Enrichment factor (EF): Enrichment factor has been calculated [5] to assess the metal enrichment in contaminated (wastewater irrigated) soil in comparison to control (groundwater irrigated) soil:

$$EF = \frac{\text{Metal concentration in contaminated soil}}{\text{Metal content in control soil}}$$

Transfer factor (TF): Transfer factor is a ratio of metal content in soil and plants, establish the pattern of metal translocation from soil to plant parts, i.e. roots (TF_R), shoots (TF_S) and fruits (TF_F). TF was calculated by dividing the metal concentrations in plant tissue with the amount of bioavailable metals in contaminated soil [24].

2.6. Statistical interpretations

Pearson's correlations (r) were calculated between bioavailable metal fractions with soil parameters (pH, CEC, OM) and also with total metal content in agricultural soil. Linear regressions were calculated between bioavailable metals in agricultural soil with metal content (mg kg^{-1} DM) in different plant parts (roots, shoots and fruits) to analyse the metal translocation from soil to plant system assuming Y-dependent variables: metal concentrations in plant parts and X-independent variables: exchangeable metal content in soil. Hierarchical clustering (Euclidean distance, nearest neighbour) of total dry matter metal content of individual species were analysed to study the interactions between metals and grouped the homogeneous metals. One way-two factor ANOVA was calculated between metal content in plant leaves with foliar biochemical constituents. Correlations (r) were also performed between leaf metal content with total chlorophyll, proteins, soluble sugars, proline and antioxidant enzymes to investigate the effect of each metal on plants biochemical constituents. All the statistical performances were done at significance level 0.05 by using XL stat-version 10, 2009.

2.7. Quality control and assurance

Care has been taken during every experimental procedure. E-mark (AR grade) standards were used for the preparation of standard curve during analysis of metals. Every analysis (related to wastewater, OCP water, soil, vegetables and biochemical parameters) was triplicate to ensure the accuracy of the experimental data/results.

3. Results and discussion

3.1. Irrigation water suitability assessment of industrial wastewater and OCP water

Assessment of irrigation suitability includes estimation of pH, EC, dissolved solids (TDS) and sodium absorption rate (SAR). EC value represents the concentrations of soluble ions while determination of sodium absorption rate (SAR) associated with salinity stress caused by exchangeable Na^+ ions present in water used for irrigation. The pH, EC, TDS and SAR values (Table 1) for both wastewater and OCP water are very much within the suitable range for the applications in agriculture [25]. Metal content in wastewater and OCP water is compared against their permissible limits for irrigation water [25]. Concentrations of Fe, Cr, Cd, Mn and Cu in wastewater are higher than the recommended standard and thereby unsuitable for use in agricultural field. In OCP water, Cr and Mn concentrations are higher than the permissible limits for agricultural applications, while rest of the metal concentrations are suitable for the use to irrigate vegetables. Groundwater quality in that area is very much appreciable for agricultural use.

3.2. Physicochemical analysis of soil irrigated with metal contaminated water

Total metal concentrations in soil are usually used as the primary pollution reference. Metal content in contaminated and control soils is represented in Table 2. Several physicochemical characteristics of the soil are known to affect the solubility and plant availability of metals which include pH, CEC, organic matter (OM) content and clay minerals [26]. Correlations between bioavailable metal content with soil pH, CEC and OM revealed strong negative correlations for most of the metals (Table 3). In alkaline pH, the metals are more firmly bounded with organic matter and their phytoavailability become reduced [27]. According to these authors, CEC increases with increase in clay content in the soil and the availability of metals decreases. Total metal concentrations of wastewater irrigated soil are compared against the control soil, and the enrichment of metals (EF) is in the sequence of Cr (4.26) > Fe (2.97) > Pb (2.93) > Mn (2.35) > Zn (2.10) > Cu (1.73) > Cd (1.45). Determination of bioavailable metal is a useful mean to predict the amounts of metal in soil environment readily available for plant uptake. Bioavailable fractions comprising 31.07–59.26% of the total metal content and rest part were in insoluble complex form (carbonate bounded or chelated with OM). Significant correlations between total metal content with their bioavailable fractions for Fe, Cd, Mn and Cu revealed their higher availability and translocation in soil–plant system.

3.3. Metal accumulation within the plant tissue of different vegetable species

Accumulation of metals in different parts of crop plants is represented in Table 4. Partitioning of metals in different plant parts

Table 1
Characterization of industrial wastewater, OCP water and groundwater (n = 6).

Parameters	Industrial wastewater	Contaminated OCP water	Underground irrigation water	FAO irrigation standards (Pescod [25])
pH	8.94–8.36 ± 0.24	7.92–7.08 ± 0.39	8.2–7.8 ± 0.21	6.5–8
EC	21.8–12.27 ± 4.19	4.17–3.69 ± 0.22	12.6–4.3 ± 3.36	750–2000
TDS	1076–872 ± 95.20	328–217 ± 46.34	620–460 ± 0.38	450
BOD	3.64–2.26 ± 0.61	5.39–4.73 ± 0.29	–	–
COD	222–152 ± 30.26	138–119 ± 8.42	–	–
NO_3-N	0.256–0.174 ± 0.04	1.16–1.02 ± 0.06	2.18–1.06 ± 0.49	–
PO_4^{3-}	0.138–0.104 ± 0.01	0.072–0.056 ± 0.01	1.46–0.078 ± 0.74	–
SAR	4.32 ± 0.20	4.44 ± 0.09	4.00 ± 1.41	1–12
Fe	8.62–7.23 ± 0.58	0.136–0.106 ± 0.013	0.12–0.06 ± 0.03	5.0
Cr	0.832–0.692 ± 0.06	0.704–0.68 ± 0.01	0.03–0.018 ± 0.01	0.1
Cd	0.034–0.024 ± 0.005	0.007–0.006 ± 0.001	ND	0.01
Pb	0.366–0.272 ± 0.039	0.024–0.018 ± 0.003	Trace–ND	5.0
Mn	0.774–0.689 ± 0.040	0.35–0.32 ± 0.018	0.088–0.05 ± 0.02	0.2
Zn	0.716–0.672 ± 0.021	0.24–0.16 ± 0.035	0.07–0.046 ± 0.01	2.0
Cu	0.763–0.704 ± 0.025	0.16–0.09 ± 0.030	0.03–0.008 ± 0.01	0.2

Water parameters = max–min ± SD; ND, not detectable; '–', not mentioned.
BOD, biochemical oxygen demand; COD, chemical oxygen demand.
EC is expressed as ms cm^{-1} at 25 °C and other parameters (except pH and SAR) are in mg L^{-1}.

Table 2
Physicochemical analysis of wastewater irrigated soil and control soil ($n = 12$).

Parameters	Contaminated soil		Control soil			
pH	6.36 ± 0.06		6.68 ± 0.12			
EC ms cm^{-1} at 25 °C	0.56 ± 0.07		0.72 ± 0.06			
OM (%)	4.21 ± 0.13		4.08 ± 0.36			
CEC	204.00 ± 12.54		168.00 ± 4.34			
Metals (mg kg^{-1} of dry soil)	Total	Bioavailable	Total	Bioavailable	Metal standards for uncontaminated soil	
Fe	258.00 ± 10.31	123.57 ± 6.33*	86.64 ± 5.32	56.33 ± 5.96	1000[a]	–
Cr	506.00 ± 20.94	219.83 ± 9.06	109.00 ± 6.0	68.50 ± 4.68	100[a]	N/A
Cd	38.00 ± 6.29	22.00 ± 3.03*	18.50 ± 3.45	11.08 ± 2.29	1.0[a]	3–6[b]
Pb	752.00 ± 5.62	42.67 ± 4.63	28.67 ± 4.69	22.10 ± 4.15	50[a]	250–500[b]
Mn	212.00 ± 11.71	65.86 ± 6.01*	86.90 ± 6.15	44.00 ± 6.45	–	–
Zn	176.00 ± 7.62	74.53 ± 4.30*	77.60 ± 4.49	52.08 ± 7.13	100[a]	300–600[b]
Cu	122.00 ± 6.48	44.67 ± 4.68	66.50 ± 3.73	36.50 ± 4.32	30[a]	135–270[b]

Soil parameters = mean ± SD.
[a] Kabata-Pendias and Pendias [46].
[b] Awashthi [47].
* $p < 0.05$.

Table 3
Correlations (r) between extractable metals with soil physical properties.

Parameters	pH	CEC	OM
Fe	−0.131	−0.168	−0.160
Cr	−0.752*	−0.716*	−0.563
Cd	0.211	0.223	0.073
Pb	−0.740*	−0.700*	−0.522
Mn	−0.959*	−0.933*	−0.700*
Zn	0.647	0.585	0.257
Cu	−0.768*	−0.810*	−0.858*

* $p < 0.05$.

is a common strategy to prevent toxicity to the above ground parts, which is due to the binding of metals with ligands and phytochelatins having high affinity for the metals [6]. Besides, interactions among the metals are also responsible for their varied accumulation and distribution within plant tissue.

All the species studied, Fe accumulation was higher in aerial parts (stem and leaves) in comparison to their roots, and the Fe content in foliar parts often cross the phytotoxic concentrations of 200 mg kg^{-1} DM [28]. Least Fe concentration was observed in fruits.

Cr content in normal plants varies 1–5 mg kg^{-1} DM and concentrations > 5 mg kg^{-1} DM considered as phytotoxic [29]. Cr concentrations in all the species were higher than the phytotoxic value, and the accumulation was higher in leaves.

Cd concentrations in different plant organs were much higher than its phytotoxic concentration, 0.5 mg kg^{-1} DM [28]. Cd content was more in roots and stems in comparison to leafy vegetative parts.

Pb is considered as the least mobile among the studied metals, and its higher accumulation was observed in roots. Pb concentrations in all the examined species were much higher than its normal plant concentrations 1–5 mg kg^{-1} DM [30,28].

Mn is considered as essential trace metals and Mn toxicity in plants can generally be associated with the Mn concentrations of 400–700 mg kg^{-1} DM [29,30]. All the species studied, Mn concentrations were well below than toxic range to cause any damage. Mn content in above ground plant parts much higher than the roots.

Normal Zn concentration in plants is 50–150 mg kg^{-1} DM [29,30] and concentration > 400 mg kg^{-1} considered as poisonous. The Zn content in all the examined species is within normal range

Table 4
Metal concentrations (mg kg^{-1} DM) in different parts of wastewater irrigated crop plants ($n = 12$, mean ± SD).

	Plant parts	Fe	Cr	Cd	Pd	Mn	Zn	Cu
Tomato (*Lycopersicum esculatum*)	Roots	185.00 ± 6.76	156.75 ± 4.57	48.75 ± 5.10	66.00 ± 5.48	50.00 ± 5.48	56.75 ± 5.25	48.25 ± 5.12
	Stem	317.50 ± 6.35	118.00 ± 3.16	52.00 ± 4.97	45.50 ± 7.33	82.00 ± 3.30	79.75 ± 5.85	87.50 ± 3.70
	Leaves	348.75 ± 13.07	225.70 ± 9.81	37.50 ± 4.80	44.00 ± 5.48	88.75 ± 6.13	84.00 ± 5.48	65.75 ± 5.80
	Fruits (Ed)	78.00 ± 6.98	34.00 ± 3.74	6.20 ± 1.28	ND	28.50 ± 3.42	36.00 ± 5.23	10.20 ± 1.72
Control species (average plant metal content)		89.67	112.33	30.66	22.50	61.00	54.33	32.67
Pea (*Pysum sativum*)	Roots	161.50 ± 9.11	134.50 ± 6.45	47.75 ± 4.43	56.00 ± 4.24	41.00 ± 5.48	49.50 ± 4.93	39.50 ± 2.89
	Stem	274.75 ± 5.91	116.25 ± 6.24	40.00 ± 2.94	43.25 ± 5.12	78.50 ± 4.93	72.00 ± 3.87	68.50 ± 6.03
	Leaves	314.00 ± 7.26	213.50 ± 4.43	35.50 ± 4.04	38.50 ± 5.20	81.75 ± 6.02	71.00 ± 5.60	64.75 ± 4.11
	Fruits (Ed)	62.00 ± 4.40	56.50 ± 4.66	9.70 ± 0.74	6.025 ± 1.13	41.00 ± 3.92	27.50 ± 4.04	16.05 ± 2.3
Control species (average plant metal content)		83.66	96.33	23.80	14.50	52.83	44.50	30.00
Beans (*Phaseolus vulgaris*)	Roots	142.25 ± 4.65	132.00 ± 4.97	30.50 ± 4.04	43.00 ± 3.16	46.00 ± 2.94	55.75 ± 3.10	40.50 ± 4.20
	Stem	222.00 ± 5.56	146.50 ± 5.80	44.00 ± 3.74	54.00 ± 0.58	72.00 ± 3.51	62.50 ± 3.70	56.70 ± 4.27
	Leaves	307.75 ± 4.35	187.00 ± 7.44	27.50 ± 4.65	30.50 ± 3.87	85.50 ± 3.87	78.00 ± 1.83	70.00 ± 2.94
	Fruits (Ed)	86.00 ± 4.55	64.00 ± 2.16	11.50 ± 2.38	8.30 ± 0.87	36.50 ± 5.32	42.00 ± 4.24	22.90 ± 3.75
Control species (average plant metal content)		98.00	126.30	28.33	25.16	59.5	46.33	35.16
Spinach (*Spinacia oleracea*)	Roots	132.50 ± 6.56	122.25 ± 7.27	44.50 ± 3.87	58.25 ± 4.86	36.00 ± 7.12	52.50 ± 6.24	44.50 ± 5.20
	Shoots (Ed)	269.75 ± 12.07	130.50 ± 11.09	42.25 ± 4.57	36.00 ± 2.90	76.00 ± 5.35	74.00 ± 5.35	78.00 ± 7.16
	Seeds	37.00 ± 4.69	24.75 ± 2.22	3.30 ± 0.54	2.13 ± 0.30	21.83 ± 3.79	16.25 ± 3.30	8.10 ± 2.17
Control species (average plant metal content)		73.00	76.00	26.5	18.5	44.25	38.0	29.5
FAO/WHO Standards [48]		450	5	0.3	5	–	60	40

FAO/WHO Standards [48] for metal concentrations in consumable vegetables, Ed, edible parts; ND, not detectable.

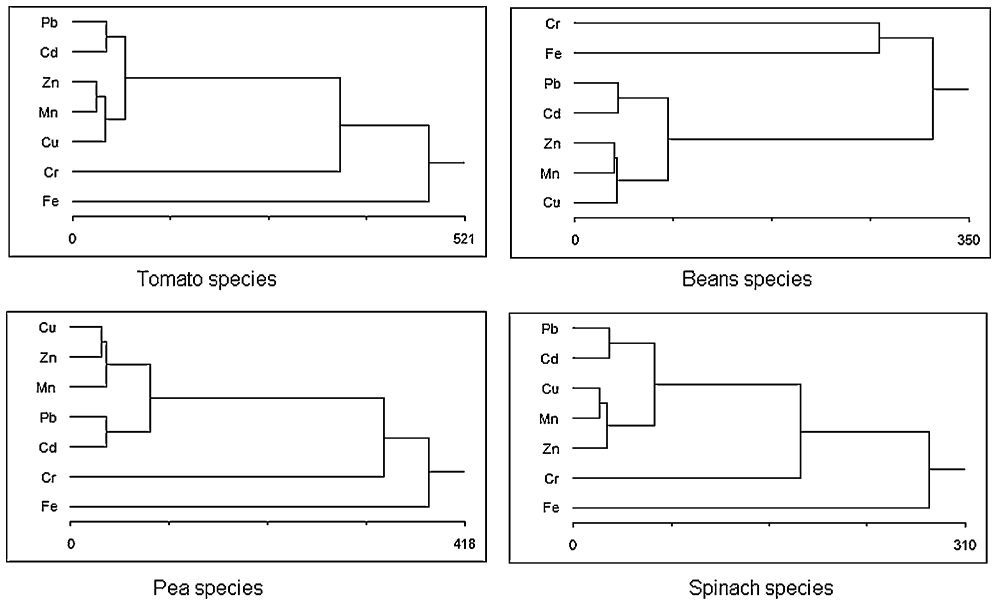

Fig. 1. Hierarchical cluster (Euclidean distance) analysis of plant metal content (DM) of individual species irrigated with metal contaminated water.

and the leafy vegetative parts exhibit the highest Zn accumulation capacity.

The mean Cu concentrations in all the examined species were much higher than the Cu content in normal plants (4–15 mg kg^{-1}) [31]. For all the studied species, higher Cu concentrations were observed in leafy vegetative parts—similar to the Mn and Zn.

The investigation showed higher metal concentrations in leafy vegetative parts of the treated plants consistent with the earlier investigations [32,4,7], while least metal concentrations were observed in fruits. The total phytomass metal accumulation in examined species is in the order of tomato > pea > beans > spinach.

Hierarchical cluster (Euclidean distance, nearest neighbour) analysis of total dry matter metal content of individual species (Fig. 1) revealed very similar scenario. From the dendrograph of individual species, two major clusters were obtained. Cluster 1 showed the association between trace metals: Mn–Cu–Zn because of similar pattern of distribution within plants, while in cluster 2, non-essential toxic metals (Cd–Pb) with lower mobility grouped together. Fe and Cr are distantly grouped and did not show any close association between themselves or with any of the clusters.

Vegetables irrigated with metal contaminated water did not show any particular phenotypic abnormalities consistent with the findings of earlier workers [1,5].

3.4. *Translocation of bioavailable metals from soil into the plant system*

The ratio of metals between soil and plants is an important criterion for the contamination assessment and selection of crop plants for cultivation on contaminated soil, and the ratio '>1' means higher accumulation of metals in plant parts than soil [7,32]. Metal in soluble form is readily uptaken by plant roots and subsequently translocate them to the aerial vegetative shoots (stem and leaves) following the linear path: soil–plant roots–aerial parts. TF values of investigated metals for different plant parts are represented in Table 5. The investigation showed high metal translocation (TF > 1) from soil to plant parts indicated higher metal uptake by all the species. Non-essential toxic elements—Cd and Pb showed lower translocation for foliage parts and tend to accumulate mostly in roots and stems ($TF_R > 1$), while Fe, Cr, Mn, Zn and Cu showed their affinity for leafy vegetative shoots ($TF_S > 1$). The higher mobility of Fe, Mn, Cu and Zn can be attributed to their higher requirement during physiological functions. The mobilization of metals from roots to shoots includes long distance translocation in the xylem and storage in the vacuoles of leaf cells and these processes affected by many factors such as phytochelatins and organic acids [33]. The translocations of metals from soil to above ground vegetative parts for different plant species are in the following sequence:

Tomato (*L. esculatum*)—Fe > Mn > Cu > Zn > Cr > Cd > Pb.
Pea (*P. sativum*)—Mn > Fe > Cu > Zn > Cr > Cd > Cd > Pb.
Beans (*P. vulgaris*)—Fe > Mn > Cu > Cr > Zn > Cd > Pb.
Spinach (*S. oleracea*)—Fe > Cd > Cu > Mn > Pb> Zn > Cr.

The higher TF_S values of Fe and Mn in all the examined species can be attributed to their higher mobility in comparison to other metals. The lowest metal translocation ($TF_f < 1$) was noticed in fruits and seeds, which can be attributed to plants inherent property to store most of metals within their vegetative tissue.

Linear regressions (R^2) were performed between soil exchangeable metals (mg kg^{-1}) with metal concentrations in different plant parts—roots, shoots and fruits (mg kg^{-1} of DM) for individual plant species to evaluate the metal translocation from soil to plant parts. High TF value is often statistically supported by higher R^2 value, expressing the higher metal translocation in soil–plant system. But contrast between TF and R^2 values is not uncommon due to interference with several other determining factors and also the interactions within the metals.

3.5. *Effect of metal stress on total chlorophyll, soluble sugars, protein and proline contents of contaminated vegetables*

Investigation of biochemical response against various metal stresses on different crop plant species is important for the identification of potential resistant variety. Results for biochemical parameters are represented in Table 6. One of the most common effects of metal stress is loss of photosynthetic pigments and is well documented by several earlier workers [1,34,35]. All the plant species from treated agricultural fields showed significant reduction ($p < 0.05$) in total chlorophyll content. In tomato plants, total chlorophyll content was negatively correlated with Cd (−0.959), Cu (−0.987), Pb (−0.988) and Mn (−0.961). Chlorophyll content in pea

Table 5
Translocation factors (TF) between soil extractable metals with metal content in plant parts (DM) along with regression (R^2) analysis.

	Fe	Cr	Cd	Pb	Mn	Zn	Cu
Tomato (*Lycopersicum esculatum*)							
TF_R	1.50	0.71	2.18	1.55	0.76	0.76	1.08
[R^2]	0.68	0.75	0.97	0.70	0.49	0.52	0.62
TF_S	2.67	0.78	2.19	1.05	1.29	1.10	1.72
[R^2]	0.93	−2.72	0.70	−0.20	0.57	0.60	0.62
TF_F	0.63	0.16	0.28	–	0.43	0.48	0.23
[R^2]	−0.52	−7.54	−3.34	–	−0.42	−3.07	−0.66
Pea (*Pysum sativum*)							
TF_R	1.31	0.61	2.17	1.31	0.62	0.66	0.88
[R^2]	0.99	0.48	0.97	0.51	0.25	0.54	0.58
TF_S	2.38	0.75	1.72	0.95	1.22	0.96	1.49
[R^2]	0.91	0.75	0.85	−0.29	0.65	0.48	0.71
TF_F	0.50	0.26	0.44	0.14	0.62	0.36	0.36
[R^2]	0.55	−1.88	−1.42	−0.49	0.61	−2.83	−0.66
Beans (*Phaseolus vulgaris*)							
TF_R	1.15	0.80	1.39	1.01	0.70	0.75	0.91
[R^2]	0.57	0.64	0.57	0.74	−0.06	0.37	0.26
TF_S	2.14	0.78	1.63	0.98	1.21	0.94	1.42
[R^2]	0.85	−0.49	0.60	0.75	0.88	0.42	0.62
TF_F	0.70	0.29	0.52	0.19	0.55	0.56	0.51
[R^2]	0.12	0.19	−2.88	−0.48	−2.04	−4.39	−0.17
Spinach (*Spinacia oleracea*)							
TF_R	1.07	0.56	2.02	1.37	0.53	0.70	1.01
[R^2]	0.74	0.26	0.90	0.50	0.10	0.06	0.78
TF_S	2.18	0.59	1.92	0.84	1.15	0.99	1.52
[R^2]	0.96	0.36	0.64	0.57	0.60	−1.16	0.86
TF_F	0.30	0.11	0.15	0.05	0.33	0.22	0.18
[R^2]	−6.45	−0.63	−1.73	−2.96	−2.48	−7.12	−1.63

TF_R: soil/root, TF_S: soil/shoots, TF_F: soil/fruits.

and bean plants showed negative correlations with Cr (−0.967), Mn (−0.951) and with Fe (−0.987), Mn (−0.997) respectively; while in spinach negative correlations were observed with Fe (−0.971) and Zn (−0.997). Decline in total chlorophyll content can be attributed to degradation of chloroplast by substitution of Mg^{2+} with metal ions or higher activity of chlorophylase. The reduction of chlorophyll was highest in pea plants.

Investigation showed significant reduction of soluble sugars in tomato, pea and spinach (Table 6) which can be corresponded with loss of photosynthetic pigments and also the higher energy requirements due to oxidative stress. Several investigations [34,35] reported decline of sugar content due to metal stress/toxicity in treated plants. Soluble sugar content in tomato plants showed significant negative correlation ($p < 0.05$) with Cu (−0.946), Pb (−0.969) and Mn (−0.996). In pea plants, negative correlations were observed with Cr (−0.985), Cu (−0.966) whereas spinach exhibited negative correlations with Cr (−0.991) and Cd (−0.965).

All the examined species from contaminated field receiving industrial discharge showed the elevated protein content collaborating with the earlier findings [1,2,36], the significant enhancement was observed in beans plants (Table 6). Protein content in beans plants showed significant positive ($p < 0.05$) cor-

Table 6
Estimation of biochemical parameters (mg g^{-1} plant tissue FM) in wastewater irrigated and control vegetables ($n = 12$).

	Total chlorophyll	Soluble sugar	Proteins	Proline
$Tomato_{(Control)}$	9.878 ± 0.083	1.207 ± 0.009	6.220 ± 0.122	2.970 ± 0.042
$Tomato_{(Treated)}$	4.104 ± 0.059	0.533 ± 0.012	6.693 ± 0.177	1.610 ± 0.048
% increase/decrease	(−) 58.453*	(−) 55.841*	NS	(+) 84.472**
$Pea_{(Control)}$	16.732 ± 0.080	1.508 ± 0.010	6.725 ± 0.138	2.124 ± 0.042
$Pea_{(Treated)}$	4.463 ± 0.079	0.582 ± 0.009	8.478 ± 0.209	1.402 ± 0.052
% increase/decrease	(−) 73.327**	(−) 61.406**	NS	(+) 49.577*
$Beans_{(Control)}$	10.742 ± 0.98	0.768 ± 0.010	7.228 ± 0.180	2.055 ± 0.058
$Beans_{(Treated)}$	3.862 ± 0.61	0.554 ± 0.011	10.128 ± 0.223	3.126 ± 0.028
% increase/decrease	(−) 64.048**	NS	(+) 40.122*	(+) 52.117*
$Spinach_{(Control)}$	7.829 ± 0.074	0.692 ± 0.010	4.510 ± 0.150	1.768 ± 0.039
$Spinach_{(Treated)}$	3.986 ± 0.026	0.430 ± 0.011	5.523 ± 0.191	3.235 ± 0.044
% increase/decrease	(−) 49.087*	(−) 37.861*	NS	(+) 82.975**

‘+’ indicates the increase and ‘−’ indicates the decrease with respect to control species. NS, not significant.
* $p < 0.05$.
** $p < 0.01$.

relation with Pb (0.989) and Zn (0.973). The increase in protein content in examined species can be attributed to the addition of essential micronutrients (Fe, Cu, Mn and Zn) through irrigation water and also higher synthesis of metal binding proteins, i.e. phytochelatins, an important strategy for survival in metal contaminated areas.

Proline accumulation, accepted as an indicator of environmental stress is also considered to have important protective roles against oxidative cellular damage [37]. As non-enzymatic cellular antioxidant, proline could be effective to maintain a balance between formation of AOS and their scavenging for detoxification of metal ions. Oxidative stress remarkably enhanced proline accumulation in plants which can be corresponded with the increased stress tolerance of plants through osmotic adjustment, stabilising proteins and sustenance of H_2O_2 at the required level [38,39]. In this study, all the species irrigated with metal contaminated water exhibit higher proline accumulation (Table 6) in foliar parts consistent with the previous findings [36,40]. Proline showed significant positive correlations ($p < 0.05$) with Fe (0.961), Cr (0.971), Zn (0.994) in tomato plants; with Cd (0.974), Pb (0.977) in pea plants; with Pb (0.996), Zn (0.991) in beans and with Cr (0.996), Cd (0.961), Pb (0.970) in spinach plants. Higher proline accumulation in plants may be associated with the decrease in plant-water potential [41]. Increased levels of heavy metals in plants have affected the permeability of membranes, causing water stress like conditions leading to an increase in proline content [42].

3.6. Effect of metal stress on antioxidant enzymes

The hyperactivity of antioxidant enzymes and accumulation of several cellular antioxidants in various plants under metal stress have been reported [8]. APX is a member of ascorbic acid-glutathione cycle and plays a significant role in the elimination of AOS and maintaining redox status of the cell [43]. APX showed significant positive correlations with Cr (0.999), Zn (0.981) in tomato, with Cd (0.957), Mn (0.979), Zn (0.968), Cu (0.974) in pea, with Cr (0.970), Cu (0.981) in beans, and with Fe (0.995), Zn (0.970), Mn (0.948) in spinach plants. APX activity was significantly higher (Fig. 2) in pea (77%) and spinach (69%).

CAT is synthesised in a tissue specific and age dependent manner and scavenge H_2O_2 during photorespiration [44]. Significant correlations found to exist with Cd (0.989), Pb (0.989), Mn (0.946) in tomato, with Fe (0.952), Cd (0.981), Pb (0.937) in pea, with Cr (0.980), Cu (0.975) in beans and Cr (0.978), Cd (0.988) in spinach. CAT activity in all the species is significantly higher (Fig. 3) than the control species. Beans showed the highest increase in CAT activity (105), followed by spinach (77%), tomato (63%) and pea (39%).

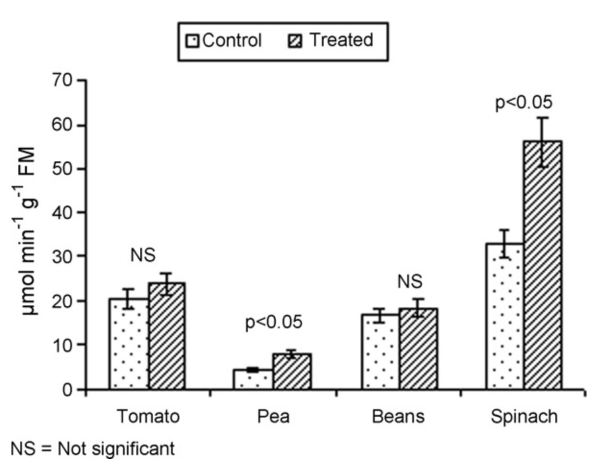

Fig. 2. Ascorbate peroxidase (APX) activity in control and contaminated/treated vegetables.

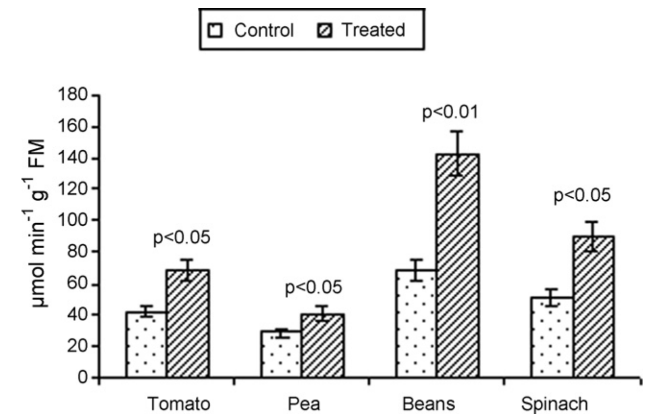

Fig. 3. Catalase (CAT) activity in control and contaminated/treated vegetables.

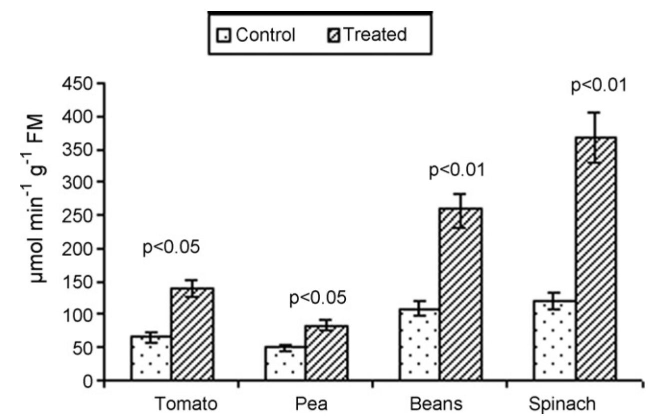

Fig. 4. Peroxidase (POD) activity in control and contaminated/treated vegetables.

POD located in cytosol, vacuole, and cell wall, both in its extracellular and intracellular form utilises H_2O_2 for oxidation of various inorganic and organic substrates [45]. POD exhibits significant correlations with Pb (0.943), Mn (0.997) in tomato plants, with Fe (0.974), Pb (0.983) in peas, with Cr (0.938), Cd (0.988), Cu (0.925) in beans and with Fe (0.974), Mn (0.984), Zn (0.964) in case of spinach. In all the vegetable species, POD activity was much higher in comparison to the APX and CAT activity (Fig. 4). The significant enhancement was observed in spinach (206%), beans (136%), tomato (109%) and pea (65%) in comparison to the control species.

4. Conclusion

This investigation reveals that OCP water in the study area is comparatively safer than the industrial wastewater for agricultural application. Different parts of vegetables irrigated with metal rich water show significantly higher metal fixing abilities. It is demonstrated that metal content is relatively higher in plant parts that possess higher metabolic activity. The metal fixation ability of fruits and seeds is weak in comparison to other vegetative parts; therefore it is advisable to grow crops/vegetables having fruits and seeds as their main edible parts. The above phytotoxic level concentration of non-essential toxic metals such as Cd, Cr, and Pb may lead to oxidative stress in cultivated vegetables resulting changes in total chlorophyll, sugar, protein, proline content with enhanced activity of antioxidant enzymes. Elevated metal content in fruits and vegetative parts of experimented vegetables, may lead to some sort of health risk to local farmers due to consumption of said vegetables. Therefore deep going study on the health risk assessment is strongly recommended.

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