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Unraveling the effects of cadmium on growth, physiology and associated health risks of leafy vegetables

Junaid Latif[1,2] · Javaid Akhtar[1] · Iftikhar Ahmad[3] · Muhammad Mahmood-ur-Rehman[4] · Ghulam Mustafa Shah[3] · Qamaruz Zaman[5] · Tariq Javaid[6] · Zia Ur Rahman Farooqi[1] · Muhammad Shakar[7] · Azka Saleem[8,9] · Muhammad Rizwan[10]

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Abstract

Vegetables are a common and important source of food and nutrition but the increasing concentration of cadmium (Cd) in the food chain via wastewater–soil–vegetable continuum is posing a threat to their growth as well as human life. This study aimed at determining the effects of variation in duration of plant exposure to different dosages of Cd on growth, physiology and potential health risks of three commonly grown leafy vegetables, viz. spinach (*Spinacia oleracea* L.), fenugreek (*Trigonella foenum-graecum* L.) and coriander (*Coriandrum sativum* L.). The experiment was a 5×3 factorial containing five doses (0, 10, 20, 30 and 40 mg Cd kg^{-1} soil) and three (25, 50 and 75 day) durations of plant exposure. The experiment was laid out in completely randomized design (CRD) with three replications. It was found that plant height, root length, leaf area and biomass were significantly affected by increasing Cd concentration up to 40 mg kg^{-1} for all the vegetables. Furthermore, Cd treatments significantly affected the photosynthetic and biochemical attributes such as chlorophyll *a*, chlorophyll *b*, carotenoids and total chlorophylls at various growth stages of vegetables tested in comparison with their respective controls. Accumulation of Cd in all the leafy vegetables, its daily intake via vegetable consumption and health risk index were significantly increased with the increase in Cd concentration. The highest values of these parameters were found at 40 mg Cd kg^{-1} soil. It could be concluded that duration of exposure and Cd dose were very important in determining Cd toxicity, which are reflected in drastic reduction in vegetable growth and physiology. Moreover, the consumption of such vegetables would have toxic effects on human health.

Keywords Coriander · Ecotoxicology · Fenugreek · Heavy metals · Photosynthetic pigment · Spinach

✉ Iftikhar Ahmad
iftikharahmad@cuivehari.edu.pk; iffises@yahoo.com

✉ Ghulam Mustafa Shah
ghulammustafashah@ciitvehari.edu.pk; gmshah1985@gmail.com

1 Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

2 College of Natural Resocures and Environment, Northwest A&F Univeristy, Yangling 712100, China

3 Department of Environmental Sciences, COMSATS University Islamabad, Vehari-campus, Vehari 61100, Pakistan

4 Institute of Horticultural Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

5 Department of Agronomy, University of Agriculture Faisalabad, Faisalabad, Pakistan

6 Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Faisalabad, Pakistan

7 Directorate of Soil Fertility and Soil Testing Institute Punjab, Lahore, Pakistan

8 Department of Bioinformatics and Biotechnology, Government College University, Faisalabad 38000, Pakistan

9 College of Life Sciences, Northwest A&F Univeristy, Yangling 712100, China

10 Department of Environmental Sciences and Engineering, Government College University, Faisalabad 38000, Pakistan

1 Introduction

Approximately 27% of national and international vegetable are being irrigated with wastewater, which includes sewage and industrial effluents (Ensink et al. 2004). This effluent has become an attractive option of irrigation for the farmers because it contains essential nutrients (N, P, Zn, Cu, Fe and Ni). Unfortunately, it also contains noxious heavy metals (Zn, Ni, Cd, Pb, Cr, As) that may have serious health concerns when permissible level of consumption is exceeded (Hu et al. 2013; Chen et al. 2014). Among the heavy metals, cadmium (Cd) has been ranked as the seventh toxic element by the Agency for Toxic Substances and Disease Registry. Soil Cd contamination in Pakistan is varied from 0.02–184 mg kg^{-1} soil reported by Waseem et al. (2014) in a classical review on pollution status of Pakistan. However, other studies reported low level of Cd 3.47 and 6 mg kg^{-1} soil based on soil type and region (Khan et al. 2011, Rehman et al. 2017). Cadmium is released into environment through various natural (volcanic eruptions, weathering) and anthropogenic (manufacturing of plastics, paint pigments, mining, batteries that contain Cd, sewage sludge, phosphate fertilizers) sources (Ahmad et al. 2016). Cd is easily absorbed by plants, and when the plants are consumed by man, it results in health impairment. For instance, itai-itai disease outbreak occurred when Japanese consumed Cd-contaminated rice as food (Nogawa et al. 2017). Now, it is known that Cd causes various diseases such as osteoporosis, renal dysfunction, lung cancer, hypertension and anemia (Zeitoun and Mehana 2014). According to World Health Organization (WHO), permissible limit of Cd is 25 μg kg^{-1} of body weight for humans, while that is 0.02 and 1 mg kg^{-1} for plants and soil, respectively (FAO/WHO 2001).

Cadmium accumulation in edible parts of vegetables is the function of Cd applied in soil (Yang et al. 2009; Anwar et al. 2016). It disrupts plant physiological metabolisms such as respiration, transpiration, nitrogen assimilation and photosynthesis (Rizwan et al. 2017). Several studies have documented toxic effects of Cd on seed germination (Ahmad et al. 2012, 2013), plant growth (Zhou and Qiu 2005; Ahmad et al. 2014, 2016), chlorophyll content (Shakya et al. 2008), CO_2 fixation (Ji et al. 2017) and inhibition of photoactivation of photosystem II by competitive binding to the essential K^+ and Ca^{2+} sites (Faller et al. 2005). A lot of literature has indicated toxicity of Cd to plants which was said to be dependent upon plant species, exposure time, concentration of metal in growth medium and soil type (Tran and Popova 2013). Previously, we have observed cultivar- and growth media-dependent response of Cd on wheat at seed germination stage (Ahmad et al. 2012, 2013). However, growth, physiology and risks of Cd accumulation in leafy vegetables cultivated in soil having various concentrations of Cd are poorly understood (Rizwan et al. 2017).

Vegetables are rich source of fats, carbohydrates, proteins, antioxidants, minerals, fiber, water and vitamins such as E and K, thiamin (B1), β-carotene (provitamin A), pyridoxine (B6), riboflavin (B2), niacin, pantothenic, folic and ascorbic acids (Prodanov et al. 2004). Consumption of leafy vegetables is being increased day by day in rural and urban community (Sobukola et al. 2010). Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant consumed as spice in diet and medicinal herb (Erum et al. 2011). Spinach (*Spinacia oleracea* L.) is an annual leafy vegetable with short growth cycle and high nutritive value (Nishihara et al. 2001). Coriander (*Coriandrum sativum* L.) is an annual herbaceous medicinal plant used as a flavoring agent and ancient medicine (Sahib et al. 2013). Cultivation and consumption rate of these vegetables is being increased annually in Pakistan and cultivation rate of spinach, fenugreek and coriander is 8820, 175 and 5453 hectares, while their production is 108,725, 527 and 3263 tonnes respectively, according to the statistics of Pakistan 2017–2018.

These vegetables are cultivated in peri-urban areas and thereby are frequently irrigated with industrial or municipal wastewater. This practice increases the risk of heavy metal accumulation in vegetables, and the accumulation will not only affect their growth and physiology but also cause various diseases in humans (Tran and Popova 2013). People who consumed those vegetables cultivated in Cd-contaminated soil are at high risk of having Cd-mediated diseases (Yang et al. 2009; Mahmood and Malik 2014). Moreover, recent studies have reported high concentration of Cd contamination of soil and food crops in Pakistan (Khan et al. 2013; Mahmood and Malik 2014). Thus, it is imperative to assess the risks of Cd accumulation in different species of vegetables. Understanding accumulation of Cd in different vegetables species is an important step toward selection of tolerant vegetable species that can be used for phyto-management of Cd-contaminated soils. Therefore, the specific objectives of the present study were to determine (1) dose- and time-dependent effect of Cd on growth and physiology of three common vegetables (spinach, fenugreek, coriander) and (2) Cd accumulation in edible parts of selected vegetables and potential health risks.

2 Materials and methods

Experimental setup – Soil was air-dried, sieved and processed for determination of basic soil properties following the standard protocols described in ICARDA. The experimental soil had pH value of 7.8 ± 0.82; EC value of 1.57 ± 0.48 dS m^{-1}; saturation percentage value of

32.57 ± 2.67%; organic matter content of 0.71 ± 0.023%; total nitrogen content of 0.052 ± 0.002%; available phosphorus content of 9.43 ± 1.24 mg kg^{-1}; and extractable potassium content of 168 ± 6.8 mg kg^{-1}, and the textural class was sandy loam (sand 55%, silt 30% & clay 15%). About 10 kg sieved Cd-contaminated soil (0, 10, 20, 30 and 40 mg kg^{-1} soil) was put in earthen pots (45 cm × 30 cm) and incubated for two weeks. Thereafter, recommended doses of N-P_2O_5-K_2O for spinach (75–60–30 kg ha^{-1}), fenugreek (30–60–40 kg ha^{-1}) and coriander (40–60–30 kg ha^{-1}) were applied to the pots containing the respective vegetable varieties. Then, ten seeds of each vegetable variety were sown per pot and irrigated with distilled water and recommended standard agronomic and cultural practices were carried out on the plants. After germination, the plants were thinned to five uniform seedlings per pot. Data on seedling growth attributes, physiological and biochemical traits were taken at first, second and third harvest at 25, 50, and 75 days of exposure (DoE), respectively.

Growth and physiological characteristics. Three randomly selected plants per pot were tagged for height measurement with the help of a measuring tape, while leaf canopy area was measured using a portable leaf area meter (Model LI-3100A, LI-COR, Lincoln, NE, USA). Thereafter, the tagged plants from each pot were harvested for measurement of root length and fresh biomass (shoot + leaves); however, to get dry biomass, plant samples were oven-dried at 70 °C till constant weight. For physiological parameters, third leaf from top of every plant was selected to determine stomatal conductance (*gs*), photosynthetic rate (*A*) and transpiration rate (*E*) using infrared gas analyzer (IRGA, model LCA-4, Analytical Development Company, Hoddesdon, England). All these parameters were recorded between at 10:00 am and 1:00 pm noon. During data recording, leaf chamber molar gas flow rate was 248 µmol s^{-1}, ambient CO_2 conc. was 352 µmol mol^{-1}, temperature of leaf chamber varied was between 36.1 and 40.4 °C, ambient pressure was 98.01 kPa, molar flow of air/leaf area was 221.06 mol m^{-2} s^{-1}, maximum value of PAR was 1050 µmol m^{-2} s^{-1} and leaf chamber volume gas flow rate was 380 mL min^{-1}. Leaf relative water content was calculated following by Barrs and Weatherley 1962. Chlorophyll and carotenoids contents were determined following the protocol of Nagata and Yamashita (1992).

Cadmium analysis. The samples of each leafy vegetable were collected from each treatment and washed with distilled water followed by drying with tissue papers before oven-drying at 70 °C. The samples were the ground to powder in a mill (IKA WERKE, MF 10 Basic, Staufen, Germany). Then, known mass of each ground sample was wet digested in a diacid mixture of HNO_3, $HClO_4$ in ratio 2:1 (Jones Jr and Case 1990). Cd concentration in the digest was measured by an atomic absorption spectrophotometer (PerkinElmer Aanalyst 100, Waltham, USA). Thereafter, the values of the oral intake of metals (mg day^{-1}) from the soil through these vegetables were determined based on the following formula (Uriah and Shehu 2014).

Daily consumption/intake of metals (DIM) = daily vegetable consumption (kg day^{-1} fresh weight) × mean vegetable metal concentration (mg kg^{-1}).

The estimated potential health risk to humans through the ingestion of these vegetables was calculated using the relation described by Uriah and Shehu (2014):

$$HRI = \frac{DIM \times C_{metal}}{RD \times B}$$

where DIM is the daily intake of metal through the vegetables (kg day^{-1}), C_{metal} is the concentration of metal in the vegetable (mg kg^{-1}), RD is the oral reference dose for the metal (mg kg^{-1} of body weight day^{-1}) and *B* is the human body weight (kg). RD for Cd was used as 0.001 mg kg^{-1} day (Zeng et al. 2015).

Statistical analysis of data – Each leafy vegetable was exposed to five levels of Cd and three exposure times (5 × 3 = 15) in CRD factorial arrangement and replicated three times. The collected data showed homogeneity of variance ($P > 0.05$) and normal distribution ($P > 0.05$) according to Levene and Shapiro–Wilk test, respectively. To determine the effects of Cd levels and DoE on growth, physiology, Cd accumulation and its risk assessment of three leafy vegetables, two-way analysis of variance was performed for each vegetable using *Statistics* v8.01. Least significant difference (LSD) test at 5% probability level was applied to determine significance between treatment means.

3 Results

Effect of Cd on growth and biomass of leafy vegetables – All vegetables tested produced taller plants, longer roots, higher leaf area and heavier biomass with time in normal soil with maximum production at 75 DoE (Table 1). These growth indices drastically reduced upon exposure to Cd. Each increment in Cd dose had significant reduction in plant height, root length and leaf area of all vegetables tested regardless of the duration of exposure (DoE). At each DoE, plant height of spinach increased, while leaf area of fenugreek and coriander was significantly reduced up to 30 mg Cd kg^{-1} soil and then dramatically increased at 40 mg Cd kg^{-1} soil. In contrast, plant height of fenugreek and coriander progressively decreased with progressive increase in Cd exposure compared to their respective controls (Cd_0).

Table 1 Effects of different levels of Cd and exposure time on growth of three leafy vegetables

Days of exposure	Cadmium (mg kg^{-1} soil)	Plant height (cm)			Root length (cm)			Leaf area (cm^2)		
		Spinach	Fenugreek	Coriander	Spinach	Fenugreek	Coriander	Spinach	Fenugreek	Coriander
25 D	Cd_0	18.96 ± 0.27^{j}	26.13 ± 0.38^{k}	13.83 ± 0.20^{k}	11.68 ± 0.22^{i}	13.74 ± 0.26^{h}	10.13 ± 0.19^{h}	329.2 ± 5.78^{i}	2.94 ± 0.05^{j}	3.25 ± 0.06^{h}
	Cd_{10}	12.81 ± 0.18^{k}	22.03 ± 0.31^{l}	8.71 ± 0.13^{l}	8.06 ± 0.15^{l}	10.64 ± 0.20^{j}	7.75 ± 0.14^{k}	267.5 ± 4.70^{j}	2.22 ± 0.04^{k}	2.53 ± 0.04^{j}
	Cd_{20}	10.76 ± 0.16^{l}	7.99 ± 0.11^{m}	7.69 ± 0.11^{m}	6.20 ± 0.12^{m}	9.20 ± 0.17^{k}	6.51 ± 0.13^{l}	216.0 ± 3.79^{k}	1.71 ± 0.03^{l}	1.81 ± 0.03^{k}
	Cd_{30}	8.7 ± 0.13^{n}	5.74 ± 0.08^{n}	6.46 ± 0.09^{n}	5.27 ± 0.10^{n}	8.47 ± 0.16^{l}	4.65 ± 0.09^{m}	174.9 ± 3.07^{l}	1.30 ± 0.02^{n}	1.30 ± 0.02^{l}
	Cd_{40}	9.73 ± 0.14^{m}	5.02 ± 0.07^{o}	5.33 ± 0.08^{o}	5.06 ± 0.10^{n}	7.23 ± 0.13^{m}	4.55 ± 0.09^{m}	138.9 ± 2.44^{m}	1.40 ± 0.02^{m}	1.40 ± 0.02^{l}
50 D	Cd_0	30.13 ± 0.31^{c}	37.28 ± 0.39^{d}	25.02 ± 0.26^{d}	17.63 ± 0.34^{d}	19.70 ± 0.32^{d}	14.52 ± 0.28^{e}	837.4 ± 13.6^{d}	4.68 ± 0.08^{f}	4.99 ± 0.08^{e}
	Cd_{10}	24.00 ± 0.26^{f}	33.19 ± 0.35^{g}	19.92 ± 0.21^{g}	14.00 ± 0.27^{g}	16.59 ± 0.29^{f}	12.65 ± 0.25^{f}	768.4 ± 12.5^{e}	3.96 ± 0.06^{g}	4.26 ± 0.07^{f}
	Cd_{20}	21.96 ± 0.23^{g}	30.13 ± 0.32^{h}	17.87 ± 0.19^{h}	12.13 ± 0.23^{h}	15.14 ± 0.26^{g}	11.41 ± 0.22^{g}	669.5 ± 10.9^{g}	3.44 ± 0.06^{h}	3.54 ± 0.06^{g}
	Cd_{30}	19.92 ± 0.21^{i}	28.09 ± 0.29^{i}	15.83 ± 0.17^{i}	10.68 ± 0.20^{j}	13.69 ± 0.21^{h}	9.748 ± 0.19^{h}	721.0 ± 11.7^{f}	3.03 ± 0.05^{j}	3.03 ± 0.05^{i}
	Cd_{40}	20.94 ± 0.22^{h}	27.07 ± 0.28^{j}	14.81 ± 0.16^{j}	9.33 ± 0.18^{k}	11.41 ± 0.27^{i}	8.296 ± 0.16^{i}	628.3 ± 10.4^{h}	3.13 ± 0.05^{i}	3.13 ± 0.05^{i}
75 D	Cd_0	38.56 ± 0.72^{a}	45.40 ± 0.60^{a}	33.43 ± 0.62^{a}	23.8 ± 0.42^{a}	25.88 ± 0.46^{a}	20.71 ± 0.37^{a}	1150.7 ± 16.6^{a}	6.76 ± 0.09^{a}	7.07 ± 0.10^{a}
	Cd_{10}	32.40 ± 0.60^{b}	41.29 ± 0.53^{b}	28.29 ± 0.53^{b}	20.19 ± 0.36^{b}	22.78 ± 0.40^{b}	18.84 ± 0.33^{b}	1047.2 ± 15^{b}	6.05 ± 0.08^{b}	6.35 ± 0.09^{b}
	Cd_{20}	30.35 ± 0.57^{c}	38.20 ± 0.53^{c}	26.23 ± 0.49^{c}	18.33 ± 0.33^{c}	21.33 ± 0.38^{c}	17.60 ± 0.31^{c}	937.6 ± 13.6^{c}	5.53 ± 0.08^{c}	5.64 ± 0.08^{c}
	Cd_{30}	28.29 ± 0.53^{e}	36.15 ± 0.47^{e}	24.17 ± 0.45^{e}	16.88 ± 0.21^{e}	19.88 ± 0.35^{d}	15.94 ± 0.28^{d}	821.8 ± 11.8^{d}	5.12 ± 0.07^{e}	5.12 ± 0.07^{d}
	Cd_{40}	29.32 ± 0.55^{d}	35.12 ± 0.41^{f}	23.15 ± 0.43^{f}	15.53 ± 0.27^{f}	17.60 ± 0.31^{e}	14.50 ± 0.26^{e}	732.6 ± 10.5^{e}	5.23 ± 0.07^{d}	5.23 ± 0.07^{d}

Data are presented as means of three replicates ± *SE*. The values having different letters within a column are statistically significant at $P \leq 0.05$

Root length of all vegetables significantly decreased at each increment in Cd dose. Similarly, fresh and dry biomass of all the vegetables decreased significantly upon exposure to Cd with maximum reduction observed at 25 DoE (Table 2). Fresh biomass of spinach was decreased up to 52% at 40 mg Cd kg^{-1} soil after 50 DoE while at same contamination level and 25 DoE led to 46% reduction as compared to their respective controls (Cd_0). In all vegetables tested, the vegetables were very sensitive to Cd toxicity at first 25 DoE. However, with increasing DoE, vegetables were better adapted to Cd stress conditions (Tables 1, 2). Results revealed that 40 mg Cd kg^{-1} soil reduced plant height of fenugreek up to 81%, root length of spinach up to 57%, leaf area up to 60% and biomass up to 78% of coriander. Therefore, these parameters were most sensitive to determine Cd toxicity with respect to tested vegetables after 25 DoE.

Effect of Cd on physiology of leafy vegetables – Variable responses of the tested vegetables to Cd were observed on relative water contents (RWC) of the tested vegetables. All vegetables showed maximum RWC after 50 DoE followed by 25 and 75 DoE (Table 2). Interestingly, spinach showed increased RWC (2–3% depending upon DoE) at 10, while no effect was observed at 20 and 30 mg Cd kg^{-1} soil. After that, there was sudden decrease in RWC (2–3% depending upon DoE) at 40 mg Cd kg^{-1} soil. In contrast, fenugreek and coriander showed reduction in RWC (up to 4% depending upon DoE) at 30 and 40 mg Cd kg^{-1} soil though except fenugreek showed 6% increase in RWC at 40 mg Cd kg^{-1} soil after 75 DoE.

Overall, the vegetables under study produced abundant Chl *a*, Chl *b* and carotenoids with time irrespective of whether they were exposed to Cd or not. Their maximum production was found at 75 DoE but carotenoids in coriander dramatically decreased at 75 DoE (Table 3). These indices reduced drastically upon exposure to Cd. With the increasing concentrations of Cd, Chl *a*, Chl *b* and carotenoids decreased significantly at each exposure time as compared to their respective controls. At each DoE, Chl *a* of all vegetables reduced gradually till toxicity level reached to 40 mg Cd kg^{-1} soil, while Chl *b* and carotenoids showed entirely different trends. The maximum reduction in Chl *b* was found in spinach at 20 mg Cd kg^{-1} soil, while in fenugreek and coriander the same result was found at 30 mg Cd kg^{-1} soil. Similarly, the maximum reduction of carotenoids was found in all vegetables at 20 mg Cd kg^{-1} soil at 25, 50 and 75 DoE except for coriander which showed similar result at 30 mg Cd kg^{-1} soil when the duration of exposure was 25 DoE. Interestingly, low dose of Cd induced slight increase in carotenoids of spinach and fenugreek at 10 mg Cd kg^{-1} soil as compared to the control (Cd_0) (Table 3). Based on our findings, Chl *a*, Chl *b* and carotenoids of spinach, fenugreek and coriander were the most sensitive parameters to determine Cd toxicity in these vegetables regardless of DoE.

There was a linear relationship between photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (*Gs*) and time irrespective of Cd treatment (Table 4). Maximum rate of *A* was observed after 75 DoE, whereas those of *E* and *Gs* were observed after 50 DoE in normal (Cd_0) and Cd-contaminated soils (Cd_{10}). Interestingly, low dose of Cd up to 10 mg kg^{-1} soil had no effect on A and E; however, increasing rate of Cd from 20 to 40 mg kg^{-1} soil led to significant reduction in A and E irrespective of DoE. After 25 DoE, transpiration rate of spinach increased up to 1% at 10 mg Cd kg^{-1} soil compared to the control (Cd_0). At each DoE, stomatal conductance of all the vegetables decreased significantly at 30 and 40 mg Cd kg^{-1} soil compared to their respective controls (Cd_0).

Effect of Cd on shoot Cd accumulation, health risk assessment and tolerance – Shoot Cd concentration of all the vegetables was a function of soil available Cd and DoE (Fig. 1). Each increment in soil Cd concentration led to significant increase in shoot Cd concentration in all the vegetables with maximum concentration observed at 40 mg Cd kg^{-1} soil except spinach which showed similar shoot Cd concentration at 30 and 40 mg Cd kg^{-1} soil. Similarly, increase in DoE also led to significant increase in shoot Cd concentration in all leafy vegetables with maximum concentration after 75 DoE.

Bioaccumulation factor (BAF) is the ratio of Cd accumulated in shoot to Cd applied in soil. Accumulation of Cd in edible parts of the three vegetables increased with DoE but decreased with increase in Cd dose (Fig. 2). In spinach, BAF increased up to 30 after 25 DoE and then decreased at 40 mg Cd kg^{-1} soil. However, a sharp decrease was observed in the same vegetable after 50 and 75 DoE. In fenugreek, BAF increased up to 20 after 25 DoE after which it gradually decreased at 30 and 40 mg Cd kg^{-1} soil. On the other hand, a sharp decrease was observed after 50 DoE. In addition to that, BAF decreased sharply up to 20 and then increased up to 30 after which it decreased at 40 mg Cd kg^{-1} soil after 75 DoE. In coriander, BAF increased up to 20 and then decreased sharply at successive increase in Cd concentrations after 25 and 50 DoE. However, the same parameter decreased sharply up to 30 and then increased at 40 mg Cd kg^{-1} soil after 75 DoE.

Tolerance index is the ratio of dry biomass of any leafy vegetables in Cd-contaminated soil to normal soil. Tolerance indices of all the tested vegetables decreased with increase in DoE and Cd dose (Fig. 2). The maximum tolerance indices of coriander (95%), fenugreek (94%) and spinach (88%) were observed at 10 mg Cd kg^{-1} soil after 50 DoE, while minimum tolerance indices of spinach (23%), fenugreek (61%) and coriander (71%) were observed at

Table 2 Effects of different levels of Cd and exposure time on biomass and relative water contents (RWC) of three leafy vegetables

Days of exposure	Cadmium (mg kg^{-1} soil)	Fresh biomass (g)			Dry biomass (g)			RWC (%)		
		Spinach	Fenugreek	Coriander	Spinach	Fenugreek	Coriander	Spinach	Fenugreek	Coriander
25 D	Cd_0	15.31 ± 0.16^{j}	7.96 ± 0.08^{h}	7.14 ± 0.07^{e}	2.14 ± 0.02^{j}	1.48 ± 0.02^{k}	1.39 ± 0.02^{i}	70.42 ± 0.51^{e}	58.36 ± 0.42^{c}	54.86 ± 0.40^{c}
	Cd_{10}	12.24 ± 0.12^{k}	7.24 ± 0.07^{j}	4.90 ± 0.05^{j}	1.72 ± 0.02^{k}	1.30 ± 0.01^{l}	0.93 ± 0.01^{j}	72.34 ± 0.52^{d}	58.35 ± 0.42^{c}	54.85 ± 0.40^{c}
	Cd_{20}	11.22 ± 0.11^{l}	5.92 ± 0.06^{m}	3.37 ± 0.03^{m}	1.57 ± 0.02^{l}	0.97 ± 0.01^{m}	0.64 ± 0.02^{k}	70.68 ± 0.51^{e}	58.37 ± 0.42^{c}	54.87 ± 0.39^{c}
	Cd_{30}	9.18 ± 0.09^{m}	5.31 ± 0.05^{n}	1.84 ± 0.02^{n}	1.29 ± 0.02^{m}	0.82 ± 0.01^{n}	0.35 ± 0.01^{l}	70.32 ± 0.49^{e}	56.02 ± 0.40^{d}	52.51 ± 0.39^{d}
	Cd_{40}	8.16 ± 0.08^{n}	4.59 ± 0.04^{o}	1.63 ± 0.02^{o}	1.14 ± 0.01^{n}	0.64 ± 0.008^{o}	0.31 ± 0.003^{l}	68.66 ± 0.49^{f}	56.01 ± 0.40^{d}	52.50 ± 0.38^{d}
50 D	Cd_0	23.29 ± 0.26^{c}	9.81 ± 0.11^{d}	8.99 ± 0.10^{c}	3.55 ± 1.98^{de}	2.88 ± 1.60^{f}	2.76 ± 1.53^{d}	80.82 ± 0.37^{b}	62.45 ± 0.29^{a}	61.25 ± 0.28^{a}
	Cd_{10}	19.20 ± 0.21^{f}	9.09 ± 0.10^{f}	6.74 ± 0.07^{f}	3.12 ± 1.76^{f}	2.70 ± 1.50^{g}	2.32 ± 1.29^{f}	82.73 ± 0.38^{a}	62.44 ± 0.29^{a}	61.24 ± 0.28^{a}
	Cd_{20}	16.85 ± 0.19^{h}	7.76 ± 0.09^{i}	5.21 ± 0.06^{i}	2.98 ± 1.65^{g}	2.36 ± 1.31^{h}	2.03 ± 1.12^{g}	81.07 ± 0.37^{b}	62.46 ± 0.29^{a}	61.26 ± 0.28^{a}
	Cd_{30}	15.93 ± 0.18^{i}	7.15 ± 0.07^{k}	3.68 ± 0.04^{k}	2.69 ± 1.49^{h}	2.21 ± 1.23^{i}	1.73 ± 0.96^{h}	80.71 ± 0.37^{b}	60.11 ± 0.28^{b}	58.91 ± 0.27^{b}
	Cd_{40}	15.32 ± 0.17^{j}	6.43 ± 0.07^{l}	3.47 ± 0.03^{l}	2.54 ± 1.41^{i}	2.03 ± 1.26^{j}	1.69 ± 0.94^{h}	79.05 ± 0.36^{c}	60.10 ± 0.28^{b}	58.90 ± 0.27^{b}
75 D	Cd_0	33.61 ± 0.31^{a}	12.18 ± 0.09^{a}	11.41 ± 0.11^{a}	4.56 ± 0.05^{a}	3.87 ± 0.04^{a}	3.75 ± 0.04^{a}	65.39 ± 0.22^{h}	55.31 ± 0.19^{e}	52.51 ± 0.18^{d}
	Cd_{10}	26.48 ± 0.25^{b}	11.47 ± 0.09^{b}	9.17 ± 0.08^{b}	4.12 ± 0.04^{b}	3.69 ± 0.03^{b}	3.30 ± 0.0^{b}	67.31 ± 0.23^{g}	55.30 ± 0.19^{e}	52.50 ± 0.18^{d}
	Cd_{20}	22.41 ± 0.21^{d}	$10.15 \pm 0.08c$	7.64 ± 0.07^{d}	3.97 ± 0.04^{c}	3.34 ± 0.03^{c}	3.00 ± 0.03^{c}	65.65 ± 0.23^{h}	55.32 ± 0.19^{e}	52.52 ± 0.18^{d}
	Cd_{30}	20.37 ± 0.19^{e}	9.54 ± 0.07^{e}	6.11 ± 0.06^{g}	3.67 ± 0.04^{d}	3.19 ± 0.03^{d}	2.70 ± 0.03^{de}	65.29 ± 0.23^{h}	52.97 ± 0.19^{f}	50.17 ± 0.17^{e}
	Cd_{40}	18.33 ± 0.17^{g}	8.82 ± 0.06^{g}	5.91 ± 0.05^{h}	3.52 ± 0.04^{e}	3.00 ± 0.03^{e}	2.66 ± 0.02^{e}	63.63 ± 0.22^{i}	58.90 ± 0.19^{b}	50.16 ± 0.17^{e}

Data are presented as means of three replicates ± *SE*. The values having different letters within a column are statistically significant at $P \leq 0.05$

Table 3 Effects of different levels of Cd and exposure time on chlorophyll contents of three leafy vegetables

Days of exposure	Cadmium (mg kg^{-1} soil)	Chlorophyll a (mg/g FW)			Chlorophyll *b* (mg/g FW)			Carotenoids (mg/g FW)		
		Spinach	Fenugreek	Coriander	Spinach	Fenugreek	Coriander	Spinach	Fenugreek	Coriander
25 D	Cd_0	4.02 ± 0.023^{e}	3.35 ± 0.007^{e}	1.86 ± 0.001^{g}	1.38 ± 0.005^{c}	1.53 ± 0.010^{c}	1.45 ± 0.003^{d}	1.43 ± 0.003^{c}	1.38 ± 0.003^{c}	1.49 ± 0.003^{a}
	Cd_{10}	2.74 ± 0.023^{k}	2.62 ± 0.006^{h}	1.25 ± 0.001^{j}	1.15 ± 0.005^{f}	1.28 ± 0.011^{e}	1.16 ± 0.003^{g}	1.48 ± 0.002^{b}	1.44 ± 0.002^{b}	1.38 ± 0.003^{d}
	Cd_{20}	2.56 ± 0.019^{l}	2.44 ± 0.006^{i}	1.16 ± 0.001^{k}	0.77 ± 0.005^{k}	0.94 ± 0.009^{i}	0.90 ± 0.003^{k}	1.05 ± 0.003^{k}	1.15 ± 0.003^{i}	1.30 ± 0.003^{f}
	Cd_{30}	2.38 ± 0.018^{m}	2.48 ± 0.005^{i}	1.07 ± 0.001^{l}	0.92 ± 0.004^{i}	0.80 ± 0.008^{k}	0.83 ± 0.003^{l}	1.27 ± 0.003^{f}	1.28 ± 0.003^{f}	1.08 ± 0.003^{k}
	Cd_{40}	2.19 ± 0.012^{n}	2.32 ± 0.003^{j}	1.01 ± 0.001^{m}	0.84 ± 0.003^{j}	0.89 ± 0.008^{j}	0.92 ± 0.002^{jk}	1.28 ± 0.002^{f}	1.12 ± 0.002^{j}	1.15 ± 0.003^{i}
50 D	Cd_0	5.21 ± 0.025^{b}	4.34 ± 0.002^{b}	2.81 ± 0.003^{c}	1.55 ± 0.006^{b}	1.73 ± 0.011^{b}	1.64 ± 0.0^{03b}	1.47 ± 0.004^{b}	1.43 ± 0.004^{b}	1.42 ± 0.003^{c}
	Cd_{10}	3.55 ± 0.025^{g}	3.39 ± 0.00^{e}	2.02 ± 0.003^{f}	1.29 ± 0.006^{d}	1.44 ± 0.10^{d}	1.31 ± 0.004^{e}	1.36 ± 0.003^{e}	1.32 ± 0.003^{e}	1.32 ± 0.004^{f}
	Cd_{20}	3.32 ± 0.020^{h}	3.16 ± 0.002^{f}	1.90 ± 0.003^{g}	0.87 ± 0.005^{j}	1.06 ± 0.009^{g}	1.02 ± 0.003^{i}	1.12 ± 0.003^{j}	1.10 ± 0.003^{k}	1.10 ± 0.003^{k}
	Cd_{30}	3.08 ± 0.019^{i}	3.12 ± 0.002^{f}	1.78 ± 0.003^{h}	1.04 ± 0.005^{g}	0.91 ± 0.009^{j}	0.9 ± 0.003^{j}	1.24 ± 0.003^{g}	1.20 ± 0.003^{h}	1.20 ± 0.003^{h}
	Cd_{40}	2.84 ± 0.013^{j}	3.00 ± 0.001^{g}	1.78 ± 0.0032^{i}	0.95 ± 0.004^{h}	1.00 ± 0.007^{h}	1.04 ± 0.003^{i}	1.19 ± 0.003^{h}	1.17 ± 0.003^{i}	1.16 ± 0.003^{i}
75 D	Cd_0	6.74 ± 0.007^{a}	5.62 ± 0.002^{a}	4.01 ± 0.001^{a}	1.85 ± 0.005^{a}	2.05 ± 0.010^{a}	1.95 ± 0.003^{a}	1.51 ± 0.013^{a}	1.47 ± 0.013^{a}	1.46 ± 0.014^{b}
	Cd_{10}	4.59 ± 0.006^{c}	4.39 ± 0.002^{b}	2.99 ± 0.001^{b}	1.54 ± 0.004^{b}	1.71 ± 0.10^{b}	1.56 ± 0.003^{c}	1.39 ± 0.011^{d}	1.36 ± 0.014^{d}	1.35 ± 0.013^{e}
	Cd_{20}	4.29 ± 0.006^{d}	4.08 ± 0.002^{c}	2.84 ± 0.001^{c}	1.04 ± 0.004^{g}	1.26 ± 0.10^{e}	1.21 ± 0.003^{f}	1.16 ± 0.009^{i}	1.13 ± 0.011^{j}	1.12 ± 0.011^{j}
	Cd_{30}	3.98 ± 0.005^{e}	4.03 ± 0.002^{c}	2.69 ± 0.001^{d}	1.23 ± 0.004^{e}	1.08 ± 0.009^{g}	1.12 ± 0.002^{h}	1.27 ± 0.009^{f}	1.24 ± 0.012^{g}	1.23 ± 0.010^{g}
	Cd_{40}	3.68 ± 0.005^{f}	3.88 ± 0.001^{d}	2.59 ± 0.001^{e}	1.13 ± 0.003^{f}	1.19 ± 0.009^{f}	1.23 ± 0.001^{f}	1.23 ± 0.011^{g}	1.20 ± 0.011^{h}	1.19 ± 0.012^{h}

Data are presented as means of three replicates ± *SE*. The values having different letters within a column are statistically significant at $P \leq 0.05$

Table 4 Dose- and time-dependent effects of Cd on physiology of three leafy vegetables

Days of exposure	Cadmium (mg kg^{-1} soil)	Photosynthetic rate (*A*)			Transpiration rate (*E*)			Stomatal conductance (*Gs*)		
		Spinach	Fenugreek	Coriander	Spinach	Fenugreek	Coriander	Spinach	Fenugreek	Coriander
25 D	Cd_0	11.51 ± 0.034^{d}	10.68 ± 0.065^{c}	7.54 ± 0.028^{d}	6.01 ± 0.0086^{d}	5.93 ± 0.023^{f}	4.80 ± 0.018^{c}	0.95 ± 0.005^{b}	0.80 ± 0.005^{b}	0.59 ± 0.003^{b}
	Cd_{10}	11.51 ± 0.034^{d}	10.68 ± 0.065^{c}	7.54 ± 0.028^{d}	6.05 ± 0.0086^{c}	5.94 ± 0.023^{f}	4.80 ± 0.018^{c}	0.93 ± 0.005^{bc}	0.79 ± 0.004^{b}	0.58 ± 0.003^{b}
	Cd_{20}	10.6 ± 0.030^{e}	9.79 ± 0.060^{f}	7.09 ± 0.026^{e}	5.01 ± 0.0074^{f}	5.79 ± 0.023^{h}	4.38 ± 0.016^{f}	0.93 ± 0.005^{bc}	0.79 ± 0.004^{b}	0.58 ± 0.003^{b}
	Cd_{30}	8.04 ± 0.018^{h}	4.26 ± 0.024^{k}	3.60 ± 0.014^{j}	4.90 ± 0.0070^{g}	5.50 ± 0.020^{i}	4.02 ± 0.013^{h}	0.79 ± 0.004^{e}	0.57 ± 0.003^{e}	0.31 ± 0.003^{f}
	Cd_{40}	7.76 ± 0.018^{i}	4.06 ± 0.026^{l}	3.49 ± 0.013^{k}	4.02 ± 0.0060^{j}	3.43 ± 0.015^{l}	3.41 ± 0.015^{k}	0.58 ± 0.004^{g}	0.48 ± 0.003^{f}	0.28 ± 0.003^{g}
50 D	Cd_0	12.01 ± 0.034^{b}	11.11 ± 0.065^{b}	7.98 ± 0.046^{b}	6.41 ± 0.0092^{a}	6.66 ± 0.0081^{a}	5.16 ± 0.018^{a}	1.07 ± 0.005^{a}	0.87 ± 0.004^{a}	0.67 ± 0.004^{a}
	Cd_{10}	12.01 ± 0.034^{b}	11.11 ± 0.065^{b}	7.98 ± 0.046^{b}	6.39 ± 0.0092^{a}	6.66 ± 0.0081^{a}	5.16 ± 0.018^{a}	1.05 ± 0.005^{a}	0.87 ± 0.004^{a}	0.66 ± 0.004^{a}
	Cd_{20}	10.54 ± 0.030^{f}	10.23 ± 0.060^{e}	7.54 ± 0.043^{d}	5.65 ± 0.0083^{e}	6.52 ± 0.0078^{b}	4.74 ± 0.016^{d}	1.05 ± 0.005^{a}	0.86 ± 0.004^{a}	0.66 ± 0.003^{a}
	Cd_{30}	8.10 ± 0.016^{g}	4.72 ± 0.024^{i}	4.07 ± 0.022^{h}	4.12 ± 0.0081^{i}	6.23 ± 0.0075^{d}	4.4 ± 0.015^{f}	0.91 ± 0.005^{c}	0.64 ± 0.003^{d}	0.40 ± 0.004^{d}
	Cd_{40}	7.74 ± 0.017^{i}	4.52 ± 0.024^{j}	3.96 ± 0.021^{i}	3.17 ± 0.0058^{l}	4.17 ± 0.0060^{j}	3.78 ± 0013^{i}	0.70 ± 0.003^{f}	0.55 ± 0.002^{e}	0.37 ± 0.004^{e}
75 D	Cd_0	12.3 ± 0.0021^{a}	11.43 ± 0.0039^{a}	8.28 ± 0.028^{a}	6.26 ± 0.0096^{b}	6.27 ± 0.0271^{c}	5.01 ± 0.0217^{b}	0.85 ± 0.023^{d}	0.73 ± 0.020^{c}	0.50 ± 0.014^{c}
	Cd_{10}	12.2 ± 0.0021^{a}	11.43 ± 0.0039^{a}	8.28 ± 0.028^{a}	6.27 ± 0.0096^{b}	6.27 ± 0.02710^{c}	5.01 ± 0.0127^{b}	0.83 ± 0.022^{d}	0.73 ± 0.019^{c}	0.50 ± 0.013^{c}
	Cd_{20}	11.86 ± 0.020^{c}	10.54 ± 0.0036^{d}	7.84 ± 0.026^{c}	4.91 ± 0.0087^{g}	6.13 ± 0.00265^{e}	4.59 ± 0.0198^{e}	0.83 ± 0.022^{d}	0.72 ± 0.019^{c}	0.40 ± 0.014^{c}
	Cd_{30}	8.03 ± 0.012^{h}	5.01 ± 0.0017^{g}	4.35 ± 0.014^{f}	4.60 ± 0.0086^{h}	5.84 ± 0.00252^{g}	4.22 ± 0.0182^{g}	0.68 ± 0.021^{f}	0.50 ± 0.017^{f}	0.22 ± 0.006^{h}
	Cd_{40}	7.52 ± 0.011^{j}	4.81 ± 0.0016^{h}	4.24 ± 0.014^{g}	3.40 ± 0.0064^{k}	3.77 ± 0.00163^{k}	3.62 ± 0.0156^{j}	0.47 ± 0.019^{h}	0.40 ± 0.013^{g}	0.19 ± 0.006^{i}

Data are presented as means of three replicates ± *SE*. The values having different letters within a column are statistically significant at $P \leq 0.05$

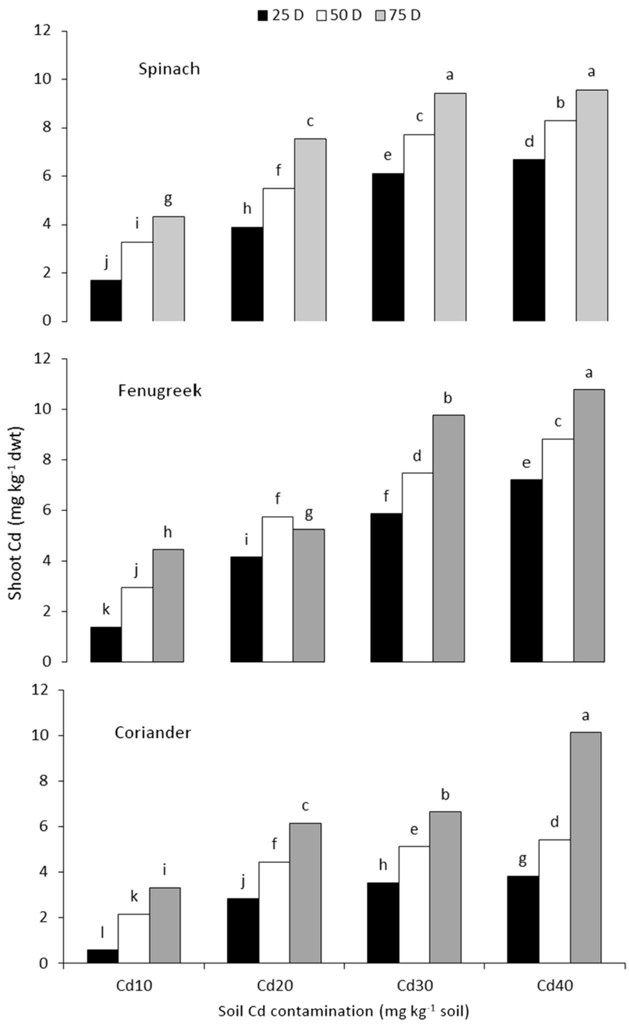

Fig. 1 Dose- and time-dependent effects of Cd on shoot Cd concentration and its bioaccumulation in three leafy vegetables. Data presented as means of three replicates with standard error, bars sharing same letter(s) are statistically nonsignificant according to HSD Tukey test at $P \le 0.05$

40 mg Cd kg^{-1} soil after 75 DoE. In general, the three vegetables followed this order of increasing Cd tolerance: coriander > fenugreek > spinach.

Daily intake of metal (DIM) via consumption of vegetables is shown in Table 5. Our results revealed a significant ($P < 0.05$) increase in DIM with increase in the level of Cd applied to soil irrespective of the vegetable type. The DIM values further increased with increase in DoE because the highest DIM value for each vegetable was found at 75 DoE and least at 25 DoE. At any given DoE and Cd level, the DIM was higher in spinach than fenugreek and coriander. Consequently, health risk index (HRI) was higher in spinach than the rest tested vegetables. The HRI also increased with increase in DoE and the levels of Cd applied to soil. However, values of HRI were < 1 for fenugreek and coriander irrespective of Cd levels and DoE. As for spinach, the HRI values were < 1 for Cd levels up to 30 mg Cd kg^{-1} soil for all the DoE. However, at the Cd level of 40 mg Cd kg^{-1} soil, the values were > 1 both at 50 and 75 DoE indicating potential health risks for this Cd level and DoE for spinach.

4 Discussion

Effect of Cd on growth and biomass of leafy vegetables – The exogenous application of different doses of Cd caused gradual reduction in plant height, root length, leaf area and biomass of three exposed vegetables (Tables 1 and 2). These findings are in line with some of the earlier studies, which concluded that plant biomass (fresh and dry weight) and growth (plant height, root length and leaf area) parameters are very sensitive to heavy metals stress in plants (Ahmad et al. 2012). All leafy vegetables suffering from Cd toxicity had relatively smaller roots and narrow yellowish leaves covered with small necrotic spots. These observations were very similar to the results of Naik et al. (2013). Growth of lateral roots of plants is usually inhibited by the presence of Cd in growth media due to abnormal growth of epidermal and cortical cells and disorderliness in cell division (Tran and Popova 2013). Biomass of all vegetables was significantly decreased with increase in dose and time of exposure to Cd (Table 2). Similar response was observed by Huang et al. (2017) who found dose-dependent reduction in biomass and growth attributes of spinach. Cd at lower concentrations retards shoot growth without toxic effects in leaves, and moderately higher concentrations of Cd severely impede root growth and led to more Cd accumulation in leaves (Baruah et al. 2017). Our results confirmed the findings of previous studies that Cd accumulation in plants is directly proportional to its toxicity in various crops such as wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.), common oak (*Quercus robur* L.), box elder (*Acer negundo* L.), common bean (*Phaseolus vulgaris* L.) (Ahmad et al. 2012, 2016; Nogawa et al. 2017). The toxic effects of Cd on studied crops may be due to fluctuations in peroxidase activity and decrease in chlorophyll contents. Furthermore, any change in the growth rate which results from increasing Cd supply must retard rate of net photosynthesis which will lead to reduction in supply of photosynthates with consequential reduction in plant growth. This might be ascribed to its effects on cell expansion or cell division or may be through its effect on RNA/DNA synthesis. Our results also supported the theory of chlorophyll reduction and of photosynthesis inhibition in the presence of Cd (Han et al. 2006). This was showcased in all the tested vegetables through showing significant reduction in Chl a, Chl b and carotenoids (Table 3). This observation might be the possible reason for decreased vegetable growth and biomass production.

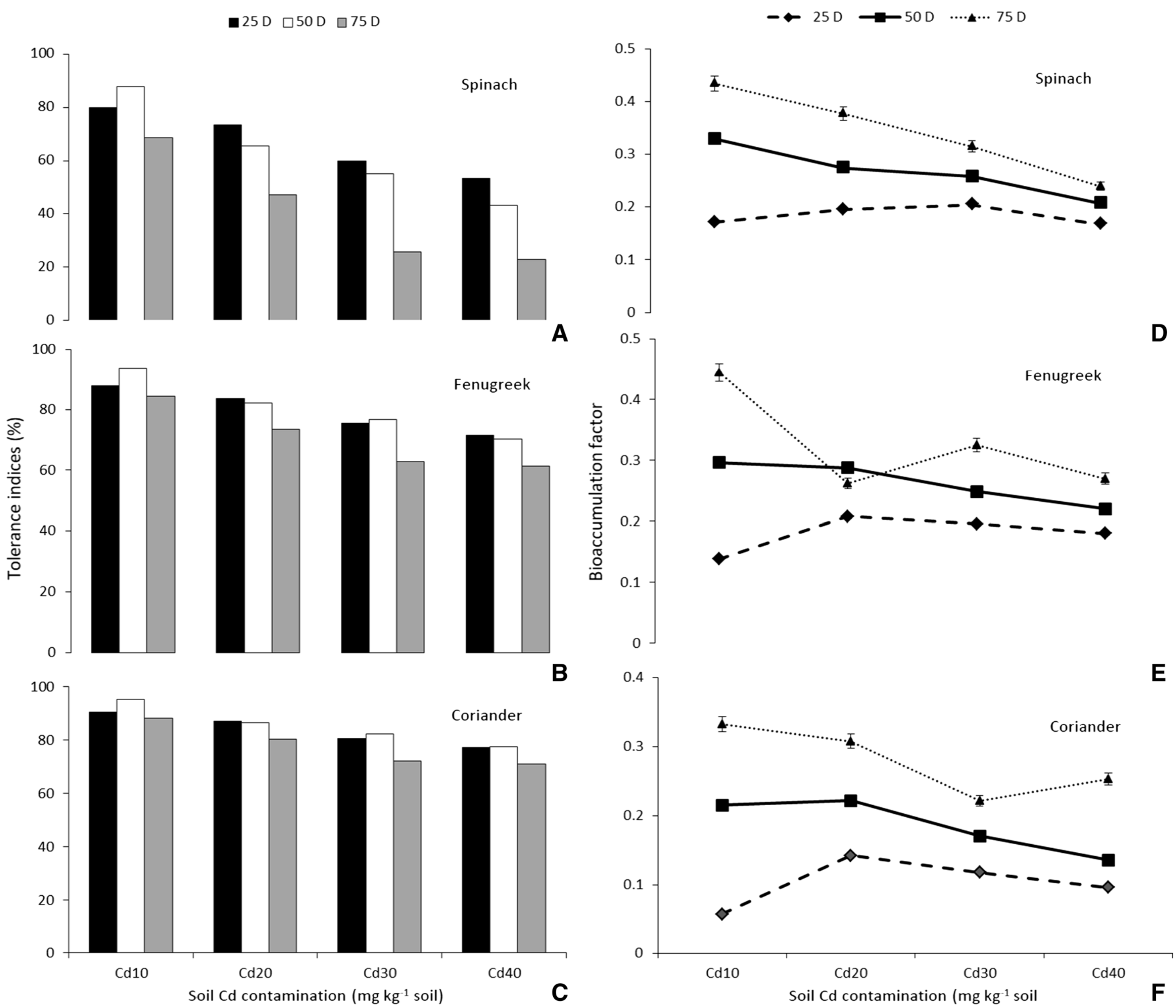

Fig. 2 Dose- and time-dependent effects of Cd on bioaccumulation and tolerance indices of Cd in three leafy vegetables spinach (**a**, **d**), fenureek (**b**, **e**) and coriander (**c**, **f**)

Effect of Cd on physiology of vegetables – Our results revealed severe effects of Cd toxicity on some physiological parameters of all tested vegetables (Tables 2 and 4). The process of photosynthesis is important for plant adaptation to stress conditions, and thus, it is, therefore, used as an indicator of plant response to stress. The results of this study showed Cd accumulation in edible part of plant. This occurrence might lead to damage of PSII reaction center in the leaf, and that consequently might lead to inhibition of photosynthesis (Tanyolac et al. 2007). Some other studies showed that reduced photosynthesis by Cd stress is due to inhibition of primary photochemical processes (Sarijeva et al. 2007). Furthermore, this occurrence could be attributed to disruptive action of heavy metals on chlorophyll synthesis, efficiency of photosystems and photosynthetic enzymes (Mobin and Khan 2007) and water balance of plant (Zhou and Qiu 2005). Although water balance of plants is an important factor for regulation of photosynthetic process (Zhou and Qiu 2005), our results did not show any toxic effect of Cd on RWC of all the tested vegetables. So, RWC status could not be a possible reason for reduced physiological parameters of exposed leafy tested vegetable types (Table 2).

Our results revealed toxic effect of Cd on chlorophyll synthesis (Table 3). This might have decreased rate of photosynthesis. This result corroborates the fact that increasing levels of Cd had negative effects on photosynthetic pigment contents (Ci et al., 2010). The inhibition of chlorophyll synthesis and photosynthetic activities might also be associated with Cd binding to calcium site, which resulted

Table 5 Daily metal intake and health risk index of three vegetables as affected by different levels of Cd and exposure time

Days of exposure	Cadmium (mg kg^{-1} soil)	Daily intake of metal (DIM) (μg/day)			Health risk index (HRI)		
		Spinach	Fenugreek	Coriander	Spinach	Fenugreek	Coriander
25 D	Cd_0	0^{k}	0^{l}	0^{m}	0^{k}	0^{l}	0^{m}
	Cd_{10}	0.00021^{j}	0.00005^{k}	0.00002^{l}	0.208^{j}	0.049^{k}	0.025^{l}
	Cd_{20}	0.00048^{h}	0.00015^{i}	0.00015^{i}	0.475^{h}	0.148^{i}	0.122^{j}
	Cd_{30}	0.00075^{e}	0.00021^{f}	0.00015^{h}	0.747^{e}	0.209^{f}	0.151^{h}
	Cd_{40}	0.00082^{d}	0.00026^{e}	0.00016^{g}	0.815^{d}	0.257^{e}	0.164^{g}
50 D	Cd_0	0^{k}	0^{l}	0^{m}	0^{k}	0^{l}	0^{m}
	Cd_{10}	0.00040^{i}	0.00011^{j}	0.00009^{k}	0.401^{i}	0.106^{j}	0.092^{k}
	Cd_{20}	0.00067^{f}	0.00021^{f}	0.00019^{f}	0.669^{f}	0.205^{f}	0.190^{f}
	Cd_{30}	0.00094^{c}	0.00027^{d}	0.00022^{e}	0.942^{c}	0.266^{d}	0.219^{e}
	Cd_{40}	0.00101^{b}	0.00031^{c}	0.00023^{d}	1.011^{b}	0.315^{c}	0.233^{d}
75 D	Cd_0	0^{k}	0^{l}	0^{m}	0^{k}	0^{l}	0^{m}
	Cd_{10}	0.00053^{g}	0.00016^{h}	0.00014^{i}	0.529^{g}	0.159^{h}	0.142^{i}
	Cd_{20}	0.00092^{c}	0.00019^{g}	0.00026^{c}	0.920^{c}	0.187^{g}	0.264^{c}
	Cd_{30}	0.00115^{a}	0.00035^{b}	0.00028^{b}	1.150^{a}	0.348^{b}	0.284^{b}
	Cd_{40}	0.00116^{a}	0.00039^{a}	0.00043^{a}	1.167^{a}	0.385^{a}	0.434^{a}

The values having different letters within column are statistically significant at $P \leq 0.05$

in deactivation of photosystem II (Faller et al. 2005). The excess Cd reduces leaf net photosynthetic rate, stomatal conductance and transpiration rate in dose- and time-dependent manner (Khan et al. 2007; Ci et al. 2010). Stomatal movement in leaves provides an opportunity for change in the rate of transpiration and partial pressure of CO_2. However, Cd toxicity severely affected stomatal openings in plants and it may depend on Cd concentration, exposure time, crop species and toxicity level suffered by plants (Wang et al. 2012; Choppala et al. 2014). Our results showed toxic effect of Cd on growth and biomass of all the vegetables types tested at low concentrations of Cd and short exposure time (i.e., 25 DoE) (Tables 1, 2), while similar exposure of the vegetables to similar conditions did not show any toxicity on *A*, *E* and *Gs* (Table 4). This observation might be the reason for root growth reduction at low concentrations and short-term exposures to Cd without harmful effects on leaves. This resulted in a reduced sink force and enhanced photosynthesis and transpiration rate. In contrast to this, negative effects on root and shoot systems cause hydropassive stomatal closure and turgor loss in plants at high concentrations and longer time exposures to Cd (Wang et al. 2012).

Effect of Cd on Cd accumulation, health risk assessment and tolerance – Application of different levels of Cd significantly increased the Cd concentration in the edible part of the vegetables (Fig. 1) and consequently daily metal intake as well as health risk index. Concentration of Cd in the leaves of all vegetables increased with increasing Cd doses and DoE. The results of the present investigation agree with those found in hydroponics, pot culture and under field conditions (Zhang et al. 2010; Ahmad et al. 2013) with the conclusion that a sharp increase in Cd concentration in plant resulted from increasing concentration of Cd in soil/water medium. However, most of the studies indicate that bioconcentration of metals in crops is genotype dependent. Also, various cultivars have different levels of tolerance to metals because of their dissimilar genetic potentials (Bauddh and Singh 2011; Ahmad et al. 2013). Each plant species has dissimilar tolerance levels to contaminants at different environmental conditions (Suñe et al. 2007). Bioconcentration of toxic elements is higher in leafy vegetables than other crops. This could be explained by their morphophysiological traits, such as number of leaves, high leaf area, transpiration rate, detoxification (e.g., synthesis of phytochelatins), efflux and uptake of metals (Xiong et al. 2014). However, we did not observe any significant effect on leaf Cd content and its bioconcentration in broad leaf spinach to narrow leaf fenugreek (Figs. 1, 2), though both parameters were different for transpiration rate (Table 4). These results implied that these vegetables might adapt to Cd exclusion mechanism to detoxify excess Cd. This might result in concentrating Cd in their roots rather than translocating it to shoot. This phenomenon has been previously reported by Alia et al. (2015) who found higher concentration of Cd in root than shoot of spinach.

Daily Cd intake via vegetables consumption increased with level of Cd application in soil, irrespective of the vegetable type. At a given level of soil Cd application, there was higher Cd intake in spinach than fenugreek and coriander. Irrespective of the vegetable type, this intake was further increased with the days of vegetable exposure with Cd, especially under levels greater than 30 mg kg^{-1}

soil. Consequently, the HRI for spinach was greater than 1, which reflected that the metal consumption lies in unsafe limits (Singh et al. 2010; Abbasi et al. 2013). On the other hand, HRI values for fenugreek and coriander suggested that Cd intake consumption via these vegetables fell within the safe limits.

Tolerance index calculated based on shoot dry biomass showed gradual decrease in Cd tolerance index with increasing Cd concentration and exposure time. Similar results have been reported by Kumar et al. (2009). They found increase in phytotoxicity and decrease in metal tolerance index with increasing soil metal contamination. Among the vegetables, coriander showed higher tolerance, while spinach showed the least tolerance to exogenous application of Cd. These differences might be Cd-induced synthesis of phytochelatins in coriander, which provide more tolerance and Cd accumulation in the vacuoles.

Cadmium toxicity to the vegetables increased with increasing concentration in growth media though response based on exposure time varied among the parameters studied. Plant growth indices (plant height, root length, leaf area and biomass) were drastically reduced at early growth stages (25 DoE), whereas exposure time did not show any drastic impact on physiological indices (chlorophyll contents, photosynthesis, transpiration and stomatal conductance). Cadmium content of leaves increased, while Cd bioconcentration decreased with increasing soil Cd contamination. However, exposure time showed inverse relation with leaf Cd contents. The tested vegetables showed Cd tolerance indices in the following order: coriander > fenugreek > spinach depending upon soil Cd contamination and exposure time. Consequently, DIM and HRI were higher in spinach as compared to other vegetables. From our findings, it is concluded that tested physiological parameters of all vegetables were decreased by increase in Cd dose alone not exposure duration. Furthermore, growth attributes (root, shoot and leaf area) of all the vegetables tested depended on Cd dose and duration of exposure. In the same vein, growth parameters tested were more sensitive to Cd and, therefore, they could be used as vital markers for determination of Cd toxicity in vegetables. Finally, it is suggested that leafy vegetables, especially spinach, should not be cultivated on Cd-contaminated soils.

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Author contributions JA, IA and QZ supervised the experiment. JL, MMR, TJ and ZRF conducted the experiment, performed statistical analysis and prepared the figures and tables. JL, AS and IA wrote the manuscript. GMS, MS and MR edited the final version of the manuscript. IA got funding from HEC. All authors have approved the final manuscript.

Compliance with ethical standards

Conflict of interest All authors have declared no conflict of interest regarding the publication of this article.

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