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Research Article

Cadmium Toxicity Affects Phytochemicals and Nutrient Elements Composition of Lettuce (*Lactuca sativa* L.)

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Lettuce varieties Bombilasta BBL and Italian 167 were treated with different concentrations of cadmium (0, 3, 6, 9, and 12 mg/L) in a nutrient film technique (NFT) system to study its toxicity on phytochemicals and nutrient elements. Antioxidants analysis which employed DPPH and FRAP, flavonoids, phenolic, vitamin C, malondialdehyde (MDA), and proline indicated significant effects of Cd treatment on the varieties tested. Different concentration levels of Cd lead to positive interactions in FRAP, phenolic, and MDA but no significant effect in flavonoids, vitamin C, and proline. Contents of macro- and microelements in the varieties were significantly affected with increase in the toxicity levels of Cd in all nutrient elements tested with interactions exhibited for iron, manganese, and zinc.

1. Introduction

Use of wastewater in agriculture is gaining wider acceptance in recent years for irrigation purposes. Essential plant nutrients and organic matter are targeted in wastewater by farmers to restore soil productivity, especially in the cultivation of vegetable crops [1]. However, heavy metals and other toxic elements which are also found in wastewater are nonessential to plants and may become toxic to them and eventually pass on to humans and other animals via food chain [2].

Cadmium is a powerful environmental pollutant of widespread occurrence that is released into the environment from phosphate fertilizers, cement factories, waste incinerators, urban traffic, heating systems, power stations, and metal working industries. David and Eric [3] estimated that about 660 metric tons of Cd per year is being applied to soils on global basis through use of phosphatic fertilizers. Uptake of Cd in plants is influenced by its bioavailability and concentration which are controlled by redox potential, organic matter, pH, temperature, and concentration of other elements [4, 5].

Higher concentrations of Cd frequently injure roots, damage photosynthetic apparatus, hinder growth of the plants, and decrease nutrients and water uptake. Stohs et al. [6] and Schützendübel et al. [7] stated that Cd directly or indirectly induces increased formation of reactive oxygen species (ROS), which interfere with the redox status of the cell and cause oxidative damage to proteins, lipids, and other biomolecules. Cadmium being a strong pollutant and a quick dissolving substance can affect all life forms. Shah and Dubey [8] reported a sharp decline in crop productivity in soils contaminated with Cd, indicating Cd as a serious problem for agriculture.

Stroiński [9] described that Cd may exert its inhibitory effect in the cells in two different ways: it may bind to specific groups of proteins and lipids inhibiting their normal function and it may induce free radical formation inducing oxidative stress. The former may occur at the transport and channel proteins of membranes disturbing the uptake of many other macro- and microelements while the latter is proved by the inactivation of antioxidant enzymes by Cd. Destruction of the cell membranes changes the ratio of essential elements and causes the decrease in their concentration; for example, Cd inhibits the transport of iron (Fe) into the shoot. Zhang and Huang [10] reported that Cd may have antagonistic or synergistic effect on the macro- and microelements in 16 wheat lines.

Plants respond to many abiotic stresses like Cd by accumulating free amino acids. Proline is one of the amino acids that accumulates in large amounts in response to environmental stresses followed by other amino acids, such as asparagine, isoleucine, leucine, methionine, and valine derived from asparatic acid [11]. Production of proline in Cd stressed plants is important in insuring the maintenance of water balance and protection of enzymes and biomolecules in addition to detoxification of reactive oxygen species. Peroxidation of polyunsaturated fatty acids of membrane lipids by reactive oxygen species is induced by Cd which eventually increases membrane fluidity and membrane permeability [12]. Increase in the levels of malondialdehyde (MDA) in cells of plants is an indication of oxidative stress otherwise known as lipid peroxidation involving oxidative degradation of polyunsaturated fatty acyl residues of membranes [13]. Plants possess antioxidative mechanism comprising antioxidative molecules and enzymes to protect themselves from oxidative damage caused by harmful reactive oxygen species like all aerobic organisms. Shah et al. [14] stated that the increased activity of antioxidative enzymes in metal exposed plants appears to serve as an important component of antioxidant defense mechanism of plants to combat metal-induced oxidative injury. Several researchers [15–18] have reported in different plant species the involvement of glutathione and ascorbic acid in the tolerance of plants to Cd phytotoxicity. Seregin and Ivanov [19] stated that the decline in net photosynthetic rate in Cd exposed plants results from the distorted chloroplast ultrastructure, restrained synthesis of chlorophylls, plastoquinone, and carotenoids, disturbed electron transport, and inhibited activities of Calvin cycle enzymes and carbon dioxide deficiency in the cells.

The aim of this study was to determine the effect of Cd treatments on phytochemicals and nutrient elements composition of lettuce, which among the two varieties is least and most accumulating of Cd, and assess element present in the varieties.

2. Materials and Methods

2.1. Planting Material and Growing Technique. Lettuce (*Lactuca sativa* L.) seeds of Italian variety 167 and Bombilasta (BBL) were planted and raised for two weeks under a rain shelter with a temperature range of 24–38°C and relative humidity of 52 to 94% under light intensity of 300 μmol m^{-2}s^{-1}. Seedlings were transferred to circulating troughs irrigated with Cooper's nutrient formulation solution [20] of mg/L : 236 N, 60 P, 300 K, 185 Ca, 50 Mg, 68 S, 12 Fe (EDTA), 2.0 Mn, 0.1 Zn, 0.1 Cu, 0.3 B, and 0.2 Mo, using a nutrient film technique (NFT) system and maintaining a pH of 5.5–6.5 and E.C. of 1.5–2.5 dS/m. Seedlings were allowed to stabilize for one week after transplanting before treatment.

2.2. Experimental Design and Treatments. The experiment was factorial, arranged in a randomized complete block design (RCBD) with three replications. Five levels of Cd (0, 3, 6, 9, and 12 mg/L) in the form of $CdCl_2$ and two lettuce varieties (BBL and Italian 167) were used. There were 21 plants of each variety per replication for each Cd level and 6 out of these plants were randomly collected at 8 weeks after treatment for determination of nutrient elements and phytochemicals.

2.3. Plant Tissue Analysis. Fresh leaf samples collected from third leaf of selected plants were put in a large brown envelope and quickly transferred to a cooler box and transported to the laboratory. Proline content was determined according to the method of Bates et al. [21], while free radical scavenging assay (DPPH) and ferric reducing antioxidant power assay (FRAP) were done according to Wong et al. [22] and lipid peroxidation (MDA) was done according to Heath and Packer [23]. Plant nutrient analysis was done according to the procedure described by Motsara and Roy [24] to analyze nutrient contents. Total phenolic acid and total flavonoid determination was conducted as described by Marinova et al. [25]. Total phenolic acid content assay was carried out using Folin-Ciocalteu agent, while total flavonoid assay was conducted using aluminium chloride colorimetric method and total vitamin C content was measured using modified method of Davies and Masten [26].

2.4. Statistical Methods. Data generated were means of three replicates. Statistical analysis was performed using statistical software package (SAS version 9.2, SAS Institute Incorporated, Cary, North Carolina, USA). Comparison among treatments was evaluated by ANOVA, and least significant difference (LSD) was used in separating means. Differences between the treatments were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Effect of Cd on Nutrient Composition. The nutrient concentrations among the varieties and Cd treatments are presented in Table 1. Essential nutrients and Cd accumulation in plant tissue depends on the lettuce varieties. Variety Italian 167 accumulated significantly higher amount of all nutrients with exception of Mg than variety BBL. Iron and Cu content in variety Italian 167 were 48.9% and 47%, respectively, higher than in BBL. Unfortunately variety Italian 167 also absorbed 31.5% more Cd than variety BBL. As a vegetable, higher content of Cd in lettuce tissue must be avoided. The toxicity effect of Cd on plant eventually will pass on to humans and other animals via food chain [2].

The degree of contamination and plant species, which have different tolerance to toxic heavy metals, determined the effect of Cd on nutrient uptake in plants. Genotypic variations in shoot Cd concentration in 146 rice accessions from rice core-collection have been examined by Ueno et al. [27] and a large variation in the shoot Cd accumulation and Cd tolerance was found. Rahat et al. [28] stated that there

Table 1: Effect of Cd concentrations on nutrient element composition in lettuces.

Treatment	Macroelements (%/DW)					Microelements (μg/g DW)				
	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	Cd
Variety										
BBL	2.59b	0.34b	5.74b	0.97b	0.52a	92.82b	60.60b	7.03b	36.53b	77.18b
Italian 167	2.99a	0.43a	6.41a	1.17a	0.53a	137.73a	75.61a	10.26a	44.75a	101.48a
Significance	**	**	**	**	ns	**	**	**	**	**
Cadmium concentration (mg/L)										
0	4.00a	0.55a	7.95a	1.53a	0.56a	152.73a	110.04a	12.68a	52.54a	0.01e
3	3.61a	0.49b	7.12b	1.28b	0.55a	104.76b	88.21b	9.78b	45.66b	74.92d
6	3.01b	0.42c	5.20c	1.05c	0.42b	86.12c	63.00c	5.53c	41.92c	95.95c
9	2.18c	0.30d	4.62d	0.90c	0.31c	59.04d	47.08d	2.66d	34.87d	120.50b
12	1.16d	0.17e	3.48e	0.59d	0.27d	29.69e	24.19e	0.57e	27.25e	152.97a
Significance	**	**	**	**	**	**	**	**	**	**
Interaction										
Variety × Cd	ns	ns	ns	ns	ns	**	*	ns	**	ns

*Significant at 5%; **highly significant at 1%; ns = not significant. Values within a column with the same letters are not significantly different ($p < 0.05$), means separation by LSD, 5%.

are significant differences in Cd tolerance among species and varieties but contradictions exist between the results of different experiments, which may be attributed to the inherent differential capacity of different species and varieties for Cd accumulation and partitioning in root and shoot and also the ability to restrict Cd in roots.

Contents of macro- and microelements in both varieties were significantly affected by Cd levels. Increase in the concentration of Cd to 12 mg/L resulted in the decrease of all macro essential elements measured with a range of 72, 69, 56, 61, and 52% for N, P, K, Ca, and Mg, respectively, compared with the control, while a decrease of 80%, 78%, 95%, and 48% was found for Fe, Mn, Cu, and Zn, respectively, indicating that Cd is a strong antagonist to these metal ions (Table 1). The toxicity effect of Cd to nutrient elements was more pronounced at 6 mg/L and at the higher concentrations. As the concentration of Cd increases, nutrients elements contents were decreased. Comparing the results with reference sufficiency ranges for plant analysis reported by Campbell [29] indicated a short fall in all macroelements except Ca. However, values obtained in microelements do not fall below the nutrient sufficiency range for lettuce. Maria et al. [30] exposed lettuce to Cd concentration of 10 and 50 μM and they found a significant decrease in leaf nutrient content of several essential elements such as Fe and Mn, but only at the highest concentration of Cd treatment, a significant reduction of Mn was observed in roots, suggesting Cd interferes with the translocation of macro- and micronutrients to the leaves.

There was no interaction effect between Cd level and variety in respect to Cd content in plant even though from our observation the varieties displayed different severity of chlorotic symptoms and plant size in response to Cd toxicity. However interaction of both factors was found significant in Fe, Mn, and Zn. Variety Italian 167 showed higher percentage of reduction in Fe (Figure 1) and Mn (Figure 2(a)) for each increment of Cd concentration as compared to BBL variety,

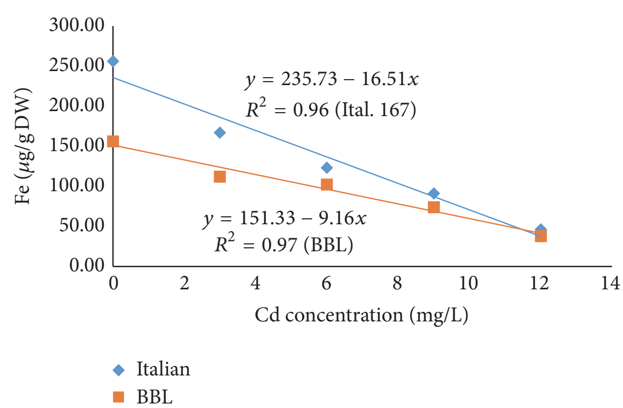

Figure 1: Interaction effect of Cd concentration and lettuce varieties on Fe content.

whereas, for Zn, higher reduction at lower Cd concentrations was exhibited by variety BBL (Figure 2(b)).

Uptake of cations like K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, and Fe^{2+} is affected by Cd. Llamas et al. [31] reported that Cd being a divalent cation may compete with Ca, Mg, or Fe in their transport across membrane. According to Perfus-Barbeoch et al. [32], members of the ZIP and NRAMP or Ca channels and transporters which are responsible for the uptake of essential elements are involved in the transport of Cd via the same route. Imbalance in the nutrient level and growth inhibition is ultimately as a consequence of the above competition between nutrients and toxic metals for binding sites in different compartments, for example, plasma membrane and cell wall. Sun and Shen [33] explained that the decrease in concentrations of Mn, Fe, Mg, S, and P in leaves of Cd-sensitive cultivars under Cd stress is revealed to be the important reason for the restraint of leaf photosynthesis and the decrease of cabbage growth.

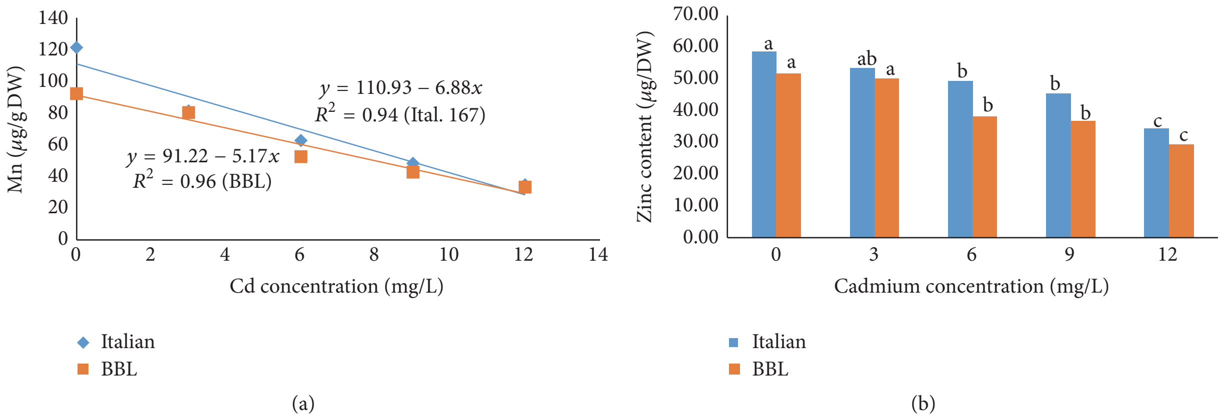

Figure 2: Interaction effect of Cd concentrations and lettuce varieties on Mn (a) and Zn (b) contents. Values within a variety with the same letters are not significantly different ($p < 0.05$), means separation by LSD, 5%.

Table 2: Effect of cadmium concentration on free radical scavenging assay (DPPH) and ferric reducing antioxidant power assay (FRAP), total flavonoids, and total phenolics in lettuce.

Treatment	DPPH scavenging (%)	FRAP assay (%)	Total flavonoids (mg CE/g)	Total phenolic (mg GAE/g)
Variety				
BBL	67.53b	63.26b	4.34b	2.71b
Italian 167	82.30a	74.22a	5.55a	4.57a
Significance	**	**	**	**
Cadmium concentration (mg/L)				
0	69.31d	63.31d	1.05e	1.20e
3	71.04d	65.25d	1.93d	1.85d
6	73.18c	68.37c	4.91c	3.55c
9	76.82b	71.12b	7.57b	5.04b
12	84.24a	75.63a	9.26a	6.57a
Significance	**	**	**	**
Interaction				
Variety × Cd	ns	ns	ns	**

Significant at 5%; **highly significant at 1%; ns = not significant. Values within a column with the same letters are not significantly different ($p < 0.05$), means separation by LSD, 5%.

3.2. Effect of Cd on Antioxidants. Determination of antioxidant activity in the Cd treated lettuce plants was conducted employing free radical scavenging assay (DPPH) and ferric reducing antioxidant power assay (FRAP) methods of Wong et al. [22]. Based on both antioxidant activity assays, variety Italian 167 has significantly higher antioxidants than variety BBL (Table 2). Significant differences were observed within the Cd treatments. There was 14.9% increase in DPPH free radical scavenging activity when stressed with 12 mg/L Cd compared with the control plants. Increase in the concentration of Cd brought about an increase in the antioxidant activity. Similar trend was observed in ferric reducing antioxidant power assay (FRAP). These two methods provided similar results as they did not reveal much differences between them, which confirmed the claim of Thaipong et al. [34] that DPPH free radical scavenging assay and FRAP assay have no significant difference in their determinations.

3.3. Effect of Cd on Phytochemicals. It would be desirable to know the nature of phytochemical in plants responded to detoxifying heavy metals. In this study, the photochemical compounds bioavailability in lettuce produced is discussed in total phenolic and flavonoid, vitamin C, malondialdehyde (MDA), and proline. The significance level effects of Cd and lettuce varieties on phytochemical compounds in leaves are presented in Tables 2 and 3.

Levels of flavonoids, phenolics, and malondialdehyde increased tremendously when subjected to higher Cd concentration of 12 mg/L, but vitamin C level significantly decreased compared with the control plants. Interaction effects of Cd and lettuce varieties were observed in total phenolics (Figure 3(a)) and lipid peroxidation (Figure 3(b)) indicating higher increase in Cd resulted in higher production of the phytochemicals in trying to protect the plants from further damage by reactive oxygen species. Italian lettuce 167 variety responded more in the production of

Table 3: Effect of cadmium concentration on vitamin C, malondialdehyde (MDA), and proline in lettuce.

Treatment	Vitamin C (mg/100 g)	MDA (nmol/g FW)	Proline (μm/g FW)
		Variety	
BBL	4.45b	0.89b	106.67b
Italian 167	5.13a	1.17a	123.07a
Significance	**	**	**
		Cadmium concentration (mg/L)	
0	6.91a	0.57d	98.44
3	6.38b	0.63d	108.65
6	5.06c	0.87c	121.41
9	3.35d	1.29b	125.26
12	2.27e	1.78a	120.60
Significance	**	**	ns
		Interaction	
Variety × Cd	ns	*	ns

*Significant at 5%; **highly significant at 1%; ns = not significant. Values within a column with the same letters are not significantly different ($p < 0.05$), means separation by LSD, 5%.

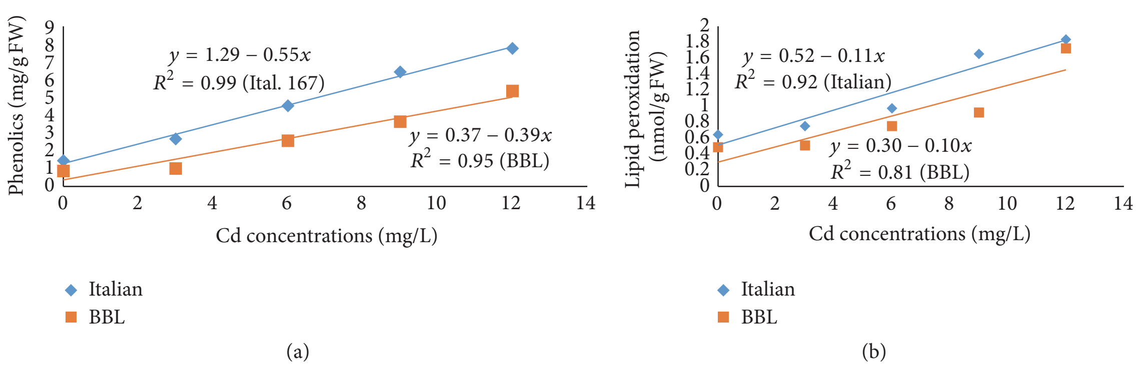

Figure 3: Interaction effect of Cd concentrations on (a) phenolic and (b) lipid peroxidation (MDA) contents of lettuce.

phytochemicals to counter the toxicity effect of Cd than variety BBL. Michalak [35] explained that phenolics, especially flavonoids, can be oxidized by peroxidase and act in the H_2O_2-scavenging, phenolic/ascorbate (ASC)/phenolic peroxidase (POX) system when treated with heavy metals. Activation of the antioxidative system of the cell is therefore as a result of heavy metal stresses which increase the reactive molecules in the stressed tissue. Nasim and Dhir [36] in an earlier study suggested the role heavy metals may play in triggering plant genes to alter the nature of secondary metabolites. Inducement of oxidative stress by heavy metals triggers signaling pathways that affect production of specific plant metabolites. Belen et al. [37] reported similar pattern of increase in the contents of phenolics and flavonoids of *Erica andevalensis* treated with acute doses of Cd. Padayatty et al. [38], Foyer [39], and Bielski et al. [40] reported that vitamin C is one of the major antioxidant agents in removing free radicals along with vitamins E and A and the minerals selenium and zinc. According to Sujogya [41], vitamin C is a second-line defense antioxidant which interacts directly with radicals like O_2^{-}, H_2O_2, and OH. Vitamin C and α-tocopherol both help to minimize the consequences of lipid peroxidation in membranes. In this study, reduction of 67% and 52% of vitamin C was observed when lettuce was exposed to 12 mg/L and 9 mg/L of Cd, respectively, as compared to control (Table 3).

Cadmium induced oxidative damage involves peroxidation of polyunsaturated fatty acids of membrane lipids by reactive oxygen species generated by Cd leading to membrane fluidity and membrane permeability [42]. As an index of lipid peroxidation under stress condition, the level of malondialdehyde (MDA) is routinely used to evaluate its effect in plant samples. Increased level of MDA to 3-fold for lettuce grown in 12 mg/L Cd over the control coincided with the findings of Shah et al. [14] in Cd stressed rice seedlings. An increase of about 1.4 to 1.6 times in malondialdehyde (MDA) content under 500 μM Cd treatment in two rice seedlings cvs Ratna and Jaya was reported by Shah et al. [14] indicating enhanced peroxidation of lipid due to Cd. Excessive accumulation of MDA when plants were subjected to Cd stress was reported by Lozano-Rodríguez et al. [43], Chaoui et al. [44], and Wu et al. [45].

According to Shah and Dubey [46] and Verma [47] one mechanism by which many plants respond to detoxifying heavy metals is the production of proline. Difference of 15% in proline content recorded between two varieties (Table 3) suggested potential effort to overcome the toxic effect of Cd in the lettuce varieties, thus protecting its delicate tissues and organs from severe damage by ROS. Srivastava et al. [48] reported that proline helps plants to recover from environmental stresses and its accumulation might be induced as a result of reactive oxygen species (ROS). Alia et al. [49] added that proline reduces oxidative damage through the mechanisms of physical quenching of singlet oxygen and chemical reaction with hydroxyl radicals. Proline also acts as a defense mechanism for survival of stressed plants by binding with metal ions due to its chelating ability. Although higher Cd levels resulted in no significant effect on proline content, there was 27% increase in proline at 9 mg/L Cd compared to control. Similar accumulation of proline in the shoots of *Brassica juncea, Triticum aestivum,* and *Vigna radiate* was demonstrated by Dhir et al. [50] in response to Cd toxicity but decreased with the exposure to Cd in hydrophytes (*Ceratophyllum, Wolffia,* and *Hydrilla*).

4. Conclusion

From the above results, it can be concluded that Cd stresses have adverse effect on phytochemicals and nutrient elements of lettuce when subjected to high concentrations. Cadmium toxicity brought about increase in the activities of antioxidants, secondary metabolites, MDA production, and proline as a defense mechanism to protect the plants against physiological and structural injuries. Nutrient imbalances and reduction in the amount of vitamin C, resulting from the toxic effect of Cd, can alone trigger oxidative stress, nutrient deficiencies, and reduction in plant growth and development of agricultural crops. Italian 167 variety absorbed more Cd than BBL variety, suggesting that Italian 167 variety should not be recommended for planting in Cd polluted environments. Viable strategies should be engaged to counteract the toxic effect of Cd in agricultural lands to protect crop plants from eventual damaging effects.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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