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Full Length Research Paper

Morphological and Physiological Changes Induced By Cadmium Toxicity in Two Varieties of Lettuce (*Lactuca sativa* L.)

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Italian 167 and Nigerian Bonbilasta (BBL) lettuce varieties were grown hydroponically in a nutrient film technique system (NFT) under the influence of cadmium concentrations of 0, 3, 6, 9 and 12 mg/L to study its toxicity effect on morphology and physiology of the lettuce. Significant effect was recorded in terms of plant height, number of leaves, fresh and dry leaf weights and dry root weight in the varieties. Despite the level of cadmium used, variety BBL was taller and recorded higher number of leaves, leaf area; fresh and dry root weights than Italian variety 167. Higher fresh and dry leaf weights were recorded by Italian variety 167. Highest cadmium concentration significantly reduced all morphological characteristics measured with interaction in plant height. Different concentration levels of cadmium has significant effect on all physiological characteristics of the two lettuce varieties studied; affecting variety BBL more than variety 167 with interactions in stomatal conductance, chlorophyll a and b and total chlorophyll.

Keywords: Cadmium toxicity, Lettuce, morphological, physiological

INTRODUCTION

Competitive demand for fresh water for domestic purposes and agriculture is increasing due to increasing global population that reaches a distressing level in many parts of the world, forcing farmers to resort to the application of wastewater in irrigation of agricultural crops. Reports (Hussain et al., 2001; Scott et al., 2004; and Hamilton et al., 2007) indicated that at least 20 million hectares in 50 countries are irrigated with raw or partially treated wastewater. Wastewater is considered a resource and a problem to both flora and fauna as it contains nutrients, such as nitrogen, phosphorus, and potassium; pathogens, high amounts of trace elements and heavy metals (Kanwar, 2000).

Lettuce was reported to be a high accumulator of Cadmium (Cd) in its leaves (Mensch and Baize, 2004; Kim et al., 1988). High accumulation of metals was found in

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lettuce, spinach, cereals, cabbage, rather than in tomatoes, corn or sweet pea (Cox, 2000). Lettuce was proposed to be an indicator crop for testing the potential human dietary risk associated with ingesting food crop grown on Cd polluted soils (Brown et al.,1996). John, et al., (2009), added that Cd ranks the highest in terms of damage to plant growth and human health. According to Santos et al., (2010), Cd is one of the most highly toxic trace pollutants for humans, animals and plants which many authors have reported its cytotoxic, mutagenic and/or carcinogenic effects in animal cells.

The occurrence of Cd is naturally in soils, but the anthropogenic emissions, mostly due to mining activities, burning of fossil fuels, metallurgical industry and the frequent use of phosphate fertilizers are the main sources of soil contamination (Singh and Agrawal, 2007). Nelson and Campbell, (1991) suggested that Cd has relative mobility in soils and does not bind strongly to organic matter. In another report by Benavides et al., (2005), stated that the degree to which plants are able to uptake Cd is conditioned by its concentration in the soil, and its bioavailability modulated by the presence of organic matter, pH, redox potential, temperature and concentrations of other elements.

A lot of research conducted on lettuce by Thys et al., (1991); Costa and Morel, (1994); Ramos et al., (2002) gave emphasis on the effects of Cd on growth of lettuce plants and on the mechanisms of Cd absorption by roots and its distribution within different organs. Recently, Ramos et al., (2002); Monteiro et al., (2009); and Zorrig et al., (2010) reported Cd interactions with other metals like Manganese, Calcium, Zinc and Iron. Among the stresses induced by Cd toxicity symptoms reported by Azevado et al., (2005) and Santos et al., (2010), includes chlorosis, necrotic lesions, wilting, disturbances in mineral nutrition and carbohydrate metabolism and ultimately reducing biomass production. A study with different levels of Cd was formulated and applied to two varieties of lettuce to study its toxicity effects on morphology and physiology of the plants.

MATERIALS AND METHODS

Two weeks old seedlings of Italian lettuce -167 and Bonbilasta (BBL) were transplanted into a trough and supplied with the Cooper's Nutrient Formulation solution (Cooper, 1979) containing (mgL^{-1}): 236 N, 60 P, 300 K, 85 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 System (NFT). The pH was maintained between 5.5 – 6.5 and E.C. of 1.5 – 2.5 s/m. Plants were subjected to different concentrations of Cd (0, 3, 6, 9, and 12 mg/L) supplied in the form of $CdCl_2$ after 9 days of transplanting. This factorial experiment was arranged in a Randomized Complete Block Design with three replications under a netted rain shelter with a temperature range of 24 - 38 ^{o}C and relative humidity of 52 to 94 % under light intensity of 300 µmol $m^{-2}s^{-1}$. Number of leaves and plant height were recorded at maturity in 8 weeks. Root parts were separated at harvest from the leaves and weighed for fresh weights and oven dried at a temperature of 65 ^{o}C for dry mass, while chlorophyll content was determined according to Coombs et al., (1985) at day 14 of exposure to Cd treatment in the young expanded leaves of lettuce. Photosynthetic rate, stomatal conductance and transpiration rate were measured in the morning between 9 am to 11 am using portable Photosynthesis System (Model LICOR – 6400). Data were analyzed using statistical software package (SAS version 9.2, SAS Institute Incorporated, Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Application of different concentrations of Cd resulted in changes in morphological and physiological characteristics of the two lettuce varieties (Tables 1 and 2). The two lettuce varieties showed similar response regarding Cd toxicity effects of stunted growth, yellowing of leaves and reduction of biomass with increase in Cd concentrations.

Highest reduction in plant height at maturity was found to be 5.27%, 26.09%, 36.34% and 45.24% respectively, after treatment with 3, 6, 9 and 12 mg/L of Cd stress compared with the control. Interaction was observed to that respect with regards to the concentration of Cd used on the varieties of lettuce. The plant heights of both varieties were linearly reduced with high dosage of Cd element (Figure 1).

Numbers of leaves were reduced highest in the order of 28.69%, 44.68%, 55.74% and 64.35% respectively over the untreated control. Similar negative effects were observed in fresh leaf weight where highest reductions of 9.68%, 31.35%, 58.09% and 78.55% respectively, were obtained. Fresh root weight was decreased by 59.84%, 65.75%, 74.01% and 81.10% respectively compared with the control. Dry leaf weight was affected and reduced to 23.26%, 35.47% and 56.40%compared with the untreated control. Highest reduction in dry root weight of 65.38%, 85.75%, 88.32% and 93.16% respectively, was similarly obtained in comparison with the untreated control.

A study on *Hordeum vulgare* by Argese et al., (2001); *Apium graveolens* by Zong et al., (2007); and *Brassica campetris* by Yang et al., (2011) have made similar reports which attributed the changes observed to high metal concentrations that damaged plant roots and inhibited nutrients uptake, consequently, inhibiting normal plant growth. Bora (1981), reported significant reduction in plant height, fresh and dry weights of various organs, leaf area, relative growth rate, root/shoot ratio that occurred in sesamum plants following Cd treatment. Stohs et al.,

Table 1: Effect of Cd Concs on Morphological Characteristics of lettuce varieties

Variety	Plant height (cm)	No. of leaves	Fresh Leaf weight (g)	Fresh Root weight (g)	Dry Leaf weight (g)	Dry Root weight (g)
Var. BBL	26.15a	25.27	85.53b	21.93	19.87b	2.63
Var. ITAL. 167	18.23b	24.60	109.80a	15.20	24.00a	1.27
Significance	**	ns	**	ns	**	ns
Cd concs. (mg/L)						
0	28.67a	40.67a	151.50a	42.33a	28.67a	7.02a
3	27.16b	29.00ab	136.83a	17.00b	28.00a	2.43ab
6	21.19c	22.50bc	104.00b	14.50ab	22.00b	1.00b
9	18.25d	18.00bc	63.50c	11.00b	18.50c	0.82b
12	15.70e	14.50c	32.50d	8.00c	12.50d	0.48b
Significance	**	**	**	*	**	*
Interaction						
Variety*Concs.	**	ns	ns	ns	ns	ns

*Significant at 5%, **highly significant at 1%, ns = not significant

Table 2: Effect of Cd concentration on physiological characteristics of lettuce varieties

Variety	Photosynthesis (mol m^{-2} s^{-1})	Stomatal Cond. (m^{-2} s^{-1})	Transpiration mL/cm^2	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Total Chlorophyll (µg/ml)
Var. BBL	12.26b	1.05b	5.65b	3.32b	3.43b	6.75b
Var. ITAL. 167	13.31a	1.45a	6.68a	4.32a	4.09a	8.41a
Significance	**	**	**	**	**	**
Cd concs (mg/L)						
0	16.17a	1.80a	8.00a	5.43a	6.07a	11.50a
3	14.77b	1.56b	7.36b	4.93b	5.58b	10.50b
6	12.84c	1.30c	6.63c	3.89c	4.05c	7.94c
9	11.22d	0.91d	5.02d	2.97d	2.06d	5.03d
12	8.91e	0.70e	3.82e	1.90e	1.03e	2.93e
Significance	**	**	**	**	**	**
Interaction						
Variety*Concs.	ns	**	ns	*	**	**

*Significant at 5%, **highly significant at 1%, ns = not significant

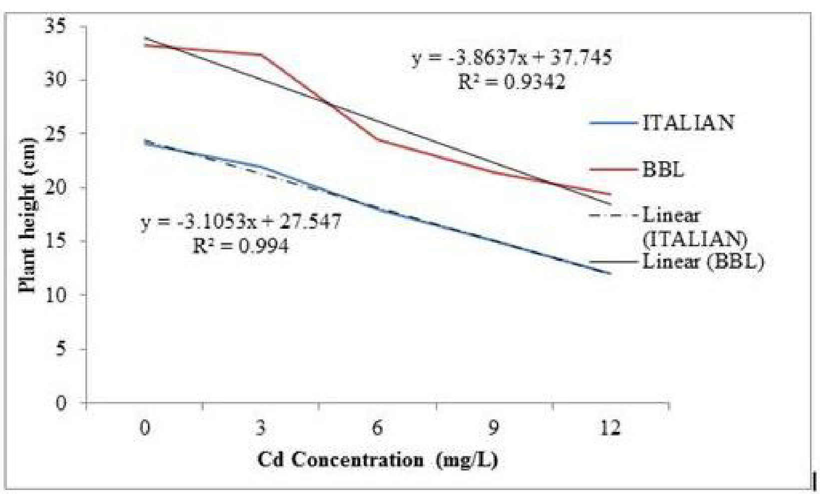

Figure 1: Interaction effect of Cd concentrations on plant height in varieties of lettuce

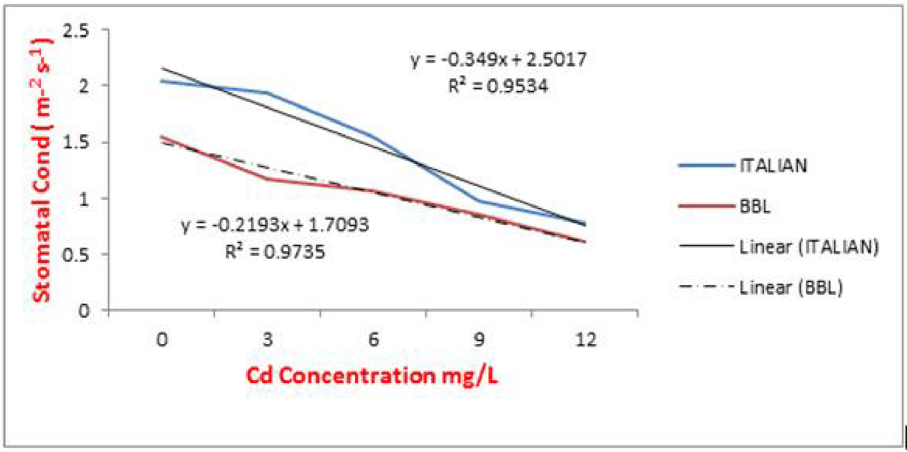

Figure 2: Interaction effect of Cd concentrations on stomatal conductance in lettuce varieties

(2000) and Schutzendubel et al., (2001) explained that Cd directly or indirectly induces increased formation of reactive oxygen species, which interfere with the redox status of the cell and cause oxidative damage to proteins, lipids and other biomolecules. This happened as a result of damages to root tips, reduction of nutrient and water uptake, impairments of photosynthesis and inhibition of growth of the plants by higher concentration of Cd in cells. In this work, roots of lettuce were more affected than other parts of the plants which may be explained as the roots being the main barriers against Cd uptake try to immobilize Cd by extracellular carbohydrates like mucilage, or callose among others (Wagner, 1993) as well as by the constituents of the cell wall (Nishizono et al., 1987). In a report by Leita et al., (1996), roots and leaves of bush been bound Cd mostly on pectic sites and histidyl groups of the cell wall. Seregin and Ivanov, (2001), suggested that the multilayer cortex appears to reduce the toxic effects of Cd on other tissues by binding most of the Cd ions in the cell wall serving as the second barrier defending plants from the toxic effects of Cd. Seregin et al., (2004), added that casparian strips found in the endodermis serve as a barrier for Cd entrance into the central cylinder.

Physiological parameters studied were significantly affected by Cd stress. Photosynthesis was reduced from 8.66%, 20.59%, 30.61%, to a maximum of 44.90% respectively, compared with the control on treatment with 0, 3, 6, 9 and 12 mg/L Cd concentrations. The decline of photosynthetic rates as suggested by Seregin and Ivanov, (2001), is due to distorted chloroplast ultrastructure; restraining the synthesis of chlorophyll, plastoquinone, and carotenoids; at the same time obstructs electron transport; inhibits enzyme activities of the Calvin cycle; and CO_2 deficiency as a result of stomatal closure when exposed to Cd stress. Negative effect of Cd on net photosynthesis (Pn) was demonstrated by Wu et al., (2003a) to be credited to a complex of physiological disturbances, mainly inhibition of the chlorophyll biosynthesis, reduction in Fv/Fm (the ratio of variable fluorescence to maximal fluorescence) and PSII, distorted stomata behavior and other photosynthetic processes in barley and tomato plants. Undesirable effect of Cd was observed in stomatal conductance amounting to 13.33%, 27.78%, 49.44% and 61.11% respectively, compared with untreated control. Similar negative trend was seen in transpiration where a reduction of 8%, 17.13%, 37.25% and 52.25% respectively, were obtained when stressed with Cd. Barcelo et al., (1988), highlighted that among the factors that causes reduced transpiration rate and stomatal conductance first is growth retardation that leads to reduced leaves area which are the major transpiring organ. Secondly, the guard cells becoming smaller occasionally or a times the guard cells becoming relatively more numerous because heavy metals generally affect leaf growth to a greater extent than the particular differentiation of stomata in Cd treated plants (Breckle, (1991). Contents of compounds maintaining cell turgor and cell wall plasticity is lowered in Cd treated plants thereby lowering the water potential is the third factor (Breckle, (1991; Leita et al., 1995); stomatal closure is the fourth factor induced by Cd as a result of increase in the content of ABA in the cells leading to the accumulation of monomers arriving at the site of cut in synthesis and the increase of the cuticle thickness, thus hindering transpiration.

Chlorophyll a, chlorophyll b, and total chlorophyll were affected by Cd treatment in a similar pattern with chlorophyll a having 9.21%, 28.36%, 45.30% and 65%; chlorophyll b was mostly affected more than chlorophyll a with 8.08%, 33.28%, 66.06% and 83.03% reduction; while total chlorophyll had a negative effect of 8.70%, 31.00%, 56.26% to a maximum of 74.52% (Table 2). Many workers have reported similar results (Singh et al., 2012; Preeti and

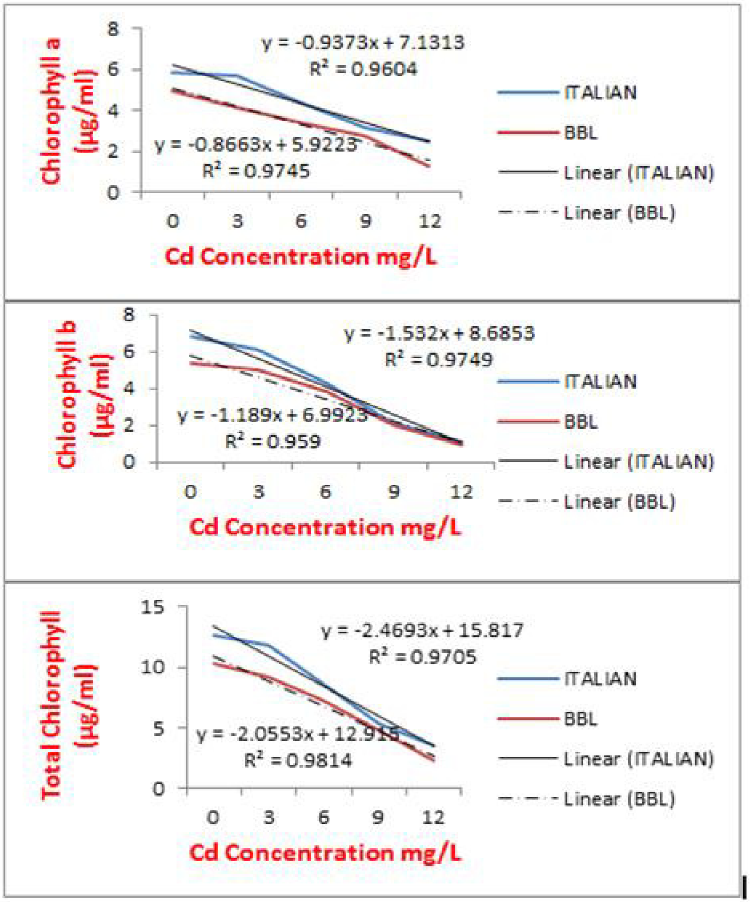

Figure 3: Interactions in chlorophyll a, chlorophyll b and total chlorophyll as a result of Cd concentrations on varieties of lettuce.

Tripathi, 2011). Reduction in chlorophyll content in lettuce and tomato plants in the presence of heavy metals was suggested to be caused by an inhibition of chlorophyll biosynthesis (Cho and Park, 2000). Increasing amounts of heavy metals in irrigation water affected chlorophyll b more than chlorophyll a in plants treated with water of the highest heavy metal content when compared with fresh water or less-contaminated industrial wastewater. Treatment with heavy metal on lettuce, cucumber and bean plants by Vassilev et al., (2007) in support of the above results, indicated that the content of all pigments get diminished and to some extent tend to changed their ratios. Sandalio et al., (2001) reported that stomatal opening, transpiration, photosynthesis are affected by Cd; and chlorosis, leaf rolls and stunting are the main symptoms of Cd toxicity in plants. Production or decreasing enzymatic and non-enzymatic antioxidants, inducing oxygen free radicals were suggested to be responsible by oxidative stress involved in Cd toxicity (Sandalio et al., 2001).

Interactions were observed in stomatal conductance, chlorophyll a, chlorophyll b and total chlorophyll. The higher the concentration of Cd in the plants the more stomatal conductance, chlorophyll a, chlorophyll b and total chlorophyll were reduced (Figure 2 and 3).There is a strong correlation in the varieties with Cd concentrations, where increase in Cd stress brought about lower performance in stomatal conductance, and lower chlorophyll a, chlorophyll b and total chlorophyll contents in the plants (Figure 2 and 3).

CONCLUSION

The present work has demonstrated the adverse effect Cd toxicity has on various aspects of plant physiological and morphological characteristics. This effect is not limited to only structural changes in the plants body but also alterations and reductions in plant biomass and ultimately affecting their productivity. A desirable means is required to minimize the uptake of heavy metals in food crops especially vegetables irrigated with polluted wastewater to protect food quality.

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