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Impact of cadmium accumulation on physiological characteristics of two cabbage varieties during phytoremediation

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Abstract

Two cabbage (*Brassica oleracea* L.) varieties, green and red, were tested by applying phytoremediate soils contaminated with cadmium. Results of this study indicated that Cd at a higher concentration has a profound impact on physiology and internal structure of plants. Cadmium accumulation was greater in roots than shoots; this discovery indicates that Cd is not easily transferred into shoots from roots. Chlorophyll and relative water content (RWC) were significantly decreased with increasing Cd concentration. The enzyme activities of CAT, SOD, and APX increased in seedlings under higher Cd concentrations. On the other hand, H_2O_2 content decreased in cadmium-stressed plants compared to the control. At high concentrations Cd-15 mM, plants showed a reduction in the number of palisade and spongy parenchyma cells and in cell size of leaves. Green cabbage had a higher capacity to uptake and translocation of Cd from contaminated soils than red.

Key words: Phytoremediation, cadmium, cabbage, antioxidant enzymes, chlorophyll.

Introduction

Heavy metal contamination is a serious issue facing the world today. It affects the biosphere in many places worldwide [39, 57]. The most problematic metals are Cd, As, Ni, Cu, Zn, Cr, Hg, Mo, Se, Fe, and B due to their potential toxicity to plants, humans, and livestock [13]. Some of the sources of heavy metal contamination are atmospheric deposition, artificial fertilizers, emission and runoff from mining and smelting operations, and compost and sewage-sludge [30]. Cadmium accumulation in soils may come from different sources, including air pollutants and soil applications of commercial fertilizers, sewage sludge, manure and lime [1, 29, 40, 42]. In polluted soils, Cd is generally present as free ions or different soluble forms, and its mobility depends on pH [12] and on the presence of chelating substances and other cations [25]. Cadmium is suggested to cause damage even at very low concentrations, and healthy plants may contain Cd levels that are toxic for mammals [16]. Moreover, it is widely recognized that Cd taken up by plants is the main source of Cd accumulation in food [38, 54]. Cabbage (*Brassica oleracea* or variants) is a leafy green biennial vegetable originating from wild cabbage (*Brassica oleracea* var. *oleracea*). It is used for its densely-leaved heads, which can be green, purple, and white [82].

Phytoextraction is a phytoremediation technique, which employs metal accumulating plants that are able to translocate chemical elements from the soil and accumulate them in harvestable parts. Here, we are discussing a possible use of white and red cabbage for the soil clean-up of cadmium by phytoextraction. Advances of phytoremediation are likely to result from more efficient plant variety selection and soil amendments as well as optimizing agronomic practices used for plant cultivation [61]. The ability of plants to accumulate elevated levels of heavy metals (hyperaccumulation) has been demonstrated in different plant species [33, 58]. Plants can accumulate Cd during plant growth, and the accumulation often occurs in edible parts, thus endangering crop yield and quality and becoming a potential hazard for human and animal health.

Overall, cabbage can be regarded not only as a valuable aliment, but also as miracle food for human health. In addition, purple cabbage contains high amounts of anthocyanins, which are known to have an important role in cancer prevention [78]. Moreover, cabbage, especially the red one, is rich in catalase [83], which play an important role in human body antioxidant defence. Overall, cabbage can be regarded not only as a valuable aliment, but also as miracle food for human health. The study evaluated the degree of stress to young plants transplanted to the contaminated soil and, should they survive under such chemical exposure, their ability to accumulate Cd in the heads.

The specific objectives of the study were: to compare the ability of two varieties of cabbage plants for cadmium uptake and accumulation in their shoots and roots and to study the anti-oxidation responses to cadmium in two cabbage varieties.

Materials and Methods

Plant culture and phytoremediation experimental design: Two cabbage cultivars, green and red, were grown in greenhouse in pots filled with local soil containing 0.56 mg Cd dm^{-3} DM of soil. Plantlets were produced from seeds and transplanted into soil at the stage of 4-6 leaves. Cadmium was added to the soil as $CdSO_4 \cdot 8H_2O$, in the different treatments (0, 1, 5, 10 and 15 mM). Control pots contained the same soil but without added Cd. The pots were filled with approximately 2 kg of soil sample. The pots filled

with polluted soil were irrigated with tap water of known heavy metal concentration. Roots and shoots were sampled, and soil samples were collected for analyses. All the treatment groups, along with control, were arranged in a completely randomized design (CRD) with five replicates in each Cd treatments group with means separated at $p<0.05$.

Plant and soil analysis: The plants were grown in pots for 57 days. After harvesting, the leaves were dried and mineralized at 450°C. The content of Cd in plants was assayed by inductively coupled plasma atomic emission spectroscopy ICP-AES JY-238 Ultrace. Cd was obtained by the ASA method, using the Varian-SpectrAA 20 and an air/acetylene flame under standard operating conditions. The concentration of Cd of the soil and water sample was determined before planting. The soil samples were oven-dried at 105°C for an hour and ground to fine particles using mortar and pestle. The ground particles were sieved using 1 mm sieve. A measured quantity (2.0 g) of the soil samples was put into an acid-ashed centrifuge bottle (scintillation bottle). Soil samples were digested using 35 ml of 0.1 M HNO_3. The scintillation bottles were attached to the lid, equilibrated in an end-over-end mechanical shaker and shaken vigorously for sixteen hours. Thereafter the samples were centrifuged at 3000 rpm for 5 min. The supernatant was decanted into the scintillation bottles for analysis. The analysis of the cadmium was done using the atomic absorption spectrophotometer (model AA6300).

Assay of chlorophyll and antioxidant enzymes: The chlorophyll content was measured photometrically with a SPAD chlorophyll meter (Konica Minolta, Japan). Activity of antioxidant enzymes and concentration of related metabolites was undertaken according to optimized protocols [23]. Fresh samples were ground in the presence of liquid nitrogen and measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany). The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured by determining ascorbic acid oxidation, one unit of APX oxidizes ascorbic acid at the rate of 1 µmol/min at 25°C. Catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decrease in absorbance of H_2O_2 at 240 nm, unit activity was taken as the amount of enzyme, which decomposes 1 µmol of H_2O_2/min. Peroxidase (POD, EC 1.11.1.7) activity was assayed using the guaiacol test, the enzyme unit was calculated as enzyme protein required for the formation of 1 µmol tetraguaiacol/min. Total superoxide dismutase (SOD, EC 1.15.1.1) activity was determined using monoformazan formation test. One unit of SOD was defined as the amount of enzyme required to induce a 50% inhibition of nitro blue tetrazolium (NBT) reduction as measured at 560 nm, compared with control samples without enzyme aliquot. The concentration of H_2O_2 was determined using potassium titanium-oxalate at 508 nm.

H_2O_2 determination: The content of H_2O_2 was measured according to the method described by Prasad *et al.* [56] with the following modification: 2 g of root tissues was ground in 50 mM K-phosphate buffer (pH 7.8). To the homogenate, 5% trichloroacetic acid (TCA) was added (TCA: mixture/1:0.7). The mixture was centrifuged at 10,000 g for 10 min. The supernatant was collected. Approximately, 1.6 ml of the resulting supernatant was mixed with 0.4 ml 50% TCA, 0.4 ml 10 mM ferrous ammonium sulfate and 0.2 mL 125 mM potassium thiocyanate. The absorbance of the reaction mixture was monitored at 480 nm.

Procedure for light microscopic study: Leaf samples were excited 2 cm above the leaf-stem intersection of second (lower) and fourth (upper) leaf of the plants for light microscopy (LM). Sections (0.5 µm) were from the resin blocks and stained with 2% toluidine blue for light microscopy. For the sulfide–silver method, the LM leaf sections embedded in paraffin were microtomed to obtain four microsections, placed on glass slides, cleaned in citrisolve, and dehydrated in an ethanol series. The sections were treated in developer solution in the dark at 22°C for 1 h. Finally, they were washed in an ethanol series, counter stained with 2% safranin, dehydrated and mounted [53].

Results and Discussion

Cd distribution in contaminated soil and its effect on plant growth: All concentrations of Cd increased cadmium accumulation in shoots and roots for both varieties. Cadmium accumulation in shoots and roots of cabbage increased as cadmium level increased (Figs 1 and 2). Green cabbage accumulated more cadmium than red variety in both shoots and roots, 10 and 15 mM $CdSO_4 \cdot 8H_2O$ resulted in greatest accumulation in shoot and root. This reduction was accompanied by a decrease in the dry weight, particularly pronounced after 57 d of growth in leaves and roots, respectively. Decrease in growth and in fresh and dry weight was also reported in Cd-treated plants of wheat, barley (*Hordeum vulgare* L.) and rice (*Oryza sativa* L.) [2, 67, 70, 84]. $CdSO_4 \cdot 8H_2O$ application of 10 and

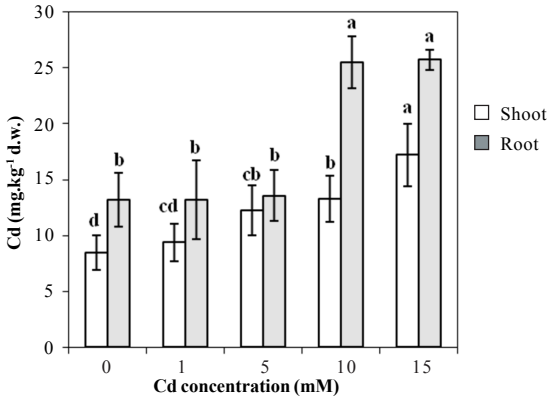

Figure 1. Cadmium accumulation in shoots and roots (in mg kg $^{-1}$ dry weight) of green cabbage at the $p < 0.05$ level.

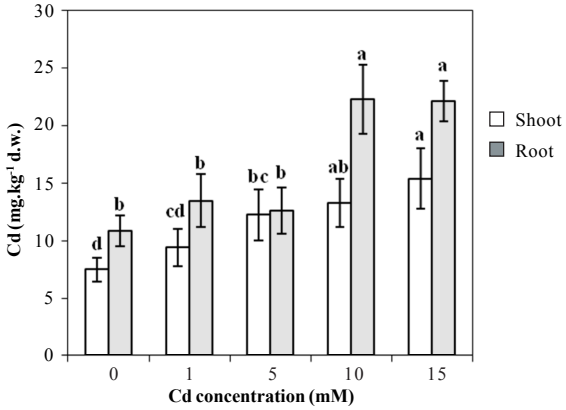

Figure 2. Cadmium accumulation in shoots and roots (in mg kg $^{-1}$ dry weight) of red cabbage at the $p < 0.05$ level.

15 mM significantly decreased shoot RWC compared to the control and the remaining treatments were equally effective (Fig. 3). Cadmium is taken up rapidly by the roots and can be loaded into the xylem for its transport into leaves. The amount of Cd accumulated in roots or translocated to leaves differs considerably among species. Most plants are sensitive to low Cd concentrations, which inhibit root and shoot growth, as a consequence of alterations in the photosynthesis rate, uptake and distribution of macronutrients and micronutrients [62]. Conversely, Cd, a non-essential heavy metal and extremely toxic, affects plant growth and development, such as reduction in photosynthesis, growth inhibition, and water and nutrient uptake [46]. It can efficiently inhibit the synthesis of proteins, such as phosphoenolpyruvate caboxylase [68].

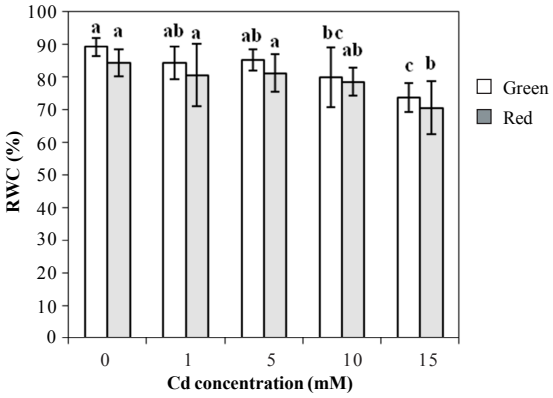

Figure 3. Relationship between relative water content (RWC %) of shoots of cabbages plants under different cadmium levels (mM).

Parameters of plant growth like biomass fresh and dry weight have been shown to be very sensitive to heavy metals in higher plants [5]. Low concentrations of Cd retard root growth without toxic effects in leaves, and moderately higher concentrations severely inhibit root growth and lead to Cd accumulation in leaves [55]. Cadmium affected root more than shoot, leading to a lower tolerance index for root. The phenomenon can be attributed to the fact that roots are the first organs receiving cadmium ions in soils via apoplastic transport, resulting in a higher Cd accumulation there [19]. Cd-exposed bean plants were distinguished by their inhibited growth and the presence of known toxicity symptoms, such as chlorosis, turning to yellowing of the first trifoliate leaves as well as some browning of the roots [18, 75].

Most of the information available about Cd physiology in plants comes from studies with the Cd-hyper accumulator *Thlaspi caerulescens* [37] and Cd-tolerant plants such as *Arabidopsis halleri* [79, 85]. It is commonly assumed that Cd, as well as other heavy metals, are taken up by transporters of essential elements, because of the lack of specificity of these proteins.

Effects of Cd on photosynthetic pigment: According to our results, the physiological responses of tested plants to cadmium exposure were much more conspicuous than was growth. The chlorophyll contents of both cabbage cultivars decreased with increasing Cd concentrations (Fig. 4). The chlorophyll content is important markers providing information on the photosynthetic ability of plants grown on media containing a heavy metal [20, 26]. Chlorophyll may be destroyed by the substitution of Mg in its

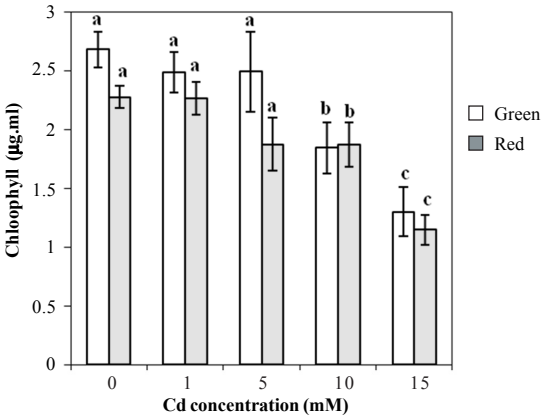

Figure 4. Impacts of Cd on the chlorophyll contents of two varieties of cabbage.

central part with Cd [32] or else the synthesis of the pigment may be disturbed at the level of its precursor, i.e. aminolevulinic acid [47, 69]. In agreement with earlier reports [71], photosynthetic activity is suppressed by heavy metals. This can be attributed to the disruptive action of metals on chlorophyll synthesis [74], on photosystem efficiency [17], on the activity of photosynthetic enzymes [45], and on plant water balance [87]. It is also attributable to chloroplast damage [8].

In addition, high concentrations of heavy metals can degrade the activities of photosynthetic enzymes and block the photosynthetic electron transport chain, resulting in reduction of chlorophyll content [73]. Vegetables growing in medium with high level of Cd showed deleterious effect in photosynthetic processes, such as chlorophyll content and photosynthesis [9, 51, 63], the activities of related enzymes [6, 49, 50, 80, 81] and photochemical reaction [35, 64]. Similar results have been obtained in other laboratory studies [27]. Continuous metabolization of chlorophyll in plants is adapted to different physiological processes. Furthermore, Cd affects the photosynthetic activity of cabbage via inhibiting chlorophyll biosynthesis and photosynthetic content. In addition, chlorophyll content was also reduced by all cadmium treatments compared to the control. From this study, it can be concluded that certain concentrations of Cd inhibit plant growth, cause chlorophyll loss, and affect photosynthetic activities. The photosynthesis of cabbage seems to be more sensitive to Cd stress probably due to more rapid damage in the cell membrane and to the biosynthesis of the photosynthetic enzymes of this species.

Effects of Cd on antioxidative enzyme activity: Antioxidative enzymes such as catalase (CAT), ascorbate peroxidase catalase (APX), and superoxide dismutase (SOD) play a key role in controlling the cellular level of these radicals and peroxides [4]. In general, the enzyme activities of CAT, APX, and SOD increased in seedlings under higher Cd concentrations compared to the control (Figs 5-7). Oxidative stress occurs when there is a serious imbalance between the production of reactive oxygen species (ROS) and antioxidant defence capacity. High level of heavy metals in plant tissues will induce exaggerated amount of free radicals and other ROS. To control the level of ROS and protect cells under stress conditions, plant tissues may produce several enzymes for scavenging ROS, including superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), and a

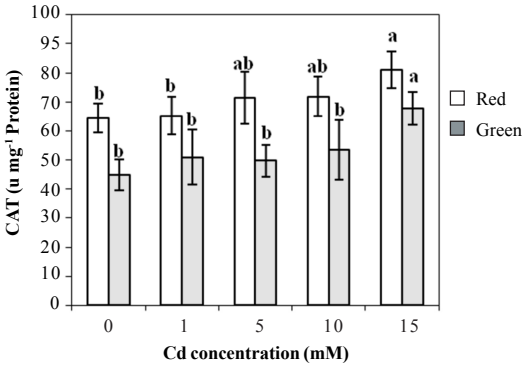

Figure 5. Effect of different Cd levels on the activity of antioxidant enzyme (CAT) in the shoot of cabbage plants.

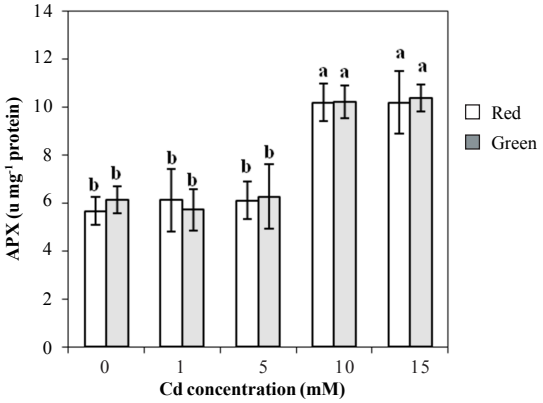

Figure 6. Effect of different Cd levels on the activity of antioxidant enzyme (APX) in the shoot of cabbage plants.

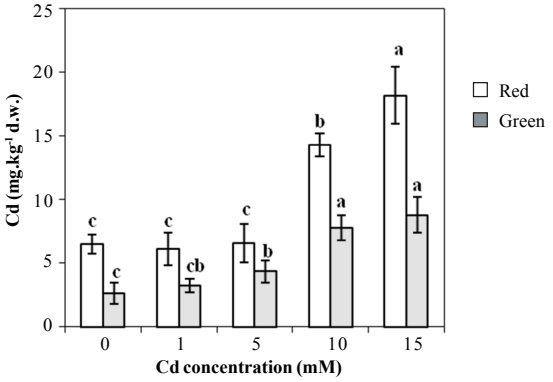

Figure 7. Effect of different Cd levels on the activity of antioxidant enzyme (SOD) in the shoot of cabbage plants.

network of low molecular weight antioxidants such as glutathione, phenolic compounds, carotenoids, and flavonoids [88]. SOD is an important antioxidant enzyme that catalyses a disproportionate amount of superoxide anion (O^{-2}) to hydrogen peroxide (H_2O_2) [14] while elevated CAT and APX activities help protect plant cell from ROS such as H_2O_2, O^{-2} and hydroxyl radicals (OH^-), which were produced in plants under stress conditions [66].

SOD is a key enzyme in the plant antioxidant defences and is uncharged of the dismutation of O_2 radicals to H_2O_2 and O_2 [3]. The analysis of total SOD activity in root extracts showed a slight increase by cadmium; however, the assay of total SOD activity in crude extracts can be subjected to interferences by compounds with mimicked SOD activity [62]. Reduction of SOD activity induced by Cd was reported in wheat [43] and bean plants [15], although the opposite effect was observed in sunflower [34], rice [31] and soybean cell cultures [65]. The enzymes SOD and CAT, and peroxidases are involved in the detoxification of O^{-2}, and H_2O_2, respectively, thereby preventing the formation of ·OH radicals. GR as well as GSH are important components of the ASC–GSH cycle responsible for the removal of H_2O_2 in different cell compartments [28, 48].

The discrepancies observed in the antioxidant activities of different plant species under Cd treatment are probably attributed to the metal concentration and period of treatment used in each case. However, the cell response to Cd is also different depending on the species, organ or tissue [10, 65], and our results could represent the cell adaptation to the metal after a long period of plant exposure. SOD is an important antioxidant enzyme that catalyses a disproportionate amount of superoxide anion (O^{-2}) to hydrogen peroxide (H_2O_2) [14], while elevated CAT and APX activities help protect plant cell from ROS such as H_2O_2, O^{-2} and hydroxyl radicals (OH^-), which were produced in plants under stress conditions [66].

In a cell, the enzyme superoxide dismutase (SOD) constitutes the first line of defence against ROS by catalysing the scavenging of $O^{-2}\cdot$ to H_2O_2, and is followed closely in importance by peroxidase (POX) and catalase (CAT) which are responsible for reduction of H_2O_2. Excess heavy metals in the soil environment cause damage to plant via growth retardation. Therefore, plant may produce more antioxidant enzyme activities to overcome cellular damages under this stress condition [41]. In this study and other reports, enzyme activity of SOD, APX, and CAT increased under heavy metal stress. This suggests that antioxidants induced in plants act to protect against stress conditions. According to Tewari *et al.* [72], plants response to heavy metals toxicity by increasing the activity of antioxidative enzymes SOD, CAT, and APX. Groppa *et al.* [21] reported that under Cd stress, the activities of the enzymes, SOD, CAT, APX, GR, and DHAR were increased in both the shoots and roots of radish [36] and pea plants [59].

Hydrogen peroxide induced by Cd excess stress: H_2O_2 content decreased in cadmium-stressed plants compared to the control (Fig. 8). Toxic concentrations of cadmium cause oxidative stress, as evidenced by the increased H_2O_2 formation and lipid peroxidation in leaves and roots of seedlings. Higher activity of catalase during a short exposure might be related to low levels of MDA, assuming the plant defense system was efficient against the stress generated by metal. Catalases and peroxidases remove the bulk of hydrogen peroxide in cells, whereas the enzymes and metabolites of the ascorbate-glutathione cycle are involved in the fine regulation of the H_2O_2 level [44].

In any case, the production of H_2O_2 may be involved in the

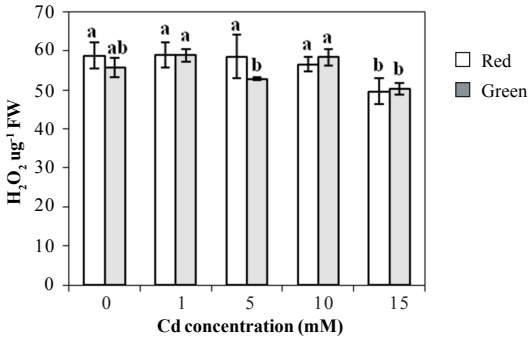

Figure 8. Effect of cadmium treatments of cabbage plants on hydrogen peroxidation.

integration of cellular processes and in the adaptation to environmental stimuli [11], since, for instance, H_2O_2 is required for cross-linking cell wall components and for regulating gene expression associated with antioxidant defence [11, 60]. During cross-linking of cell wall polymers, hydrogen peroxide is consumed in the formation of lignin precursors catalysed by POD [44] and under normal condition only a meager POD activity was detected in the meristem and elongation zone of the shoots. The peroxidase isoenzymes are located in the vacuole, the cell wall, and the cytosol, whereas H_2O_2 scavenging in the chloroplasts depends on the ASC-glutathione cycle [44]. Peroxidase induction is considered to be a general response to heavy-metal excess in plants [76]. Our results suggest that in leaves the Cd-induced increase in enzyme activity may efficiently remove H_2O_2, thus contributing to reduce the H_2O_2 accumulation in this organ.

Effect of Cd on structural changes of plant: Light micrographs of high level of Cd- treated leaves showed Cd precipitates as black deposits along the walls of xylem and phloem vessels compared to their respective controls (Figs 9 and 10). The Cd-treated plants showed changes in leaf structure with an increase in metal concentration. The treatment Cd 15 mM showed significant foliar structural changes compared to the control. The light micrographs obtained from the leaf samples of cadmium-treated plants showed changes in the distribution of chloroplasts in palisade and spongy parenchyma cells. Light micrographs of cadmium-treated plant showed thickly stained areas along the walls of xylem and phloem vessels compared to the control. Structural changes in leaves include shrinkage of epidermal, palisade, and spongy parenchyma cells and a decrease in starch content and relative water content. The cadmium accumulation in leaves of plants treated with Cd 15 mM was seen as depositions in light micrographs and as electron dense depositions along the walls of vascular bundles in light micrographs. These depositions were either simple salts of cadmium or large organic molecules, such as proteins and carbohydrates complexes, with mercury. Apart from these physiological and morphological changes, Cd accumulation also results in structural changes in leaves, stems and roots. Structural changes in leaves include shrinkage of epidermal, palisade and spongy parenchyma cells.

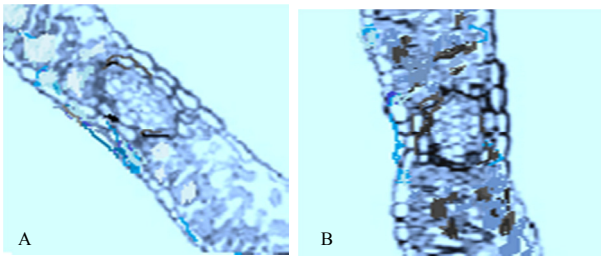

Figure 9. Light micrographs showing the transverse of section of control (A) and (B) Cd treated leaves of green cabbage.

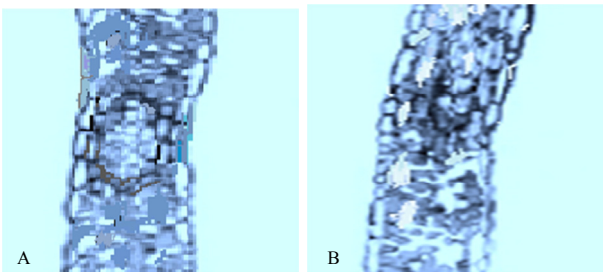

Figure 10. Light micrographs showing the transverse of section of control (A) and (B) Cd treated leaves of red cabbage.

Uptake and accumulation of metals at higher concentrations can be cytotoxic in some plant species, causing structural and ultrastructural changes affecting the growth and physiological wellbeing of the plants [7, 24, 77, 86]. Heavy metals such as zinc, hyperaccumulation results in a decrease in mesophyll cell size in *Arabidopsis halleri* [86] while Cd accumulation causes a breakdown of chloroplasts in bush bean plants [7] and decreases plant growth in *Brassica juncea* [22]. This study found that higher cadmium concentrations (Cd 15 mM) resulted in decreased translocation or uptake of metals and water from roots and stems to leaves. All physiological and biochemical processes in plants may be negatively affected by heavy metals when plants are exposure to cadmium-contaminated soil, water or air [52].

Conclusions

Cabbage was seriously affected by cadmium at high concentrations. The cabbage plant may be grown directly in soils contaminated with moderate amounts of cadmium. Further studies need to be conducted to establish the maximum amount of cadmium that the plants may tolerate, and the ability of cabbage plants to germinate and grow in such soil. Green cabbage had a higher capacity to uptake and translocation of Cd from contaminated soils than red. Conversely, cabbage accumulated significant amounts of cadmium from the soil, and its cadmium concentrations increased with time as plants grew, especially in the roots. Moreover, excess cadmium stress increased antioxidant enzyme activities of APX, CAT and SOD. Under excess cadmium stress, the induced antioxidant enzyme activity of cabbage seedlings may function as a protective mechanism to shield the plants from toxicity and exacerbated growth. Our results systematically illustrate the physiological implications of structural alterations caused by cadmium at higher concentrations. Plants of the Brassicaceae family are considered useful for phytoremediation owing to their tolerance to high concentrations of heavy metals, which may be hyperaccumulated in the tissues.

Acknowledgments

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