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Ameliorative Effect of Chelating Agents on Photosynthetic Attributes of Cd Stressed Sunflower

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Abstract

Excessive input of heavy metals in water reservoir and cultivated land primarily affects the growth and yield of crops. The aim of this work was to study the mechanism of Cd toxicity and damage to photosynthetic pigments and their efficiency and the potential of natural (OA) and synthetic chelators (EDTA) in assisting the phytoextractor sunflower plant. The pot experiment was laid out in a complete randomized way for Cd, chelators and hybrids at seedling, vegetative and reproductive stages with three replications. Cd affects the gas exchange parameters directly or indirectly by affecting the light and dark reactions, while indirect effect includes inhibition of chlorophyll and carotenoids biosynthesis and degradation and alteration in Chl a/b ratio. Among two chelators, natural chelator OA found to be very supportive in ameliorating the Cd tocixity by phytoextractor in assistance to sunflower hybrid Hysun-33.

Keywords

Sunflower, Organic Chelate, Photosynthesis, Metal Toxicity

1. Introduction

Environmental pollution has become a serious problem at global level due to human activities [1] [2]. Urbanization and industrialization are proving hazardous to environment due to greater production of industrial wastes especially metallic elements [3] [4] [5].

Among metals, cadmium (Cd) is the most toxic pollutant of water, soil and atmosphere [6]. Naturally it occurs in soil due to weathering of sedimentary parent rocks [7]. It exists in the form of sulphides, oxides, and carbonates in lead, zinc and copper ores [8]. The industrial sources include phosphate fertilizers, nickel-cadmium batteries, pigments, plastics, ceramics and solar cells. The major culprits are phosphate fertilizers, Cd containing municipal waste and sewage sludge [9] [10] [11]. Other sources include burning of fossil fuels, mine tailings, waste and slag of smelter and urban refuse [8].

The irrigation of agricultural land with Cd containing waste water reduced the concentration of photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids [12] [13] [14]. Exposure of plants to Cd causes leaf chlorosis which is most simple and visual indicator of toxicity of Cd [15] as it disrupts the structure and function of chloroplast by inhibiting the functioning of chlorophyll biosynthesizing enzymes [16] [17].

The higher Cd concentrations decreased the photosynthetic activity [18] by reducing chlorophyll synthesis, by reacting with porphobilinogen deaminase and d-ALA dehydratase −SH group, resulting in prophyrins and ALA (the intermediates of cholorophyll biosynthesis) [19] [20] and by producing chlorophyllide through protochlorophillide photoreduction [21].

Chelating agents either synthetic (EDTA, DTPA, HEDTA) or natural (oxalic acid, citric acid, acetic acid) are commonly used as amendments to induced phytoextraction [22]. EDTA is the most extensively used synthetic chelator for induced phytoextraction which dissolves the bonds between the metal and soil particles and promotes metal solubility and bioavailability for easy uptake by plants [23] [24]. Although the practice of using synthetic chelators such as EDTA, DTPA is very effective for phytoextraction of metals but their persistence in soil and low rate of degradation increases the risk of metals leaching and contamination of ground water [24] [25].

Oxalic acid is a cheap environment friendly and quickly biodegradable natural chelator that can be used in phytoextraction without having a danger of metal leaching and contamination of ground water [26] [27]. Its efficient role in Cd mobilization, translocation and phytoextraction has been extensively observed [28] [29].

This research was planned for comparative study of ameliorative potential of natural and synthetic chelators on photosynthetic attributes of two Cd treated sunflower hybrids.

2. Material and Methods

A pot experiment was performed in Old Botanical Garden, University of Agriculture, Faisalabad, Pakistan. The prevailing climatic conditions at the time of experiment were 34˚C with 66% relative humidity in month of July 2015. Pots were filled with 10 kg soil and properly irrigated with water for maintaining suitable moisture content. Ten surface sterilized achenes (with 0.1% mercuric chloride) of two sunflower hybrids (Hysun-33 and FH-533) were sown at 1 inch depth in plastic pots. Treatments *i.e.* 0, 250 and 450 mg·Cd/kg soil along with and without EDTA and OA @ 1 g/kg soil each were applied in the rooting medium. Experiment was completely randomized with three factors factorial and three replications. After complete germination, four plants were kept for determination of chlorophyll and carotenoids pigments and gas exchange attributes. All the physiological and photosynthetic attributes were determined at seedling, vegetative and reproductive stages of plants.

Gas exchange parameters includes net assimilation rate (*A*), transpiration rate (*E*), sub-stomatal CO_2 concentration (*Ci*), stomatal conductance (*gs*) and water use efficiency (*A*/*E*) were measured from a fully expanded youngest leaf by using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). The specifications/adjustments of IRGA were as follows: leaf surface area 11.35 cm^3, ambient CO_2 concentration (Cref) 342.12 μmol/mol, temperature of leaf chamber (Tch) varied from 39.2˚C to 43.9˚C, leaf chamber volume gas flow rate (v) 396 ml/min, leaf chamber molar gas flow rate (U) 251 μmol/sec, ambient pressure (P) 99.95 kPa, molar flow of air per unit leaf area (Us) 221.06 mol/m^2/sec, PAR (Q leaf) at leaf surface was maximum up to 918 μmol/m^2.

The concentration of chlorophyll (a, b and total) was calculated following the method of Arnon [30] and whereas carotenoids were calculated following the method of Davis [31]. Fresh leaves (0.2 g) were grind well and extracted in 80% acetone at 4˚C. The extract was centrifuged at 10,000 rpm for five minutes at 4˚C. The optical density of the supernatant was measured at 663, 645 and 480 nm on spectrophotometer (Hitachi-220 Japan).

3. Results

3.1. Photosynthetic Pigments

The chlorophylls concentration (chl-a, chl-b, Total) significantly ($P < 0.01$) reduced in the presence of Cd in growth medium in both hybrids. Hybrids varied significantly at all three growth stages. FH-533 showed maximum reduction of 92% in chlorophyll-a contents (chl-a), 96% in chl-b, 80% in chl-T and 96% in carotenoids by application of 450 mg·Cd/kg at seedling stage while in Hysun-33 this reduction was 67%, 76%, 55% and 90% respectively as compared to control. EDTA alone significantly affected chlorophylls and carotenoids at reproductive stage and caused 12%, 21%, 13% and 14% reduction in chl-a, chl-b, chl-T and carotenoids content of Hysun-33 and and 14%, 22%, 20% and 23% of FH-533 respectively (**Figure 1**). The OA alone significantly reduced 6%, 10%, 8% and 23% chl-a, chl-b, chl-T and carotenoids in Hysun-33 and 16%, 8%, 8% and 15% in FH-533 only at seedling stage (**Figure 1**).

Cadmium addition @ 250 and 450 mg/kg in growth medium in the presence of 1 g·EDTA/kg imposed more severe effects on photosynthetic pigments of FH-533 than Hysun-33 as compared to control treated plants at vegetative and reproductive stages (**Figure 1**). Cd × OA interaction was significant for seedlings of two hybrids. In the presence of 1 g·OA/kg soil maximum reduction was observed in combination with 450 mg·Cd/kg soil which caused reduction in chlorophylls and carotenoids contents of Hysun-33 and FH-533 respectively (**Figure 1**).

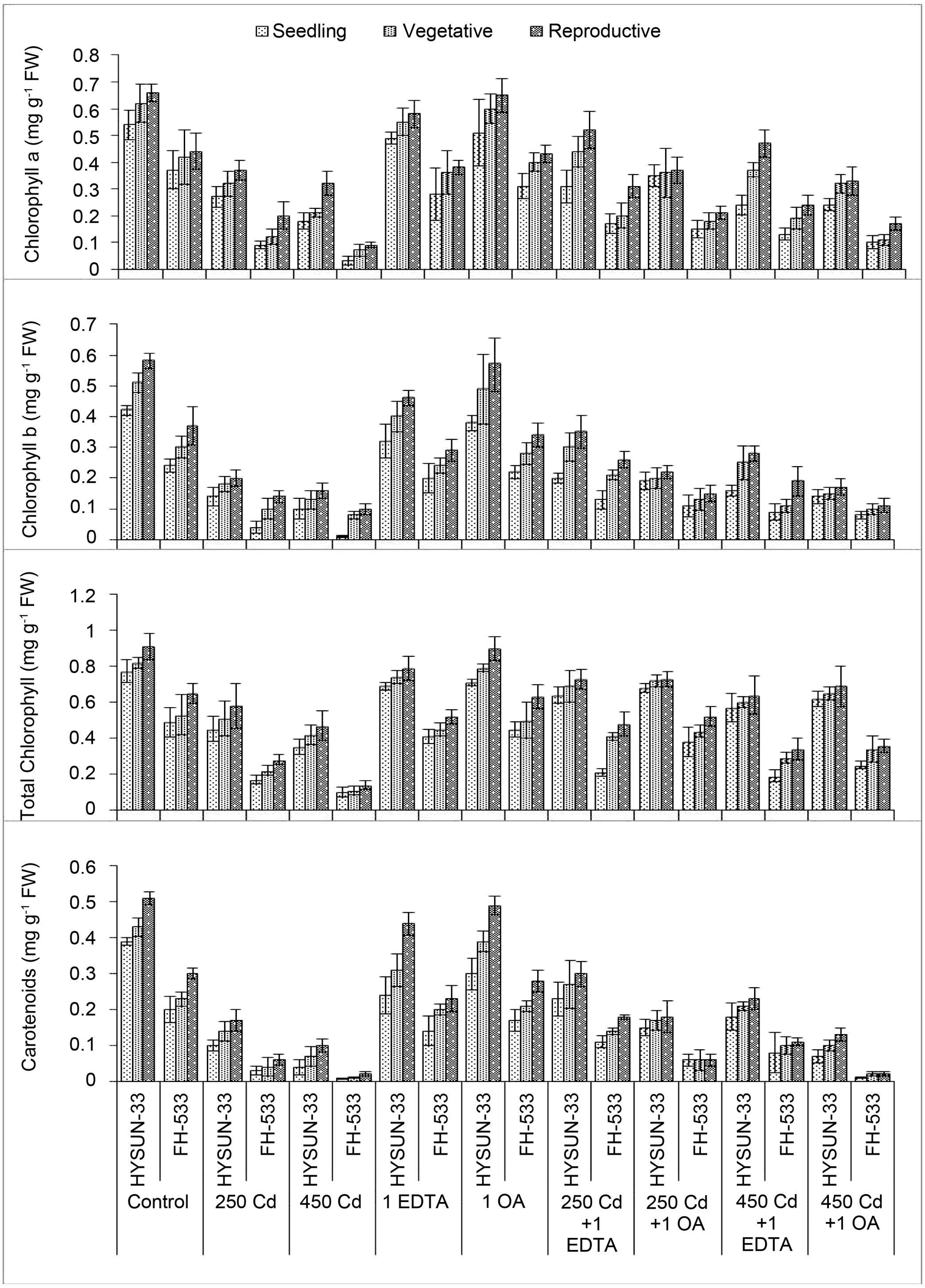

Figure 1. Effect of 250 and 450 mg/kg Cd along with EDTA (1 g/kg) and OA (1 g/kg) on pigments concentration of two sunflower hybrids (HYSUN-33 and FH-53) at seedling, vegetative and reproductive stages.

3.2. Gas Exchange Attributes

Statistical analysis revealed that cadmium significantly ($P < 0.01$) affected the photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs) and sub-stomatal CO_2 concentration (Ci) of treated plants at all three stages *i.e.*

seedling, vegetative and reproductive stages. EDTA and OA alone had significant effects on gas exchange attributes only at seedling stage. Hybrids differed significantly throughout the studied period; Hysun-33 had better *A*, *E*, and *Ci* as compared to FH-533. Cadmium application @ 250 and 450 mg·Cd/kg resulted in decrease of gas exchange attributes at seedling stage and this reduction was greater at reproductive stage of both the hybrids. EDTA significantly reduced the physiological attributes at seedling stage in both the selected hybrids *i.e.*, Hysun-33 and FH-533 but found significant at reproductive stage for *Ci* (**Figure 2**).

Cd × EDTA interaction was also significant for these parameters at seedling stage, so the addition of EDTA @ 1 g/kg in growth medium containing 250 and 450 mg·Cd/kg slightly affected them as compared to Cd alone (**Figure 2**). OA impact alone was statistically significant ($P < 0.01$) at vegetative and reproductive stage of two hybrids for *Ci* and OA plus Cd interactive effect was proved significant for *A*, *E* and *gs* only at seedling stage. The impact of OA alone was mild as

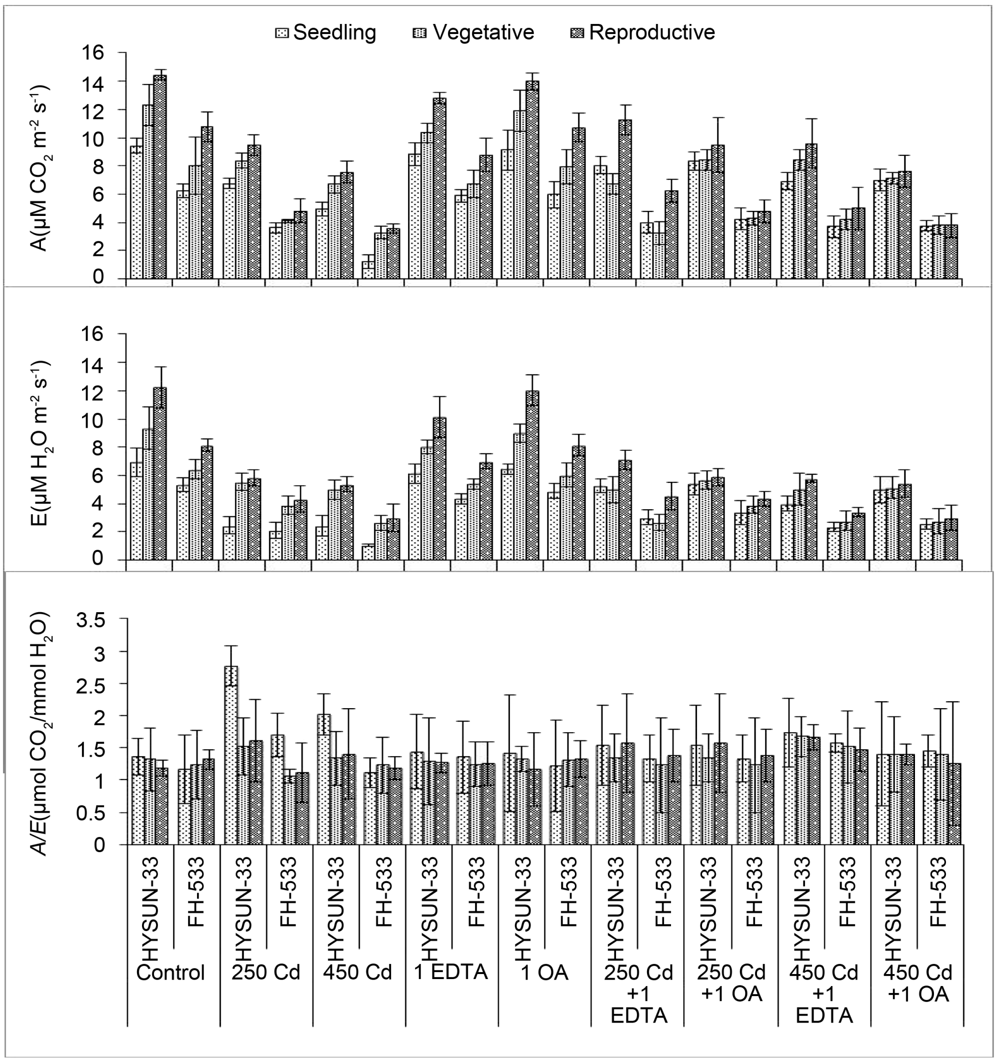

Figure 2. Effect of 250 and 450 mg/kg Cd along with EDTA (1 g/kg) and OA (1 g/kg) on net assimilation rate (*A*), transpiration rate (*E*) and water use efficiency (*A*/*E*) of two sunflower hybrids (HYSUN-33 and FH-53) at seedling, vegetative and reproductive stages.

compared to combined application with Cd and 1 g·OA/kg + 450 mg·Cd/kg affected the gas exchange attributes of two hybrids greater than 1 g·OA/kg + 250 mg·Cd/kg respectively (**Figure 2** and **Figure 3**).

From analysis of variance it is clear that all the main factors *i.e.*, Cd, OA and EDTA proved non-significant alone and in combination for water use efficiency (*A*/*E*) of sunflower hybrids throughout the studied. Only significant factor was hybrid at seedling stage. Minimum water use efficiency 1.11 μmol·CO_2/mmol·H_2O was measured in FH-533 under 250 mg·Cd/kg application at vegetative stage and maximum 3.19 μmol·CO_2/mmol·H_2O was maintained by Hysun-33 under 250 mg·Cd/kg application at seedling stage.

All the interactions for hybrids, Cd and chelating agents remained statistically non-significant ($P > 0.05$) for pigments and gas exchange attributes at seedling, vegetative and reproductive stages.

3.3. Discussion

a. Pigments

Reduction in the concentration of photosynthetic pigments such as chl-a, chl-b, chl-T and accomplice pigments like carotenoids has been found a common symptom of metal toxicity in a number of species [12] [13] [14]. Decline in

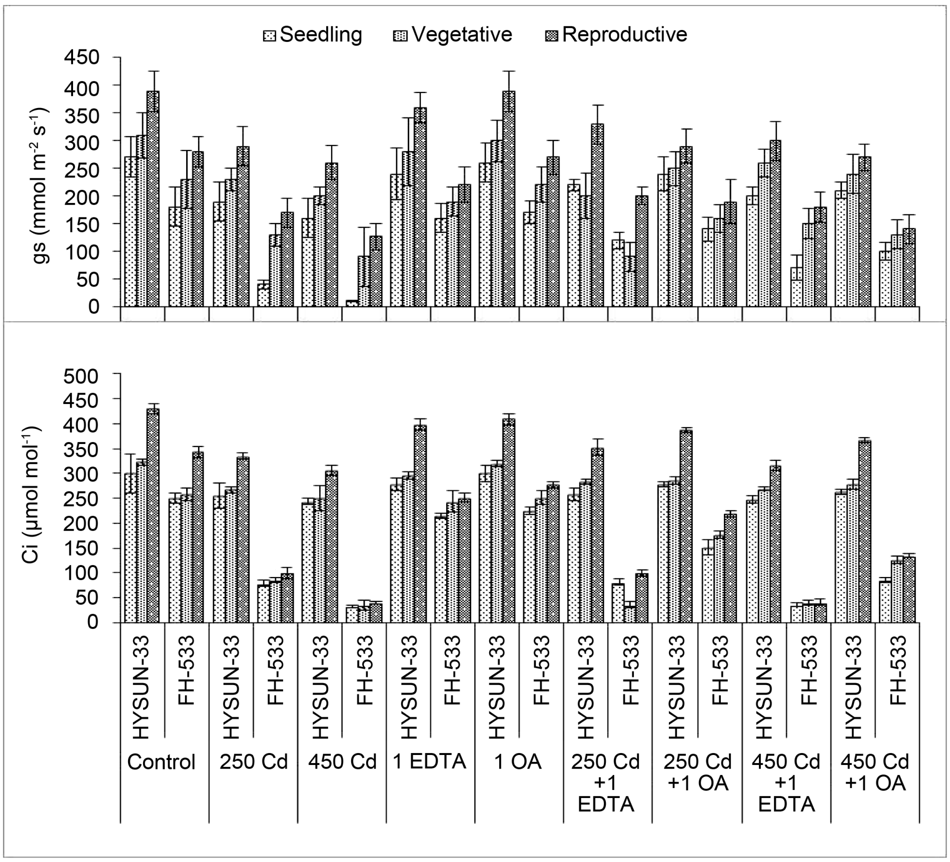

Figure 3. Effect of 250 and 450 mg/kg Cd along with EDTA (1 g/kg) and OA (1 g/kg) on sub-stomatal CO_2 concentration (*Ci*) and stomatal conductance (*gs*) of two sunflower hybrids (HYSUN-33 and FH-53) at seedling, vegetative and reproductive stages.

photosynthetic pigments (Chlorophyll a, b and carotenoids) was noticed in sunflower under stress condition [30]. Exposure of plants to Cd causes leaf chlorosis which is most simple and visual indicator of Cd toxicity [15].

In present study Cd caused significant reduction in chl-a, chl-b, chl-T and accomplice pigment carotenoids especially at seedling stage as in pea plants [31] [32]. Cadmium reduced the chlorophyll concentration [33] [34] by removing the Mg ion from its binding position in chlorophyll [35] resulting in degradation of chlorophyll molecule [36] or by inhibiting the activities of enzymes involved in cholorophyll biosynthesis like protochlorophyllide reductase [37] porphobilinogen deaminase [38] aminolevulinic dehydratase [39] thus causing deficiency in Fe^{2+} and Mg^{2+} supply necessary for chlorophyll synthesis, and also causing inhibition of carbonic anhydrase activity due to Zn^{2+} scarcity [40].

Both chealtors *i.e.*, EDTA and OA helped in reducing the damaging effects of Cd on pigments; however OA had given more assistance in ameliorating the damaging effects of Cd than EDTA. The sensitive hybrid FH-533 experienced more reduction in pigment contents than Hysun-33 (**Figure 1**). EDTA improved pigment status of *Arundo donax* L. grown in Cd, Pb and arsenic contaminated soil [41] and *Tribulus terrestris* [42].

Chelators treated plants might be of increased concentration of Fe in the shoots which have influence on chlorophyll b structure furthermore formation of metal-chelator complex which is incapable to penetrate the membranes of plant hence chelators decrease the metal mobility and then its toxicity [43].

Broadly, in interpretation of the high redox potential of Cd it is interpreted that during biosynthesis of photosynthetic pigments the reductive steps are inhibited due to Cd stress. In addition the activity of vital enzyme protochlorophyllide reductase, responsive for protochlorophyll reduction into chlorophyll known to be repressed [44].

b. Gas exchange parameters

In the present investigated study photosynthetic rate (*A*), transpiration rates (*E*), stomatal conductance (*gs*) and substomatal CO_2 concentration (*Ci*) were decreased and water use efficiency (*A*/*E*) increased under the treatment of Cd, EDTA and OA in combination or by their separate application in all harvest but this increasing and decreasing pattern in gas exchange parameters were more dominating at seedling stage. These trends are more pronounced when Cd was separately added in growth medium. According to present findings EDTA and OA application along with Cd helped in improving the gas exchange parameters of sunflower hybrids. The comparison between chelating agents showed that OA proved more helpful in reducing the adverse effects of Cd on gas exchange parameters than EDTA (**Figure 2**). EDTA influenced gas exchange parameter *i.e.*, *A*, *E*, *gs*, *Ci* and *A*/*E* of *Tribulus terrestris* growing in metal contaminated soil [42]. In present study the improved efficiency of gas exchange parameters by chelators (EDTA, OA) application might be due to formation of chelate with Cd which reduced the noxious effects of Cd [45]. Transpiration is a vital process for the enhancement of water soluble components or uptake of contaminants and flux to the upper plant parts [42]. The greater *A*/*E* in (Cd+ chelator) treated plants can be interpreted as plants effort to improve their water regime. Improved *A*/*E* is largely a meaning of reduced water use then overall/net improvement in production of plant or biochemistry of assimilation [46].

Cd induced negative effects on *A*, *E* and *Ci* in *Brassica napus* [47], Pea and barley [48], Maize [49]. Reduction in photosynthetic rate due to Cd toxicity might be the result of reduction in chlorophyll contents by its reaction with porphobilinogen deaminase and d-ALA dehydratase –SH group, resulting in prophyrins and ALA the intermediates of cholorophyll biosynthesis [19] [20] by producing chlorophyllide through protochlorophillide photoreduction [21]. In mitochondria and chloroplast for heme and chlorophyll synthesis ALA formation is the rate controlling and regulating step during tetrapyrrole biosynthetic pathway [50]. Cd restricts ALA synthesis reduce the chlorophyll synthesis and many other photosynthesis related reactions [16] [51] by causing Fe insufficiency [51]. The heme and chlorophyll biosynthesis is interrupted by Cd, as it reacts with the –SH functional groups like ALA dehydratase, ALA synthase, protochlorophyllide reductase and PBG deaminase [52].

Low Cd concentration increased transpiration rate of plants, however, decreased due to higher Cd concentrations. In *Phragmites australis* 50 µM Cd had no effect on this parameter, while reduction was noticed with 100 µM Cd treatments [53]. Higher Cd concentrations caused structural abnormalities in stomata resulting in rudimentary flawed stomata with reduced size and number, leading to depreciate stomatal conductance and modified rate of transpiration [54].

4. Conclusion

Cd affects the gas exchange parameters directly or indirectly by effecting the light and dark reactions. The direct effect of Cd on light reactions is on photosynthesis O_2 evolution, photophosphorylation and reduction of NADP, while indirect effect includes inhibition of chlorophyll biosynthesis and degradation and alteration in Chl-a and b ratio.

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