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Determination of EC_{50} of Cd and Evaluation of Growth and Biochemical Response of Palak Plants (*Beta Vulgaris*) to Different Cd Treatments

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This study was designed to evaluate the growth, biochemical response and Cd accumulation pattern of Palak (*Beta vulgaris*), variety of All green H1 plants for a range of Cd treatments [control (0), 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L) at two sampling stages, 25 days after germination (25 DAG) and 50 DAG. The present research also quantified the EC_{50} value of Cd for 50% biomass inhibition in palak plants. Increasing Cd concentration had pessimistic effects on growth and biomass. Plant height, biomass, total leaf area and yield decreased significantly on increasing Cd treatment at the two sampling stages ($p<0.05$). EC_{50} for biomass reduction was found to be 27.42 mg/L. Total phenols, thiols and MDA content elevated on increasing Cd concentration. Photosynthetic pigments, chlorophyll a and b reduced significantly by 50.81% and 48.88%, respectively at highest 50 mg/L Cd treatment. A significant interaction ($p<0.05$) of Cd treatment × plant age was found on Cd content analysis at the two sampling stages, Cd content increased with increasing treatment duration.

Keywords: Cadmium stress, Chlorophyll, Growth and biomass, Interaction

Introduction

Heavy metal contamination has become a common problem in soil and aquatic ecosystems. It is the result of unprecedented bioaccumulation of heavy metals in the environment.[1] High bioavailable metal concentration in soil and water may prove deleterious to human, plants and aquatic life. Cd is one of those heavy metal that is considered toxic for plants, and its phytotoxicity is attributed to its high mobility in soils. Due to this property, its uptake is facilitated in plants along with other micronutrients like Zn, Cu and Fe.[2] Use of chemical fertilizers, fungicides and waste water irrigation accounts for anthropogenic Cd sources in the soil. From soil, the heavy metals enter the aquatic ecosystems through runoff water. Glyphosate is commonly used herbicides by farmers which causes desorption of Cd from soil, which then is carried through water in the aquatic ecosystems.[3] In many agricultural lands and in suburban areas, Cd contaminated water from industries and sewage sludge is used for irrigation of cereals and vegetable crops and thus results in transfer of Cd into these crops.[4] Leafy vegetables show a good potency to accumulate heavy metals, particularly in their leaves. The present work is carried on Palak or Indian spinach (*Beta vulgaris* var. All green H1). It is a leafy vegetable which is a good source of iron and its market depends upon its local production. In Varanasi, this vegetable is generally grown in the sub urban areas and is mostly irrigated by heavy metal contaminated waste water.

Uptake of excessive amount of Cd by plants leads to abnormal morphological and biochemical responses, resulting in altered plant growth characteristics.[5] Cd toxicity is responsible for reduction in plant biomass and inhibition of root growth. Cd and other heavy metals are responsible for generation of reactive oxygen species (ROS) in plant cells, which is termed as oxidative stress. Cd stress stimulates overproduction of oxygen free radicals such as superoxide radicals (O_2°), hydroxyl radical (OH°) and hydrogen peroxide (H_2O_2).[6] As a result of this, a range of biochemical defence mechanisms come into action. Biochemical studies have shown that heavy metal stress leads to lipid peroxidation of plant cell membrane, manifested in the increased MDA (malonialdehyde) content.[7] In response to Cd induced oxidative stress, plants have evolved a series of enzymatic and non enzymatic antioxidative systems, whose efficiencies and activities are reported to increase under Cd stress. Research findings have confirmed that total phenolic content and flavinoids increased under Cd stress.[8] Superoxide dismutase (SOD) activity increased in

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pepper leaves and pea seedlings when provided Cd stress.

It has also been reported that Cd toxicity has suppressed photosynthetic ability of plants. Photosynthetic activity has direct relation with plant growth and development. Cd toxicity affects photosynthesis by inhibiting biosynthesis of chlorophyll by hindering the uptake and translocation of mineral nutrients required for chlorophyll synthesis.[9]

As per World Health Organization (WHO), the permissible limit of Cd in the soil is 4 mg/kg.[10] To assess the toxicity of heavy metals, EC_{50} is an important parameter. EC_{50} determines the effective concentration of a toxicant causing 50% inhibition of a response. The study of parameters such as bioconcentration and translocation factor helps to evaluate how effective the plant is in accumulating and transferring the heavy metal from soil. Soil heavy metal threshold serves as a connecting link between soil pollution and food crop safety. Many remedial measures are being undertaken to mitigate Cd toxicity like phytoremediation using hyper accumulator plants, chemical extraction, bioleaching, adsorption using nanoparticles like CuO to adsorb heavy metals.[11] Ensuring vegetable crop safety is of utmost importance, as vegetables form an important part of human diet. This study was designed with the aim: (a) To estimate the EC_{50} of Cd with respect to biomass in Palak plants, (b) To assess the efficiency of defense machinery of Palak plants at different Cd concentrations, (c) To analyze Cd bioaccumulation in different plant parts and translocation efficiency.

Materials and methods

Experimental Setup, Treatment and Soil Analysis

The present experiment was conducted in the Botanical Garden of Banaras Hindu University, Varanasi. The experimental set up was in randomized block design with six treatments [control (0), 10, 20, 30, 40 and 50 mg/L], each with three replicates. Every pot was filled with 4 kg soil and primary nutrients nitrogen, phosphorous and potassium were added. The seeds of tested Palak (*Beta vulgaris*) variety, All Green H1, were obtained. Total 10 seeds were sown at regular spacing in each pot. After emergence, the seedlings were thinned to five seedlings per pot. First treatment was given at 5 days after germination (DAG) and subsequently repeated at every 5 days interval up to 45 days. At every treatment 20 ml of Cd solution was given to each pot. Physicochemical analysis of soil was done prior to sowing. Soil type of the botanical garden was alluvial with pH of 7.42 ± 0.01, organic carbon content (%) 0.68±0.03, available N 186.08 kg/ha, Olsen P 25.42 kg/ha available K 49.67 mg/kg available Cd 0.09 mg/kg.

Growth and Biochemical Analysis

Plants were sampled in triplicates for growth analysis at two sampling stages, 25 DAG and 50 DAG. Root growth, shoot growth and total plant growth was measured using the meter scale. Fresh weight was measured on the electronic balance. Numbers of leaves were counted and total leaf area was measured using Leaf Area meter. For biomass analysis, the plants were oven dried and then weighed on the electronic balance. To calculate yield, fresh weight of Palak leaves were measured at the time of final harvesting.

Biochemical analysis was also done at 25 DAG and 50 DAG. Total phenol content was determined by method of Bray and Thorpe[12] using Folin-Ciocalteau reagent. Thiol was estimated with the protocol of Fahey and Brown (1978).[13] Lipid peroxidation was measured in terms of Malanoldialdehyde content following the protocol of Heath and Packer (1968).[14] By extracting leaf tissues in 5 ml 5% TCA, 1 ml of supernatant was obtained. To this 4 ml of TBA was added and boiled for 30 minutes and then cooled immediately. Golden yellow colour developed, OD was taken at 600 nm.

Chlorophyll (a and b) and Carotenoids were quantified in leaf extracts using the formula of Machlachlan and Zalik (1963).[15] The result was expressed in terms of dry weight (d.w). In 10 ml 80% acetone, 1 g leaf sample was crushed. The extract was filtered using muslin cloth. OD was determined for chlorophyll a and b at 645 nm and 663 nm and expressed as mg/g d.w. Protein content quantization was done according to Lowry *et al* (1951)[16] using Bovine serum albumin as reference material. SOD activity was determined using methodology of Beauchamp and Fridorich (1971).[17] In 100 mM Phosphate buffer (pH=7.8) 0.1 g leaf tissue was crushed and then centrifuged for 25 minutes at 15,000 rpm at 4°C. Activity was expressed as unit/g f.w.

Measurement of Photosynthetic Rate

Net photosynthetic rate was measured at the two sampling stages using a portable photosynthetic unit (LI-COR, LI-6200, INC) at ambient conditions.

Determination of Cd Content in Plant

The dried plant samples were analyzed for Cd content at both 25 DAG and 50 DAG. Dried plant and soil samples were grinded until they passed through a 2 mm mesh sieve. In 10 ml of Aqua regia - $HCLO_4$ mixture (v:v, 4:1), 0.1 g of dried plant samples were digested at 150°C until a clear solution was obtained.[18] The solution was filtered and Cd concentration in the filtrate was determined by Atomic Absorption Spectrophotometer and expressed in mg/kg d.w. Translocation factor (TF) was calculated to determine the Cd transfer efficiency of the plants. TF = Cd content in shoot/Cd content in root.

Statistical Analysis

The statistical analysis was performed using SPSS software (version 20). The data were subjected to Duncan's multiple range tests to separate the means using significance level of $p < 0.05$. Cd treatment × age interaction was evaluated using two-way analysis of variance (ANOVA). EC_{50} was calculated for biomass using linear regression equation.

Results and Discussion

Effect of Cd Stress on Phenolic Content and Thiol Content in Palak Leaves

Estimation of total phenols at two samplings is shown in Fig. 1a. At 25 DAG, at 30, 40 and 50 mg/L Cd treatment, total phenol content increased significantly by 19.65%, 24.01% and 38.42% respectively, over control. However, at 50 DAG, on providing 10, 20, 30, 40 and 50 mg/L Cd treatment, total phenol content increased significantly by 35.29%, 32.99%, 117.90%, 144.7% and 146.54%, respectively, as compared to control. Thus, total phenolic content increase was found to be more at 50 DAG. Phenolics act as biomarkers for heavy metal stress.[19] They act as antioxidants, preventing evolution of free radicals as a result of Cd stress.[20] A recent finding has indicated that on providing Cd stress, total phenolic content increased in blueberry plantlets (*vaccinium corymbosum* L.) aftermath of which, a decrease in oxidative damage was observed.[21]

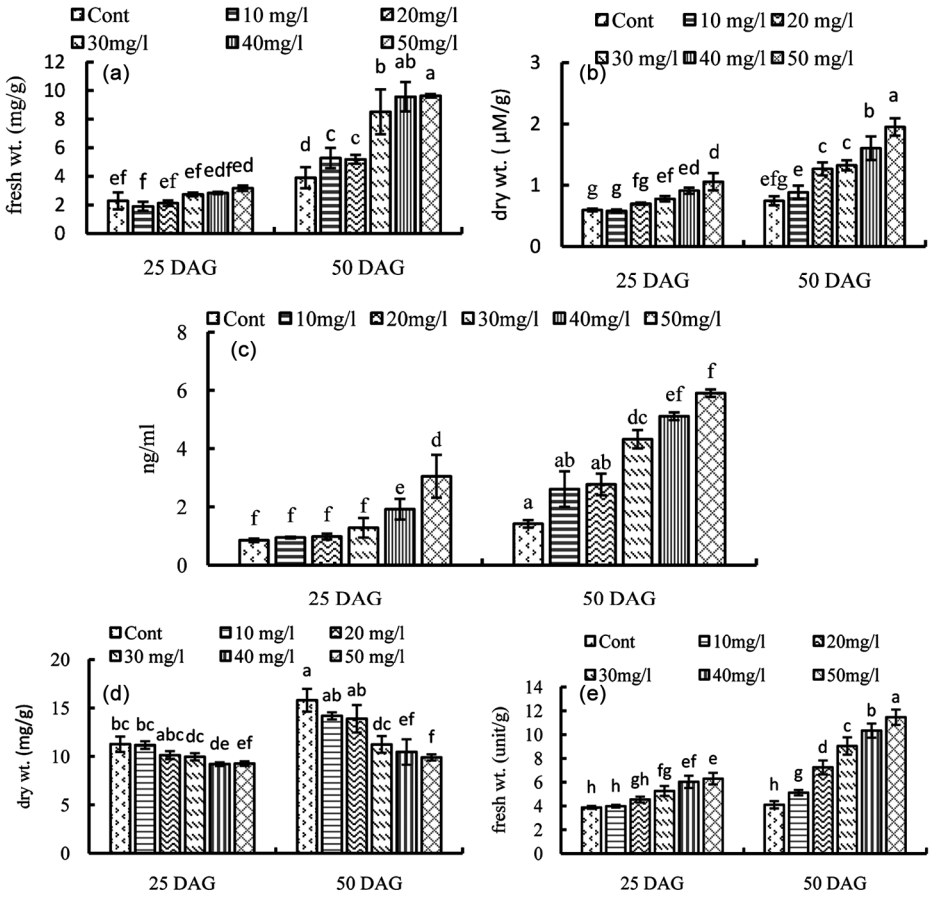

Fig. 1 — Effect of varying Cd concentration on (a) total phenols, (b) total thiols, (c) lipid peroxidation, (d) Protein content, & (e) superoxide dismutase (SOD) activity at two sampling stages (25 DAG and 50 DAG)

Thiols possess redox properties. High thiol content indicates presence of abiotic stress, various thiols act as metal chelators binding heavy metals and thus reducing their damage.[22] At first sampling (25 DAG), at 30, 40 and 50 mg/L Cd concentration given, the significant rise in thiol content was found to 30%, 51.66% and 76.66% respectively as compared to control as shown in Fig. 1b. However, no significant effect was observed at 10 and 20 mg/L Cd concentration. At 50 DAG, at 20, 30, 40 and 50 mg/L Cd, the significant increase in thiol content was found to be 69.33%, 77.33%, 113.33% and 160%, respectively when compared to no Cd treatment. Thus, percentage increase in thiol content was found to be more at second sampling.

Lipid Peroxidation of Membranes in Response to Cd Stress

Malonaldihyde (MDA) content representing peroxidation of membrane lipids is shown in Fig. 1c. At 25 DAG, at Cd concentration of 40 and 50 mg/L, MDA content increased significantly by 125.88% and 258.8% respectively, with respect to control, else no significant effect was observed at 10, 20 and 30 mg/L Cd. However, at 50 DAG, at all the Cd concentration of 10 to 50 mg/L, significant increase in MDA content was found to be 83.80%, 95.07%, 204.92%, 259.85% and 316.19% respectively, with respect to control. Higher is the degree of lipid peroxidation, more is the production of free radicals, which is manifested in increased MDA content.[23] Similar results were obtained when 0.3 mM of Cd treatment, increased lipid peroxidation in seedlings of mung bean.[24] MDA content increase was significantly more at 50 DAG as compared to 25 DAG. Thus, MDA content showed a significant dose × duration interaction.

Soluble Protein Content in Palak Leaves

At 25 DAG, no significant effect was observed in protein content at the five treatments. However, at 50 DAG, at 30 to 50 mg/L Cd treatment, a significant decrease of 28.84%, 33.77% and 37.31% respectively, was observed with respect to control (Fig. 1d). Reduced protein content in palak leaf extracts hints towards inhibition of enzyme action by Cd. It causes inactivation of many enzymes required for protein synthesis.[25] It has been reported that Cd inhibits amino acid mobilization at protein synthesis sites.

Determination of SOD (Superoxide Dismutase) Activity in Palak

Among enzymatic antioxidants, Superoxide Dismutase (SOD) is the first one to come into action against reactive oxygen species (ROS). Higher SOD activity was observed in leaves at 25 DAG and 50 DAG (Fig. 1e). Further, the activity was recorded more at 50 DAG. At first sampling, at 30, 40 and 50 mg/L Cd treatment the activity in the leaves increased significantly by 35.82%, 55.41% and 62.62% respectively as compared to control. At second sampling, the significant increase in activity of SOD was 53.60%, 91.73%, 119.06% and 143% respectively as compared to control at 20 – 50 mg/L Cd treatment. SOD activity has also been found to be increased in *Koelreuteria paniculata* under Cd stress.[26] The increase in SOD activity is due to accumulation of H_2O_2 as a result of Cd stress, which triggers the antioxidative enzyme activity.

Photosynthetic Pigment (Chlorophyll a, b and Carotenoids) Response to Cd Stress in Palak Leaf Extracts

The responses of chlorophyll and carotenoids have been shown in Fig. 2(a–d). From the results, it is clear that increasing Cd treatment negatively affected the total chlorophyll content (a and b) and carotenoids). At 25 DAG, a significant reduction of 26.41% and 33.96% in chlorophyll a was noticed at 40 mg/L and 50 mg/L Cd treatment. At the same Cd concentrations, the reduction in chlorophyll b was 35.41%. At 50 DAG, at 30–50 mg/L, the significant decrease in chlorophyll a was 24.59%, 45.90% and 50.81% over control. However, significant reduction in chlorophyll b content was recorded for all the five Cd treatment when compared to control. Thus, chlorophyll b was affected more than chlorophyll a. On combining the total chlorophyll content decreased. Similarly, a decrease of 50% and 32% in chlorophyll a and chlorophyll b respectively has been reported in *Coronopus didymus* when treated with 400 mg/kg Cd.[27] Decreased chlorophyll activity may be attributed to disrupted chloroplasts and photosynthetic apparatus due to heavy metal stress.[28] ROS generation promotes peroxidation of chloroplasts membranes due to Chlorophyllase activity which becomes active under heavy metal stress.[29] Carotenoid content is shown in Fig. 2(d). At first sampling, no significant effect was observed upto 40 mg/L Cd treatment. At second sampling, a significant decrease of 22.22% and 29.77% was observed at 40 and 50 mg/L Cd treatment. Thus, the observations confirm that chlorophyll is more sensitive to Cd toxicity as compared to carotenoids. This may be due to the reason that carotenoids act as antioxidants as compared to chlorophyll under stress conditions.[30] However, reduction in carotenoid content at high Cd concentration might be due to deformed chloroplasts

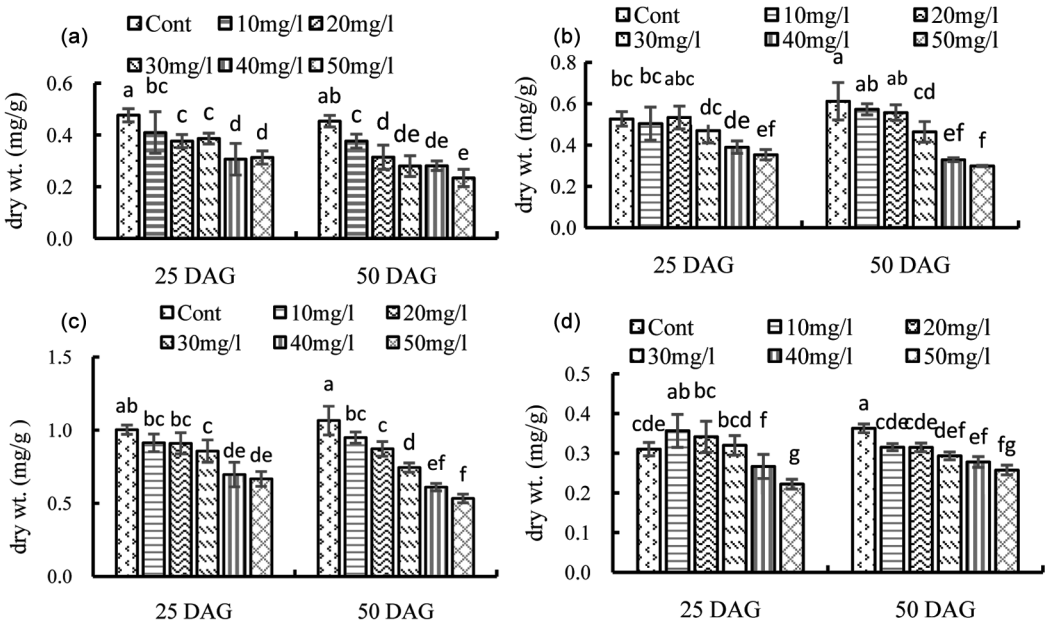

Fig. 2 — Photosynthetic pigment (chlorophyll a, b and carotenoids) content in response to Cd stress in Palak leaf extracts at two sampling stages (25 DAG and 50 DAG): (a) chlorophyll a, (b) chlorophyll b, (c) Total chlorophyll, & (d) Carotenoids

resulting in irregular thylakoids, which are the sites of carotenoid production.

Photosynthetic Activity under Cd Stress

Photosynthetic activity declined under Cd stress (Fig. 3). At first sampling, at 30, 40, and 50 mg/L Cd treatment, a significant decrease of 28.93%, 46.04% and 54.01% was observed with respect to control. At second sampling, at the same Cd treatment mentioned above, a significant decrease of 24.14%, 40% and 51.88% was there over control. Thus, at Cd concentration below 30 mg/L no significant effect was observed at both sampling stages. Reduction in chlorophyll content and total leaf area due to Cd stress is responsible for decrease in photosynthetic rate. Inhibition of chlorophyll synthesis or its destruction due to Cd toxicity is held responsible for reduced photosynthetic activity.[31]

Growth Response to Cd Stress

The effect of elevated Cd concentration on growth characteristics of Palak plants is shown in Table 1. Results show that increased Cd concentration had deleterious effects on morphological parameters. Cd treatment significantly affected the growth of palak plants. At 25 DAG, when treated with 30, 40, and 50 mg/L Cd, the plant height decreased significantly by 12.28%, 23.90% and 25.89%, respectively over control. At 50 DAG, at same Cd concentration stated above, plant height decreased significantly by

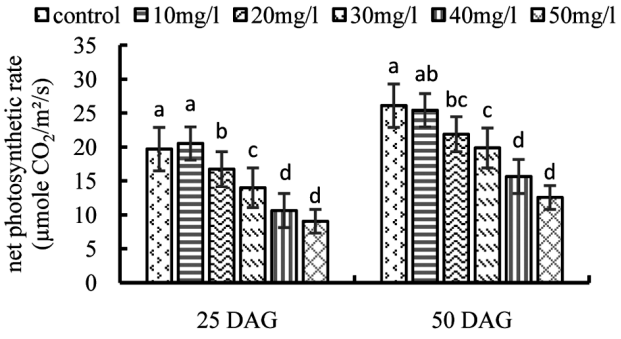

Fig. 3 — Photosynthetic activity under Cd stress at 25 DAG and 50 DAG

12.79%, 19.11% and 26.97%, respectively, as compared to control. The plants did not show any significant effect at 10 and 20 mg/L Cd treatment. Thus, increasing Cd concentration had reductive effect on plant height at both the sampling stages. The decrease in plant height is due to the reason that Cd competes with essential minerals required for growth and development of plant, and replaces them, thus hampering the growth of plants.[32] It has been demonstrated that Cd treatment of 5 mg/kg in soil reduced concentration of essential nutrient elements like K, Ca and Zn in pea seedlings.[33]

Effect of Cd Stress on Biomass of Palak Plants and Quantification of EC_{50}

Biomass analysis (Table 1) at first sampling showed that on increasing Cd concentration from 10 to 20, 30,

Table 1 — Comparative study of growth, biomass and morphological features at 25 DAG and 50 DAG; Each value is mean of triplicates ± SE (standard error), calculated at significance level $p \leq 0.05$

			25 DAG			
Parameter	control	10 mg/l	20 mg/l	30 mg/l	40 mg/l	50 mg/l
Plant growth (cm)	17.1±0.15^{a}	17.7±0.85^{a}	16.6±0.23^{a}	15±0.20^{b}	14.5±1.46^{c}	13.2±0.25^{d}
Biomass (g)	1.72±0.20^{a}	1.22±0.09^{b}	1.08±0.07^{bc}	0.89±0.03^{cd}	0.78±0.06^{de}	0.64±0.13^{e}
Number of leaves	13.67±1.52^{ab}	14.00±2.64^{a}	12±1^{ab}	11.67±1.52^{ab}	10.33±1.53^{b}	10.33±0.58^{b}
Total leaf area (cm^2)	209±2^{a}	195.33±6.03^{ab}	183.33±6.11^{b}	160.67±16.26^{c}	142±9.16^{d}	130.67±4.04^{d}
			50 DAG			
Parameter	control	10 mg/l	20 mg/l	30 mg/l	40 mg/l	50 mg/l
Plant growth (cm)	25±1.23^{a}	24.8±0.36^{ab}	24.1±0.81^{b}	22±0.41^{c}	19.1±1.77^{d}	18.6±0.57^{e}
Biomass (g)	3.59±0.43^{a}	3.47±0.26^{a}	3.00±0.07^{b}	1.84±0.26^{c}	1.32±0.21^{d}	1.09±0.09^{d}
Number of leaves	21.66±2.08^{a}	20.33±2.52^{a}	19±2^{ab}	18±1^{b}	13.66±1.53^{c}	12.33±2.08^{c}
Total leaf area (cm^2)	298±10.81^{a}	265±21^{b}	230±17.09^{c}	198.6±10.60^{d}	187±12.29^{d}	172.66±7.09^{d}
Yield (g/pot)	11.04±1.07^{a}	9.24±0.52^{b}	8.33±1.17^{bc}	7.24±0.24^{cd}	6.61±0.64^{d}	5.03±0.10^{e}

40, and 50 mg/L, a significant decrease of 28.65%, 37.42%, 47.95%, 54.97%, and 60.22%, respectively was observed as compared to control. At second sampling, a significant reduction of 16.43%, 48.75%, 63.23% and 69.63% was observed at 20, 30, 40, and 50 mg/L Cd treatment, respectively. On the basis of dry matter response to Cd stress at second sampling stage, dose versus biomass response graph (Fig. 4) was plotted and EC_{50} was calculated using linear regression equation. EC_{50} value was found to be 27.4 mg/L Cd treatment. At this concentration the dry matter content reduced to half (50% reduction) when compared to control. Reduced biomass is due to reduced chlorophyll content and reduced photosynthetic activity, so less biomass fixation.[34] Also, due to reduction in total leaf area, photosynthetic ability of the plant may have been affected resulting in less carbon fixation and thus reduced biomass.

Number of Leaves and Total Leaf Area

Number of leaves and total leaf area was affected significantly at higher doses of Cd as shown in Table 1. At second sampling at Cd concentration of 30, 40, and 50 mg/L, the significant decrease in total number of leaves was 16.90%, 36.93% and 43.07%, respectively, as compared to control. Response of total leaf area at 25 DAG showed a significant reduction of 24.56%, 33.33% and 38.65% at 30, 40 and 50 mg/L Cd concentration when compared to control. At 50 DAG, at the same Cd treatment, percentage reduction was 33.33%, 37.24% and 42.055, respectively, over control. In pepper plants (*Capsicum annum*) total leaf area reduced due to Cd stress.[35]

Yield of Palak

Yield was determined at the time of final harvesting (50 DAG) shown in Table 1. The yield per pot at 10, 20, 30, 40, and 50 mg/L was 83.69%, 75.45%, 65.58%, 59.87% and 48.56% over control, respectively. Yield declined at elevated Cd concentration. Decline in chlorophyll contents, increased membrane lipid peroxidation, reduced carbon fixation, more utilization of the photosynthates towards repair mechanisms etc are some of the factors which were responsible for reduction in yield upon Cd stress. Research conducted on lady finger (*Abelmoschus esculentus*) showed that yield decreased by 22%, 39% and 46% when treated with 10, 50 and 100 mg Cd per Kg in soil, respectively.[36]

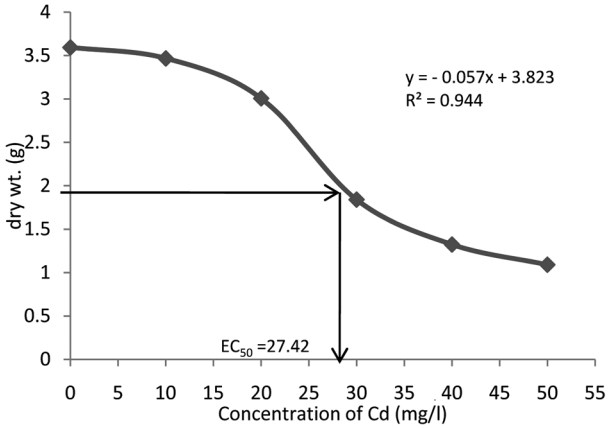

Fig. 4 — EC_{50}: Effective concentration of Cd causing 50% inhibition of Palak biomass as compared to control group

Cd Concentration in Plants

Cd content analysis in Palak plants is shown in Table 2. Cd concentration in root and shoot of the plants showed that Cd concentration × age of plant interaction was significant. At first sampling, Cd content in root was more as compared to shoot. At highest Cd treatment of 50 mg/L Cd, shoot Cd concentration was 5.38 mg/kg dw. Whereas root Cd

Table 2 — Shoot and Root Cd content in Palak plants at 25 DAG and 50 DAG; Each value is mean of triplicates ± SE, calculated at significance level $p \leq 0.05$

	25 DAG		50 DAG		
Treatment	Shoot Cd ($mgkg^{-1}$)	Root Cd ($mgkg^{-1}$)	Shoot Cd ($mgkg^{-1}$)	Root Cd ($mgkg^{-1}$)	TF Cd shoot / Cd root
Control	1.23±0.14e	2.15±0.06^{e}	1.50±0.14^{e}	3.37±0.10^{e}	0.44
10 mg/l	2.58±0.22^{e}	4.63±0.25^{d}	3.18±0.58^{e}	7.35±0.61^{d}	0.43
20 mg/l	4.01±0.13^{bc}	7.00±1.01^{cd}	8.13±0.22^{d}	11.27±1.45^{c}	0.72
30 mg/l	5.31±0.23^{a}	8.75±0.29^{b}	18.60±0.28^{c}	15.98±0.12^{b}	1.16
40 mg/l	5.67±0.22^{a}	10.08±0.22^{ab}	23.47±0.67^{b}	18.07±0.22^{a}	1.30
50 mg/l	5.38±0.06^{ab}	10.23±0.31^{a}	28.95±0.12^{a}	18.70±0.12^{a}	1.55

content was 10.23 mg/kg dw. But, at 50 DAG, the Cd content in shoot was found to be more as compared to root. At Cd concentration of 10–50 mg/L, the Cd content in shoot of palak at 50 DAG was 3.18, 8.13, 18.60, 23.47 and 28.95 mg/kg, respectively.

It can be seen that Cd accumulation in the root and shoot of Palak plants differed with respect to time. High Cd concentration in the roots at 25 DAG may be because dose duration was not enough for the plants to transport it to the shoot, so most of the Cd accumulated in the root. Also, root being the primary absorption site, employs mechanism to chelate Cd in root vacoules. It has been reported that *Arabis paniculata* accumulated Cd within the root cells due to sequestration of Cd by organic compounds.[37] At 50 DAG, the Cd content in root and shoot of Palak increased proportionately with increase in the duration of treatment given. This effect may be due to the reason that duration period was more, so plants accumulated sufficient Cd and transported it to the leaves. Similar effects were observed in *Boerhavia diffusia*, the accumulation of Cd, Pb, Cr and Cd increased gradually with time.[38]

Translocation factor (TF): At 50 DAG, the value of TF was found to be >1 at Cd > 20 mg/L which indicates a high tendency to accumulate metals (Table 2).

Conclusions

In the present research conducted on Indian Palak (*Beta vulgaris* var. All green H1), it was observed that Cd concentration and duration had significant effects on plant morphology and biochemical responses at both the sampling stages. Plant growth and biomass analysis reflected that plant height, number of leaves, total leaf area, biomass and yield reduced significantly at higher doses of Cd (>30 mg/L). On varying Cd concentration from 10 to 50 mg/L, a significant increase in total phenols, thiols, lipid peroxidation and SOD activity was observed at both the sampling stages. However, significant reduction in total chlorophyll content was observed at higher concentration of Cd treatment along with the reduced photosynthetic rate at 30 to 50 mg/L Cd treatment at both the sampling stages. The negative effect of Cd doses on the rate of photosynthesis was manifested in biomass accumulation and yield, which also declined with increasing Cd doses. BCF and TF value was > 1 which is indicative of high heavy metal accumulation capacity. The present research study has shown that Cd accumulation in Palak leaves was above threshold consumption limit of 1.5 mg/kg.

On the basis of inference drawn from the present work, further ameliorative measures will be explored to ameliorate EC_{50} Cd toxicity in Palak plants.

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