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EFFECTS OF CADMIUM AND LEAD ON PHYSIOLOGICAL TRAITS OF CANOLA

HAMID MEHRPANAH[1], ANDREAS MELIKYAN[2], SOHEIL KOBRAEE[*3]

1: PhD student at the Armenian National Agrarian University.

2: PhD in Agricultural sciences at the Plant & Vegetable Growing Program in Agronomy Department National Agrarian University.

[*]3: Department of Agronomy and Plant Breeding, Kermanshah Branch**,** Islamic Azad University, Kermanshah, Iran.

[*]Corresponding author: E Mail: Kobraee@yahoo.com; Tel: 00988317243181

ABSTRACT

Chlorophyll and biochemical contents variation can be used as a valuable index for evaluation of plants tolerance to environmental constraints. Three years greenhouse studies were conducted as randomized complete design in Kermanshah, Iran. Pots experiments were carried out at 2008 until 2010 years in order to investigate the effects of cadmium (Cd) and lead (Pb) effects on some physiological traits of canola (*Brassica napus* L.) plants. In this experiment two cultivars (Hyola 60 and Hyola 401) were used. Treatments were 50, 100 and 150 mg/L Cd with control and 50,100 and 300 mg/L Pb with control. Results showed that the plant exhibited a decline in chlorophyll content and carotenoids with Cd and Pb but Cd was found to be more detrimental than Pb treatment in *B. napus*. The protein content was decreased by Cd (150 mg/L) to 95% and 44% by Pb (300 mg/L) at the adult stage. According to our results H401 cultivar was more susceptible to heavy metals and H60 cultivar had more tolerate to heavy metals and it is better use this cultivar for phytoremediation purposes.

Keywords: Cd, Pb, Heavy Metal Toxicity, *Brassica napus*.

INTRODUCTION

Environmental pollution is one of the most important factors that limit plant photosynthesis [1]. It has shown that photosynthesis reduction in such conditions is associated with malfunction of biochemical reactions [2]. Cd and Pb is absorbed by plants, accumulated in different parts which cause growth inhibition and change in morphological, physiological and biochemical characteristics of plants [3]. They affect morphological and physiological traits related to photosynthesis and finally impress plant biomass. Therefore, the plants get weak and its tolerance to biotic and abiotic stresses is declined. Decreased transpiration and increased temperature occur in plant by reduction leaves area. Cd and Pb general toxic effects are decreasing number of chloroplasts per cell, chlorophyll and carotenoid contents [4]. There are different opinions about the primary site of the Cd and Pb damage effect on active pigments [5] photosystem II water splitting enzymes [6], Calvin's cycle enzymes [7] and electron transport [8]. Therefore, exposure of plants to Cd and Pb, increase fluorescence and decrease PSII photochemical efficiency [9]. The present experiment was undertaken to investigate a change in the level of biochemical aspects, total protein and pigment content in *Brassica napus* treated with Cd and Pb in order to contribute to an understanding of *B. napus* adaptation to environmental pollution. This crop may further be useful in soil reclamation through the process of phytoremediation. However, selection of *B. napus* would be of great importance to reclaim the soil with lesser impact on plant metabolism and hence the yield. The aim of this experiment was to determine level of these heavy metals that has harmful effect on chlorophyll and pigment content in canola.

MATERIALS AND METHODS

Pots experiments were carried out at the private greenhouse of sahneh, near Kermanshah province of Iran during three spring seasons 2008 and 2010 in order to investigate the response of two cultivars canola to Cd and Pb soil pollution. The soil used in the experiment was collected from the surface layer (0-30 cm) of south sahneh which had no pollution of heavy metals. Physical and chemical properties of the soil were determined by soil test. Air dried soil was sieved by 4 mm sieve and then 5.8 kg soil is put to each pot (23 cm in height and 21 cm in diameter). Seeds of *B. napus* were obtained from agricultural organization of Kermanshah were sown in the earthen pots, containing homogenously mixed soil with farm yard manure and the pots were watered daily and kept in Micro model (green house) under natural photoperiod of 12 to 13 h and temperature of 28±4 °C. Care was taken to avoid drainage of solution during the treatment by giving water slightly less than field capacity. After germination the plants were treated with Cd ($CdCl_2$), Pb [$Pb(NO_3)_2$] as pollutants. *B. napus* was subjected to three different concentrations of Cd (50, 100, 150 mg/L) or Pb (50, 100, 300 mg/L). The plant leaves were collected at adult growth stages (60 days) for analysis. Pots containing 5 plants and each treatment was replicated 5 times (each replicate is a plant) were taken for all the treatments arranged in completely randomized block design (CRD). All the chemicals were of analytical grade reagent (Merck). The chlorophyll a and b were determined according to the method of [10] and carotenoids according to [11]. The fresh leaves were cut into 0.5 cm segments and extracted over night with 80% acetone at -10°C. The extract was centrifuged at 14000 x g for 5 min and the absorbance of the supernatant was read at 480, 645, 663 nm using a spectrophotometer (Hitachi -220). Total soluble proteins were determined using the method of [12]. Total soluble sugars were determined according to the method of [13]. Analysis of variance (ANOVA) for all the measured variables was performed by MSTAT-C Ver. 2.10, Inc., Michigan state university. The treatment means were separated using Duncan's multiple range test (DMRT) taking $P<0.05$ as significant.

RESULTS AND DISCUSSION

-Chlorophyll and Carotenoid Content

Table 1 and 2 shows the effect of metal concentration Pb and Cd on total chlorophyll, chlorophyll ”a” and chlorophyll ”b” content. With increase in cadmium concentration total chlorophyll and other its components decreased. The effect of 150 mg/l cadmium on total chlorophyll was more pronounced in both cultivars. As you seen in table 1 with 150mg/l cadmium total chlorophyll decreased by 4.31 and 3.31 mg/g/fw in H60 and H401, respectively (about 40.46 and 49.54% decreased compared control treatment). Lower decrease of total chlorophyll contents belong to 50 mg/l. Also the effect of lead on total chlorophyll and other components was shown in table (2). These results show that 300 mg/l pb decrease total chlorophyll to 4.96 and 3.80 mg/g/fw in H60 and H401, respectively (about 29.34 and 40.53%). Similar decrease in chlorophyll content under heavy metal stress was reported earlier in cyanobacteria, unicellular chlorophytes (*Chlorella*), gymnosperms, such as *Picea abies* and angiosperms, such as *Zea mays*, *Quercus palustrus* and *Acer rubrum* [14]. In addition, 50 mg/l had less effect on total chlorophyll. Pb proved to be less toxic as compared to Cd and in both cultivar. Also, different concentration of lead and cadmium on H60 cultivar was less than H401 and the other hand H60 was more tolerate to H401.

-*Carotenoid,Soluble Protein ans sugar Content*

Table 3 and 4 shows the effect of metal concentration Pb and Cd on caretenoids, soluble proteins and soluble sugars content. Based on results obtained, with increase of Cd and Pb concentration all this compounds decreased. In all treatments, the effects of 150 and 300 mg/l Cd and Pb respectively on these traits were stronger. Although carotenoid contents in H401 was more than H60 cultivar, but with increase Cd and Pb it decrease in H401 was more than H60 with 150 and 300 mg/l Cd and Pb carotenoid contents to 1.30, 1.42 for H60 and H401 cultivars (about 37.56 and 40.64%) and 1.57, 1.63 for H60 and H401 cultivars (about 33.55 and 36.26%). Indeed, the difference between them is slight but percent decrease of carotenoid in H401 was more than H60. The most common effect of Cd toxicity in plants is stunted growth, leaf chlorosis and alteration in the activity of many key enzymes of various metabolic pathways. In our study, with varied concentrations of Cd and Pb the decline in chlorophyll content in plants exposed to Cd^{2+} and Pb^{2+} stress is believed to be due to (a) inhibition of important enzymes, which associated with chlorophyll biosynthesis; (b) impairment in the supply of Mg^{2+}, Fe^{2+}, Zn^{2+} and Mg^{2+}. The decrease in chlorophyll content was also reported in sunflower [15]. Our studies of soluble protein content coincides with the findings of [16] who found decrease in soluble protein content in *B. juncea* when grown on various amendments of tannery waste containing heavy metals.

CONCLUSIONS

In conclusion, our results indicated that the exposure of *B. napus* to Cd and Pb results is an decrease in pigment content and at lower concentration of heavy metals increase in protein was observed but at higher concentrations it was decreased. In all parameters its value decrease of H60 is less than H401 and in other hand it could be said H60 cultivar from standpoint of biochemical traits was more tolerant to other cultivar and this cultivar is more suitable for phytoremediation activity.

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Table 1: Effect of varying cadmium concentrations on total chlorophyll, chlorophyll "a" and chlorophyll "b" (mg g^{-1} fresh leaf tissue) of canola cultivars

Cd treatment	Total Chl (mg/g/fw)		Chl a (mg/g/fw)		Chl b (mg/g/fw)	
(mg/L)	H60	H401	H60	H401	H60	H401
0	7.24	6.56	5.47	4.84	3.57	2.90
50	6.88	5.28	5.03	3.53	3.06	1.67
100	5.14	3.94	3.87	2.72	2.70	1.46
150	4.31	3.31	3.60	2.53	2.45	1.33

Table 2: Effect of varying lead concentrations on total chlorophyll, chlorophyll "a" and chlorophyll "b" (mg g^{-1} fresh leaf tissue) of canola cultivars

Pb treatment	Total Chl (mg/g/fw)		Chl a (mg/g/fw)		Chl b (mg/g/fw)	
(mg/L)	H60	H401	H60	H401	H60	H401
0	7.02	6.39	5.52	4.41	3.28	2.48
50	6.15	4.72	4.60	3.24	3.11	1.63
100	5.45	4.18	4.06	2.85	2.76	1.50
300	4.96	3.80	4.02	2.82	2.66	1.45

Table 3: Effect of varying cadmium concentrations on carotenoids, soluble proteins and total soluble sugars (mg g^{-1} fresh leaf tissue) of canola cultivars

Cd treatment	Carotenoids (mg/g fw)	Soluble proteins (mg/g fw)	soluble sugars (mg/g fw)

(mg/L)	H60	H401	H60	H401	H60	H401
0	2.13	2.44	2.05	1.87	92.73	102.07
50	1.83	2.09	1.81	1.65	91.55	101.4
100	1.67	1.83	1.69	1.54	69.33	76.82
150	1.30	1.42	1.28	1.11	43.90	41.65

Table 4: Effect of varying cadmium concentrations on carotenoids, soluble proteins and total soluble sugars (mg g^{-1} fresh leaf tissue) of canola cultivars

Pb treatment	Carotenoids (mg/g fw)		soluble proteins (mg/g fw)		soluble sugars (mg/g fw)	
(mg/L)	H60	H401	H60	H401	H60	H401
0	2.04	2.09	1.77	1.63	88.61	94.18
50	1.76	1.93	1.67	1.52	79.77	88.39
100	1.68	1.84	1.66	1.52	70.26	77.85
300	1.57	1.63	1.32	1.09	58.88	60.03