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Original article

The effect of cadmium stress on photosynthetic pigments and secondary metabolites in borage (*Borago officinalis* L.)

P. Sheikhzadeh[1*], N. Zare[1], Sh. Abootalebi[2]

1. Associate Professor, Department of Plant Production and Genetics, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran

2. Ph.D. Student, Department of Plant Production and Genetics, Faculty of Agriculture and Natural Resources, Mohaghegh Ardabili University, Ardabil, Iran

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Extended abstract

Introduction

Heavy metals are one of the most important abiotic stresses which can have detrimental effects on the growth, metabolic pathways, and physiological and biochemical characteristics of plants. Today, the accumulation of heavy metals in agricultural lands has an increasing trend that can affect the production and quality of agricultural products as well as human health. Among heavy metals, cadmium (Cd) is one of the most important worldwide environmental pollutants. It can rapidly be taken up by plants and accumulates in plant tissues, and easily enter the food chain; so this heavy metal is a serious threat to humans, animals, plants, and environmental sustainability. Secondary metabolites play vital protective and adaptive roles in plants in response to biotic and abiotic stresses. In this study, the effect of cadmium stress on the physiological characteristics and secondary metabolite production, and cadmium accumulation in borage (*Borago officinalis* L.) was investigated under hydroponic conditions.

Materials and methods

Borage seeds were germinated in Petri dishes, and the 5–6 cm seedlings were then transferred to hydroponic containers containing half of the Hoagland nutrient solution with continuous aeration. The cultures were maintained in a growth chamber with 16 hours of light and 25±2 °C. Cadmium treatments were applied at five levels (0, 81, 162, 243, and 324 µM cadmium) using cadmium nitrate (Cd (NO3)2 .4H2O) at the 6-7 leaves stage. European borage seedlings were sampled at five-time intervals (12, 24, 48, 72, and 108 hours after cadmium stress) treatments and cadmium content, physiological characteristics, and secondary metabolites of leaf samples were measured.

Results and discussion

The results showed that the amount of photosynthetic pigments, chlorophyll index (SPAD), chlorophyll fluorescence, and secondary metabolites in borage leaves were significantly influenced by cadmium stress. With increasing cadmium concentration and exposure duration, the absorption and accumulation of cadmium in borage leaves increased significantly. Cadmium stress reduced the amount of chlorophyll a and b, total chlorophyll, and carotenoids at all sampling times in comparison with the control treatment. The maximal quantum efficiency of photosystem II and the chlorophyll index (SPAD) were decreased with increasing the cadmium concentration and exposure duration so that the lowest

[*] Corresponding author: Parisa Sheikhzadeh; E-Mail: sheikhzadehmp@gmail.com

value of these traits was observed at 108 hours after treatment with 324µM cadmium. With increasing the cadmium stress severity, the amount of secondary metabolites including anthocyanin, phenol, and total flavonoids and also the amount of soluble sugars were significantly increased in the borage leaves. The highest amount of these metabolites was observed at 108 hours after treatment with 324 µM cadmium.

Conclusion

In general, the results of this study showed that increasing the concentration and duration of cadmium stress negatively influenced plant photosynthesis by reducing the content of photosynthetic pigments and increasing chlorophyll fluorescence. On the other hand, increasing the concentration and duration of cadmium stress, increased the cadmium absorption and accumulation in the borage leaves as well as the amount of secondary metabolites.

***Keywords*:** Efficiency of photosystem II, Heavy metals, Medicinal plant, Secondary metabolites

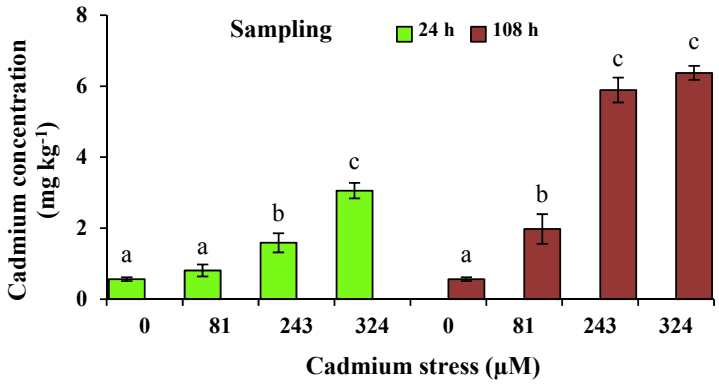

Fig. 1. The effect of different concentrations of cadmium on cadmium accumulation in borage leaves at 24 and 108 hours after treatment. The data are the mean of 3 replicates and the error bars indicate the standard error. Distinct letters in each sampling time indicate a significant difference at the 5% level of probability.

Table 1. Analysis of variance of the effects of cadmium stress on photosynthetic pigments in borage leaves at different exposure duration

Sampling time	SOV	df	Mean of Squares			
			Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
12 h	Cadmium stress	4	0.166 ns	0.243**	0.638**	0.218**
	Error	10	0.115	0.019	0.105	0.027
	CV (%)		*2.34*	*5.03*	*1.88*	*3.86*
24 h	Cadmium stress	4	3.105**	0.141*	4.360**	0.751**
	Error	10	0.174	0.029	0.124	0.047
	CV (%)		*2.92*	*6.19*	*2.08*	*5.48*
48 h	Cadmium stress	4	5.297**	0.365**	8.392**	1.797**
	Error	10	0.045	0.009	0.044	0.009
	CV (%)		*1.50*	*3.52*	*1.25*	*2.26*
72 h	Cadmium stress	4	17.009**	0.383**	21.764**	3.067**
	Error	10	0.016	0.049	0.043	0.008
	CV (%)		*0.95*	*8.19*	*1.29*	*2.34*
108 h	Cadmium stress	4	54.587**	2.255**	77.481**	5.427**
	Error	10	0.071	0.030	0.135	0.024
	CV (%)		*2.34*	*7.63*	*2.69*	*4.50*

*, ** and ns: significant at a probability level of 5% and 1%, and non-significant at probability level of 5%, respectively

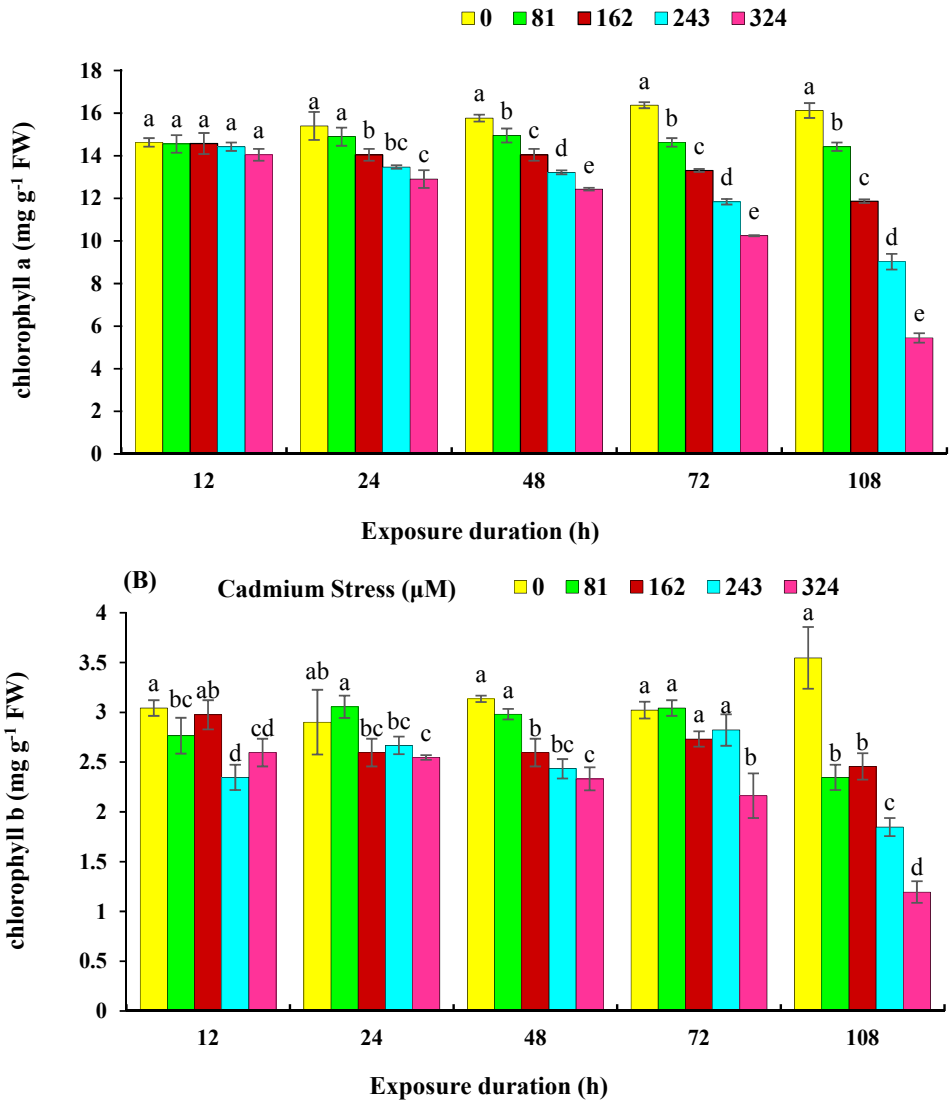

Fig. 2. The effect of different concentrations of cadmium on the chlorophyll a (A) and chlorophyll b (B) content in borage at different exposure times. The data are the mean of 3 replicates and the error bars indicate the standard error. Distinct letters in each sampling time indicate a significant difference at the 5% level of probability.

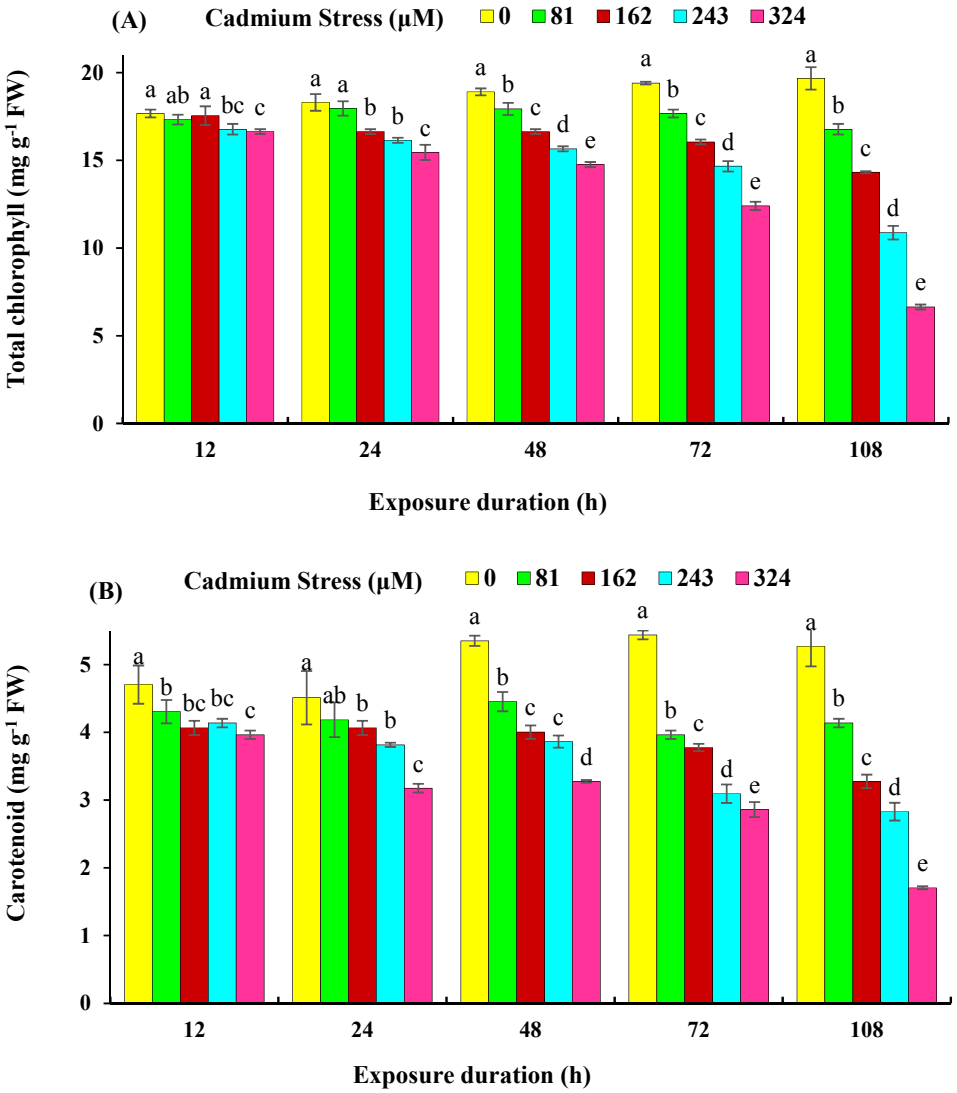

Fig. 3. The effect of different concentrations of cadmium on the total chlorophyll (A) and carotenoids (B) content at different times after treatment in borage. The data are the mean of 3 replicates and the error bars indicate the standard error. Distinct letters in each sampling time indicate a significant difference at the 5% level of probability.

Table 2. Analysis of variance of the effect of concentrations of cadmium on chlorophyll fluorescence and chlorophyll index (SPAD) in different exposure duration

Sampling time	SOV	df	Mean of Squares				
			F_0	F_m	F_v	II F_v/F_m	Cholorophyll Index
24 h	Cadmium stress	4	1.767^{ns}	927.233^{ns}	930.733^{ns}	0.00007**	4.048**
	Error	10	6.267	2017.800	2002.467	0.00001	0.425
	CV (%)		*1.13*	*3.96*	*1.48*	*0.44*	*2.19*
48 h	Cadmium stress	4	31.933**	7359.733**	6424.733*	0.0001**	30.701**
	Error	10	3.800	1087.067	1129.00	0.00003	0.455
	CV (%)		*0.87*	*2.91*	*3.69*	*0.70*	*2.50*
108 h	Cadmium stress	4	265.900**	68145.900**	60653.100**	0.001**	42.876**
	Error	10	25.333	3314.600	3438.133	0.00005	0.519
	CV (%)		*2.27*	*5.41*	*7.01*	*0.90*	*2.74*

*, ** and ns: significant at a probability level of 5% and 1% and non-significant at probability level of 5%, respectively

Table 3. The effect of different concentrations of cadmium on minimum fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv), maximal quantum efficiency of photosystem II (Fv / Fm) in borage at different exposure duration

Sampling time	Cadmium concentration (µM)	F_0	F_m	F_v	F_v/F_m
24 hours	0	219.0 ±1.47 a	1144.0±12.16 a	923.0 ±11.01 a	0.814±0.0014 a
	81	220.0 ±2.08 a	1135.7±11.69 a	915.7 ±10.89 a	0.813±0.0017 a
	162	220.0 ±2.57 a	1140.0±28.02 a	883.3 ±48.84 a	0.81±0.0032 a
	243	220.6 ±1.45 a	1148.3±24.16 a	928.3 ±22.24 a	0.809±0.0020 a
	324	221.0 ±1.15 a	1126.7±13.77 a	907. 7±14.71 a	0.801±0.0017 b
48 hours	0	217.0±2.22 c	1173.7±18.85 a	948. 7±19.83 a	0.817±0.0012 a
	81	220.3±0.89 bc	1172.3±12.17 a	948.0±13.01 a	0.814±0.0028 ab
	162	222.6±1.45 ab	1145.0±11.06 ab	922.3±12.17 a	0.813±0.0011 ab
	243	224.3±1.88 a	1104.3±23.39 bc	884.0±24.26 ab	0.804±0.0046 bc
	324	225.0±1.35 a	1057.7±25.27 c	840.7±24.12 b	0.794±0.0046 c
108 hours	0	206.67±1.45 c	1192.7±29.97 a	961.3±30.88 a	0.820±0.0014 a
	81	219.67±2.33 b	1177.7±8.64 a	950.3±9.17 a	0.820±0.0017 a
	162	220.33±3.53 b	1134.7±15.16 a	914.3±17.94 a	0.821±0.0046 a
	243	227.33±2.72 ab	955.0±11.67 b	735.3±9.68 b	0.801±0.001 b
	324	231.33±3.84 a	853.0±26.72 b	646.3±16.23 b	0.789±0.0078 b

Data are mean of 3 replicates ± standard error. In each sampling time, means followed by the same letter(s) are not significantly different ($p \leq 0.05$).

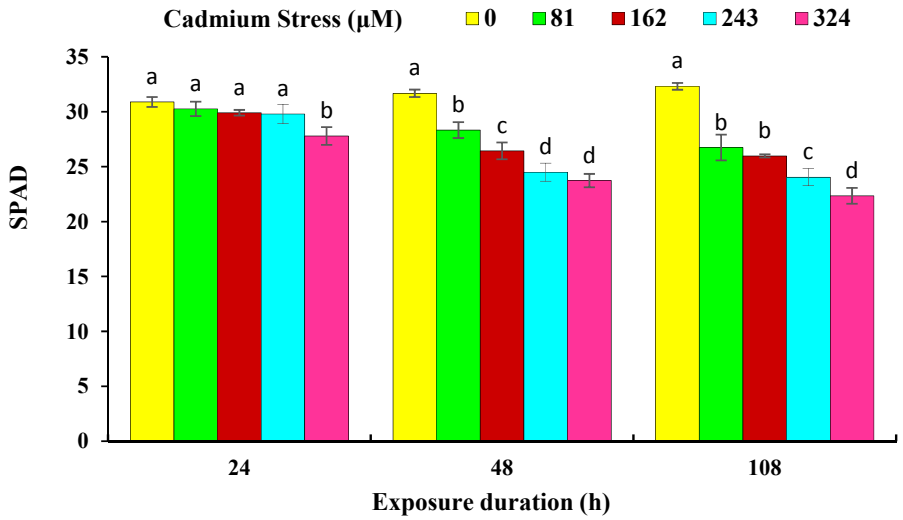

Fig. 4. The effect of cadmium concentration on chlorophyll index (SPAD) in borage at different exposure duration. The data are the mean of 3 replicates and the error bars indicate the standard error. Distinct letters in each sampling time indicate a significant difference at the 5% level of probability.

Table 4. Analysis of variance of the effect of cadmium stress on secondary metabolites and soluble sugars in borage at different exposure duration

Sampling time	SOV	df	Mean of Squares			
			Anthocyanin	Phenol	Flavonoid	soluble sugar
12 h	Cadmium stress	4	0.109 ^{ns}	206.943**	20.487**	0.00002 ^{ns}
	Error	10	0.042	6.710	1.168	0.00005
	CV (%)		*7.37*	*20.46*	*6.41*	*14.41*
24 h	Cadmium stress	4	0.128*	322.982**	101.119**	0.002**
	Error	10	0.028	11.909	3.839	0.00004
	CV (%)		*6.01*	*21.88*	*9.61*	*18.21*
48 h	Cadmium stress	4	0.290**	1160.196**	147.755**	0.041**
	Error	10	0.020	4.723	3.223	0.00008
	CV (%)		*4.65*	*6.97*	*7.73*	*8.88*
72 h	Cadmium stress	4	0.075*	1226.450**	75.193**	0.072**
	Error	10	0.037	4.127	4.610	0.0001
	CV (%)		*6.58*	*6.01*	*9.40*	*5.40*
108 h	Cadmium stress	4	0.181*	1618.484**	179.229**	0.202**
	Error	10	0.036	6.088	7.088	0.0001
	CV (%)		*6.28*	*6.66*	*9.08*	*2.85*

*, ** and ns: significant at a probability level of 5% and 1% and non-significant at probability level of 5%, respectively

Table 5. The effect of cadmium concentration on the amount of secondary metabolites and soluble sugars in borage at different exposure duration

Time after treatment	Cadmium concentration	Anthocyanin	Phenol	Flavonoid	Soluble Suger
	µM	µmol g^{-1}	---------- mg g^{-1} ----------		
12 hours	**0**	2.60±0.16 a	2.05±0.33 c	13.07±0.41 c	0.0133±0.003 a
	81	2.65±0.10 a	6.53±0.72 c	15.34±0.53 b	0.0151±0.004 a
	162	2.69±0.06 a	14.47±2.26 b	17.61±0.32 a	0.0155±0.003 a
	243	2.91±0.14 a	17.73±2.28 b	18.99±0.93 a	0.0186±0.005 a
	324	3.05±0.07 a	22.50±0.4 a	19.23±0.72 a	0.0200±0.004 a
24 hours	**0**	2.51±0.11 c	2.14±0.33 d	13.09±0.68 c	0.0137±0.002 d
	81	2.70±0.07 bc	9.14±0.92 c	18.63±0.41 b	0.0144±0.003 d
	162	2.77±0.06 abc	17.92±3.18 b	19.11±0.47 b	0.0334±0.004 c
	243	2.84±0.12 ab	20.82±2.67 b	22.15±1.04 b	0.0532±0.004 b
	324	3.08±0.07 a	28.84±1.26 a	28.96±2.11 a	0.0694±0.005 a
48 hours	**0**	2.68±0.06 b	2.52±0.32 l e	14.57±0.66 d	0.0179±0.002 d
	81	2.89± 0.08 ab	20.91±0.56 d	19.4±1.31 c	0.0215±0.004 d
	162	2.94±0.07 ab	36.97±2.26 c	22.2±0.78 c	0.0536±0.005 c
	243	2.98±0.09 ab	43.22±0.89 b	27.11±1.13 b	0.1298±0.006 b
	324	3.12±0.10 a	52.19±1.23 a	32.78±1.37 a	0.2977±0.007 a
72 hours	**0**	2.67±0.07 c	3.36±0.32 e	16.12±0.54 c	0.0504±0.002 d
	81	2.76±0.10 c	26.91±0.56 d	19.58±1.44 c	0.0825±0.003 c
	162	3.08±0.13 b	35.85±1.54 c	23.53±1.24 b	0.1040±0.007 c
	243	3.31±0.12 ab	49.20±0.94 b	26.69±1.32 ab	0.2758±0.005 b
	324	3.36±0.09 a	54.15±1.78 a	28.24±1.41 a	0.4145±0.004 a
108 hours	**0**	2.73±0.10 c	3.36±0.16 e	18.81±0.83 d	0.1199±0.005 e
	81	2.90±0.14 bc	27.44±0.64 d	26.39±1.40 c	0.1580±0.005 d
	162	2.97±0.04 bc	38.46±1.86 c	29.44±1.36 bc	0.2645±0.005 c
	243	3.11±0.09 ab	52.84±1.81 b	31.95±2.54 b	0.5231±0.006 b
	324	3.39±0.13 a	63.11±1.71 a	39.95±0.90 a	0.7278±0.009 a

In each column, means which followed by the same letter(s) are not significantly different ($p \leq 0.05$)