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Original Research Article

The pigment content of *Sesuvium portulacastrum* L. under copper and zinc stress

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A B S T R A C T

Keywords

Sesuvium portulacastrum, copper, zinc, chlorophyll, carotenoid.

The present work deals with the effect of copper and zinc on the pigment content of *Sesuvium portulacastrum* L. The *Sesuvium portulacastrum* plants are grown in pots containing the soil amended with various levels of copper and zinc (control, 100, 200, 300, 400, 500 and 600 mg kg^{-1}). All pots were irrigated as and when necessary. The experiments were replicated five times. The pigment contents of plant samples were analyzed at four different intervals (30, 60, 90 and 120^{th} day). The results indicated that the various pigments analyses such as the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of *Sesuvium portulacastrum* increased at low levels (copper, 100-200 mg kg^{-1} and zinc, 100-300 mg kg^{-1}) and decreased further with an increase in the soil metal levels (copper, 300-600 mg kg^{-1} and zinc, 400-600 mg kg^{-1}).

Introduction

Heavy metals and metalloids are an increasing environmental problem worldwide. Agricultural soils are contaminated with heavy metals by human- induced activities and creating severe problems (Ahmad *et al*., 2011; Ali *et al*., 2011). Since the beginning of the industrial revolution, pollution of the biosphere with toxic metals has accelerated dramatically. Plants are one pathway for toxic metal mobilization into human food chain and paradoxically they may also provide an elegant means of reducing this spread. Elements such as Cu, Zn, Ni, Co, Fe, Mo and Mn are essential mineral nutrients and play a significant role in gene expression, biosynthesis of proteins, nucleic acids, growth substance, chlorophyll and secondary metabolites, and carbohydrate and lipid metabolism (Rengel,1999; Parmar *et al*., 2012).

Heavy metals contamination of soil and groundwater causes major environmental and human health problems. The commonly used methods for extraction of heavy metals from the environment are expensive. Phytoremediation is one of the effective technologies for this goal. Hence in this technology testing and introducing new plants are very important. In addition salinity is also common in different parts of the world. Therefore, investigating the survival of salt- tolerant halophytes under heavy metal stress seems pertinent (Shevyakova *et al*., 2003; Vahedi, 2013).

The present investigation deals with the effect of copper and zinc on pigment (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) content of *Sesuvium portulacastrum*.

Materials and Methods

Plant cutting

The experimental plant, the *Sesuvium portulacastrum* L. belongs to the family Aizoaceae is one of the important halophytic plants of India. Plant cuttings of *Sesuvium portulacastrum* used in the experiments were collected from T.S. pettai village nearer to pichavaram mangrove forest [11˚ 43′ N and 79˚ 77′ E] on the south east coast of Tamil Nadu, India. Plant cutting with each 5 cm length with uniform thickness were chosen for experimental purpose.

Pot culture experiments

The experiments were conducted during January-April 2012. *Sesuvium portulacastrum* L. plants were grown in pots in untreated soil (control) and in soil to which copper and zinc had been applied (100, 200, 300, 400, 500 and 600 mg kg^{-1} soil). The inner surfaces of pots were lined with a polythene sheet. Each pot contained 3 kg of air dried soil. The copper and zinc as finely powdered ($CuSO_4 .5H_2O$, $ZnSO_4 .7H_2O$) was applied to the surface soil and thoroughly mixed with the soil. Ten plant cuttings were planted in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of six per pot, after a week of planting. Each treatment including the control was replicated five times.

Sample collection

The plant samples were collected at thirty days interval, up to four months viz., 30, 60, 90 and 120th day for the measurement of pigments. Six plants from each replicate of a pot was analyzed for its various parameters and the average was calculated. These mean values were used for statistical analysis. Chlorophyll a, Chlorophyll b, total chlorophyll and carotenoid contents in plant samples were estimated by the following methods.

Estimation of chlorophyll (Arnon, 1949)

Hundred milligram of fresh leaf was ground in a mortar and Pestle with 20 ml of 80% acetone. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was saved. The pellet was reextracted with 5 ml of 80% acetone each time, until it become colourless. All the supernatants were pooled and utilized for chlorophyll determination. Absorbance was measured at 645nm and 663nm in spectrophotometer. The chlorophyll content was determined by using the following formula.

$$\text{Chlorophyl a (mg g}^{-1}) = \frac{12.7\,A_{663} - 2.69\,A_{645}}{a \times 1000 \text{ x } w} \times V$$

$$\text{Chlorophyl b(mg g}^{-1}) = \frac{22.9\,A_{645} - 4.68\,A_{663}}{a \times 1000 \times w} \times V$$

$$\text{Total chlorophyl l (mg g}^{-1}) = \frac{20.2\,A_{645} + 8.02\,A_{663}}{a \times 1000 \times w} \times V$$

Where,

a = length of light path in the cell (1 cm)
v = volume of the extract in ml and
w = fresh weight of sample in gram

Estimation of carotene (Kirk and Allen, 1965)

The same chlorophyll extract was measured at 480nm, in spectrophotometer to estimate the carotene.

$$\text{Carotene (mg g}^{-1}) = \frac{(A_{480} + 0.114\,A_{663}) - .638\ A_{645}}{a \times 1000 \times w} \times V$$

Results and Discussion

Pot culture experiments were conducted at Botanical garden in Annamalai University during January to April, 2012. Periodical observations were made to record the pigment contents of treated and untreated *Sesuvium portulacastrum* L. The data gathered from periodical observations were processed, statistically analyzed and the results are presented in the form of tables.

Photosynthetic pigments (mg g^{-1} fr. wt.)
Chlorophyll a

Results on the effect of copper and zinc on chlorophyll a content of *S. portulacastrum* are presented in Table 1. The chlorophyll a content of copper treated *S. portulacastrum* increased considerably up to 200 mg kg^{-1} of soil (viz., 1.12, 1.19, 1.39 and 1.32) in all the sampling days. Copper concentration beyond this level showed a gradual decrease in chlorophyll a. The lowest chlorophyll a content was recorded at 600 mg kg^{-1} of copper level (viz., 0.586, 0.612, 0.662 and 0.647) in all the sampling days.

In zinc treated *S. portulacastrum* higher chlorophyll a content was observed at 100, 200 and 300 mg kg^{-1} of soil (viz., 1.07, 1.12, 1.28, 1.25; 1.14, 1.17, 1.43, 1.36 and 1.20, 1.25, 1.46, 1.41 respectively) in all the sampling days. The chlorophyll a content of *S. portulacastrum* showed a decreasing trend with increase in zinc level. The chlorophyll content showed a progressive trend up to 90th day and it gradually declined afterwards due to the senescence of leaves. Significant variations (F values at 1 per cent level) were observed with treatment, sampling days and interaction between treatment and sampling days in both copper and zinc treated *S. portulacastrum* plants.

Chlorophyll b

The effect of copper and zinc on chlorophyll b content of *S. portulacastrum* is presented in Table 2. Chlorophyll b content of *S. portulacastrum* leaves increased at lower concentrations (100-200 mg kg^{-1}) of copper (viz., 1.02, 1.09, 1.25 and 1.20; viz., 1.10, 1.18, 1.32 and 1.30) in all the sampling days and decreased further with an increase in the copper level. The minimum chlorophyll b content was observed at 600 mg kg^{-1} of copper level (viz., 0.570, 0.626, 0.690 and 0.680) in all the sampling days. Significant variations (F values at 1 per cent level) were observed with treatment, sampling days and interaction between treatment and sampling days in copper treated *S. portulacastrum* plants.

The chlorophyll b content of zinc treated *S. portulacastrum* leaves increased with increasing concentration of applied zinc in soil up to 300mg kg^{-1}(viz., 1.18, 1.27, 1.40 and 1.38). For further higher concentration, the chlorophyll b content decreased. Minimum chlorophyll b content of *S. portulacastrum* was observed at 600 mg kg^{-1} of zinc level (viz., 0.678, 0.696, 0.783 and 0.756) in all the sampling days. At all concentrations chlorophyll a was always higher than the chlorophyll b content. Analysis of variance calculated showed significant F test values at 1 per cent level for treatment and sampling days and non-significant for interaction between treatment and sampling days in zinc treated *S. portulacastrum* plants.

Total chlorophyll content

The effect of copper and zinc on total chlorophyll content of *S. portulacastrum* is presented in Table 3. Total chlorophyll content of *S. portulacastrum* leaves increased at lower concentrations (100-200 mg kg^{-1}) of copper (viz., 1.120, 1.510, 1.930 and 1.740; 1.250, 1.610, 2.045 and 1.900) in all the sampling days and decreased further with an increase in the copper level**.** The minimum total chlorophyll content was observed at 600 mg $kg^{-1}$of copper level (viz., 0.588, 0.778, 0.926 and 0.856) in all the sampling days. The total chlorophyll content of zinc treated *S. portulacastrum* leaves increased with increasing concentration of applied zinc in soil up to 300 mg kg^{-1}(viz., 1.302, 1.740, 2.205 and 1.985). For further higher concentrations, the total chlorophyll content of zinc treated *S. portulacastrum* plants decreased. Minimum total chlorophyll content of *S. portulacastrum* was observed at 600 mg kg^{-1} of zinc level (viz., 0.615, 0.796, 0.986 and 0.920) in all the sampling days. The chlorophyll content showed a progressive trend up to 90th day and it gradually declined afterwards due to the senescence of leaves. Significant variations (F values at 1 per cent level) were observed for treatment, sampling days and interaction between treatment and sampling days in both copper and zinc treated *S. portulacastrum* plants.

Carotenoid content

The effect of copper and zinc on carotenoid content of *S. portulacastrum* is presented in Table 4. The carotenoid content of *S. portulacastrum* plant was found to be the highest at 200 mg kg^{-1} of copper level (viz., 0.778, 0.910, 1.120 and 1.068) in all the sampling days. Increase in copper level significantly decreased the carotenoid content of *S. portulacastrum* leaves. The lowest carotenoid content of *S. portulacastrum* was observed at 600 mg kg^{-1} of soil (viz., 0.398, 0.463, 0.559 and 0.556) in all the sampling days.

Carotenoid content of *S. portulacastrum* leaves increased at 100, 200 and 300 mg kg^{-1} of zinc level in the soil (viz., 0.736, 0.887, 1.106, 1.04; 0.793, 0.951, 1.164, 1.100 and 0.839, 0.998, 1.210, 1.150 respectively) in all the sampling days. Further increase in zinc level significantly decreased the carotenoid content of *S. portulacastrum* leaves. F values calculated were significant at 1 per cent level for treatment, sampling days and interaction between treatment and sampling days in both copper and zinc treated *S. portulacastrum* plants.

Maximum photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of *S. portulacastrum* were observed at 100- 200 mg kg^{-1} of copper and 100-300 mg kg^{-1} of zinc level in the soil in all the sampling days. Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as change in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity(Parekh, 1990).

Table.1 Effect of copper and zinc on Chlorophyll-a content (mg g^{-1} fresh weight) of *Sesuvium portulacastrum* L.

Metals added in the soil(mg kg^{-1})	Copper				Zinc			
	Sampling days				Sampling days			
	30	60	90	120	30	60	90	120
Control	0.916	0.944	1.09	1.05	0.916	0.944	1.09	1.05
100	1.05(+14.63)	1.10(+16.52)	1.26(+15.60)	1.23(+17.14)	1.07(+16.81)	1.12(+18.64)	1.28(+17.43)	1.25(+19.05)
200	1.12(+22.27)	1.19(+26.06)	1.39(+27.52)	1.32(+25.71)	1.14(+24.45)	1.17(+23.94)	1.43(+31.19)	1.36(+29.52)
300	0.813(-11.24)	0.828(-12.29)	0.980(-10.09)	0.912(-13.14)	1.20(+31.01)	1.25(+32.41)	1.46(+33.94)	1.41(+34.28)
400	0.751(-18.01)	0.764(-19.07)	0.870(-20.18)	0.873(-16.86)	0.826(-9.82)	0.841(-10.91)	0.992(-8.99)	0.934(-11.05)
500	0.656(-28.38)	0.682(-27.75)	0.810(-25.69)	0.770(-26.67)	0.766(-16.37)	0.780(-17.37)	0.877(-19.54)	0.886(-15.62)
600	0.586(-36.03)	0.612(-35.17)	0.662(-39.42)	0.647(-38.38)	0.615(-32.86)	0.660(-30.08)	0.725(-33.49)	0.716(-31.81)

Comparison of significant effects	F test	CD P= 0.05	F test	CD P= 0.05
Metal levels	**	0.0139	**	0.0305
Sampling days	**	0.0483	**	0.0225
Interaction	**	0.0337	**	0.0596

Average of five replications

Figures in parentheses represent per cent reduction (-) over control

Table.2 Effect of copper and zinc on Chlorophyll-b content (mg g^{-1} fresh weight) of *Sesuvium portulacastrum* L

Metals added in the soil(mg kg^{-1})	Copper				Zinc			
	Sampling days				Sampling days			
	30	60	90	120	30	60	90	120
Control	0.866	0.922	1.03	0.998	0.866	0.922	1.03	0.998
100	1.02(+17.78)	1.09(+18.22)	1.25(+21.36)	1.20(+20.24)	1.08(+24.71)	1.12(+21.47)	1.26(+22.33)	1.20(+20.24)
200	1.10(+29.33)	1.18(+27.98)	1.32(+28.15)	1.30(+30.26)	1.13(+30.48)	1.22(+32.32)	1.37(+33.01)	1.31(+31.26)
300	0.776(-10.39)	0.809 (-12.25)	0.915(-11.65)	0.863(-13.53)	1.18(+36.26)	1.27(+37.74)	1.40(+35.92)	1.38(+38.28)
400	0.731(-15.59)	0.765(-17.03)	0.865(-16.02)	0.799(-19.94)	0.782(-09.70)	0.825(-10.52)	0.935(-09.22)	0.881(-11.72)
500	0.665(-23.21)	0.689(-25.27)	0.776(-24.66)	0.735(-26.35)	0.746(-13.86)	0.780(-15.40)	0.882(-14.68)	0.826(-17.23)
600	0.570(-34.18)	0.626(-32.10)	0.690(-33.01)	0.680(-31.86)	0.678(-21.71)	0.696(-24.51)	0.783(-23.98)	0.756(-24.24)

Comparison of significant effects	F test	CD P= 0.05	F test	CD P= 0.05
Metal levels	**	0.3024	**	0.6230
Sampling days	**	0.9369	NS	NS
Interaction	**	0.6225	NS	NS

Average of five replications

Figures in parentheses represent per cent reduction (-) over control

Table.3 Effect of copper and zinc on total Chlorophyll content (mg g^{-1} fresh weight) of *Sesuvium portulacastrum* L.

Metals added in the soil(mg kg^{-1})	Copper				Zinc			
	Sampling days				Sampling days			
	30	60	90	120	30	60	90	120
Control	0.970	1.300	1.620	1.470	0.970	1.300	1.620	1.470
100	1.120(+15.46)	1.510(+16.15)	1.930(+19.13)	1.740(+18.37)	1.131(+16.60)	1.545(+18.85)	1.970(+21.60)	1.770(+20.41)
200	1.250(+28.86)	1.610(+23.85)	2.045(+26.23)	1.900(+29.25)	1.266(+30.51)	1.665(+28.08)	2.143(+32.28)	1.930(+31.29)
300	0.860(-11.34)	1.140(-12.31)	1.402(-13.46)	1.310(-10.88)	1.302(+34.23)	1.740(+33.85)	2.205(+36.11)	1.985(+35.03)
400	0.730(-24.74)	0.975(-25.01)	1.253(-22.65)	1.160(-21.09)	0.872(-10.10)	1.150(-11.54)	1.425(-12.04)	1.330(-09.52)
500	0.638(-34.23)	0.842(-35.23)	1.102(-31.97)	0.980(-33.33)	0.770(-20.62)	1.003(-22.85)	1.315(-18.83)	1.183(-19.52)
600	0.588(-39.38)	0.778(-40.15)	0.926(-42.84)	0.856(-41.77)	0.615(-36.60)	0.796(-38.77)	0.986(-39.13)	0.920(-37.41)

Comparison of significant effects	F test	CD P= 0.05	F test	CD P= 0.05
Metal levels	**	0.0506	**	0.0181
Sampling days	**	0.0267	**	0.0410
Interaction	**	0.0705	**	0.0637

Average of five replications

Figures in parentheses represent per cent reduction (-) over control

Table.4 Effect of copper and zinc on carotiond content (mg g^{-1} fresh weight) of *sesuvium portulacastrum* L.

Metals added in the soil (mg kg^{-1})	Copper				Zinc			
	Sampling days				Sampling days			
	30	60	90	120	30	60	90	120
Control	0.636	0.751	0.929	0.861	0.636	0.751	0.929	0.861
100	0.715(+12.42)	0.869(+15.71)	1.095(+17.88)	1.025(+19.05)	0.736(+15.72)	0.887(+18.11)	1.106(+19.05)	1.04(+20.79)
200	0.778(+22.33)	0.910(+21.17)	1.120(+20.56)	1.068(+24.04)	0.793(+24.68)	0.951(+26.63)	1.164(+25.30)	1.100(+27.76)
300	0.566(-11.01)	0.660(-12.12)	0.808(-13.02)	0.772(-10.34)	0.839(+31.92)	0.998(+32.89)	1.210(+30.25)	1.150(+33.56)
400	0.504(-20.75)	0.612(-18.51)	0.720(-22.50)	0.680(-21.02)	0.572(-10.06)	0.663(-11.72)	0.812(-12.59)	0.775(-09.99)
500	0.476(-25.16)	0.551(-26.63)	0.698(-24.86)	0.628(-27.06)	0.486(-23.58)	0.570(-24.10)	0.741(-20.24)	0.671(-22.07)
600	0.398(-37.42)	0.463(-38.35)	0.559(-39.83)	0.556(-35.42)	0.412(-35.22)	0.502(-33.15)	0.605(-34.88)	0.549(-36.24)

Comparison of significant effects	F test	CD P= 0.05	F test	CD P= 0.05
Metal levels	**	0.0050	**	0.0077
Sampling days	**	0.0029	**	0.0078
Interaction	**	0.0078	**	0.0031

Average of five replications

Figures in parentheses represent per cent reduction (-) over control

Increased chlorophyll ratios due to environmental stress have been reported in spinach leaves (Delfine *et al*., 1999). A high chlorophyll ratios also indicates a change in the PSII/ PSI ratio of leaves (Anderson, 1986).The increased chlorophyll content was obviously due to copper and zinc at a low level which act as a structural and catalytic components of proteins, enzymes and as cofactors for normal development of chlorophyll biosynthesis. This would be evident from the study of Ouzounidou (1994a), Mocquot *et al*. (1996) Umebese and Motajo, (2008) and Mysliwa-Kurdziel and Strazalka (2002) under copper treatment and Agarwala *et al*. (1977), Stiborova *et al*. (1986) and Balashouri and Prameeladevi (1995) under zinc treatment.

In excess levels, copper (300-600 mg kg^{-1}) and zinc (400-600mg kg^{-1}) become toxic to plants and a decrease in photosynthetic pigments was observed. These results are comparable with the reports of Lidon and Henriques (1992), Romeu-moreno and Mas (1999), Mysliwa-Kurdziel and Strazalka (2002) under copper treatment and Bonnet *et al*. (2000), Dube *et al*. (2003), Ismail and Azooz (2005) and Sharma *et al*. (2009) under zinc treatment.

Higher level of these metals brought about a marked depression in photosynthetic pigments in *S. portulacastrum* plants. It might be due to excess supply of copper and zinc which interferes with the chlorophyll biosynthesis. The synthesis of photosynthetic pigments depends on the adequate supply of iron (Brown, 1956). The excess supply of copper and zinc seems to induce physiological iron and Mg deficiency and lowering of chlorophyll biosynthesis (Agarwala *et al*., 1977; Lanaras *et al*., 1993; Siedlecka and Krupa, 1999). Several experiments were performed to reveal the site (s) of metal action in the process of chlorophyll biosynthesis. Mechanism of the inhibition is not yet understood thoroughly; however, sulfhydryl group interaction has been proposed as the mechanism for the inhibition of δ-aminolevulinic acid synthase, aminolevulinic acid dehydrogenase and protochlorophyllide reductase (Mysliwa-Kurdziel, 2004). Various abiotic stresses decrease the chlorophyll content in plants (Ahmad *et al*., 2007). It has been proposed that Cu at toxic concentration interferes with enzymes associated with chlorophyll biosynthesis and protein composition of photosynthetic membranes (Lidon and Henriques, 1991; Singh *et al*., 2007).

The results indicated that the various pigments such as the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of *Sesuvium portulacastrum* increased at low levels (copper, 100-200 mg kg^{-1} and zinc, 100-300 mg kg^{-1}) and decreased further with an increase in the soil metal levels (copper, 300-600 mg kg^{-1} and zinc, 400-600 mg kg^{-1}). From the present investigation it was concluded that the copper, 100-200 mg kg^{-1} and zinc, 100-300 mg kg^{-1} increased the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of *Sesuvium portulacastrum*.

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