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## Changes in Biochemical Variations of *Sesuvium portulacastrum* under Copper and Zinc Treatment

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### ABSTRACT

The present work deals with the ecophysiological studies on the biochemical contents of the effect of copper and zinc on *Sesuvium portulacastrum* L. The *Sesuvium portulacastrum* plant are grown in pots in a spilt plot design with copper and zinc concentration levels as main treatments (control, 100, 200, 300, 400, 500 and 600 mg/kg<sup>-1</sup>). The experiments were replicated five times. The *Sesuvium portulacastrum* plants are raised in pots. The copper and zinc were mixed with (1:2) the sand and applied to the pot soil (10 kg /acre). The two heavy metals copper and zinc were applied in the soil mixed. Pots were irrigated as and when necessary. The plant samples were analyzed at four different intervals (30, 60, 90 and 120<sup>th</sup> DAS). The results indicates that the heavy metals (copper and zinc) application, at the six rates (100, 200, 300, 400, 500 and 600 mg kg<sup>-1</sup>) caused reduction in various biochemical contents such as (Amino acid, Proline, Protein, Total sugar and Starch) when applied copper and zinc. Increasing in various bio chemical contents such as (Amino acid, Proline, Protein, Total Sugar and Starch) copper 200 mg kg<sup>-1</sup>, zinc 300 mg kg<sup>-1</sup> only increased low concentration and higher concentration is decreased the all biochemical contents *Sesuvium portulacastrum*.

**Keywords:** *Sesuvium portulacasrtum*, Copper, Zinc, Amino acid, Proline, Protein, Total Sugar and Starch

## 1. INTRODUCTION

All plants, terrestrial and aquatic have the ability to acquire and accumulate metal ions such as Fe, Cu, Mn and Zn which are essential for plant growth and development. Some plants can also accumulate nonessential toxic metal ions. All plants show a certain reaction to the increasing of toxic elements concentration in soil, depending upon the sensitivity of plants, exposure intensity and chemical species. Some species of plants disappear from such lands, while other, on the contrary is stimulated by these elements. On lands containing – metals some plant species (metalophytes) have developed tolerance towards metals and others (hyper accumulators) are characterized by the capacity to accumulate high quantities of metals in their tissues. Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic and various radio nuclides from soil (Baker *et al.*, 2000; Marchiol *et al.*, 2004; Cherysafopoulou *et al.*, 2005). Garbisu and Alkorta (2001) reviewed one phytoremediation category, phytoextraction, for its ability to remove heavy metals from soil using its ability to uptake metals which are essential for plant growth (Fe, Mn, Cu, Mg, Mo and Ni). Some metals with no known biological functions (Cd, Cr, Pb, Co, Ag, Se and Hg) accumulated (Baker and Brooks, 1989; Rashkin *et al.*, 1994; Jayakumar *et al.*, 2010; Jayakumar *et al.*, 2013).

## 2. MATERIALS AND METHODS

### 2. 1. Plant cutting

The experimental plant, the *Sesuvium portulacastrum* L. belongs to the family Aizoaceae which 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. Plants cutting with each 5 cm length with uniform thickness were chosen for experimental purpose.

### 2. 2. 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<sup>-1</sup> 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<sub>4</sub>·5H<sub>2</sub>O, ZnSO<sub>4</sub>·7H<sub>2</sub>O) 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.

### 2. 3. Sample collection

The plant samples were collected at thirty days interval, up to four months viz., 30, 60, 90 and 120<sup>th</sup> day for the measurement of various morphological growth parameters. The biochemical (amino acid, proline and protein) copper and zinc content of the plants were estimated at all the four sampling periods. Six plants from each replicate of a pot were

analyzed for its various parameters and the average was calculated. These mean values were used for statistical analysis.

### **3. BIOCHEMICAL ANALYSIS**

#### **3. 1. Estimation of total free amino acids (Moore and Stein, 1948)**

One ml of ethanol extract was taken in 25 ml test tubes and neutralized with 0.1N sodium hydroxide using methyl red indicator. One ml of ninhydrin reagent was added (800 mg stannous chloride in 500 ml of citrate buffer, pH 5.0, 20g ninhydrin in 500 ml methyl cellosolve; both solutions were mixed). The contents were boiled in a water bath for 20 minutes, and 5 ml of diluent solution (distilled water and *n*-propanol mixed in equal volume) was added, cooled and diluted to 25 ml with distilled water. The absorbance was measured at 570 nm in a spectrophotometer. The standard graph was prepared using leucine.

#### **3. 2. Estimation of proline (Bates *et al.*, 1973)**

##### **3. 2. 1. Extraction**

Five hundred mg of plant material was taken in a pestle and mortar and homogenized with 10 ml of 3 per cent aqueous sulphosalicylic acid. Then the homogenate was filtered through Whatman No. 2 filter paper. The residue was re-extracted two times with 3 per cent sulphosalicylic acid and pooled. The filtrates were made upto 20 ml with 3 percent sulphosalicylic acid and used for the estimation of proline.

##### **3. 2 .2. Estimation**

2 ml of extract was taken in a test tube and 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added to it. The mixture was incubated for one hour at 100 °C in a water bath. The tubes were transferred to an ice bath to terminate the reaction. Then to each test tube 4 ml of toluene was added and mixed vigorously using a test tube stirrer for 10-20 seconds. The toluene containing the chromophore was separated from the aqueous phase with the help of separating funnel and the absorbance was measured at 520 nm in a spectrophotometer using an appropriate blank. The proline content was determined from a standard curve prepared with proline and the results were expressed in milligram per gram dry weight.

#### **3. 3. Estimation of protein (Lowry *et al.*, 1951)**

Fresh tissue weighing 0.5 g was macerated in 20 per cent trichloroacetic acid using mortar and pestle. The homogenate was then centrifuged at 600 rpm 30 minutes and the supernatant was discarded. To the pellet, 5 ml of 0.1N NaOH was added and centrifuged for 30 minutes. The supernatant was saved for the estimation of protein. To 0.5 ml of protein extract, 5 ml of copper reagent (C) was added (Reagent C: mixture of reagent A and B at 50:1 ratio; reagent A: 2 per cent Na<sub>2</sub>CO<sub>3</sub> in 0.1N NaOH; reagent B: equal volume of 1percent CuSO<sub>4</sub> and two percent of sodium potassium tartarate). The tubes were shaken well and allowed to stand in dark for 10 minutes at room temperature. Properly diluted Folin-ciocalteu reagent (0.5 ml) was added to this solution and mixed thoroughly. The absorbance was read at

660 nm in a spectrophotometer against an appropriate blank. Bovin serum albumin was used as the standard.

### 3. 4. Estimation of total sugars (Nelson, 1944)

Plant samples were treated with 80 percent boiling ethanol for taking extractions (5 ml extract representing 1 g of tissue). Five readings for each sample were taken. One ml of ethanol extract taken in the test tubes was evaporated in a water bath. To the residue, 1 ml of distilled water and 1 ml of 1 N sulphuric acid were added and incubated at 49 °C for 30 minutes. The solution was neutralised with 1 N sodium hydroxide using methyl red indicator. One ml of reagent 'C' (Nelsons reagent) was added to each test tube prepared by mixing reagent A and reagent B in 25:1 ratio (Reagent A: 25 g sodium carbonate, 25 g sodium potassium tartarate, 20 g of sodium bicarbonate and 200 g anhydrous sodium sulphate in 1000 ml: Reagent B: 15 g cupric sulphate in 100 ml of distilled water with 2 drops of concentrated sulphuric acid). The test tubes were heated for 20 minutes in a boiling water bath, cooled and 1ml of arsenomolybdate reagent (25 g ammonium molybdate, 21 ml of concentrated sulphuric acid, 5 g sodium arsenate dissolved in 475 ml of distilled water and incubated at 37 °C in a water bath for 48 hours) was added. The solution was thoroughly mixed and diluted to 25 ml and measured at 495 nm in a Spectrophotometer. The reducing sugar contents of the unknown samples were calculated from glucose standard.

### 3. 5. Estimation of starch (sumner and somers, 1949)

The ethanol insoluble residues taken from ethanol extraction were dried at 60 °C for 4 hours in an oven. To 200 mg of the powdered residue, 3 ml of 6 N HCl was added and autoclave at 100 °C for an hour. The flask was cooled and volume was raised to 25 ml with distilled water. One ml of aliquot was drawn and, neutralized with 1N NaOH and sugar was estimated by Nelson's method (Nelson, 1944).

## 4. RESULTS

### 4. 1. Amino acids

The amino acid content of copper treated *S. portulacastrum* shoot at different ages, under different concentration of applied copper is shown in Table 1. The amino acid content of *S. portulacastrum* shoot was found to be the highest at 200 mg kg<sup>-1</sup> of copper level (viz., 10.91, 16.72, 22.93 and 21.20) and the same was lowest at 600 mg kg<sup>-1</sup> of copper level (viz., 4.80, 7.50, 10.56 and 9.65) in all the sampling days. Amino acid content of zinc treated *S. portulacastrum* shoot increased with increased concentration of applied zinc in the soil up to 300 mg kg<sup>-1</sup> (viz., 11.40, 16.98, 24.10 and 21.82) in all the sampling days. For further higher concentrations, the amino acid content of zinc treated *S. portulacastrum* decreased. The lowest amino acid content was recorded at 600 mg kg<sup>-1</sup> of zinc treated *S. portulacastrum* (viz., 5.70, 7.96, 10.76 and 9.96) in all the sampling days. In both the treatments amino acid content is higher on the 90<sup>th</sup> day. Variance of mean values for treatment, sampling days and interaction between them are significant at 1 per cent level both in copper and zinc treated *S. portulacastrum* plants.

**Table 1.** Effect of copper and zinc on amino acid content (mg g<sup>-1</sup> fresh weight) of *Sesuvium portulacastrum* L.

Metals added in the soil (mg kg <sup>-1</sup> )	Copper				Zinc			
	Sampling days				Sampling days			
	30	60	90	120	30	60	90	120
Control	8.25	12.38	17.05	15.56	8.25	12.38	17.05	15.56
100	10.38 (+25.82)	15.61 (+26.09)	21.66 (+27.04)	19.94 (+28.15)	10.52 (+27.51)	16.30 (+31.66)	22.43 (+31.55)	20.63 (+32.58)
200	10.91 (+32.24)	16.72 (+35.06)	22.93 (+34.49)	21.20 (+36.25)	11.06 (+34.06)	16.89 (+36.43)	23.78 (+39.47)	21.56 (+38.56)
300	7.23 (-12.36)	10.99 (-11.23)	15.22 (-10.73)	13.64 (-12.34)	11.40 (+38.18)	16.98 (+37.16)	24.10 (+41.35)	21.82 (+40.23)
400	6.15 (-25.45)	9.46 (-23.59)	12.90 (-24.34)	11.85 (-23.84)	7.32 (-11.27)	11.26 (-09.05)	15.52 (-08.97)	13.98 (-10.15)
500	5.65 (-31.51)	8.12 (-34.41)	11.43 (-32.96)	10.08 (-35.22)	6.40 (-22.42)	9.78 (-21.01)	13.24 (-22.35)	12.34 (-20.69)
600	4.80 (-41.82)	7.50 (-39.42)	10.56 (-38.06)	9.65 (-37.98)	5.70 (-30.91)	7.96 (-35.70)	10.76 (-36.89)	9.96 (-35.99)

Comparison of significant effects	F test	CD P = 0.05	F test	CD P = 0.05
Metal levels	**	0.0344	**	0.0426
Sampling days	**	0.0305	**	0.0318
Interaction	**	0.0807	**	0.0841

Average of five replications

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

#### 4. 2. Proline

The proline content of *S. portulacastrum* at different stages of shoot is furnished in Table 2. The proline content of *S. portulacastrum* shoot increased with increased concentration of copper level. Maximum proline accumulation was recorded at 600 mg kg<sup>-1</sup> of copper level (viz., 3.02, 3.55, 4.20 and 4.03) in all the sampling days. Minimum proline content was observed at 100 mg kg<sup>-1</sup> of copper (viz., 1.65, 1.87, 2.28 and 1.98). The proline content of *S. portulacastrum* shoot increased with increased concentration of zinc level.

Maximum proline accumulation was recorded at 600 mg kg<sup>-1</sup> of zinc level (viz., 2.80, 3.38, 3.95 and 3.78) in all the sampling days. Minimum proline content was observed at 100 mg kg<sup>-1</sup> of zinc level (viz., 1.60, 1.85, 2.24 and 1.97) in all the sampling days. F test values for treatment, sampling days and interaction were significant for both in copper and zinc treated *S. portulacastrum* plants.

**Table 2.** Effect of copper and zinc on proline content (mg g<sup>-1</sup> fresh weight) of *Sesuvium portulacastrum* L.

Metals added in the soil (mg kg <sup>-1</sup> )	Copper				Zinc			
	Sampling days				Sampling days			
	30	60	90	120	30	60	90	120
Control	2.15	2.56	3.05	2.88	2.15	2.56	3.05	2.88
100	1.65 (-23.25)	1.87 (-26.95)	2.28 (-25.24)	1.98 (-31.25)	1.60 (-25.58)	1.85 (-27.73)	2.24 (-26.56)	1.97 (-31.60)
200	1.79 (-16.74)	1.99 (-22.26)	2.33 (-23.61)	2.16 (-25.01)	1.70 (-20.93)	1.96 (-23.44)	2.29 (-24.92)	2.13 (-26.04)
300	2.39 (+11.16)	2.82 (+10.16)	3.40 (+11.47)	3.16 (+9.72)	1.82 (-15.35)	2.10 (-17.97)	2.38 (-21.97)	2.31 (-19.79)
400	2.56 (+19.07)	3.16 (+23.44)	3.67 (+20.33)	3.58 (+24.30)	2.37 (+10.23)	2.80 (+09.37)	3.36 (+10.16)	3.13 (+08.68)
500	2.85 (+32.56)	3.44 (+34.37)	3.98 (+30.49)	3.85 (+33.68)	2.52 (+17.21)	2.95 (+15.23)	3.61 (+18.36)	3.48 (+20.83)
600	3.02 (+40.46)	3.55 (+38.67)	4.20 (+37.70)	4.03 (+39.93)	2.80 (+30.23)	3.38 (+32.03)	3.95 (+29.51)	3.78 (+31.25)

Comparison of significant effects	F test	CD P = 0.05	F test	CD P = 0.05
Metal levels	**	0.5601	**	0.0151
Sampling days	**	0.4038	**	0.0362
Interaction	**	1.0683	**	0.0957

Average of five replications

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

### 4. 3. Protein

The protein content of copper and zinc treated *S. portulacastrum* is shown in Table 3. The protein content of copper treated *S. portulacastrum* shoot increased appreciably with increasing concentration of applied copper in the soil up to 200 mg kg<sup>-1</sup> of copper level (viz., 15.22, 17.52, 22.05 and 19.86). For further higher concentrations of zinc, the protein content of *S. portulacastrum* decreased. The minimum protein content was observed at 600 mg kg<sup>-1</sup> of copper level (viz., 6.72, 8.10, 10.19 and 9.16) in all the sampling days. F test values for treatment, sampling days and interaction were significant in copper treatment *S. portulacastrum* plants. The protein content of zinc treated *S. portulacastrum* shoot increased with increasing concentration of applied zinc in the soil up to 300 mg kg<sup>-1</sup> of zinc (viz., 15.82, 18.25, 23.10 and 21.10). For further higher concentrations of zinc, the protein content of *S. portulacastrum* decreased. The lowest protein content was recorded at 600 mg kg<sup>-1</sup> of zinc treated *S. portulacastrum* (viz., 7.30, 8.56, 10.89 and 10.22) in all the sampling days. F test values of zinc treated *S. portulacastrum* were non-significant for treatment and sampling days and significant for interaction between them.

**Table 3.** Effect of copper and zinc on protein content (mg g<sup>-1</sup> fresh weight) of *Sesuvium portulacastrum* L.

Metals added in the soil (mg kg <sup>-1</sup> )	Copper				Zinc			
	Sampling days				Sampling days			
	30	60	90	120	30	60	90	120
Control	11.26	13.13	16.43	15.16	11.26	13.13	16.43	15.16
100	14.20 (+26.11)	16.23 (+23.61)	20.42 (+24.28)	18.58 (+22.56)	14.49 (+28.68)	17.16 (+30.69)	21.56 (+31.22)	19.12 (+26.12)
200	15.22 (+35.17)	17.52 (+33.43)	22.05 (+34.20)	19.86 (+31.01)	15.47 (+37.39)	17.98 (+36.94)	22.70 (+38.16)	20.62 (+36.01)
300	9.80 (-12.97)	11.36 (-13.48)	14.61 (-11.08)	13.56 (-10.55)	15.82 (+40.50)	18.25 (+38.99)	23.10 (+40.60)	21.10 (+39.18)
400	8.52 (-24.33)	10.32 (-21.40)	12.99 (-20.94)	11.78 (-22.29)	10.18 (-09.59)	11.63 (-11.42)	14.78 (-10.04)	13.65 (-09.96)
500	7.76 (-31.08)	9.10 (-30.69)	10.96 (-33.29)	10.28 (-32.19)	8.84 (-21.49)	10.45 (-20.41)	12.83 (-21.91)	11.74 (-22.56)
600	6.72 (-40.32)	8.10 (-38.31)	10.19 (-37.98)	9.16 (-39.58)	7.30 (-35.17)	8.56 (-34.80)	10.89 (-33.72)	10.22 (-32.58)

Comparison of significant effects	F test	CD P = 0.05	F test	CD P = 0.05
Metal levels	**	0.0353	NS	NS
Sampling days	**	0.0327	NS	NS
Interaction	**	0.0865	**	0.0844

Average of five replications

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

#### 4. 4. Total sugars

The results on the effect of different level of copper and zinc on the total sugar content of the *S. portulacastrum* are given in Table 4. The total sugar content of *S. portulacastrum* shoot was found to be highest at 200 mg kg<sup>-1</sup> of copper level (viz., 12.76, 16.58, 24.42 and 19.98) in all the sampling days. With further increase in copper level there was a gradual decrease in the total sugars content which occurred in all the sampling days. Zinc treatment at low levels (100, 200 and 300 mg kg<sup>-1</sup> of soil) increased the total sugars content of shoot of *S. portulacastrum* (viz., 11.98, 15.78, 23.62, 19.72; 12.75, 16.80, 25.48, 20.64 and 13.11, 17.06, 25.69, 20.96 respectively) in all the sampling days. With further increase of zinc level (400, 500 and 600 mg kg<sup>-1</sup>), the total sugar content of *S. portulacastrum* was reduced in all the sampling days. The total sugar content of *S. portulacastrum* showed a progressive trend up to the growth stage (30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day) and gradually declined on 120<sup>th</sup> day due to the senescence of leaves. F values calculated for treatment, sampling days and interaction between treatment and sampling days were significant at 1 per cent level in both copper and zinc treated *S. portulacastrum* plants.

**Table 4.** Effect of copper and zinc on total sugar content (mg g<sup>-1</sup> fresh weight) of *Sesuvium portulacastrum* L.

Metals added in the soil (mg kg <sup>-1</sup> )	Copper				Zinc			
	Sampling days				Sampling days			
	30	60	90	120	30	60	90	120
Control	9.43	12.62	18.72	15.38	9.43	12.62	18.72	15.38
100	11.92 (+26.40)	15.89 (+25.91)	22.94 (+22.54)	19.21 (+24.90)	11.98 (+27.04)	15.78 (+25.04)	23.62 (+26.17)	19.72 (+28.22)
200	12.76 (+35.31)	16.58 (+31.38)	24.42 (+30.45)	19.98 (+29.91)	12.75 (+35.21)	16.80 (+33.12)	25.48 (+36.11)	20.64 (+34.20)
300	8.33 (-11.66)	11.06 (-12.36)	16.75 (-10.52)	13.52 (-12.09)	13.11 (+39.02)	17.06 (+35.18)	25.69 (+37.23)	20.96 (+36.28)



400	7.26 (-23.01)	9.86 (-21.87)	14.76 (-21.15)	11.98 (-22.11)	8.41 (-10.82)	11.30 (-10.46)	16.96 (-09.40)	13.68 (-11.05)
500	6.48 (-31.28)	8.74 (-30.74)	13.24 (-29.27)	11.06 (-28.09)	7.33 (-22.27)	9.98 (-20.92)	14.38 (-23.18)	12.15 (-21.01)
600	5.83 (-38.18)	7.66 (-39.30)	11.88 (-36.54)	9.62 (-37.45)	5.86 (-37.86)	8.18 (-35.18)	12.25 (-34.56)	9.84 (-36.02)

Comparison of significant effects	F test	CD P = 0.05	F test	CD P = 0.05
Metal levels	**	0.0355	**	0.0220
Sampling days	**	0.0325	**	0.0333
Interaction	**	0.0860	**	0.0882

Average of five replications

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

#### 4. 5. Starch

The results on the effect of different concentrations of copper and zinc on starch content of *S. portulacastrum* shoot are presented in Table 5. There was a gradual decrease in the starch content with increasing copper level. The maximum decrease of starch content was recorded at 600 mg kg<sup>-1</sup> of copper level (viz., 5.44, 6.84, 9.62 and 7.98) in all the sampling days. However, the starch content increased up to 200 mg kg<sup>-1</sup> of copper level (viz., 12.26, 15.38, 20.86 and 17.81) in all the sampling days. In zinc treated *S. portulacastrum* at 100, 200 and 300 mg kg<sup>-1</sup> of soil increased starch content was observed (viz., 11.65, 14.68, 20.21, 17.32; 12.38, 15.56, 21.15, 17.73 and 12.59, 15.69, 21.28, 17.98 respectively). Zinc treatment beyond this level decreased the starch content of *S. portulacastrum*. The lowest starch content was recorded at 600 mg kg<sup>-1</sup> of zinc level (viz., 5.83, 7.32, 9.58 and 8.23) in all the sampling days. F test values were significant (at 1 per cent level) for treatment, sampling days and interaction treatment and sampling days both in copper and zinc treated *S. portulacastrum* plants.

**Table 5.** Effect of copper and zinc on starch content (mg g<sup>-1</sup> fresh weight) of *Sesuvium portulacastrum* L.

Metals added in the soil (mg kg <sup>-1</sup> )	Copper				Zinc			
	Sampling days				Sampling days			
	30	60	90	120	30	60	90	120
Control	9.10	11.22	15.42	13.05	9.10	11.22	15.42	13.05
100	11.48 (+26.15)	14.43 (+28.61)	19.62 (+27.24)	16.93 (+29.73)	11.65 (+28.02)	14.68 (+30.84)	20.21 (+31.06)	17.32 (+32.72)

200	12.26 (+34.47)	15.38 (+37.08)	20.86 (+35.28)	17.81 (+36.47)	12.38 (+36.04)	15.56 (+38.69)	21.15 (+37.16)	17.73 (+35.86)
300	7.96 (-12.53)	9.88 (-11.94)	13.78 (-10.63)	11.60 (-11.11)	12.59 (+38.35)	15.69 (+39.84)	21.28 (+38.01)	17.98 (+37.78)
400	7.23 (-20.55)	9.05 (-19.34)	12.10 (-21.53)	10.11 (-22.53)	8.11 (-10.88)	10.09 (-10.07)	13.98 (-09.34)	11.72 (-10.19)
500	6.18 (-32.09)	7.76 (-30.84)	10.66 (-30.87)	8.94 (-31.49)	7.28 (-20.01)	9.16 (-18.36)	12.36 (-19.84)	10.26 (-21.38)
600	5.44 (-40.22)	6.84 (-39.04)	9.62 (-37.61)	7.98 (-38.85)	5.83 (-35.93)	7.32 (-34.76)	9.58 (-37.87)	8.23 (-36.93)

Comparison of significant effects	F test	CD P = 0.05	F test	CD P = 0.05
Metal levels	**	0.0352	**	0.0439
Sampling days	**	0.0255	**	0.0302
Interaction	**	0.0675	**	0.0799

Average of five replications

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

## 5. DISUSSION

### 5. 1. Amino acids and proteins

Amino acids and protein content of *S. portulacastrum* were higher at low level of copper (100-200 mg kg<sup>-1</sup>) and zinc (100-300 mg kg<sup>-1</sup>) in the soil than in the control plants. Further, the values decreased with a gradual increase in copper (300-600 mg kg<sup>-1</sup>) and zinc (400-600 mg kg<sup>-1</sup>) level in all the sampling days. The decrease in amino acid and protein content in excess copper and zinc treated *S. portulacastrum* is similar to the observations reported by Mocquot *et al.* (1996), Liao *et al.* (2000), Mazen (2004), Guo *et al.* (2007), Zengin and Kirbag (2007) and Al-Hakimi and Hamada (2011) under copper treatment and Ren *et al.* (1993), Ghosh and Srivastava (1994) and Upadhyay and Singh (1995) under zinc treatment and Jayakumar and Vijayarengan (2014) under cobalt treatment. Protein and amino acids are regarded to play a significant role in metal chelation, by which heavy metal detoxification and tolerance in plants take place (Hall, 2002). The inhibitory action of excess copper and zinc on amino acid and protein content may be due to binding of metals with sulfhydryl group of protein, causing deleterious effect in the normal protein form (Manivasagaperumal *et al.*, 2011). It is thought that decrease in protein content under heavy metals stress may be due to increase in protease activity (Palma *et al.*, 2002), various structural and fragmentation of protein (John *et al.*, 2009), DNA-protein cross-links (Atesi *et al.*, 2004), interaction with thiol residues of proteins and replacement them with heavy metals in metalloproteins (Pal *et al.*, 2006). Ferritins, iron containing proteins in plants are known to bind a variety of divalent metal ions (Zn, Cu, Cd, Pb and Be) *in vitro* and *in vivo*.

Similar reduction in amino acid and protein content was also reported by Nagoor (1999) in *Zea mays* and Kumar and Sadhna (1999) in *Albizia lebbek*, Muthuchelian *et al.* (2001) in *Erythrina variegata*, Gardea-Torresdey *et al.* (2004) in *Convolvulus arvensis*, Jayakumar and Vijayarengan, (2006) in Black gram, Romero-Puertas *et al.* (2007) in pea, Jayakumar *et al.*, (2007) in soybean plants, Dinakar *et al.* (2008) in *Brassica juncea*. Jayakumar *et al.*, (2008a, 2008b, 2009) and Hamid *et al.* (2010) in *Phaseolus vulgaris* due to other metals.

## 5. 2. Proline

A marked increase in proline content was observed in plants treated with excess of copper and zinc in contrast with the lower level of copper (100-200 mg kg<sup>-1</sup>) and zinc (100-300 mg kg<sup>-1</sup>) treatment. This would be evident from the study of Kastori *et al.* (1992) in Pb, Cd, Cu and Zn, Bassi and Sharma (1993a) in copper and zinc, Zengin and Munzuroglu (2005) in Cd, Cu and Pb, Backor *et al.* (2004), Azooz *et al.* (2012) in Cu and Azooz *et al.* (2011) in Zn and Pb. The increase in proline content due to different stress, particularly drought and salinity, is a common observation. The accumulation of proline in plants is a general response to some abiotic stress (Jain *et al.*, 2001; Fariduddin *et al.*, 2009; Jaleel and Azooz, 2009). But it is the fact that metal stress also increases the proline content which has been meagerly reported in the literature. It may be argued that proline accumulation helps to conserve nitrogenous compounds and protect the plant against heavy metal stress. These results also support the view that proline acts as a membrane stabilizing agent under stress conditions (Poschenrieder and Barcelo, 2004; Manivasagaperumal *et al.*, 2011). Our results of increased proline content corroborated with the findings of Bassi and Sharma (1993a), who also found that copper proved to be a stronger inducer of proline accumulation than Zn in wheat seedlings. It has been determined that, as a response to heavy metals generated stress, plants increase their proline (Zengin and Kirbag, 2007; Jayakumar *et al.*, 2010). The considerable accumulation of proline as a result heavy metals stress has been reported by Dhir *et al.* (2004), John *et al.* (2008) and Pant *et al.* (2011).

## 5. 3. Sugars and starch

Sugar and starch contents showed a decreasing trend with progressive increase in copper and zinc concentration in *S. portulacastrum*. However 100-200 mg kg<sup>-1</sup> of copper and 100-300 mg kg<sup>-1</sup> of zinc level produced positive effect on the sugar and starch contents, which is in consonance with the findings of Lanaras *et al.* (1993) and Singh *et al.* (2007) under copper treatment, Shrotri *et al.* (1979), Samarakoon and Rauser (1979), Farshian *et al.* (2007) and Hamid *et al.* (2010) under zinc treatment and Greger and Lindberg (1986) under cadmium treatment and Jayakumar *et al.*, (2014) under cobalt treatment. The reduction of sugar and starch content might be due to the imbalance which might eventually lead to depletion of carbohydrate reserves (Murata *et al.*, 1969). The decline in the carbohydrate content with respect to the high level of zinc may be due to its role on the enzymatic reactions related to the cycles of carbohydrate catabolism (Rabie *et al.*, 1992; Manivasagaperumal *et al.*, 2011). The decrease in the total carbohydrate content corresponded with the photosynthetic inhibition or stimulation of the respiration rate (Zengin and Kirbag, 2007). Al-Lahham *et al.* (2007) showed that translocation of heavy metals in tomato is dependent upon the carbohydrate partitioning, which is under control by the effects of heavy metals.

## 6. CONCLUSION

From the present research deals with biochemical changes in *S. portulacastrum* under copper and zinc treatment, all the biochemical constituents of *S. portulacastrum* for beneficial value of low concentration of zinc (100-200 mg kg<sup>-1</sup>) and copper (100-300 mg kg<sup>-1</sup>) treatment, due to increase the concentration of zinc (above 200 mg kg<sup>-1</sup>) and copper (above 300 mg kg<sup>-1</sup>) decrease the biochemical constituents in *S. portulacastrum*.

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