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Interactive effects of copper and zinc accumulation in *Portulaca olearcea* stem cuttings, through hydroponics

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ABSTRACT

The present study was designed to assess the effect of Copper and Zinc ions on the restoration potential of stem cuttings of Portulaca oleracea to document the accumulation capacity of the plant cuttings at different concentrations of the Copper and Zinc metal solutions and to assess the interactive effect these metals on the biomorphometric parameters of Portulaca oleracea The twigs maintained at hydroponics culture was continuously monitored for 40 days for certain parameters such as root initiation, sprouting, number of adventitious and lateral roots produced, root elongation (the length of the first regenerated root) and magnitude of stem decay. The results showed that the sprouting, adventitious and lateral root development started only on the 14th day of the treatment respectively and the stem decay had also got initiated on 18th day of treatment at higher concentration levels of Copper and Zinc exposed stem cuttings. The stem decay was prominent at 20 mg/l of Zinc irrespective of the copper concentration. Hence the magnitude of toxicity was found to be higher when the concentration of Zinc primarily gets increased along with Copper. These shows considerable interactive effects exist when the plants are exposed to both the metals in an ideal environment. Hence its bioconcentration factor is expected to have promising applications of Portulaca oleracea towards the phytoremediation of heavy metals in the environment.

Keywords: *Portulaca oleracea*, Bioaccumulation, Copper, Zinc, hydroponics

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INTRODUCTION

Heavy metals (elements with an atomic density greater than 6 g/cm^3) are one of the most persistent pollutants in water. Unlike other pollutants, they are difficult to degrade, but can accumulate throughout the food chain, producing potential human health risks and ecological disturbances. Their presence in water is due to discharges from residential dwellings, groundwater infiltration and industrial discharges. The discharge of wastewater containing high concentrations of heavy metals to receiving water bodies has serious adverse environmental effects. Their occurrence and accumulation in the environment is a result of direct or indirect human activities, such as rapid industrialization, urbanization and anthropogenic sources [1-4].

The contamination of heavy metals to the environment, i.e., soil, water, plant and air is of great concern due to its potential impact on human and animal health. Cheaper and effective technologies are needed to protect the precious natural resources and biological lives. Substantial efforts have been made in identifying plant species and their mechanisms of uptake and hyper accumulation of heavy metals in the last decade. There are genetic variations among plant species and even among the cultivar of the same species. The mechanisms of metal uptake, accumulation, exclusion, translocation, osmoregulation and vary with each plant species and determine its specific role in phyto remediation [5Variations exist for hyper accumulation of different metals among various plant species and within populations. These variations do not correlate with either the metal concentration in the soil or the degree of metal tolerance in the plant [6].

Among several plants investigated, *Portulaca oleracea* was found to have the greater capability of accumulating the metals such as Cu, Zn, Cd, Pb etc. It *is* an ornamental plant of medicinal value, as it contains high levels of fatty acids, vitamin E and omega-3-fatty acids and is known to reduce the incidence of cancer and heart diseases [7]. The leaves of the plant were shown to regenerate both roots and shoots in the sterile distilled water medium. The seedling so developed, established well when transplanted onto the soil medium [8]. Makesh Kumar *et al*. [9] reported the effect of Hg, Cd, Cu, Zn and Pb on the restoration ability of the stem cuttings of the plant, *Portulaca oleracea*. The effect of Cu, Cd, Zn, Hg and Pb on the restoration potential of leaves of the medicinal plant, *P. oleracea* has been investigated [10]. The interactive effects of Se and Hg on the regeneration potential of the leaves have also been studied [11]. Preliminary investigation carried out by Deepa *et al.*, [12] revealed another dimension of the plant i.e. its potentials to extract and accumulate copper and zinc from soil.

Though bioaccumulation of heavy metal by several plant species had been studied widely, the interactive effect of these heavy metals upon bio accumulation, on the plants needs further investigation. Hence the present study is carried out to investigate the potential of *Portulaca oleracea* in extracting copper and zinc from aqueous solutions and to assess the interactive effect of these metals on the growth and biomass of the referred plant species.

MATERIALS AND METHODS

Sample collection and Preparation:

Healthy, well grown Portulaca oleracea plants were collected washed thoroughly and plants of equal size and length were segregated. The effect of soluble forms of Copper and Zinc(Copper sulfate and Zinc chloride) on the regeneration ability of *P. oleracea* and the amount of uptake of these metals were studied by using twigs of approximately equal length and thickness (10 cm and 4mm respectively). The stem cuttings were prepared by removing the leaves and roots using sharp stainless steel knife from the segregated healthy plants propagated under natural conditions.

Copper sulfate ($CuSO_4 \cdot 5H_2O$) and Zinc Chloride ($ZnCl_2$) form the sources of Copper and Zinc respectively. The stock solution of Cu was prepared by dissolving known quantity of the metal salt in distilled water. Required concentrations (0.1, 0.25, 0.5, 1, 2.5, 5, 10 mg/l) of Cu were prepared by diluting the stock solution (1000 mg/l) appropriately with distilled water. Similarly the stock solution of Zn was prepared by dissolving known quantity of the metal salt in distilled water. Required concentrations ((1, 2.5, 5, 10 and 20 mg/l) of Zn were prepared by diluting the stock solution (1000 mg/l) appropriately with distilled water. Distilled water served as the medium for the growth of control plants. Replicates were maintained for each set and three twigs per container were kept.

Experimental Setup:

The experiment was conducted by submerging the lower part of the twig (approximately 4–5 cm) in the treatment containers (each containing 150 ml of known concentration of the heavy metals, Copper and Zinc). Four to five twigs were submerged in each container. Two containers with distilled water were maintained as control. The solutions from all the containers were changed weekly once to enhance the uptake of metal ions (Fig 1). The twigs were observed continuously for 40 days for certain parameters such as root initiation, sprouting, number of leaves generated, root elongation (the length of the first regenerated root), number of adventitious and lateral roots produced, initiation time and magnitude of stem decay.

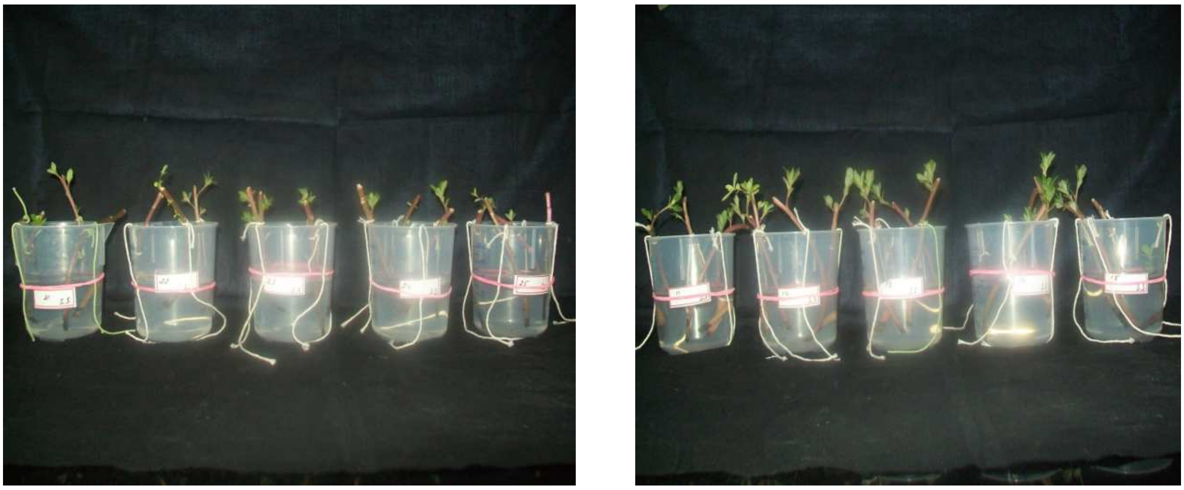

Fig 1: Experimental setup

RESULTS AND DISCUSSION

In the present study *P. oleracea was* exposed to increasing concentrations of Copper along with zinc ions ranging from 0. 1 mg/l to 10 mg/l of Cu and 1 mg/l to 20 mg/l of Zn. A container with distilled water, without any metal was maintained as a control .The exposed plants were observed for sprouting, adventitious and lateral root development, magnitude of increase in length of the roots and stem decay. The interactive effect of Copper and Zinc on the biometric parameters of the plant was also assessed.

Interactive effects of Copper and Zinc on *P. oleracea* stem cuttings

Unlike the effect of individual metal, the combined effect of Copper and zinc has considerable detrimental effect on the regeneration potential of the *P. oleracea* stem cuttings. The formation of root initiated only on the 14^{th} day of treatment at 0.1 mg/l Cu+ 1 mg/l Zn Concentration level, whereas it initiated on the 18^{th} day of treatment at 0.1 mg/l Cu+ 2.5 mg/l Zn and 0.1 mg/l Cu+ 5 mg/l Zn concentration levels, respectively. But at 0.1 mg/l Cu+ 10 mg/l Zn concentration level, the root initiation was on the 22^{nd} day of treatment and there was no any considerable change in the initiation till the end of observations. According to Helal *et al*., [13] soil irrigation with water containing high concentrations of sodium chloride causes an increase in the solubility of cadmium and copper. The adsorption of copper and zinc is greater at high pH. However pH regulation was not carried out in the present study.

Kidd and Monterroso [14] investigated the efficiency of *Alyssum serpyllifolium* ssp. *lusitanicum* (Brassicaeae) for use in phytoextraction of poly metal-contaminated soils. The plant was grown on two mine spoil soils, one contaminated with Cr (283 mg/kg) and the other moderately contaminated with Cr (263 mg/kg), Cu (264 mg/kg), Pb (1433 mg/kg) and Zn (377 mg/kg). The results suggest that *A. serpyllifolium* could be suitable for phytoextraction uses in poly metal-contaminated soils, provided that Cu concentrations were not phytotoxic. Uneo *et al.,* [15] studied the interaction between Zn and Cd in *T. caerulescens* in solution culture and in pot soil. Results from long term (4 weeks) and short term (1 week) solution culture experiments indicate that Cd accumulation in the shoot was not affected by the supply of a 4~10-fold excess of Zn, whereas the Cd concentration of the roots decreased with increasing Zn concentrations in the solution. Copper being a micronutrient very sparse studies have been conducted toward their interaction with other metals. But still the phyto toxic effect of Zinc at higher concentrations (> 10 mg/l) were well established in various studies conducted elsewhere [16-21]. This fact was evident in the present study also (Fig 2 & 3)

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Fig(2.a)The stem cuttings exposed to Copper+ Zinc (1+20,2.5+1, and 0.1+5mg/l)

Fig(2.b)The stem cuttings exposed to Copper+ Zinc (0.1+1, 0.1+2.5 and 2.5+2.5mg/l)

Fig(2.a)The stem cuttings exposed to Copper+ Zinc (1+2.5, 1+5 and1+10mg/l)

Fig(2.b)The stem cuttings exposed to Copper+ Zinc (10+2.5,10+5, and10+10mg/l)

Fig (3a)The decay of stem cuttings

Fig (3 b) Root initiation and elongation

There was no root initiation at other higher combination of Cu and Zn concentration levels. This was comparable with the study done by Deepa, [22] The increase in the number of roots along the days of treatment was considerably got lowered with increasing concentrations of zinc from 1 mg/l (29 roots on 22nd day of treatment) to 10 mg/l (6 roots on 22nd day of treatment), (Table 1). However the length of the adventitious roots was less than 2 cm even on the 22nd day of treatment. It was less than 1 cm at 5 and 10 mg/l of Zinc along with 0.1 mg/l of Cu respectively (Table 2). Copper can act in either stimulating or inhibitory mode depending on its availability [23] and such an ambivalent act is evident in the present study as there is an increase in the numbers of roots produced at the lower treatment concentrations and complete inhibition at still higher concentrations. Despite of this the studies documenting the interactive effect of Copper and Zinc on the growth potential of plants are very sporadic.

Table 1: Number of adventitious roots (Mean ± SD) developed in plants treated with Copper and Zinc solutions at varying concentrations at frequent interval of days

No of days → Cu Concn (mg/L)↓	No of days → Zn Concn (mg/L)↓	14	18	22	26	30	34
0.1	1.0	6±2.22	19±8.63	23±10.32	25±11.87	27±13.07	29±13.62
0.1	2.5	NRD	7±3.95	8±4.97	9±5.45	10±5.72	10±5.81
0.1	5	NRD	3±1.70	6±3.28	8±4.90	8±4.90	8±4.90
0.1	10	NRD	NRD	5±4.75	6±5.75	6±6	6 ±6.25

Table 2: Length of adventitious roots in cm (Mean ± SD) developed in plants treated with copper and lead Zinc solutions at varying concentrations at frequent interval of days

No of days → Cu Concn (mg/L)↓	No of days → Zn Concn (mg/L)↓	14	18	22	26	30	34
0.1	1.0	0.39±0.15	0.53±0.08	1.47±0.22	1.59±0.17	1.69±0.23	1.87±0.27
0.1	2.5	NRD	0.50±0.21	1.02±0.41	1.10±0.42	1.17±0.46	1.29±0.51
0.1	5	NRD	0.48±0.28	0.53±0.31	0.58±0.34	0.60±0.36	0.65±0.38
0.1	10	NRD	NRD	0.15±0.15	0.18±0.18	0.20±0.20	0.25±0.25

Foot Note: NRD - No Root Development

Table 3: Number of sprouts (Mean ± SD) developed in plants treated with copper and lead solutions at varying concentrations at frequent interval of days

No of days → Cu Concn (mg/L)↓	No of days → Zn Concn (mg/L)↓	14	18	22	26	30	34
Control	Control	0.75±0.25	0.5±0.28	0.5±0.5	0.25±0.25	0.25±0.25	0.25±0.25
0.1	1.0	1±0.25	1±0.29	1±0.50	0±0.25	0±0.25	0±0.25
0.1	2.5	2±0.50	2±0.41	3±0.29	3±0.29	3±0.29	3±0.0
0.1	5	4±0.85	4±0.65	3±0.65	2±0.58	1±0.48	1±0.48
0.1	10	3±1.0	2±0.95	2±0.95	2±0.75	1±0.63	1±0.48
0.1	20	2±0.82	1±0.48	1±0.75	1.0 ±0.48	1±0.48	1±0.50
1	1.0	2±1.75	1±1.25	1±0.75	1±0.75	1±0.50	1±0.25
1	2.5	2±0.50	1±0.41	1±0.41	1±0.25	1±0.29	1±0.27
1	5	1±0.50	1±0.50	0±0.25	0±0.25	NS	NS
1	10	1±0.58	1±0.25	1±0.25	1±0.25	NS	NS
1	20	2±0.65	1±0.41	1±0.48	1±0.48	1±0.29	0±0,25
2.5	1.0	1±0.50	1±0.50	0±0.25	0±0.25	NS	NS
2.5	2.5	1±0.25	1±0.25	1±0.29	1±0.29	0±0.25	0±0.25
2.5	5	1±0.75	1±0.71	1±0.48	1±0.29	1±0.29	NS
2.5	10	1±0.29	1±0.29	0±0.25	0±0.25	NS	NS
2.5	20	0±0.25	0±0.25	0±0.25	NS	NS	NS
5	1.0	1±0.25	1±0.24	1±0.29	0±0.25	0±0.25	NS
5	2.5	1±0.75	1±0.58	1±0.58	1±0.48	1±0.29	0±0.25
5	5	1±0.75	1±0.58	1±0.48	1±0.29	0±0.25	NS
5	10	1±0.75	1±0.50	1±0.50	0 ±0.25	0±0.25	NS
5	20	1±0.75	1±0.50	1±0.50	0 ±0.25	0±0.25	1±0.0
10	1.0	2±0.41	2±0.48	1±0.41	1±0.48	1±0.29	0 ±0.25
10	2.5	3±0.95	3±0.85	2±0.71	2±0.50	2±0.41	1±0.25
10	5	3±1.11	3±0.87	2±0.55	1±0.63	1±0.41	0±0.25
10	10	1±0.48	1±0.48	1±0.25	1±0.25	1±0.25	NS
10	20	4±0.48	3±0.41	2±0.41	1±0.25	1±0.25	1±0.29

Foot Note: NS - No Sprouting

Table 4: Length of stem decay in cm (Mean ± SD) of the plants treated with copper and lead solutions at varying concentrations at frequent interval of days

No of days → Cu Concn (mg/L)↓	No of days → Zn Concn (mg/L)↓	14	18	22	26	30	34
Control	Control	ND	ND	0.6±0.20	0.85±0.29	7.4±2.48	7.4±2.48
0.1	1.0	0.68±0.06	1.33±0.09	2.60±0.07	3.45±0.06	4.55±0.06	5.73±0.09
0.1	2.5	0.60±0.06	0.78±0.05	0.95±0.06	1.70±0.04	2.50±0.04	3.08±.009
0.1	5	ND	ND	ND	ND	ND	ND
0.1	10	ND	ND	ND	ND	ND	ND
0.1	20	1 ±0.05	1±0.07	3±0.09	3±0.10	4±0.07	5±0.12
1	1.0	ND	ND	ND	ND	ND	ND
1	2.5	ND	ND	ND	ND	ND	ND
1	5	ND	ND	ND	ND	ND	ND
1	10	ND	ND	ND	ND	ND	ND
1	20	1±0.05	1±0.05	2±0.06	3. ±0.04	4±0.05	4±0.06
2.5	1.0	ND	ND	ND	ND	ND	ND
2.5	2.5	ND	ND	ND	ND	ND	ND
2.5	5	ND	ND	ND	ND	ND	ND
2.5	10	ND	ND	ND	ND	ND	ND
2.5	20	1±0.09	3±0.20	3. ±0.11	4±0.09	5±0.06	6±0.09
5	1.0	ND	ND	ND	ND	ND	ND
5	2.5	ND	ND	ND	ND	ND	ND
5	5	ND	ND	ND	ND	ND	ND
5	10	ND	ND	ND	ND	ND	ND
5	20	1±0.06	1±0.07	1±0.14	2±0.07	3. ±0.05	3±0.09
10	1.0	ND	ND	ND	ND	ND	ND
10	2.5	ND	ND	ND	ND	ND	ND
10	5	ND	ND	ND	ND	ND	ND
10	10	ND	ND	ND	ND	ND	ND
10	20	1±0.04	2±0.14	3±0.18	4±0.19	4±0.09	6±0.03

Foot Note: ND - No Decay

A few terrestrial plants like *Thlaspi caerulescens* [24]and aquatics like *Ceratophyllum demersum* [25]show potential to hyperaccumulate more than one metal. In the present study, we tried to observe the potentiality of Portulaca plants to grow and accumulate high amount of more than single toxic metal investigated viz., Cu and Zn. Previous studies on this plant by Kumar *et al.*, [9], Thangavel and Subburam [10], Thangavel *et al.*,[11]. Anandi *et al.*, [26] and Deepa *et al.* [12] concentrated primarily on investigating the ability of the plants to regenerate under stress conditions exerted by Cu, Hg, Cd, Zn, Pb, Se and Al. They demonstrated that metal treatment reduced the capacity of regeneration and toxicity order was reported to be Cd>Cu>Al>Zn>Hg>Se>Pb. Deepa *et al.*, [12] studied accumulation of Cu by plants and showed that plants could regenerate up to a maximum of 1,600 µg g−1 dw Cu in soil and accumulated >1,000 µg g−1 dw copper.

With reference to sprouting, one to three sprouts were observed in all the cuttings and it started drying in almost all the plants after 20 days of treatment. However at the combinations of 0.1 mg/l Cu+ 2.5 mg/l; Zn, 0.1 mg/l Cu+ 5 mg/l Zn and 0.1 mg/l Cu+ 10 mg/l Zn, the sprouting process was fairly better with 3 to 4 sprouts on the 14th day of treatment (Table 3). The stem decay was highly found at the concentration of 10 mg/l of Zinc irrespective of the copper concentration in the solution which is around 4 cm at the end of 34 days (Table 4). Hence the interactive effect of copper and zinc was evident with observable deleterious effect when compared to control treatment. Hence the observations should be viewed with concern as it will be toxic to the plants on exposure in a long run.

CONCLUSION

The results of the present findings established that both Copper along with Zinc ions in aqueous solutions interferes with the regeneration potential of the stem cuttings by reducing the root growth and increasing their decay. The magnitude of toxicity was found to be higher at a combination of Copper and zinc at the concentration levels beyond 0.1 mg/l for Copper and 2.5 mg/ l for Zinc. The stem decay was prominent at 20 mg/l of Zinc irrespective of the copper concentration. As the symptoms of toxicity to the plant were dependent on the concentration level, as well as the on the interactive effect of both the metals, the regeneration potential of stem cuttings and the interactive effect of the metals on the plant's growth can be used for the in situ monitoring of copper and zinc contamination in various components of environment. However, the metal analysis could not be completed at stipulated time which is expected to reveal the exact Cu and Zn accumulating capacity of *Portulaca oleracea*. Thus further investigations in the present study are expected to have promising applications towards the phytoremediation of heavy metals in the environment.

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