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Influence of graded levels of essential heavy metals and their interaction effects on the bio-concentration factor of Mn, Cu and Zn in tuberose cv. 'Prajwal'

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Abstract

Heavy metals are pollutants of great concern due to their toxicity and immutable nature. As a result of human actions such as mining, industrial activity and agricultural practices, heavy metals find their way into the soil. Transfer of heavy metals from polluted soils to plants is the major pathway of human exposure to soil contamination. Bio Concentration Factor of Mn, Cu and Zn from soils were calculated in the present investigation based on the phytoremediation capability. Phytoremediation is an emerging cost-effective, non-intrusive and aesthetically pleasing technology in which herbaceous plants and trees are used to remediate and remove the pollutants from soils. Three essential heavy metals viz., MnSO₄, CuSO₄ and ZnSO₄ at different graded levels of concentration were used in the present investigation with the main objective to study the phytoremediation capability of tuberose cv. 'Prajwal'. Data recorded on phytoremediation of tuberose cv. 'Prajwal' were analyzed statistically using OPSTAT software and least significant difference was used to differentiate the treatments. An increase in the concentration of MnSO₄ recorded a significant increase in the bio-concentration factor of Mn in tuberose cv. 'Prajwal' during both the years of study as well as in the pooled data mean in comparison to all other treatments. CuSO₄ @ 300 mg kg⁻¹ soil recorded significantly highest bio-concentration factor of Cu (656.4, 17.0 and 336.7 respectively during 2018-19, 2019-20 and the pooled data mean). ZnSO₄ @ 400 mg kg⁻¹ soil recorded significantly highest bio-concentration factor of Zn in tuberose cv. 'Prajwal' (262.66, 89.98 and 176.32 respectively during 2018-19, 2019-20 and the pooled data mean). Bio-concentration factor confirmed that tuberose cv. 'Prajwal' had been a potential phytoremediator of essential heavy metals from soil.

Keywords: MnSO₄, CuSO₄ and ZnSO₄, bio concentration factor, tuberose, phytoremediation

Introduction

Heavy metals are harmful to humans, animals and tend to bio-accumulate in the food chain. Activities such as mining and smelting of metal ores, industrial emissions and applications of insecticides and fertilizers are contributed to elevated levels of heavy metals in the environment (Alloway, 1994) ^[1]. Foliar uptake of atmospheric heavy metal emissions has also been identified as an important pathway of heavy metal contamination in crops (Bassuk, 1986 and Salim *et al.*, 1992) ^[5, 26]. Presence of high concentration of both essential and non-essential heavy metals are considered to affect the plant growth and development adversely and sometimes even lead to death under extreme conditions, thus heavy metal toxicity has been considered as one of the major abiotic stresses leading to hazardous effects in plants as many of them are toxic even at very low-level concentrations. Industrial revolution accelerated the biosphere with heavy metals. A common response of heavy metal toxicity is the excessive accumulation of reactive oxygen species (ROS) which can cause peroxidation of lipids, oxidation of proteins, inactivation of enzymes, DNA damage and/or interact with other vital constituents of the plant cells (Bohra *et al.*, 2015) ^[7]. Metal deposition in environment is either due to natural processes or as a result of human activities which include application of chemical fertilizers, pesticides, mining processes, industrialization, vehicular exhausts and several other factors.

Plants adopt various cellular mechanisms to avoid/minimize the detrimental effects of heavy metal toxicity in a variety of different ways. On exposure to heavy metals, plants initially try to prevent entry of metal into the root cells by implementing avoidance strategy, thus restricting the uptake and transport of heavy metals into the plant system.

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When heavy metals manage to enter the root cells, they are detoxified at intracellular level through processes like cell wall binding, plasma membrane exclusion, chelation and sequestration of heavy metals by ligands such as phytochelatins and metallothioneins, induction of heat shock proteins, biosynthesis of polyamines and production of anti-oxidants (Dalvi and Bhalerao, 2013 and Bohra *et al.*, 2015) [9, 7]. Excess of heavy metals also activate oxidative stress defence mechanisms and the synthesis of stress related proteins in plants. This inherent ability of plants to tolerate and accumulate toxic contaminants can be explored to treat the metal contaminated areas efficiently.

Interest in phytoremediation as a method to solve environmental contamination has been growing rapidly in the recent years. This green technology that involved 'tolerant plants' has been utilized to clean up the soil and ground water from heavy metals and other toxic organic compounds (Alberto and Sigua, 2013) [12]. More recently, use of plants in metal extraction (phytoremediation) appeared as a promising alternative in the removal of heavy metals excess from soil and water (Glass, 2000) [12]. Ting *et al.*, (1991) [33] reported that uptake of cadmium and zinc by algae *Chlorella vulgaris*. *Lemna minor* was used to reclaim the lead contaminated water (Rahmani and Sternberg, 1999) [24]. Prasad *et al.* (2001) [23] noticed that bioaccumulation of cadmium and copper by *Lemna trisulca*. A plant with an abnormally high level of metal accumulation is called a hyper accumulator (Jaffre *et al.*, 1976) [15]. These plants can even prosper on contaminated soils and accumulate extremely high contents of trace elements in the above ground biomass (Baker, 1987) [4]. A large number of plant species were already tested to find out their ability to accumulate potentially toxic elements to a high extent in the above ground biomass. In general, metals are required for a variety of metabolic processes in all organisms, however many metals can be toxic, but plants have evolved systems to regulate the uptake and distribution of metals. The uptake of metal occurs primarily through the roots of plants therefore this becomes the primary site for regulating their accumulation. In extreme conditions, where the metal concentration is very high in soil, leaves, roots and shoots play an important role by accumulating the metal more than required by their physiology (Girdhar *et al.*, 2014) [13]. Basic characteristics of hyper accumulator include i) plant which can not only accumulate high concentrations of heavy metals in their root/shoots but also grow actively producing large biomass in order to remove a greater amount of metal (Shevyakova *et al.*, 2008 and Schwitzguebel *et al.*, 2002) [27, 28], ii) plants should have unique features such as (a) nutrition-competitive ability (b) fast growth (c) high efficiency of photosynthesis (d) short life cycle and anti-pest capability (e) tolerance to high metal concentrations (f) highly effective metal accumulating biomass and (g) easy to harvest.

Materials and Methods

The present investigation was carried out during Rabi-2018 to kharif-2020 at the College of Horticulture, Dr. YSR Horticultural University, Anantharajupeta, Annamayya district of Andhra Pradesh, India. The location is situated at an altitude of 162 meters (531 feet) above the mean sea level. The geographical location was 13°59' North latitude and 79°19' East longitude. Poly bags of 12 kg capacity were

used in the present investigation and bags were filled with 10 kg of virgin soil brought from uncultivated barren land. Soil was dried, grounded and sieved through 2 mm sieve and then 3 samples of soil were collected at random just before filling the poly bags. Further, regularly soil samples were collected from poly bags at every 90 days interval and the samples were used for analysis of essential heavy metals content present in the soil. The experimental material used for the present study was tuberose single type cultivar 'Prajwal'. 'Prajwal' also known as 'Arka Prajwal' is a cross between 'Shringar' x 'Mexican Single' varieties released by Indian Institute of Horticultural Research, Bangalore. It bears single type flowers on tall and stiff spikes. Pinkish red tinge is observed on the bud before opening. The variety has been reported as tolerant to high concentrations of heavy metals and has the ability to extract heavy metals from contaminated soils. The experiment was laid out in a completely randomized design with three replications under open field conditions as pot culture experiment. The experiment consisted of 10 treatments, viz., T₁ = RDF+MnSO₄ @ 1,000 mg/ kg soil, T₂ = RDF+MnSO₄ @ 2,000 mg/kg soil, T₃ = RDF+MnSO₄ @ 3,000 mg/ kg soil, T₄ = RDF+CuSO₄ @ 100 mg/kg soil, T₅ = RDF+CuSO₄ @ 200 mg/kg soil, T₆ = RDF+CuSO₄ @ 300 mg/kg soil, T₇ = RDF+ZnSO₄ @ 200 mg/kg soil, T₈ = RDF+ ZnSO₄ @ 400 mg/kg soil, T₉ = RDF+ ZnSO₄ @ 600 mg/kg soil, T₁₀ = Control (No RDF and no essential heavy metals). One bulb was planted in each poly bag and there were 300 plants in each replication. Poly bags of size 13" x 15" with 12 kg capacity were used in the present study. Poly bags were filled with 10 kg of growing medium containing red earth, sand and farmyard manure in the ratio of 2:1:1. Calculated quantities of heavy metals as per the concentration were incorporated into the growing medium just before filling the poly bags and all the poly bags were left over on polythene mulch sheet for ten days to get settle down the growing medium. Based on the calculated area of poly bag (12.56 m²), NPK fertilizers were applied in the form of Urea @ 540 g, SSP @ 1570 g and MOP @ 410 g per annum of the crop growth period at every three months interval as per the recommended package of practice suggested by Dr. Y.S.R. Horticultural University, Venkataramannagudem, West Godavari district of Andhra Pradesh. A day before planting all the selected tuberose bulbs were soaked in carbendazim solution at a concentration of 0.1% for half an hour and the bulbs were dried in shade. Bulbs weighing about 40 g were planted at a depth of about 3-4 inches in soil filled in poly bags and watered with rose can immediately after planting. Five plants were randomly selected and tagged with a label in each replication of the treatment to record the non-destructive observations which include vegetative growth, physiological, floral and flower yield parameters. In case of destructive sampling, one plant at each stage of observation recording was removed carefully by tearing the polythene bag and removed the soil adhered to the root system of the plant, washed thoroughly with clean water to remove the soil particles adhered to the plant and dried in shade for recording the observations on growth analysis and estimation of heavy metals in different parts of the plant at regular intervals of 90 days i.e., at 90, 180, 270 and 360 days after transplanting. Bio-concentration factor was defined as the ratio of heavy metal concentration in the plant roots to that in soil (Malik *et al.*, 2010) [20].

Results

Significant differences were noticed in the bio-concentration factor of Mn in tuberose cv. 'Prajwal' by soil application of graded levels of essential heavy metal concentrations (Table 1). Among the graded levels, MnSO_4 recorded significantly higher levels of bio-concentration factor of Mn in tuberose cv. 'Prajwal'. An increase in the concentration of MnSO_4 recorded a significant increase in the bio-concentration factor of Mn during both the years of study as well as in the pooled data mean. Among the graded levels of CuSO_4 , an increase in the concentration of CuSO_4 recorded significant decrease in the bio-concentration factor of Mn up to 200 mg kg^{-1} soil and thereafter significant increase in the bio-concentration factor of Mn was observed up to 300 mg kg^{-1} soil during 2018-19 and the pooled data mean, whereas non-significant differences were noticed in the bio-concentration factor of Mn during 2019-20. Similar kind of trend was noticed in the bio-concentration factor of Mn by soil application of graded levels of ZnSO_4 as noticed with the graded levels of CuSO_4 during 2018-19 and the pooled data mean, whereas non-significant differences were noticed in the bio-concentration factor of Mn during 2019-20. Among all the treatments, significantly lowest bio-concentration factor of Mn (3.81, 1.60 and 2.71 respectively during 2018-19, 2019-20 and the pooled data mean) was noticed with untreated control plants. Based on the critical analysis of these results, it may be concluded that soil application of graded levels of MnSO_4 recorded significantly higher levels of bio-concentration factor of Mn in comparison to all other graded levels of essential heavy metal concentrations during both the years of study as well as in the pooled data mean.

Significant differences were observed among the intervals of observation recorded with respect to the bio-concentration factor of Mn by soil application of graded levels of essential heavy metal concentrations during 2018-19 and the pooled data mean, whereas non-significant differences were noticed in the bio-concentration factor of Mn during 2019-20. Among the intervals of observation recorded, significantly highest level of bio-concentration factor of Mn (22.35 and 18.03 respectively during 2018-19 and the pooled data mean) was recorded during the initial 90 DAP interval, whereas significantly lowest level of bio-concentration factor of Mn (5.06 and 7.68 respectively during 2018-19 and the pooled data mean) was recorded at 360 DAP interval during both the years of study as well as in the pooled data mean. Further, it was noticed that non-significant differences were noticed in the bio-concentration factor of Mn in between the intervals of 90 DAP and 180 DAP interval during 2018-19 and the pooled data mean. Based on the critical analysis of these results, it may be concluded that bio-concentration factor of Mn was found almost stable up to 180 DAP interval and thereafter a significant decrease was noticed till the end of the investigation. Non-significant differences noticed in the bio-concentration factor of Mn during 2nd year of study i.e., 2019-20 might be attributed to the almost exhaustion of the element by roots during the 1st year of study i.e., 2018-19.

Data pertaining to the interaction effects between graded levels of essential heavy metal concentrations and the intervals of observation recorded with respect to the bio-concentration factor of Mn was found significant during 2018-19 and the pooled data mean. Among the interaction effects, significantly highest level of bio-concentration factor of Mn (150.72 and 103.43 respectively during 2018-

19 and the pooled data mean) was recorded by soil application of MnSO_4 @ 3000 mg kg^{-1} soil at 180 DAP interval followed by soil application of MnSO_4 @ 3000 mg kg^{-1} soil at 270 DAP interval (52.59 and 52.01 respectively during 2018-19 and the pooled data mean), whereas significantly lowest level of bio-concentration factor of Mn (1.83 and 1.60 respectively during 2018-19 and the pooled data mean) was recorded in the untreated control plants at 360 DAP interval.

Data pertaining to the influence of graded levels of soil applied essential heavy metal concentrations and their interaction effects with intervals on bio-concentration factor of Cu in tuberose cv. 'Prajwal' were presented in Table 2. Significant variation was noticed in the bio-concentration factor of Cu by soil application of graded levels of essential heavy metals during 2018-19, 2019-20 and the pooled data mean. Among the graded levels of essential heavy metal concentrations, soil application of CuSO_4 @ 300 mg kg^{-1} soil recorded significantly highest bio-concentration factor of Cu (656.4, 17.0 and 336.7 respectively during 2018-19, 2019-20 and the pooled data mean) followed by soil application of CuSO_4 @ 200 mg kg^{-1} soil, whereas soil application of CuSO_4 @ 100 mg kg^{-1} soil recorded significantly lowest bio-concentration factor of Cu (48.4, 6.4 and 27.4 respectively during 2018-19, 2019-20 and the pooled data mean). Among the graded levels of MnSO_4 , significantly lowest bio-concentration factor of Cu was noticed with the soil application of MnSO_4 @ 2000 mg kg^{-1} soil (57.2, 3.4 and 30.3 respectively during 2018-19, 2019-20 and the pooled data mean), whereas soil application of lowest and highest concentrations of MnSO_4 were found at par with each other during 2018-19 and 2019-20. Among the graded levels of ZnSO_4 , significantly highest bio-concentration factor of Cu was noticed with the soil application of ZnSO_4 @ 400 mg kg^{-1} soil (57.8, 5.4 and 31.6 respectively during 2018-19, 2019-20 and the pooled data mean), whereas soil application of lowest and highest concentrations of ZnSO_4 were found at par with each other during 2018-19 and the pooled data mean. Among all the treatments, significantly lowest bio-concentration factor of Cu was noticed with untreated control (12.1, 1.8 and 7.0 respectively during 2018-19, 2019-20 and the pooled data mean).

Significant differences were noticed among the intervals of observation recorded with respect to bio-concentration factor of Cu in tuberose cv. 'Prajwal' by soil application of graded levels of essential heavy metal concentrations during 2018-19, 2019-20 and the pooled data mean. Among the intervals of observation recorded, significantly highest bio-concentration factor of Cu (266.36, 7.99 and 137.17 respectively during 2018-19, 2019-20 and the pooled data mean) was recorded at 90 DAP interval, whereas significantly lowest bio-concentration factor of Cu (7.10, 4.98 and 6.04 respectively during 2018-19, 2019-20 and the pooled data mean) was recorded at 360 DAP interval. Based on the critical analysis of these results, it may be concluded that bio-concentration factor of Cu was found higher at the initial 90 DAP interval and up to 180 DAP interval during 2018-19 and thereafter a drastic reduction was noticed in the bio-concentration factor of Cu even during 2019-20.

Data pertaining to the interaction effects between graded levels of soil applied essential heavy metal concentrations and the intervals of observation recorded with respect to bio-concentration factor of Cu was found significant during

2018-19, 2019-20 and the pooled data mean. Among the interaction effects, significantly highest bio-concentration factor of Cu (1400.1, 21.6 and 710.9 respectively during 2018-19, 2019-20 and the pooled data mean) was recorded by CuSO_4 @ 300 mg kg^{-1} soil at 90 DAP interval followed by CuSO_4 @ 300 mg kg^{-1} soil at 180 DAP interval (1209.0, 21.6 and 615.3 respectively during 2018-19, 2019-20 and the pooled data mean), whereas significantly lowest bio-concentration factor of Cu (1.5, 0.7 and 1.1 respectively during 2018-19, 2019-20 and the pooled data mean) was recorded with the untreated control at 360 DAP interval during 2018-19, 2019-20 and the pooled data mean.

Significant differences were noticed in the bio-concentration factor of Zn in tuberose cv. 'Prajwal' by soil application of graded levels of essential heavy metal concentrations during 2018-19, 2019-20 and the pooled data mean (Table 3). Among the graded levels of essential heavy metal concentrations, soil application of graded levels of ZnSO_4 recorded significantly higher bio-concentration factor of Zn in comparison to other essential heavy metal concentrations during both the years of study as well as in the pooled data mean. Among the graded levels of ZnSO_4 , soil application of ZnSO_4 @ 400 mg kg^{-1} soil recorded significantly highest bio-concentration factor of Zn (262.66, 89.98 and 176.32 respectively during 2018-19, 2019-20 and the pooled data mean). The lower and higher concentrations of ZnSO_4 were found at par with each other in the pooled data mean, whereas in the data recorded during 2018-19 and 2019-20 they have recorded marginal differences in the bio-concentration factor of Zn. Among the graded levels of MnSO_4 , an increase in the concentration of MnSO_4 recorded a significant decrease in the bio-concentration factor of Zn during 2018-19 and the pooled data mean. Among the graded levels of CuSO_4 , significantly lowest bio-concentration factor of Zn was recorded with CuSO_4 @ 100 mg kg^{-1} soil, whereas its higher concentrations recorded non-significant differences in the bio-concentration factor of Zn in tuberose cv. 'Prajwal' during 2018-19, 2019-20 and the pooled data mean. Among all the treatments, significantly lowest bio-concentration factor of Zn was recorded with the untreated control (20.94, 17.48 and 19.21 respectively during 2018-19, 2019-20 and the pooled data mean).

Significant differences were noticed among the intervals of observation recorded with respect to the bio-concentration factor of Zn by soil application of graded levels of essential heavy metal concentrations during 2018-19, 2019-20 and the pooled data mean. Among the intervals of observation recorded, significantly highest bio-concentration factor of Zn (132.53, 49.87 and 91.20 respectively during 2018-19, 2019-20 and the pooled data mean) was recorded at 180 DAP, whereas significantly lowest bio-concentration factor of Zn (35.36, 26.03 and 30.69 respectively during 2018-19, 2019-20 and the pooled data mean) was recorded at 360 DAP interval.

Data pertaining to the interaction effects between graded levels of essential heavy metal concentrations and the intervals of observation recorded with respect to the bio-concentration factor of Zn was found significant during 2018-19, 2019-20 and the pooled data mean. Among the interaction effects, significantly highest bio-concentration factor of Zn (532.94, 152.56 and 342.75 respectively during 2018-19, 2019-20 and the pooled data mean) was recorded by ZnSO_4 @ 400 mg kg^{-1} soil at 180 DAP interval followed

by ZnSO_4 @ 400 mg kg^{-1} soil at 270 DAP (383.93, 130.84 and 257.38 respectively during 2018-19, 2019-20 and the pooled data mean), whereas significantly lowest bio-concentration factor of Zn (9.64, 7.87 and 8.75 respectively during 2018-19, 2019-20 and the pooled data mean) was recorded in the untreated control plants at 90 DAP interval.

Discussion

Based on the critical analysis of these results, it may be concluded that with the progress of the investigation, bio-concentration factor of Mn recorded significant reduction which may be attributed to the absorption of Mn element through roots from soil, hence recorded significant decrease in the bio-concentration factor of Mn in tuberose cv. 'Prajwal'.

Based on the critical analysis of these results, it may be concluded that bio-concentration factor of Cu was found higher during the initial reading recorded at 90 DAP and to certain extent at 180 DAP only and thereafter a drastic reduction in the bio-concentration factor of Cu was noticed which may be attributed to the exhaustion and utilization of Cu element in the physiological processes of plant system. Copper as a cofactor for many enzymes involves in both respiration and photosynthesis, thus Cu had been considered as a vital element in the plant's growth and development. However, excessive usage or consumption of this metal has been considered to be highly toxic (Mateos-Naranjo *et al.* 2015) [18]. Rosenzweig (2002) [25] explained that plants and other organisms regulate their intracellular Cu levels by regulating Cu uptake and by reducing the intracellular free Cu concentration through metallochaperones which are soluble Cu-binding proteins that deliver Cu to the sites where it is in need within the cell (Halloran and Culotta, 2000) [14]. Bio-concentration factor of Cu was found significantly highest during the initial period of experimentation and significant reduction was noticed with the progress of the experiment. Further, it may be noticed that soil application of higher concentration of CuSO_4 recorded significantly highest bio-concentration factor in comparison to all other treatments including its lower concentrations. The present result was found in close conformity with the earlier findings of Frederick and Ching (2014) [11] and Mkumbo *et al.* (2012) [22] in tomato and Mellem *et al.* (2012) [21] in *Amaranthus dubius*.

Based on the critical analysis of these results, it may be concluded that significantly higher bio-concentration factor of Zn was recorded at the initial 90 and 180 DAP intervals during the 1st year of study i.e., 2018-19 and thereafter a significant reduction was noticed in the remaining intervals of 2018-19 as well as 2019-20. Zinc had been considered as one of the necessary trace elements for plant's successful growth and development. Further, Zn was found as a structural element in some of the enzymes such as polyphenol oxidase, ascorbic acid oxidase and cytochrome oxidase (Samecka-Cymerman and Kempers AJ, 2001) [29]. McGrath *et al.* (1997) [19] reported that when plants absorbed Zn, the heavy metal, it was then transformed from the insoluble to soluble Zn^{2+} state and thus accumulated into the plant system. Significantly higher bio-concentration factor of Zn recorded at 180 DAP in comparison to 90 DAP interval and then onwards a significant reduction in the bio-concentration factor of Zn was recorded by soil application of graded levels of essential heavy metal concentrations.

Table 1: Influence of soil applied essential heavy metals (Mn, Cu and Zn) on bio-concentration factor of Mn in *Polianthes tuberosa* cv. Prajwal

Treatment (mg of element kg ⁻¹ soil)	Bio-concentration factor of Mn														
	2018-2019					2019-2020					Pooled (2018-2020)				
	I ₉₀	I ₁₈₀	I ₂₇₀	I ₃₆₀	Mean	I ₉₀	I ₁₈₀	I ₂₇₀	I ₃₆₀	Mean	I ₉₀	I ₁₈₀	I ₂₇₀	I ₃₆₀	Mean
MnSO ₄ 1000	25.81	20.60	13.45	6.23	16.52	10.13	9.97	3.73	50.64	18.61	17.97	15.28	8.59	28.43	17.57
MnSO ₄ 2000	43.12	8.09	13.90	11.41	19.13	21.95	21.18	15.17	24.71	20.75	32.53	14.63	14.53	18.06	19.94
MnSO ₄ 3000	43.05	150.72	52.59	2.81	62.29	51.34	56.15	51.43	11.66	42.65	47.19	103.43	52.01	7.23	52.46
CuSO ₄ 100	37.58	14.37	3.83	3.72	14.87	7.97	7.51	12.74	1.78	7.50	22.77	10.94	8.28	2.75	11.18
CuSO ₄ 200	8.57	2.20	6.21	3.92	5.22	8.17	8.11	6.92	2.33	6.38	8.37	5.15	6.56	3.12	5.80
CuSO ₄ 300	22.02	4.88	3.70	5.01	8.90	6.01	5.70	5.43	1.40	4.63	14.01	5.29	4.56	3.20	6.76
ZnSO ₄ 200	12.85	4.28	9.65	2.06	7.21	17.61	17.17	16.63	3.63	13.76	15.23	10.72	13.14	2.84	10.48
ZnSO ₄ 400	11.80	6.36	5.10	1.75	6.25	5.17	5.28	4.11	1.49	4.01	8.48	5.82	4.60	1.62	5.13
ZnSO ₄ 600	14.12	7.04	15.13	11.95	12.06	7.18	4.07	4.14	4.00	4.10	10.65	5.55	9.63	7.97	8.45
Control	4.61	4.03	4.78	1.83	3.81	1.59	1.64	1.82	1.38	1.60	3.10	2.83	3.30	1.60	2.71
Mean	22.35	22.25	12.83	5.06		13.71	13.67	12.21	10.30		18.03	17.96	12.52	7.68	
Factor	T	I	T × I			T	I	T × I			T	I	T × I		
SEm±	0.28	0.18	0.57			8.29	5.24	16.59			0.07	0.04	0.14		
CD at 5%	0.81	0.51	1.62			23.39	NS	NS			0.19	0.12	0.39		

Table 2: Influence of soil applied essential heavy metals (Mn, Cu and Zn) on bio-concentration factor of copper in *Polianthes tuberosa* cv. Prajwal

Treatment (mg of element kg ⁻¹ soil)	Bio-concentration factor of Cu														
	2018-2019					2019-2020					Pooled (2018-2020)				
	I ₉₀	I ₁₈₀	I ₂₇₀	I ₃₆₀	Mean	I ₉₀	I ₁₈₀	I ₂₇₀	I ₃₆₀	Mean	I ₉₀	I ₁₈₀	I ₂₇₀	I ₃₆₀	Mean
MnSO ₄ 1000	181.1	35.4	66.3	4.9	71.9	3.1	6.1	3.8	4.1	4.3	92.1	20.8	35.0	4.5	38.1
MnSO ₄ 2000	170.3	29.7	12.9	15.9	57.2	2.3	3.5	3.6	4.1	3.4	86.3	16.6	8.3	10.0	30.3
MnSO ₄ 3000	193.3	136.8	8.0	1.6	84.9	9.4	1.2	4.2	4.5	4.8	101.3	69.0	6.1	3.1	44.9
CuSO ₄ 100	165.9	14.8	8.6	4.5	48.4	6.6	6.4	6.9	5.7	6.4	86.2	10.6	7.7	5.1	27.4
CuSO ₄ 200	195.7	129.7	11.2	21.8	89.6	17.7	7.1	6.8	10.8	10.6	106.7	68.4	9.0	16.3	50.1
CuSO ₄ 300	1400.1	1209.0	10.6	5.9	656.4	21.6	21.6	16.6	8.1	17.0	710.9	615.3	13.6	7.0	336.7
ZnSO ₄ 200	65.7	58.5	5.7	3.9	33.4	2.2	3.8	3.9	4.5	3.6	34.0	31.1	4.8	4.2	18.5
ZnSO ₄ 400	171.3	40.8	13.0	5.9	57.8	11.6	1.2	4.6	4.2	5.4	91.4	21.0	8.8	5.0	31.6
ZnSO ₄ 600	109.6	8.5	4.6	5.3	32.0	3.5	8.6	3.4	3.1	4.6	56.5	8.6	4.0	4.2	18.3
Control	10.7	30.1	6.2	1.5	12.1	2.1	2.2	2.2	0.7	1.8	6.4	16.2	4.2	1.1	7.0
Mean	266.4	169.3	14.7	7.1		8.0	6.2	5.6	5.0		137.2	87.7	10.2	6.0	
Factor	T	I	T × I			T	I	T × I			T	I	T × I		
SEm±	3.20	2.02	6.40			0.19	0.12	0.39			0.46	0.29	0.93		
CD at 5%	9.03	5.71	18.07			0.55	0.34	1.10			1.31	0.83	2.63		

Table 3: Influence of soil applied essential heavy metals (Mn, Cu and Zn) on bio-concentration factor of zinc in *Polianthes tuberosa* cv. Prajwal

Treatment (mg of element kg ⁻¹ soil)	Bio-concentration factor of Zn														
	2018-2019					2019-2020					Pooled (2018-2020)				
	I ₉₀	I ₁₈₀	I ₂₇₀	I ₃₆₀	Mean	I ₉₀	I ₁₈₀	I ₂₇₀	I ₃₆₀	Mean	I ₉₀	I ₁₈₀	I ₂₇₀	I ₃₆₀	Mean
MnSO ₄ 1000	103.18	124.94	35.99	40.61	76.18	80.60	58.91	42.57	12.86	48.73	91.89	91.92	39.28	26.73	62.45
MnSO ₄ 2000	79.16	91.60	56.71	30.88	64.58	25.87	41.51	17.57	21.99	26.74	52.51	66.55	37.14	26.43	45.66
MnSO ₄ 3000	22.77	33.27	9.86	19.04	21.23	29.80	28.25	46.85	22.73	31.91	26.28	30.76	28.35	20.88	26.57
CuSO ₄ 100	22.55	27.46	18.90	43.05	27.99	23.29	33.17	14.67	33.92	26.26	22.92	30.32	16.79	38.49	27.13
CuSO ₄ 200	58.71	145.57	24.79	75.60	76.16	13.61	37.46	29.35	43.87	31.07	36.16	91.52	27.07	59.74	53.62
CuSO ₄ 300	63.18	125.45	60.47	34.69	70.95	27.83	48.76	18.50	24.89	29.99	45.50	87.10	39.48	29.79	50.47
ZnSO ₄ 200	92.65	171.74	20.27	17.56	75.56	61.81	31.78	14.86	29.91	34.59	77.23	101.76	17.56	23.73	55.07
ZnSO ₄ 400	110.45	532.94	383.93	23.34	262.66	56.72	152.56	130.84	19.81	89.98	83.58	342.75	257.38	21.57	176.32
ZnSO ₄ 600	134.43	32.64	31.59	45.40	61.01	84.28	30.37	41.67	35.83	48.03	109.35	31.50	36.63	40.61	54.52
Control	9.64	39.72	10.97	23.44	20.94	7.87	35.94	11.58	14.55	17.48	8.75	37.82	11.27	18.99	19.21
Mean	69.67	132.53	65.35	35.36		41.16	49.87	36.84	26.03		55.42	91.20	51.09	30.69	
Factor	T	I	T × I			T	I	T × I			T	I	T × I		
SEm±	3.58	2.26	7.16			0.89	0.56	1.79			1.13	0.71	2.27		
CD at 5%	10.10	6.39	20.21			2.53	1.60	5.06			3.20	2.02	6.40		

Zinc had been considered as an element involved in numerous aspects of cellular metabolism of plants. Zinc is required for the catalytic activity of many enzymes and

plays a key role in the immune function and protein synthesis, thus Zn supports the normal growth and development of plants. Chary *et al.* (2008) [8] reported

varied concentrations of Zn accumulated in the leafy vegetables irrigated with waste water in and around Hyderabad. Begum and Harikrishna (2010) ^[6] also noticed similar kind of observation with respect to the concentrations of Zn accumulated in the leafy vegetables grown in the peri-urban areas of Bangalore. Lokeshwari and Chandrappa (2006) ^[17] noticed appreciable amounts of Zn accumulated in the leafy vegetables and appears to consume higher amounts of Zn uptake from the continued irrigation of sewage water. The present result was also found in close conformity with the earlier findings of An *et al.* (2006) ^[3] in *Pteris vittata*, Li *et al.* (2015) ^[16] in aquatic plants, Zhuang *et al.* (2007) ^[34] in different plant species in the field, Mkumbo *et al.* (2012) ^[22] in tomato, Subhashini *et al.* (2013) ^[30] in weeds, flowering plants and grasses, Subhashini and Swamy (2014) ^[31] and Subhashini and Swamy (2015) ^[32] in *Canna indica* and Dotaniya *et al.* (2017) ^[10] in spinach.

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