Research Article

Bioaccumulation of heavy metals in tomatoes (*Lycopersicum esculentum*) grown in soil amended with different biofertilizers in Sargodha, Punjab Pakistan

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Abstract

Effects of different biofertilizer on growth of *Lycopersicum esculentum* was performed in a pot experiment. Sugarcane press mud, poultry waste and farmyard manure was used in the farming of *Lycopersicum esculentum* as a bio fertilizer. Four pots were selected to check the effects of bio fertilizer such as press mud (T1), poultry waste (T2) and farmyard manure (T3), fourth one was control condition (T0). T1 contain 50% sugarcane press mud and 50% garden soil, T2 contain 50% poultry waste and 50% garden soil and T3 contain 50% farmyard manure. Heavy metals concentration in *L. esculentum* samples for cadmium, cobalt, chromium, copper, lead, ferric, manganese and zinc were assorted from 0.354 to 0.379, 2.0091 to 3.96, 0.685 to 1.202, 2.188 to 6.778, 0.0529 to 0.17, 1.368 to 5.031, 0.025 to 0.157 and 2.717 to 5.173 mg/kg, respectively. BCF estimations of metals in vegetables and soil were seen in safe limit. Cu demonstrated the BCF most extreme level (6.82) and Cd demonstrated the lower esteem (0.087). DIM value assessed for *L. esculentum* samples was found inside safe limit. The most elevated HRI was found for Cd at T3 and the least for Cr at T2 treatment. PLI esteem was discovered the most elevated for Cd (2.90) and the least for Pb (0.0028).

Keywords: Bio fertilizer; Health risk; Heavy metal; *Lycopersicum esculentum*; Vegetable

Introduction

Tomato (*Lycopersicum esculentum* L.) is very essential vegetable contain important nutrient [1]. The cultivation of tomatoes occurs in tropical and subtropical climates in all over the world [2]. The carbohydrates, proteins and fats are present in low range in tomatoes nutritionally. Tomatoes contain dietary fibers, glucose, Na and K [3]. Tomatoes contain essential Vitamin E, B₁₂, vitamin C and vitamin D, Ca, Fe and Mg are also present in it. In local India it is frequently use as vegetable, chutney, curry and pickle. It is very important because the juice of tomatoes can be used to reduce body fat and cholesterol content in blood [2].

These days, most vegetables producer began the act of utilizing diverse non-regular natural organic manures to improve the profitability of crops and to satisfy the necessity of sustenance deficiency of food on the planet. There are some significant rich natural which are utilized in substitution of inorganic manure. These composts, for example, press mud, poultry waste, and farmyard manure contain less amount of heavy metals when compared with other fertilizer and their utilization is so easy. Major source of contamination of foodstuff are heavy metals [4]. Significant amount of metals effectively collected in green vegetables when contrasted with grain or fruits [5]. Different Vegetables contain diverse range of traces and lethal metals in their parts such as fruits, leaves and underground stem portion. Heavy metals accretion in vegetables may guide hazard to all creature wellbeing [6]. Plants amass traces metals in their roots and stem portion. Taking-up of various heavy metals by plants grown in soil related with the long time utilization of chemical fertilizer compost, pesticides and water system with sewage and local wastewater.

Materials and Methods

Study area

Study area The experiment was conduct dated from January to April 2017 at institution of higher education (University of Sargodha) Sargodha situated in northeast region of Pakistan, (33°8'0" N, 74°7'0" E).

The multipurpose pot experiment was conducted to examine the agronomical growth performance of tomatoes grown in sugarcane press mud, farmyard manure and poultry waste-amended soil at area of the section of Botany, University of Sargodha, Punjab Pakistan. Tomatoes were grown in the first week of February 2017. Ten seeds were sow in pot. Each pot containing clay and loamy soil amended with 50% press mud, poultry waste and farmyard manure. Physical condition such as temperature was 25-20°C day/night; 55-60% humidity is very suitable for seed germination. Press mud, poultry waste and farmyard manure were added in three different treatments. Press mud, poultry waste and farmyard manure were applied as 50% and 50% rested pots fill with loamy clay garden soil. 12 pots were designed for three different treatments. Four control ports were used to compare the effects of organic fertilizer. Each treatment contains three replicates. T0: control, T1: Poultry waste, T2: press mud, T3: farmyard manure. Each pot contains ten seeds, and then they were watered with fresh ground. Plants were harvested at after 90 days when crop is fully repined.

Physico-chemical properties soil texture

Loam (Sand= 62.2%, Silt= 22.6%, Clay= 9.2%) EC: 0.5 ds/M and pH: 6.4. Litmus paper can be used to measure the pH of soil this method employs the special strip of paper that change the color based on pH of sample solution.

Soil samples were managed from central part of the pots. Firstly soil samples dried in open air and oven dried for 24 hours at 75°C. The samples were sealed in paper bags with name. Plant samples after collection were clean with distilled H₂O to deplete out impurities. Fine pieces of samples were cut in to small section so these samples could be dried in open air and sun to obtain appropriate dry weight. After 4 days all samples were shifted in to an oven at 75°C for 48 hour to remove all moisture contents in crop edible parts. All samples of vegetable and soil subjected to digestion by using of conc. HNO₃ and H₂O₂. The dried samples of 1g were taken in a flask and add 5 ml of acid in flask and remained it for 12. For

every sample 4 ml of H₂O₂ was added into mixture and put them on heat for 1 hour and added further H₂O₂ until colorless fumes is obtain and transparent material is obtain. After it cools the mixture and the filtered it by using Whatman #42 filter paper. The, by adding double distilled water made up to 50 ml and further use it for analysis. Metals were analyzed using atomic absorption spectrophotometer (6300, Shimadzu, Japan). The levels of metals were compared with the standard values provided by WHO/FAO and USEPA for validation and assessment of degree of contamination.

PLI can be calculated by using the following formula [7].

PLI= concentration of metal in samples soil/metals contents in reference soil

BCF (Bioconcentration factor) was found as:

Bio concentration factor= (Metals in vegetables) / (Metals in soil)

Sajjad *et al.* [8] describes the formula to calculate the average DIM for vegetables samples.

DIM = (C_{metal} × C_{factor} × D_{food intake}) / W_{average body weight}

Where C is the metal absorption (mg/kg) in tested samples, C_{factor} is constant value (0.085) which used to convert the fresh weight in to dry weight. Daily intake of vegetables for adult (0.345 kg/person/day) and children (0.17 kg/person/day) in Pakistan, while W_{average body weight} is the body weight of fully developed (63 kg) and kids (30 kg). The tolerable range for daily intake of metals is given in (Table 1).

The HRI can be defined as the ratio of the daily intake of metals in the food crops to the oral reference dose (R_{fd}) (EPA, 2002) and was calculated using the following equation:

Health risk index (HRI)= DIM/R_{fd}

HRI >1 for any metal in food crops indicates that the consumer is at health risk.

Oral reference dose (R_{fd}) is presented in (Table 2).

Data were analyzed with SPSS (ver. 17.0, SPSS Inc., Chicago, Ill.). Data were subjected

to one-way analysis of variance (ANOVA). Mean and standard error (r value) of soil and crop parameters with sugarcane press mud, poultry waste and farmyard manure treatments were calculated with MS Excel (ver. 2007, Microsoft Redmond Campus, Redmond, WA) and graphs produced with Sigma plot (ver. 12.3, Systat Software, Inc., Chicago, IL, USA).

Results and Discussion

Heavy metal concentrations in soil samples

The reference values for metals in soil are given in (Table 3). Analysis of variance results showed that the treatments have a non-significant effect on the absorption of Cd, Co, Cr, Zn and Fe whereas noteworthy outcome on the Cu concentration in the soil (Table 4). The concentration of heavy metals in soil were in order as among three different amended soils the metal levels were observed show lower than permissible values at all three contaminated soil. As a result of T1, T2 and T3 treatments, Zn, Fe, Cu and Co showed higher concentrations while those Pb, Cd, Mn and Cr showed the lower concentrations (Table 5). Clay topsoil finished soil controls the bioavailability of micronutrients proficiently [9]. In the present examination, mud loamy soil was collected from three unique locales to detect the physiochemical properties of soil tests. Uptake of micronutrients by vegetables is based on soil pH, that particularly increase the access of Mn, Zn, Fe and Cu to food crops [10]. In the present study, high EC range was found in the soil samples. EC values at three sites were between 0.88-1.35. The range (%) of organic matter at different locations was ranged from 0.57 to 0.78. Mojiri [11] reported the lower values as compared to the present study. Permissible maximum limits of the Cd, Co, Cr, Cu, Pb, Fe, Mn and Zn accumulation in soil were reported as 3, 65, 100, 50, 300, 21000, 2000 and 200 mg/kg, respectively by USEPA [12]. Among three different amended soils the metal levels were observed lower than permissible values at all three contaminated soil.

Table 1. Daily intake limits by different standards at tolerable range

Tolerable daily intake		
Metal	(mg/kg/day)	Source
Cd	0.004	[13]
Co	0.78	[14]
Cr	0.58	[15]
Cu	3	[16]
Pb	0.318	[17]
Fe	51	[16]
Mn	1.43	[18]
Zn	60	[17]

Table 2. Oral references dose for metals

Metal	Cd	Co	Cr	Cu	Pb	Fe	Mn	Zn
RfD (mg/kg/day)	0.001	0.043	1.5	0.04	0.0035	0.7	0.041	0.37

Source: [14]

Table 3. Reference value (mg/kg) of metals in soil

Metal	Cd	Co	Cr	Cu	Pb	Fe	Mn	Zn
Reference values	1.49	9.1	1.5	8.39	8.15	56.9	46.75	44.19

Source [13, 15, 19]

Table 4. Means squares of analysis of variance for heavy metals in soil collected from experiment site

Df		Mean squares							
Metal		Cd	Co	Cr	Cu	Pb	Fe	Mn	Zn
Site	2	0.342ns	0.348ns	0.432ns	1.542**	31.49ns	0.519ns	0.324*	1.118ns
Error	6	0.546	0.183	0.158	0.072	11.16	0.645	0.602	0.308

**=significant at 0.001 level ns= non-significant

Table 5. Heavy metal concentrations (mg/kg $\pm$ SE) in soil collected from three sites

Metal	Treatment				Maximum permissible limits
	T0	T1	T2	T3	
Cd	0.448 $\pm$.0037	0.332 $\pm$.0687	0.457 $\pm$.0043	0.394 $\pm$.011	0.5
Co	0.537 $\pm$.0265	0.455 $\pm$.093	0.562 $\pm$.0565	0.488 $\pm$.0055	1
Cr	0.135 $\pm$.0145	0.146 $\pm$.0045	0.779 $\pm$.0066	0.1896 $\pm$.0045	50
Cu	1.222 $\pm$.0064	1.310 $\pm$.0419	1.251 $\pm$.0061	1.254 $\pm$.0053	20
Pb	0.1791 $\pm$.015	0.1266 $\pm$.0204	0.1083 $\pm$.0164	0.1416 $\pm$.0166	10
Fe	1.868 $\pm$.7593	5.682 $\pm$.150	5.682 $\pm$.1502	0.7196 $\pm$.0133	1000
Mn	0.537 $\pm$.0062	0.023 $\pm$.0044	0.0829 $\pm$.032	0.0529 $\pm$.0271	30
Zn	4.535 $\pm$.571	17.633 $\pm$.1120	12.692 $\pm$.0229	10.713 $\pm$.2714	50

PML = permissible maximum limits; S.E= standard error; source: [20]

Heavy metal concentration in vegetables

Analysis of variance results showed that the treatments have a noteworthy consequence going on the meditation of Co, Cr, Zn, Pb, Fe and Mn although negative outcome on the concentration of Cd and Zn exhibited in vegetable (Table 6). Among different metals, Cu concentration was found highest in the T1 treatment and Mn concentration was found lowest in the T1 treatment. The range of metals was between 0.0258-6.778 (Table 7). Beforehand, Khan *et al.* [21] recommended high convergences of Zn, Cu, Fe, and Ni in vegetable develops in soil sullied with poultry squander. On the basis of these reasons, the current examination showed high concentrations of toxic metals that are metals (Mo, Fe, Zn, Cd, Co, Pb, Cu) in soil. As compared to present study Mapanda *et al.* [5] observed high range of different toxic metals in Cu, Zn, Pb, and Cd values. Permissible maximum limits of the Cd, Co, Cr, Cu, Pb, Fe, Mn and Zn accumulation in plants were reported as 2, 50, 50, 20, 10, 425, 500 and 100 mg/kg, respectively by WHO [6], FAO/WHO [22], Standard Guidelines in Europe [20]. The concentration of heavy metal accumulation in samples of *L. esculentum* showed the lower values as compared to these permissible maximum limits. The concentration of Cu was found highest in poultry waste treatment and the concentration of Mn was found lowest in poultry waste treatment. Use of these organic wastes as bio fertilizer can increase the potential of heavy metals uptake by vegetables from soil. Soil is main source of heavy metals which store the large amount of heavy metals. Mean concentration of heavy metals such as Cd, Co, Cr, Pb, Cu, Fe, Mn, and Zn was found lower than reported by Khan *et al.* [23].

Pollution load index

The PLI at T1 treatment was lower as compared to T0, T2 and T3, respectively. Among different metals, the PLI value of Cd was found highest at T2 and the PLI value of Pb was found lowest at T2. The PLI values of metals ranged from 0.0028 to 2.90 (Table 8). The PLI gives the information about assessment of heavy metals pollution and its status. In the present

study, the PLI in press mud treatment (T1) was lower as compared to that observe in press mud and farmyard manure. Concentration of Cd was found highest in poultry waste amended soil and concentration of Pb was found lowest in poultry waste amended soil. Cd pollution in soil is very dangerous, unsecure and its contamination is harmful for human and animals [24].

Bio concentration factor

Bio concentration factor for all metals was found lower at T2 treatment as compared to the other treatments. Among different metals at three treatments, Cu showed the maximum BCF value while Cd showed the minimum BCF value. The BCF values of metals ranged from 0.087 to 6.82 (Table 9). High Zn concentration of vegetables can be due to effect of other heavy metals [25]. Cu showed the maximum value for bio concentration factor at T1 treatment. The BCF was minimum for Cd in the present investigation. The uptake of heavy metals by vegetables is mostly affected by environmental factor and plant age [26].

Correlation coefficient

According to the results of the statistical analysis, Cd showed positive and significant correlation, while Co, Pb, Fe, Mn and Zn illustrated optimistic and significant association. Cr and Cu showed negative and non-significant correlation among mud and vegetables (Table 10).

Daily intake of metals (DIM) and Health risk index (HRI)

The uppermost DIM worth was predictable as 0.389mg for Cu in T1 treatment and the minimum DIM rate was expected as 0.0003 for Mn at T3 treatment. The range of HRI value of *L. esculentum* samples among different treatments was between 0.0003-2.170. The highest HRI was found for Cd at T3 treatment and the lowest for Cr at T2 treatment (Table 11). DIM value of Zn was the lowest and value of Mn was the highest in the present study and these values were higher than the values reported by Harmanescu *et al.* [27] in Romania. Harmanescu *et al.* [27] also determined the daily intake of Cd and Pb via vegetables grown on a mining area in Romania. The values of

DIM were determined high for Cu at T1 treatment as 0.0389 mg/kg/day and the lowest DIM value was estimated for Mn as 0.0003 at T3 treatment in the present study. Daily intake of Cd and Pb was higher while the qualities watched for Cr were bring down in the present

examination when contrasted with the qualities represented by Singh *et al.* [19] in India. DIM of Cu was higher in the present research than the qualities revealed by Maleki and Zarasvand [28].

Table 6. Analysis of heavy metal concentrations in *L. esculentum* at different treatments

Metal	Soil	Vegetable
Cd	1.116*	0.001 ns
Co	48.177*	5.390**
Cr	2.319*	0.357***
Cu	196.660**	23.680*
Pb	1.098*	0.020***
Fe	104.060 ns	17.888***
Mn	2.171 ns	0.042***
Zn	292.402**	6.587 ns

**=significant at 0.001 level ns= non-significant

Table 7. Heavy metal concentration (mg/kg $\pm$ SE) in edible part of *L. esculentum* grown at different treatments

Metal	Treatment				Maximum permissible limit
	T0	T1	T2	T3	
Cd	0.354 $\pm$.0309	0.377 $\pm$.0160	0.373 $\pm$.0430	0.378 $\pm$.0430	0.5
Co	2.098 $\pm$.397	2.0091 $\pm$.066	3.96 $\pm$ 1.142	2.116 $\pm$.3706	1
Cr	1.136 $\pm$.0027	1.202 $\pm$.01485	0.6858 $\pm$.202	1.173 $\pm$.0169	50
Cu	3.549 $\pm$ 1.292	6.778 $\pm$.7317	5.153 $\pm$ 1.404	2.188 $\pm$.0913	20
Pb	0.167 $\pm$.0303	0.0954 $\pm$.0057	0.1704 $\pm$.0145	0.0529 $\pm$.0117	10
Fe	5.031 $\pm$.7602	2.297 $\pm$.8371	1.368 $\pm$.43098	1.397 $\pm$.4639	1000
Mn	0.102 $\pm$.0069	0.0258 $\pm$.00828	0.0758 $\pm$.0211	0.1570 $\pm$.0286	30
Zn	2.717 $\pm$.640484	5.1733 $\pm$ 2.709	4.5595 $\pm$.624	4.3425 $\pm$.973	50

PML: permissible maximum limits, S.E: standard error, Source: [6]

Table 8. Pollution load index for heavy metals in soil grown with *L. esculentum*

Metal	Treatment			
	T0	T1	T2	T3
Cd	2.872	2.90	2.8758	1.552
Co	0.089	0.044	0.0678	0.045
Cr	0.225	0.218	0.2183	0.23
Cu	0.119	0.118	0.2530	0.425
Pb	0.0057	0.0028	0.0080	0.011
Fe	0.4800	1.6432	0.5604	0.437
Mn	0.0060	0.0052	0.0050	0.0074
Zn	0.367	0.3043	0.400	0.261

Table 9. Bio concentration factor for vegetable/soil system

Metal	Treatment			
	T0	T1	T2	T3
Cd	0.0827	0.0881	0.087	0.16
Co	2.5821	4.9303	6.412	5.09
Cr	3.3659	3.6702	2.094	3.4
Cu	3.5401	6.8292	2.426	0.613
Pb	0.5099	0.5870	0.373	0.078
Fe	1.2858	0.1715	0.299	0.391
Mn	0.3610	0.1053	0.321	0.449
Zn	0.1673	0.3846	0.257	0.375

Table 10. Correlation among top soil and vegetables samples

Metal	Soil/vegetable
Cd	1.000*
Co	.796
Cr	-.984
Cu	-.346
Pb	0.946
Fe	0.601
Mn	0.925
Zn	0.865

Table 11. DIM and HRI for human of heavy metals by consumption of vegetables samples collected from experiment site

Metal	Treatment	T0	TO	T2	T3
Cd	DIM	0.0020	0.0021	0.0021	0.0021
	HRI	2.0355	2.1677	2.1447	2.1735
Co	DIM	0.0120	0.0115	0.0227	0.0121
	HRI	2.0355	0.2686	0.5295	0.2829
Cr	DIM	0.0065	0.0069	0.0039	0.0067
	HRI	0.0043	0.0046	0.0026	0.0044
Cu	DIM	0.0204	0.0389	0.0039	0.0125
	HRI	0.5101	0.9743	0.0985	0.3145
Pb	DIM	0.0009	0.0005	0.0009	0.0003
	HRI	0.2743	0.1567	0.2799	0.0869
Fe	DIM	0.0289	0.0132	0.0078	0.0080
	HRI	0.0413	0.0188	0.0112	0.0114
Mn	DIM	0.0005	0.0001	0.0004	0.0009
	HRI	0.0143	0.0036	0.0106	0.0220
Zn	DIM	0.0156	0.0297	0.0261	0.0249
	HRI	0.0422	0.0803	0.0708	0.0674

Conclusion

The present investigation was intended to explore the potential human health risks related with the utilization of vegetables yields sullied with harmful heavy metals. There was a considerable amount of heavy metals in the *L. esculentum* samples which could be attributed to the accumulated metals in the soil. But the BCF and PLI indicators indicated the vegetable to be safe for human consumption owing to the fact that metal concentrations were in safe limits.

Authors' contributions

The experiment was conceived and designed by: ZI Khan, K Ahmad & M Munir, Collected the samples and analysis were executed by: P Akhter, IS Malik & M Nadeem, Collected data was analyzed statistically by: H Bashir, Initial draft of the manuscript was prepared and edited by: S Nazar, A Ashfaq, M Sohail, R Sultana, H Memona, U Farooq, S Ullah & M Sana.

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