

Heavy metals assessment in vegetables irrigated with waste water: A case study in District Sargodha, Pakistan

ABSTRACT

Ameer Khan, Sadaf Javed, Amin Shah, Maria Nawaz, Farzana Shaheen and Humera Nawaz

Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan

An experiment was conducted to evaluate the heavy metals toxicity in vegetables irrigated with wastewater. Different experimental sites were selected from district Sargodha, Pakistan. Samples of wastewater, contaminated soil and vegetables irrigated with wastewater were collected and analyzed. Concentration of heavy metals such as Cadmium (Cd), Copper, (Cu), Chromium (Cr), Zinc, (Zn) and Iron (Fe) were determined from soil, water and vegetables samples irrigated with wastewater and fresh water. Cadmium concentration were present from 0.1 to 0.3 $\mu\text{g g}^{-1}$, chromium concentration from 0.03 to 0.11 $\mu\text{g g}^{-1}$, Copper concentration from 0.2 to 0.7 $\mu\text{g g}^{-1}$, iron concentration were present from 2.6 to 4 $\mu\text{g g}^{-1}$ and Zinc concentration were present from 0.1 to 0.3 $\mu\text{g g}^{-1}$ in vegetables irrigated with waste water. Heavy metals concentrations recorded in vegetables were within the safe limit except cadmium as recommended by WHO.

Keywords: Experimental sites, radish, spinach, contaminated soil, heavy metals, wastewater

Comment [m1]: It should substitute by was

Comment [m2]: Idem

Comment [m3]: Idem

INTRODUCTION

In Pakistan, shortage of surface water supply is a serious issue. This shortage of water is being compensated by wastewater (sewage and industrial effluents) to grow vegetables especially in areas around the big cities [1]. Disposal of sewage water and industrial wastes is a great problem. Often it is drained to the agricultural lands where it is used for growing crops including vegetables. Industrial or municipal wastewater is mostly used for the irrigation of crops due to its easy availability, disposal problems. This concern is of great importance, where untreated

31 sewage is applied for longer periods to grow vegetables in urban lands. Some metals are essential
32 micronutrients at low concentration and at high concentration they cause growth inhibition of
33 plants [2].Irrigation of agriculture soils through wastewater may not only result in soil
34 contamination, but also affect food quality and safety [3].Irrigation with wastewater becomes the
35 source of heavy metals in crops and vegetables. Some of the heavy metals are essential but the
36 others are not essential for proper plant growth. These sewage effluents are considered not only a
37 rich source of organic matter and other nutrients but also they elevate the level of heavy metals
38 like Fe, Mn, Cu, Zn, Pb, Cr, Ni, Cd and Co in receiving soils [4]. Therefore, as the concentration
39 of heavy metals increased in the soil they could be transferred in body through food and water
40 and accumulate in the body of animals and may lead to the disruption of numerous biological and
41 biochemical processes in the human body [5].Food and water are the main sources of our
42 essential metals; these are also the media through which we are exposed to various toxic metals.
43 Heavy metals are easily accumulated in the edible parts of plants. The main objective of this study
44 was to determine the concentrations of Cu, Zn, Cd, Cr, and Fe vegetables irrigated with
45 wastewater.

46 Permissible limits [15] of the metals ($\mu\text{g/g}$) recommended by WHO

47

Metals	Cd	Cu	Cr	Zn	Fe
Permissible Limits($\mu\text{g/g}$)	0.1	73.3	2.3	100	425

48

49

50 MATERIALS AND METHODS

51 Study area and sampling

52 Experimental sites were selected in Sargodha region to evaluate the level of heavy metals
53 toxicity from soil, vegetables irrigated with wastewater. The samples of wastewater,
54 contaminated soil and different vegetables (spinach and radish) were collected from the
55 experimental sites (Silanwali, Kot **Frid** and Isataqlalabad) to determine the level of heavy metals
56 from vegetables irrigated with wastewater.

Comment [m4]: It should be substituted by Farid

57 **Collection of samples**

58 Samples of contaminated soil, wastewater and vegetables irrigated with wastewater were
59 collected during growing seasons from three sites of District Sargodha. A sample of each
60 vegetable was same size and age group.

Comment [m5]: I consider that the authors have to include the sample number used in the experiment.

61 **Sample Preparation:**

62 All the collected samples of vegetables (spinach and radish) were washed with double distilled
63 water to remove pollutants. The edible parts of each sample of vegetable were weighed and air-
64 dried to reduce the water content. All the samples were then oven-dried in a hot air oven at 60–
65 70°C for 24 h, to remove all moisture. Dried samples were powdered using a pestle and mortar
66 and sieved through muslin cloth.

67

68 **Digestion:**

69 Dry material was digested according to the methods [6]. For each vegetable, three powdered
70 samples from each source of irrigation (0.1 g each) were accurately weighed and placed in
71 digestion tube and 3ml of concentrated H₂SO₄ was added in the digestion tubes. Samples were
72 incubated overnight and then placed in the digestion chamber and heated at 300°C for 30
73 minutes. Fumes from the samples were emitted. The Digestion tubes were removed from the
74 digestion chamber and cooled and put 0.1 ml of H₂O₂ along the sides of digestion tubes and
75 placed digestion tube again in the digestion chamber and fumes were emitted from digested
76 material and again removed digestion tubes from digestion chambers and put H₂O₂ into the
77 digestion tubes and place in digestion chamber. Repeated the process until samples become
78 transparent. Distilled water was added into the extract material up to volume 50ml and filtered
79 for determination of heavy metals.

80

81 **Standards**

82 Standard solutions of heavy metals (1000 mg/l) were procured from Merck. Solutions of varying
83 concentrations were prepared for different heavy metals by diluting the standards.

84 **Heavy metal determination:**

85 From the digested material heavy metals Cadmium (Cd), Copper, (Cu), Chromium (Cr), Zinc,
86 (Zn) and Iron (Fe) were analyzed from atomic absorption spectrophotometer model number AA-
87 6300.

88 **Statistical analysis:**

89 The data were analyzed by analysis of variance technique. Difference for variance
90 character was compared by using least significance difference at 0.05 levels [7].

91

92 **RESULTS**

93 Analysis of variance of the data of cadmium, chromium, copper, iron and zinc concentration in
94 normal and wastewater, contaminated and non- contaminated soil and vegetables irrigated with
95 wastewater is presented in (Table 1 and 2). The concentration of different heavy metals observed
96 from normal and wastewater are presented in (Table. 3). Cadmium, copper and zinc
97 concentration was observed high in waste water collected from Kot Farid (Table 3). Chromium
98 was observed from the wastewater collected from Istaqlalaabad (Table 3). The data of heavy
99 metals observed from contaminated and non- contaminated soil is presented in (Table. 4).
100 Cadmium concentration was observed high from the soil samples collected from Silanwali
101 (Table. 4). Chromium was observed high from the soil samples collected from Kot Farid (Table.
102 4). Copper was observed high from the soil samples collected from Silanwali and KotFarid
103 (Table. 4). Zinc concentration was observed high from the samples collected from Istaqlalabad
104 and Silanwali (Table 4). Data of different heavy metals collected from vegetables is presented in
105 (Table5). Cadmium and chromium was observed high in spinach collected from Istaqlalabad and
106 KotFarid (Table 5). Copper concentration was observed high in spinach collected from
107 Silanwali, KotFarid and Istaqlalabad (Table5). Iron was observed high from the samples of
108 spinach collected from Sialanwali. Zinc concentration was observed high from spinach samples
109 collected from Silanwali (Table 5). Overall, heavy metals were observed high from the spinach
110 samples irrigated with waste water.

Comment [m6]: The authors do not comment anything about Tables 1 and 2.

111 Table 1. Mean square from the analysis of variance of the data of cadmium, chromium and
 112 copper concentration for radish (*Raphanussativus*) and spinach (*Spinaciaoleracea*) irrigated
 113 with normal and wastewater from District Sargodha.

Comment [m7]: I consider that the statistical analysis used in this case is not the most indicated, since the authors are working on two different plant species and not on two varieties of the same species; so the factorial method should not be used.

		Cadmium			Chromium			Copper		
SOV	D.F	Water	Soil	Vegetable	Water	Soil	Vegetable	Water	Soil	Vegetable
Sites	2	3.57*	13.7*	0.685*	1.48*	36.90*	0.10*	1.05*	24.98*	1.95*
Vegetable	1	8.20*	49.94*	5.39*	1.13*	111.69*	1.21*	0.12*	108.59*	7.22*
Interaction	2	2.93*	2.51*	0.56*	0.78*	16.70*	0.29*	3.30*	0.46*	0.45*
Error	12									
Total	17									

114
 115 Table 2. Mean square from the analysis of variance of the data of Iron and Zn concentration for
 116 radish (*Raphanussativus*) and spinach (*Spinaciaoleracea*) irrigated with normal and waste water
 117 from District Sargodha.

		Iron			Zn		
SOV	D.F	Water	Soil	Vegetable	Water	Soil	Vegetable
Sites	2	6.11*	91.79*	0.10*	1.96*	0.72*	9.09*
Vegetable	1	1.20*	563.63*	1.21*	8.45*	2.10*	19.00*
Interaction	2	2.76*	23.0*	0.29*	4.99*	1.02*	5.78*
Error	12						
Total	17						

118
 119 Table 3. Heavy metals concentration ($\mu\text{gg-1}$) in waste and normal water collected from different
 120 experimental sites of District Sargodha

	Water		
Sites	Silanwali	Kot Farid	Istaqlal Abad

	Waste water	Normal water	Waste water	Normal water	Waste water	Normal water
Cadmium	0.065±0.001	0.019±0.002	0.07±0.004	0.05±0.002	0.056±0.002	0.034±0.005
Chromium	0.05±0.002	0.03±0.001	0.04±0.002	0.02±0.001	0.08±0.003	0.06±0.002
Copper	0.06±0.001	0.04±0.001	0.07±0.004	0.03±0.001	0.05±0.001	0.04±0.001
Iron	1.6±0.005	1.2±0.02	2.1±0.04	1.5±0.06	1.6±0.3	0.5±0.04
Zinc	8.2±0.2	6.7±0.03	10.2±1.2	8.3±2.1	9.7±1.3	7.5±1.7

121

122

123 Table 4. Heavy metal concentration (µgg-1) in contaminated and non- contaminated soil
124 collected from different experimental sites of District Sargodha

	Soil					
Sites	Silanwali		Kot Farid		Istaqlal Abad	
	Contaminated soil	Non-contaminated soil	Contaminated soil	Non-contaminated soil	Contaminated soil	Non-Contaminated soil
Cadmium	0.13±0.004	0.08±0.03	0.06±0.004	0.04±0.007	0.05±0.009	0.04±0.01
Chromium	0.07±0.02	0.08±0.03	0.14±0.07	0.06±0.004	0.16±0.02	0.06±0.012
Copper	0.08±0.02	0.05±0.008	0.08±0.02	0.06±0.003	0.1±0.008	0.07±0.02
Iron	1.6±0.5	1.0±0.04	1.7±0.5	1.0±0.06	1.8±0.01	1.2±0.7
Zinc	0.07±0.02	0.03±0.02	0.075±0.02	0.04±0.007	0.07±0.02	0.03±0.002

125

126

127 Table 5. Heavy metal concentration (µgg-1) in radish (*Raphanussativus*) and spinach
128 (*Spinaciaoleracea*) irrigated with wastewater collected from different experimental sites of
129 District Sargodha.

130

Sites	Silanwali		Kot Farid		Istaqlalabad	
	Radish	Spinach	Radish	Spinach	Radish	Spinach
Cadmium	0.3±0.002	0.48±0.001	0.1±0.005	0.29±0.0007	0.1±0.003	0.65±0.004
Chromium	0.03±0.002	0.11±0.005	0.02±0.001	0.08±0.003	0.06±0.001	0.089±0.006
Copper	0.23±0.005	0.7±0.01	0.2±0.004	0.7±0.0004	0.5±0.002	0.7±0.005
Iron	3.5±0.2	4.0±0.02	3.4±0.005	2.6±0.02	3.5±0.4	3.0±0.08
Zinc	0.17±0.06	0.8±0.03	0.2±0.04	0.3±0.01	0.1±0.004	0.25±0.01

131 DISCUSSION

132 As the population is increasing day by day and demand of food also has been increased. To meet
133 the demand of food, vegetables are being grown around the big cities by using a wastewater as
134 irrigation source. Wastewater contains different heavy metals, which disturb the food quality and
135 safety. It has been observed from the current scenario that majority of the population is suffering
136 from malnutrition and therefore, different disease have been diagnosed due to nutrients
137 deficiency. The heavy metals accumulation and translocation potential varied from metal to metal
138 and from plant to plant. Heavy metals are included in the main category of environmental
139 pollutants as they can remain in the environment for long periods; their accumulation is
140 potentially hazardous to humans, animals and plants [8]. Cadmium is well known as a highly
141 environmental element due to its great toxicity and highly mobility from soil to plants and food
142 chain [9]. Elevated levels of heavy metals in sewage water have been reported by many other
143 scientists in Pakistan and they noted excessive concentration of Cu, Mn, Pb and Cd in sewage
144 water samples. [10] Reported higher contents of Zn, Cu, Pb, Ni, Cd and Cr in wastewater samples
145 from Rawalpindi Area and from Peshawar [11]. Genotypic effect, environmental effect and their
146 interaction effects highly affect heavy metals uptake in crop genotypes [12]. The uptake of heavy
147 metal by vegetables is not only affected the plant species but also affect the physicochemical
148 characteristics of soil. [13] Collected spinach, bitter gourd, okra, pumpkin and egg plant samples
149 and observed that they were contaminated with heavy metals. Similarly, [14] collected spinach
150 and turnip samples from market and found that these vegetables were unsafe for eating due to
151 higher heavy metal concentration. It has been investigated by many scientists that heavy metals
152 heavy metals are accumulated in edible portion of vegetables and found that Zn, Cu, Pb, Ni, Cd
153 and Cr contents were higher than the safe limits [16]. It has been noted that irrigation with waste

Comment [m8]: To eliminate

154 water contaminated the leaf and fruit parts of the vegetables and increased the levels of heavy
155 metals than the recommended levels of WHO [17]. Due to copper toxicity plant appears stunted
156 are usually bluish in colour and eventually turn yellow or brown. Chromium toxicity in plants
157 observed at multiple levels from reduced yield through effects on leaf and root growth and
158 inhibition of enzymatic activities and mutagenesis.

159 CONCLUSION

160 From the experimental study it is observed that waste water was the main source of pollution to
161 soil and irrigation with waste water containing high amount of cadmium leads to increase the
162 concentration of cadmium in vegetables.

163

164

165 LITERATURE CITED

166

167 1. Khan, A., Shahid J, Atif M, Tahir M, Abid N and Abdul M. Heavy metal status of soil and
168 vegetables grown on peri-urban area of Lahore district. Soil Environ. 2013: 49-54, 2013.

169 2. Marshall.: Enhancing food chain integrity. Quality assurance mechanism for air pollution
170 impacts on fruits and vegetables systems. Crop post harvest program, final. Tech
171 Rep. 2004: 7530-7545.

172 3. Muchuweti, M., Birkett J.W., Chinyanga E., Zvauya R., Scrimshaw M. D and. Lester J. N..
173 Heavy metal content of vegetables irrigated with mixture of wastewater and sewage
174 sludge in Zimbabwe: implications for human health. Agriculture, Ecosystem and
175 Environment. 2006; 41-48.

176 4. Singh, K.P., Mohan D., Sinha S and. Dalwani R. Impact assessment of treated/ untreated
177 wastewater toxicants discharged by sewage treatment plants on health, agricultural, and
178 environmental quality in the wastewater disposal area. Chemosphere. 2004; 227-255.

179 5. Parashar, P and. Prasad F. (2013): Study of Heavy Metal Accumulation in Sewage Irrigated
180 Vegetables in Different Regions of Agra District, India," Open Journal of Soil Science,
181 Vol. 3 No. 1, 2013, pp. 1-8. doi: 10.4236/ojss.2013.

- 182 6. Allen, S E, Grimshaw HM and Rowland A P.: Chemical analysis. In: Methods in Plant
183 Ecology, 2nd ed., eds. P. D. Moore and S. B. Chapman.1986; 285–344. Oxford:
184 Blackwell Scientific Publications.
- 185 7.Steel, R. G. D. and Torrie, J. H. Principles and procedures of statistics. McGraw Hill Book Co,
186 New York.,1980: 876-920.
- 187 8. Benavides M P, Gallego S M, Tomaro M L. Cadmium toxicity in plants. Braz. J. Plant
188 Physiol., 2005; 21-34.
- 189 9. Vig, K., Megharaj M., Sethunathan N., Naidu R.: Bioavailability and toxicity of cadmium to
190 microorganisms and their activities in soil. A review. Adv. Environ. Res., 2003;121-135.
- 191 10. Jagtap, M.N., Kulkarni M.V and Puranik P.R. Flux of heavy metal in soils irrigated with
192 urban waste water. American Eurasian Journal of Agricultural and Environmental
193 Sciences.2010; 487-493.
- 194 11. Ehsan I.U., Perveen S., Shah Z., Nazif W., Shah S.S and Shah H.U. Study on accumulation
195 of heavy metals in vegetables receiving sewage water. Journal of the Chemical Society of
196 Pakistan .2011; 220-226
- 197 12. Zeng F. R., Mao, Y., Cheng, W.D., Wu F. B and Zhang G.P.: Genotypic and environmental
198 variation in chromium, cadmium and lead concentrations in rice. Environmental
199 Pollution.2008; 309–314.
- 200 13. Farid, S. Toxic element concentration in vegetables irrigated with untreated city effluents.
201 Science,Technology and Development. 2003; 58-60.
- 202 14. Ronaq, R.N., I. Haider, M. Qadir and N. Hasnain. Studies on the distribution of heavy and
203 toxic metals (Copper, Lead, Zinc and Cadmium) in different vegetables using Atomic
204 Absorption Spectroscopy. Proceedings of 5th National Chemical Conference, Islamabad,
205 Pakistan.2005; 63-64.

- 206 15. FAO/WHO.: Expert Committee on Food Additives, Summary and conclusions, 53rd Meeting,
207 Rome (1999).
- 208 16. Ahsan, I., S. Z.Perveen, W. Shah, W. Nazif, S.S. Shah and H.U. Shah. 2011. Study on
209 accumulation of heavy metals in vegetables receiving sewage water. Journal of the
210 Chemical Society of Pakistan 33(2): 220-227.
- 211 17. Khan, A., J. Shahid, M. Atif, M. Tahir, M., N. Abid, and A. I. Majeed.2013. Heavy metal
212 status of soil and vegetables grown on peri-urban area of Lahore district. Soil Environ.
213 32(1): 49-54.

214