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HEAVY METAL CONTAMINATION IN WATER, SOIL AND A POTENTIAL VEGETABLE GARLIC (*ALLIUM SATIVUM* L.) IN PUNJAB, PAKISTAN

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Abstract

Heavy metal contamination in soil, water, and garlic (*Allium sativum* L.) (watered with canal, ground and sewage waters) in a semi-arid region was investigated in this study. A sub-urban area of district Khushab, Pakistan was chosen as the study site to assess the risks associated with the consumption of this vegetable supplied with three different types of water for irrigation. Sewage water had higher contents of metals and metalloids (Cu, Ni, Se, Mo, As, Fe and Zn) than in other waters. Mean metal concentrations were below the permissible values, but those of Pb and Mo exceeded their respective limits. Metal correlation for the vegetable and soil was significantly positive except for Cu. The range of bio-concentration factor varied between 0.06-20.51 mg/kg. The sewage water had the highest pollution load index. Zinc had the highest daily intake value (0.199), while Se had the lowest value (0.003). The range for health index stood between 0.261-73.44 mg/kg. Metals like Zn, Ni and Cu had enrichment factor higher than 1.0 which raised serious health concerns. It has been a routine to irrigate crops with sewage water but proper management of wastewater is required prior to its supply to the fields. Hazardous quotient (HQ) indicated alarming levels of different metals with respect to public health due to utilization of this vegetable receiving wastewater irrigation.

Key words: Metals, Pollution load index, Health risk index, Waste water.

Introduction

As the good quality water resources are being vanished, municipal wastewater is frequently used for crop irrigation in urban and semi-urban localities. Mostly, the sewage water is contaminated with heavy metals and metalloids. Amid this polluted water usage, it is not unexpected that heavy metal accumulation would take place in soil of the fields (Khan *et al.*, 2008) and then be taken up by the plants growing therein (Singh *et al.*, 2010). This pollution is of great concern for farmers and agriculturists as it contaminates the food chain both directly and indirectly (D'Mello, 2003; Gholizadeh *et al.*, 2009; Khair, 2009; Ahmad *et al.*, 2014). Continual usage of this metalloid rich wastewater may raise the levels of metals in crops and consumers who ultimately utilize these as a food source. This could cause severe health issues (Marshall *et al*., 2007).

Garlic (*Allium sativum* L.) is a typical spice used in Pakistani households and other parts of the world. It has multiple medicinal uses and is used with all types of cooked foods all over the world by human populations (Makheja & Beiley, 1990; Ali, 1995; Borek, 2001). Therefore because of its importance worldwide, the metal uptake capacity was assessed to consider the risks associated with metal accumulation potential of garlic.

This study aimed to find out the concentrations of metals and metalloids in the vegetables, soil and irrigation water used for crop cultivation. This study would highlight the potential hazards of consuming contaminated garlic grown on soils watered with sewage. The concentrations of Pb, Se, Ni, Cu, Mo, As, and Fe were determined mainly to understand the metal translocation from soil to plants.

Materials and Methods

Study area: A semi-urban area of Khushab city, Punjab, Pakistan, was selected for the present study. River Jhelum flows adjacent to the city and its geographical location is 32.30ºN and 72.34ºE. The summer (25-49˚C) and winter (5-23 ˚C) temperatures vary considerably and extreme conditions exist in both seasons. Rainfall per annum is recorded as 526 mm (Appendix 3). Three sites namely Joiya, Talokar and Khushab were selected for sampling which were irrigated with ground water, canal water and municipal wastewater, respectively. Three replicates of samples from soil, water and garlic were taken from each site.

Collection of samples: Water samples (up to 100 ml) were collected from the three sites and 1.0 ml conc. HNO_3 was added to each water sample to prevent microbial activity. Garlic grown sites were chosen for soil sampling. Each sample weighed 1 kg was collected from 20 cm depth. The samples were kept in open air and sunlight to eliminate moisture. Afterwards, an oven was used to dry the samples; the samples were kept there for 4-5 days at 72°C. The samples were then crushed using an electric machine. The garlic samples were separately collected and thoroughly cleansed with distilled water. The samples were divided into two parts i.e., root and shoot. A convection oven was used to dry the samples wherein they were placed for 3 days at 70°C. Thereafter, they were powdered and passed through a 1 mm fine sieve for further analyses.

Sample processing: The digestion of water samples was carried out using 10 ml of conc. HNO_3. A hot plate was used and the heating continued till the solution turned clear. Filtration was done using a Whatman filter paper (no. 42). Then, 50 ml volume was made by adding distilled water to the digested sample.

The soil sample (1.0 g) was taken in a flask along with H_2SO_4 and H_2O_2 (1:2 ratio) in a digestion chamber. The flask was taken out after the evaporation stopped, and after cooling, H_2O_2 (2 ml) was added to it. The flask was again placed in a digestion chamber till the mixture had been colorless. After cooling and filtration, the solution was poured in a clean and labeled plastic bottle with distilled water to make final volume up to 50 ml. The pH and EC of soil were determined following Mathieu & Pielain (2003). The organic content was determined by Anne method which is a modification of the Walkey-Black method (McLean, 1982).

The garlic vegetable samples (1.0 g) were processed by adding a mixture of H_2SO_4 and H_2O_2 in 1:2 ratio in a glass flask. The sample digestion was carried out till the solution became colorless. Final volume of 50 ml was made using distilled water and kept in pre-washed bottles.

Analysis: The metal concentrations (Ni, Cu, Pb, Fe, Zn and Mo)were determined using an atomic absorption spectrophotometer, Perkin-Elmer AAS-5000 (Perkin-Elmer Corp. 1980)While Se in soil and vegetable samples were determined by a fluorometric following Watikinson (1966) and total As,by a flow injection hydride generation AAS (Perkin Elmer Aanalyst 400) using arsenate as a standard (Welsch, 1990).

Statistical analysis: The metal concentration in each category of samples was analyzed by a one-way ANOVA using the statistical software, SPSS 17. The correlation between the vegetable and soil for metals was calculated as well. The means were compared at probability levels of 0.05, 0.01 and 0.001 levels following Steel and Torrie (1980).

Other parameters like bio-concentration factor (BF), pollution load index (PLI), health risk index (HRI) and metal enrichment factor (EF) were also determined (Table 1).

Results

Statistical analysis of the data showed that Se, Ni, Pb, As and Fe had significant variation in concentration with respect to the sites in water regimes (Table 2). The metal concentrations were higher in sewage water than that in canal and ground waters (Table 3). Though most concentrations were below the permissible range at all sites, but those of Pb and Mo exceeded the permissible limits.

The soil had a loamy texture. The soil pH varied from 7.5-8.5 and EC from 0.85-1.05 dS m^{-1}. The organic matter of the soil was from 0.58-0.79% as presented in Table 4. The Zn, As, Pb, Cu, Ni and Mo concentrations varied significantly among the sampling sites, while Se had no significant change (Table 5). The site irrigated with sewage water had higher metal and metalloid content than the other two sites. The sequence of metal concentrations in the soil was: As > Fe > Pb > Ni > Mo > Cu > Zn > Se (Table 6). It was observed that Se had no significant change in the plant tissues obtained from the three sites while a significant variation was noticed in Cu, Zn, As, Pb, Mo and Ni concentrations as revealed by ANOVA (Table 7). The vegetables obtained from the site receiving sewage water had higher metal contents. The observed order of metal concentration was: Fe > Zn > Cu > Mo > Ni > Pb > As > Se (Table 8).

Table 2. One-way ANOVA of metal concentration in water at three different sites.

Metals and metalloids	Sites
As	0.001***
Cu	0.001ns
Fe	0.033***
Mo	0.002ns
Ni	0.004***
Pb	0.008***
Se	0.002***
Zn	0.001ns

*** = Significant at 0.001 level, ns = Non-significant

Bio-concentration factor varied considerably at all sites (Table 9). The order of metal concentration in canal water was: Zn > Cu > Mo > Fe > Ni > Se > Pb > As. The metal concentration in the sewage and ground waters had similar order as for canal water: Zn > Cu > Mo > Ni > Fe > Se > Pb > As. The metal correlation between soil and plant was significant and positive except for Cu which had a non-significant correlation (Table 10). The pollution index followed the similar trend in all treatments. The observed order was as follows: As > Pb > Ni > Fe > Mo > Cu > Se > Zn (Table 11).

Table 1. Formulas for parameters.

Parameter	Formula	References
Bio-concentration factor	$\frac{\text{Concentration of metal in vegetable}}{\text{Concentration of metal in soil}}$	(Cui *et al.*, 2004)
pollution load index (PLI)	$\frac{\text{Metal concentration in investigated soil}}{\text{Reference value of the metal in soil}}$	(Liu *et al.*, 2005)
Daily intake of metal (DIM)	$C_{metal} \times D_{food\ intake} / B_{average\ weight}$	
Health risk index (HRI)	DIM / R_fD	(USEPA, 2002)
Enrichment factor (EF)	$\frac{[(M)^{veg}/(M)^{soil}]sample}{[(M)^{veg}/(M)^{soil}]standard}$	(Buat-Menard & Chesselet, 1979)

Table 3. Metal and metalloid concentrations in water of *Allium sativum* treated with canal, ground and sewage water.

Metals	Mean ± S.E.			Maximum permissible level in (μg/g)
	GWI	CWI	SWI	
As	0.016 + 0.002	0.017 + 0.001	0.024 + 0.001	0.1
Cu	0.023 + 0.012	0.031 + 0.002	0.032 + 0.004	0.2
Fe	0.675 + 0.012	0.711 + 0.017	0.868 + 0.009	5
Mo	0.068 + 0.011	0.071 + 0.006	0.098 + 0.007	0.01
Ni	0.095 + 0.006	0.115 + 0.010	0.168 + 0.007	0.2
Pb	0.233 + 0.014	0.268 + 0.007	0.337 + 0.017	0.1
Se	0.013 + 0.001	0.015 + 0.001	0.022 + 0.001	0.02
Zn	0.613 + 0.016	0.637 + 0.057	0.646 + 0.051	2

Source [a] WWF-February, 2007

Table 4. Physico-chemical properties of soil.

Soil properties	Site-I	Site-II	Site-III	Mean squares
pH	8.56 ± 0.17	8.21 ± 0.05	7.57 ± 0.22	0.75*
EC	0.85 ± 0.02	0.99 ± 0.01	1.05 ± 0.08	0.03***
Organic matter	0.58 ± 0.05	0.76 ± 0.11	0.79 ± 0.04	0.03***
Soil texture	Loamy	Loamy	Loamy	

Table 5. One-way ANOVA of metal concentration in soil at three different sites.

Metals and metalloids	Sites
As	40.05**
Cu	1.646***
Fe	77.78**
Mo	8.958***
Ni	2.671***
Pb	62.02***
Se	1.014ns
Zn	1.998***

** and***= Significant at 0.01 and 0.001, levels; ns = Non-significant

Table 7. One-way ANOVA of metal concentration in vegetables at three different sites.

Metals and metalloids	Sites
As	0.861***
Cu	24.36**
Fe	107.7**
Mo	1.599***
Ni	2.553**
Pb	0.787***
Se	0.019ns
Zn	16.14**

, *= Significant at 0.01 and 0.001 levels; ns = Non-significant

Table 6. Metal and metalloid concentrations in soil of *Allium sativum* treated with canal, ground and sewage water.

Metals	Mean ± S.E.			Maximum permissible level in soil (μg/g)
	GWI	CWI	SWI	
As	40.31 + 0.525	43.35 + 0.425	47.58 + 2.38	20
Cu	2.364 + 0.027	2.975 + 0.31	3.838 + 0.138	100
Fe	34.43 + 1.539	38.45 + 0.277	44.55 + 1.537	50000
Mo	3.313 + 0.105	4.943 + 0.106	6.772 + 0.216	40
Ni	6.368 + 0.127	7.136 + 0.079	8.245 + 0.203	50
Pb	28.18 + 0.491	30.44 + 0.908	36.94 + 1.254	100
Se	1.598 + 0.028	1.681 + 0.025	2.644 + 0.323	10
Zn	1.683 + 0.071	2.315 + 0.046	3.306 + 0.046	300

PML =Permissible maximum limit (Chiroma *et al.*, 2014); S.E = Standard Error
GWI= Ground water irrigation, CWI= Canal water irrigation, SWI= Sewage water irrigation

Table 8. Analysis of variance for metals and metalloids concentrations in garlic vegetables treated with canal, ground and sewage water.

Metals	Mean ± S.E.			Maximum permissible level in (μg/g)
	GWI	CWI	SWI	
As	2.778 + 0.108	3.475 + 0.091	3.831 + 0.077	7
Cu	11.32 + 0.369	13.97 + 0.697	17.02 + 0.871	73
Fe	31.73 + 1.925	39.08 + 0.766	43.61 + 1.091	425
Mo	6.755 + 0.065	7.121 + 0.148	8.161 + 0.171	5
Ni	6.305 + 0.155	7.221 + 0.238	8.151 + 0.265	67
Pb	4.973 + 0.087	5.425 + 0.045	5.995 + 0.081	0.30
Se	0.546 + 0.024	0.623 + 0.006	0.705 + 0.015	-
Zn	34.61 + 0.208	36.74 + 0.731	39.25 + 0.796	100

PML =Permissible maximum limit (Chiroma *et al.*, 2014) S.E = Standard Error
GWI= Ground water irrigation, CWI= Canal water irrigation, SWI= Sewage water irrigation

Table 9. Bio-concentration factor for vegetable/soil system.

Study sites	Bio-concentration factor							
	Mo	**As**	**Se**	**Fe**	**Cu**	**Zn**	**Ni**	**Pb**
GWI	2.036	0.069	0.342	0.922	4.789	20.51	0.991	0.176
CWI	1.441	0.081	0.371	1.016	4.698	15.86	1.011	0.178
SWI	1.205	0.081	0.266	0.978	4.434	11.87	0.988	0.162

Table 10. Correlation between soil and vegetable.

Metals and metalloids	**As**	**Cu**	**Fe**	**Mo**
Soil-vegetable	0.878 **	0.887ns	0. 940**	0.840**
Metals and metalloids	Ni	Pb	Se	Zn
Soil-vegetable	0.858**	0.911**	0.841**	0.923**

Table 11. Pollution load index for metals and metalloids in soil.

Study sites	Pollution Load Index							
	Mo	**As**	**Se**	**Fe**	**Cu**	**Zn**	**Ni**	**Pb**
GWI	0.364	13.43	0.055	0.605	0.282	0.038	0.702	3.458
CWI	0.543	14.45	0.057	0.675	0.355	0.052	0.787	3.735
SWI	0.744	15.86	0.091	0.783	0.457	0.075	0.910	4.533
Ref. values (mg kg^{-1})	3.0	29.0	0.7	56.90	8.39	44.19	9.06	8.15

(Dutch Standards, 2000; Singh *et al*., 2010; Dosumu *et al*., 2005)

Table 12. Health risk intake (HRI) and daily intake of metals (DIM mg/kg/day) of metal contents via intake of *Allium sativum* from wastewater irrigated sites.

Study sites	Metals and metalloids								
	Hazard quotient	**Mo**	**As**	**Se**	**Fe**	**Cu**	**Zn**	**Ni**	**Pb**
GWI	DIM	0.039	0.016	0.003	0.182	0.065	0.199	0.036	0.028
	HRI	4.315	53.25	0.628	0.261	1.628	0.537	1.813	8.169
CWI	DIM	0.041	0.019	0.004	0.224	0.081	0.211	0.042	0.032
	HRI	4.548	66.61	0.717	0.321	2.009	0.571	2.076	8.913
SWI	DIM	0.046	0.022	0.004	0.251	0.098	0.226	0.047	0.034
	HRI	5.214	73.44	0.811	0.358	2.446	0.609	2.343	9.849

Table 13. Enrichment factor of metals and metalloids in *Allium sativum*.

Study sites	Enrichment Factor (EF)						
	Mo	**As**	**Fe**	**Cu**	**Zn**	**Ni**	**Pb**
GWI	0.763	0.264	0.141	3.885	15.08	6.562	0.311
CWI	0.824	0.296	0.135	3.241	13.43	6.864	0.338
SWI	0.686	0.375	0.145	3.839	10.07	6.326	0.280
Ref_{veg}	5	7	425.5	10	60	1.5	5
Sources of vegetable Ref	Chiroma *et al.* (2014)	Chiroma *et al.* (2014)	FAO/WHO (2007)	GB15199-94	FAO/WHO (2007)	FAO/WHO (2011)	FAO/WHO (2011)
Ref_{soil}	3	29	56.9	8.39	44.19	9.06	8.15
Sources of soil Ref	Dutch Standards, (2000)	Dutch Standards, (2000)	Dosumu *et al.* (2005)	Singh *et al.* (2010)	Singh *et al.*(2010)	Singh *et al.*(2010)	Singh *et al.*(2010)

Daily metal intake was also estimated. The highest value was observed for Zn and the lowest for Se at all sites. Highest values were obtained for the sewage water treatment. The health risk index due to consumption of garlic had a range of 0.261-73.44 mg/day. Following order was observed in ground water and canal water treatments: As > Pb > Mo > Ni > Cu > Se > Zn > Fe. The sewage water treatment had a different order which was: As > Pb > Mo > Cu > Ni > Se > Zn > Fe. HRI was higher than 1.0 for Pb, Mo, As, Cu and Ni while it was lower than 1.0 for Fe, Zn and Se (Table 12). The enrichment factor during ingestion of *A. sativum* had a range of 0.124-15.37. The order of metals and metalloids in ground water treatment was: Fe > As > Pb > Mo > Cu > Ni > Zn, while for canal and sewage water treatments, the order was: Fe > Pb > As > Mo > Cu > Ni > Zn (Table 13).

Discussion

Domestic and industrial wastewaters are used for irrigating crops which tend to raise metal concentrations in agricultural soils (Arora *et al*., 2008). The present study reinforced this important finding that wastewater contained high amounts of metals. Higher ranges of Ni and Cu and lower ranges of Zn and Pb were observed in a study conducted on treated and untreated wastewater in Varanasi (Singh *et al*., 2004) as compared to the metal concentrations reported in our study. However, soil differential ability to absorb and adsorb different metals mainly depends on soil pH (Turner, 1994; McBride *et al*., 1997), as it is believed to be associated with the balance of adsorption, speciation of metals and exchange of solid particles as well as solubility of different substances

(Cavallaro & McBride, 1984; Sauve *et al*., 1997). Similarly, Mapanda *et al*. (2005) reported higher values of metalloids in the soil.

The values for As exceeded the safe limit within the soil as suggested by Chiroma *et al.* (2014). This increase in As concentration could be due to higher uptake of this metal by soil. The concentrations of As (41.5-47.8), Cu (3.15-3.63), Fe (34.4-41.9), Mo (4.79-5.85), Ni (1.95-3.70), Pb (27.5-33.8), Se (1.68-2.73), and Zn (4.25-6.25) in the canal and sewage waters were in agreement to those reported in Ahmad *et al.* (2014), while those of Mo, Ni and Zn were significantly lower. The levels of metals in the garlic's edible part at all sites irrigated with the three different types of water were below the permissible level except for As (Chiroma *et al.*, 2014), thus toxicity level of As was higher. The values recorded in the current investigations for Se were found to be lower than those recognized by Rayman (2000). The Se accumulation in soil as well as in plants is an environmental concern which could occur due to a variety of industrial sources, mining, and geochemical processes (Shardendu *et al.*, 2003). The tissue Cu, Ni and Zn concentrations reported by Chao *et al.* (2007) were reported to be lower than those found in the present study which demonstrated that these metals were absorbed largely as compared to the other metals.

The bio-concentration factors for As, Se, Cu, Ni, Fe, Zn, Mo and Pb were within the range of estimations done by Ahmad *et al*. (2014) from the same area, and the treatments were also similar. However, the current findings showed lower levels of As, Cu, Mo, Ni and Pb, higher of Zn and Se, and similar of Fe. Only the edible part of the garlic was considered for bio-concentration factor; other organs like shoot, root and leaves were not analyzed in the present study. The factor value for metals was higher for garlic than that for the soil. This discrepancy could be due to variability of metal absorption by plants and variation in metal sources (Tsafe *et al.*, 2012). This could be also due to different agricultural practices and environmental factors common to these sites.

Extent of metal contamination is determined by pollution load index. The current results showed that Pb and As pollution in the soil had the greatest possibility to cause health safety issues. These high values are attributed to vehicular exhaust and the industrial discharge (Abou Donia, 2008). Similarly, high PLI values for metals (>1) were found in a study by Maiz *et al*. (2000). The present results showed that the study area was highly polluted with As and Pb, moderately polluted with Ni, Fe, Mo, Cu, and less polluted with Zn and Se at all three sites. Khan *et al*. (2017) recorded almost similar PLI values for the metals under investigation.

The standards of the Integrated Risk Information System have the recommended values of R_fD for Fe Mo, Cu, Se, Ni, and Zn of 0.70, 0.009, 0.04, 5×10^{-3}, 0.02, 3×10^{-4}, and 0.37 mg kg^{-1} day^{-1}, respectively (USEPA, 2010). The R_fD value for Pb is 0.0035 mg kg^{-1} day^{-1} (Anon., 1993). The normal metal ingestion per day is 0.345 kg of the vegetable with normal body weight of 60 kg for adults (Ge, 1992; Wang *et al.*, 2005). Adults normally show reduced response instance, nausea, weakness of joints, and failure of memory when exposed to Pb dose above the R_fD (Anon., 2009). A predictable exposure is acquired by dividing daily intake of heavy metals by reference values. Human health is considered under risk if the index exceeds 1.0 (Anon., 2002).

Enrichment factor (EF) determined elemental depletions in the soil. The EF values lower than 1.0 (Mo, As, Fe and Pb) indicated metal leaching (Loska *et al*., 2005).The present results of EF were higher for Zn, Ni, Cu and Pb while lower for Mo and Fe.

Conclusion

The garlic vegetable growing in soil irrigated with sewage water had considerably higher amount of metals examined. Thus, the consumption of this vegetable could be hazardous for public health. The high pollution index indicates the potential problems that could arise with continued utilization of this vegetable. Suitable management of wastewater prior to its application to fields is extremely necessary.

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Declaration of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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