MONITORING OF HEAVY METAL CONTAMINATION IN HUMAN BLOOD-POPULATION EXPOSED TO DIFFERENT WASTEWATER IRRIGATED AGRICULTURAL FARMS AND SOURCE APPORTIONMENT IN PAKISTAN

Z. Iqbal[a], M. Raza[b], M. Gul[c], R. Haider[d*], T. I. Qureshi[e] and F. Bashir[f]

[a]Environmental Protection Agency Punjab, Ferozepur road Lahore, Pakistan
[b]Department of Agriculture and Resource Economics, MNS- University of Agriculture Multan, Pakistan
[C] School Education Department, Punjab, Pakistan.
[d]Environmental Protection Agency Punjab, Ferozepur road Lahore, Pakistan
[e] Department of Environmental Engineering, NFC- Institute of Engineering & Technology, Multan, Pakistan
[f]PakistanCouncil of Scientific and Industrial Research, Center for Environmental Protection Studies, Laboratories Ferozpur Road, Lahore
[*]Corresponding author E-mail: rizwanchemist@gmail.com

Acknowledgements: The authors acknowledge the cooperation of Rauf Ahmed from the Pakistan Council for Science and Industrial Research lab Lahore, Pakistan for help during sample analysis.
Funding source: The authors declare that there is no source of funding in this study.

ABSTRACT: The study was designed to assess the heavy metal contents in human blood of farmers in six agricultural farms irrigated with untreated industrial effluents, urban wastewater, canal and tube well water and source apportionment in peri urban area of Multan city,Pakistan. Human blood samples (n=30) and wastewater, soil, Brassica and Maize plants, Chicken liver, groundwater and raw milk (each n=30) were analyzed for cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni) and lead (Pb) by Inductively Coupled Plasma-optical Emission Spectrometry (ICP- OES). Mean contents of Cd, Cr, Mn and Ni exceeded the WHO safe limits in human blood of population exposed to wastewater farms and all metals were within safe limits at canal and tube well water farms. Contamination level of human blood at industrial wastewater irrigated farm was highest and was lowest at tube well water farm. Total contents of metals in blood were 100 to 350 times higher at wastewater irrigated farms than that at canal water farm. The multivariate statistical analysis indicated that the wastewater, soil and foodstuff grown at respective farms were common sources of human blood contamination. The human population exposed to wastewater irrigated fields may suffer to serious health implications due to blood contamination with heavy metals exceeding safe limits. Use of properly treated wastewater in agricultural farms may safeguard the health of public and ecosystem.

Key words: Heavy metals; wastewater irrigation; human blood contamination; source apportionment.

(Received 18.09.2024 Accepted 03.12.2024)

INTRODUCTION

The humans and other living organisms are directly linked with their environment for survival (Irshad *et al*. 2015). The rapid industrialization, urbanization and their resulting wastes are main causes of increasing water, soil and air pollution (Åkesson *et al*. 2008). Industrial waste contain different chemical compounds and heavy metals (Speight 2017). Some elements having high toxicity level like Cd, Cr and Pb are retained in soil, and cause serious health implications to animals and humans via dust ingestion, skin contact and intake contaminated food grown on such soils (Kusin *et al*.2018 ; Yang *et al*.2018). Heavy metals are natural component of earth crust and are present in natural soil at very low concentration and are considered safe for ecosystem. Such natural soil is considered as unpolluted/uncontaminated/preindustrial soil (MEF 2007). Past and current industrial developments and commercial activities, improper waste treatment and its disposal, over 200 year's period, are main causes of soil contamination which has raised the heavy metal concentration in soil (Chen *et al*. 2005; EC 2013).About 20 million ha (7 percent) of land is irrigated with wastewater in the world (UN Water 2015).The application of even treated wastewater in irrigation without precautionary measures can lead to accumulation of nutrients, pathogenic microorganisms, salts and heavy metals in land (Qadir *et al*. 2010).Untreated wastewater is being used to grow various crops in vicinities of all the cities and this mode of disposal of wastewater is considered as the cheapest way (Qadir *et al*. 1997). The soils irrigated with wastewater and vegetables grown on such soil were contaminated with heavy metals above permissible limits. The regular intake of vegetables grown with untreated wastewater may accumulate the heavy metals in human body to the toxic level.There is complicated relationship between soil contaminants and human health. Three million contaminated soil sites are estimated in Europe which needs immediate remediation. (van Liedekerke *et al*. 2014).

The humans are consumers of all food stuffs like ground water, agricultural produces like vegetables, meat, milk etc and are exposed to their respective living environmental conditions and remain in contract with air and soil. The humans living in polluted areas are exposed to contaminants and heavy metals accumulate in their body. The accumulated heavy metal in soils are depleted through erosion, leaching and plant uptake (A Kabata-Pendias 2010). The intake of heavy metals by humans via contaminated food stuffs is one of the pathway and pose risk to human health (Khan *et al*. 2008). Inhalation and ingestion of contaminated soil dust and particles is direct pathway exposure to population living in contaminated area (Cambra *et al*. 1999). The heavy metals accumulate in human organs and do not eliminate from the body (ATSDR 2012). The chronic exposure to chromium, cadmium and lead causes lungs damage, kidney diseases and fragile bones (ATSDR 2017).

The above scenario reflects that the human population exposed to contaminated environment intake heavy metals in their body and blood. The contamination level depends on the level of exposed environmental conditions. It is hypothesized that the human population residing in wastewater irrigated agricultural farms may intake the heavy metals in their blood through different pathways. Therefore, the objectives of this study were to i) investigate the concentration of Cadmium (Cd), Chromium (Cr), Copper (Cu), Manganese (Mn), Nickel (Ni) and Lead (Pb) in human blood of population residing at agricultural farms under different qualities of wastewater irrigation ii) identify the sources of heavy metals in human blood using multivariate statistical analysis. The novelty of study that this is the first study conducted in Pakistan.

MATERIALS AND METHODS

Study area: Multan is 5^{th} largest populous district of Punjab having current population 2.8 million. It is located at 30.2^o north, 71.4^o east on east bank of Chenab River in the geographic center of Pakistan (NESPAK, 2017; Iqbal *et al*. 2019). The climate is cold in winter and very hot in summer with maximum mean temperature in summer 42 and 21^OC in winter, and annual rainfall 186 millimeters (Abbas *et al*. 2014).

Multi discipline environmental issues are prevailing in study area due to old and current urbanization and industrialization. Haphazard industrial clusters were established and are operational within city area and Multan Industrial Estate (MIE) on an area of 1300 acres having more than 300 industrial units is functional on west side of the city. The untreated wastewater of Multan industrial estate is being used to irrigate the agricultural land. The urban wastewater having effluents of industrial units located in city area is being discharged into WASA sewerage system which is disposed of in water bodies used directly to irrigate land for agricultural produce (Iqbal *et al*. 2019; Iqbal *et al* 2020). The same practice is being carried out in other cities and towns of Pakistan (Ensink *et al*. 2004; Van Der Hoek *et al*. 2002). Therefore the finding of this study may be replicated in other cities of Pakistan.

Six agricultural farms, major agricultural produce producer were selected in suburb of Multan City as representative farms. A- farm under untreated industrial effluents irrigation, B and D farms under untreated urban wastewater , C- farm under mixed water (canal water + urban untreated wastewater), E- farm under canal water (control area) and F- farm under tube well water irrigation (Figure 1).

Sampling and analytical methodology: The human blood samples were collected through medical officer of concerned basic health unit (BHU). The persons visited their concerned BHU for their treatment and diagnostic purpose and medical officer took blood samples for pathological diagnose with standard protocol and method. A portion of such blood sample (each 5 ml) in designed vials for preservation was taken from medical officer with the consent of concerned person. Total 30 human blood samples of locally born farmers (Male age 50-60 years) (each site n=5) were collected and placed in ice boxes and freezed till analyses

Composite samples of wastewater/water used for irrigation, soil, drinking water from hand pumps, Maize and Brassica plants, domestic chicken liver and buffalo's milk (Each n=30) were collected from each farm according to standard methods for determination of selected metals contents to use the data for statistical analyses. Samples were assigned proper identity with inventory.

Analytical analyses of samples for selected metals was performed in center of environmental protection studies laboratories, Pakistan Council of Scientific and Industrial Research, Lahore accredited for ISO with ICP-OES Perkin Elmer, USA, Optima DV 5300 in accordance with standard guidelines and methods (APHA, 2005; ASTM, 2007; AOAC, 2002).

Multivariate statistical analyses was performed for source apportionment of the heavy metals in human blood including i) ANOVA, ii) Pearson correlation matrix (PCM), iii) Hierarchical cluster analyses (HCA), and iv) Principal component analysis (PCA).

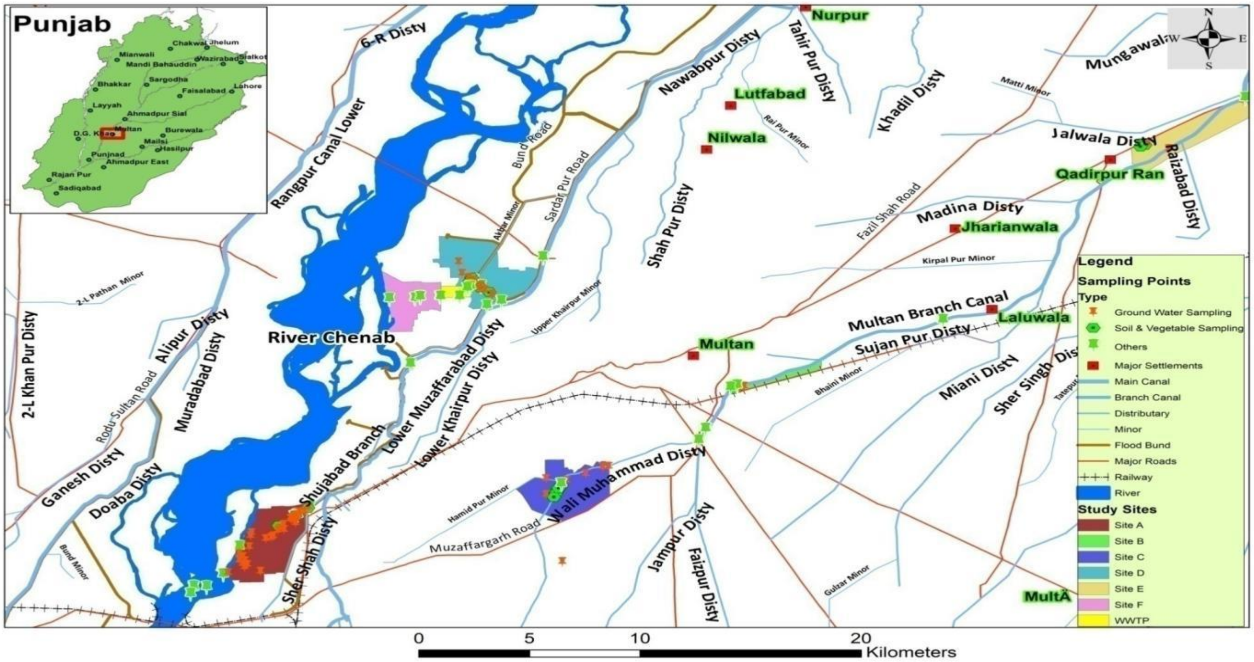

Figure 1 Study area of Multan City, Pakistan.

RESULTS

Concentration of the selected metals in soil, groundwater, wastewater, Maize and Brassica plants, livers of domestic chicken, and raw milk of buffaloes across all farms were systematized for statistical analysis (data not shown).The results (Table 1) showed that mean concentrations of Cd, Cr, Mn and Ni in human blood exceeded WHO (1996) safe limits at wastewater irrigated farms (A, B, C, D) and mean contents of all metals were highest at untreated industrial effluents irrigated farm (A). Mean concentrations of Cr and Ni were slightly higher than the safe limits at canal water irrigation farm (E). All the metals were within safe limits in human blood exposed to canal water irrigation farm (F). The results further revealed that the contamination level of Cd in blood was 67, 123, 37 and 60 times higher at farm-A, B, C and D respectively than that at farm-E. The contamination level of Cr was 105, 205, 135 and 105 times higher at farm-A, B. C and D respectively than that at farm-E. The contamination level of Cu was 38, 30, 17 and 36 times higher at farm-A, B, C and D than that at farm-E. The contamination level of Mn was 854, 354, 152 and 778 times higher at farm-A, B, C and D than that at farm-E. The contamination level of Ni was 325, 400, 173 and 267 times higher at farm-A, B, C and D than that at farm-E. The contamination level of Pb was 20, 15, 18 and 15 times higher at farm-A, B, C and D than that at farm - E.

Table 1 Mean contents of heavy metals (μg/L) in human blood- population across all agricultural farms.

Farm		*Cd*	*Cr*	*Cu*	*Mn*	*Ni*	*Pb*	*Total metals*
A	Mean	20	21	55	1230	390	8	1724
	± SD	0.71	1.22	0.71	7.07	7.07	0.71	
B	Mean	37	41	43	510	480	6	1117
	± SD	0.71	0.71	0.71	7.07	45.28	0.71	
C	Mean	11	27	24	218	204	7	491
	± SD	0.71	0.71	0.71	1.41	11.40	0.71	
D	Mean	18	21	52	1120	320	6	1537
	± SD	1.22	0.71	0.71	7.07	7.07	0.71	
E	Mean	0.3	0.2	1.44	1.44	1.2	0.4	5.0
	± SD	0.06	0.05	0.29	0.29	0.29	0.06	
F	Mean	0.2	0.136	1.28	0.804	0.816	0.2	3.44
	± SD	0.06	0.033	0.22	0.09	0.07	0.05	
MRL (μg/l)		0.3[a]	0.16[b]	800[a]	8[a]	1[a]	50[a]	859.5

a WHO (1996), b ATSDR, (2000)

eding WHO limits Below WHO limits

The total contents of metals (µg/L) across all farms were 1724.0, 1117.0, 491.0, 1537.0, 4.982 and 3.436 (Figure 2). Order of total contents of metals was as: A > D >B > C > E > F. The blood of population at farm-F contained lower contents of heavy metals than that at farm-E. The blood of human population exposed to wastewater irrigation farms was 100 to 350 times more contaminated than that at canal and tube well water irrigation farms. It might be due to the presence of higher concentration of heavy metals in wastewater, soil and other linked pathways and contaminated food stuff grown with wastewater like vegetables, ground water, milk, chicken liver which provided chance to transmit the same in human body.

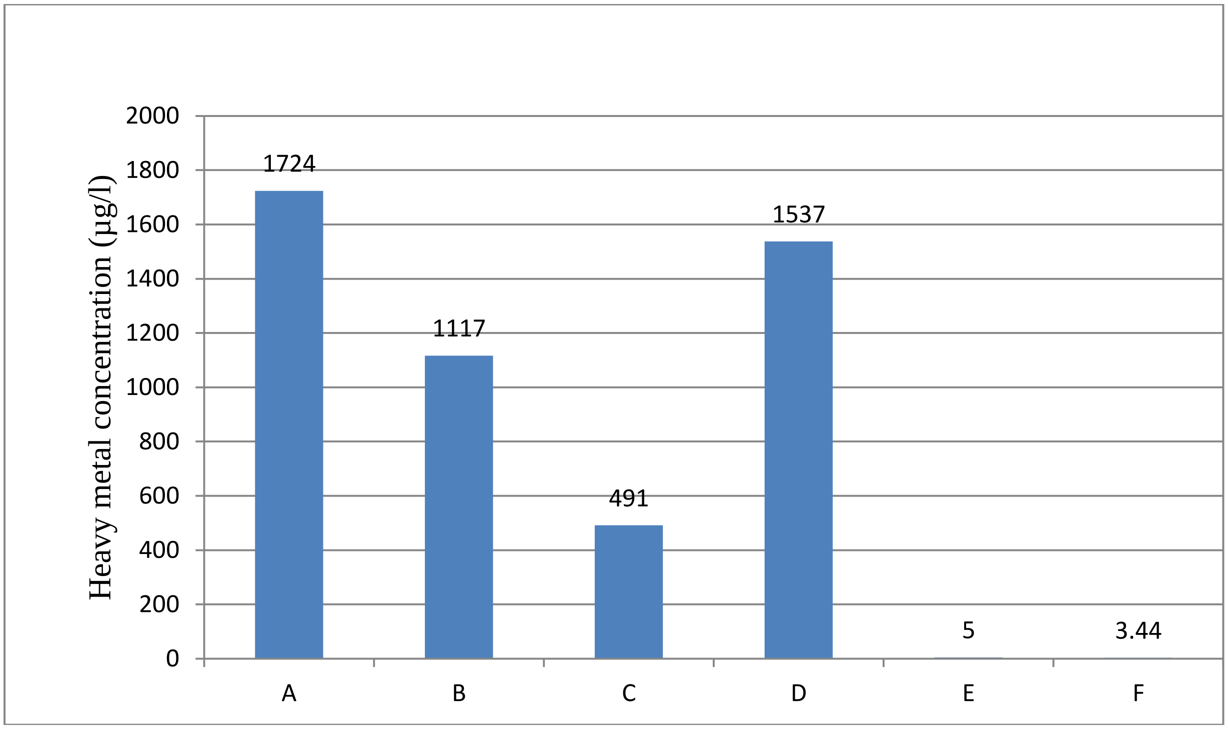

Figure 2 Comparison of total heavy metals concentration in human blood of population across all agricultural farms.

Source apportionment: Normally the concentrations of elements emerging from similar or same source tend to show significant correlation (Jiang *et al*. 2014). Therefore Pearson correlation coefficients of heavy metals were computed. Hierarchical Cluster Analyses (HCA) reveals the formation of groups of elements originating from same or similar source. Principal Component Analyses (PCA) show the relationship between the elements and formation of groups of elements. The positive principal component loadings indicate the anthropogenic source and negative loadings show the natural source (Rodriguez *et al*. 2018). HCA and PCA are often coupled for confirmation of results (Soliman *et al*. 2015). Hence HCA and PCA both were performed.

PCM between metals within blood (Table 2) indicated highly significant positive correlation between all pairs of the metals at the level of 0.01 showing same source disseminating the human blood contamination across all farms.

PCM (Table 2) between metals of wastewater and human blood indicated highly significant positive correlation in most of the pairs at 0.01level showing the wastewater and water irrigating the farms is common source for human blood contamination across all farms.

PCM (Table 3) between metals of soil and human blood indicated highly significant positive correlation in most of the pairs at 0.01level showing the contaminated soil as common source for human blood contamination across all farms.

PCM between human blood metals and food stuff metals (Table 4) showed highly significant positive correlation in most of the metal pairs at 0.01 and significant positive correlation at 0.05 which indicated that the consumption of contaminated food stuff grown at respective sites contributed the metal contamination in human blood across all sites.

HCA was shown with dendogram which exhibited similarities (Figure 3) indicating the formation of two groups of the heavy metals in human blood. Group 1 contained Cd, Ni and Cr, and group 2 contained Cu, Mn and Pb.

Table 2 PCM between heavy metals within human blood and metals of wastewater

	Cd	*Cr*	*Cu*	*Mn*	*Ni*	*Pb*	*wwCd*	*wwCr*	*wwCu*	*wwMn*	*wwNi*	*wwPb*
Cd	1	**0.922****	**0.794****	**0.568****	**0.957****	**0.704****	**0.861****	-0.102	**0.837****	**0.607****	0.346	0.312
Cr		1	**0.729****	**0.449***	**0.900****	**0.807****	**0.788****	-0.239	**0.871****	**0.421***	0.181	0.19
Cu			1	**0.938****	**0.919****	**0.872****	**0.786****	0.189	**0.707****	**0.751****	**0.651****	**0.554****
Mn				1	**0.745****	**0.739****	**0.629****	0.361	**0.486****	**0.771****	**0.757****	**0.638****
Ni					1	**0.840****	**0.876****	-0.035	**0.852****	**0.704****	**0.476****	**0.473****
Pb						1	**0.721****	-0.113	**0.778****	**0.560****	**0.431***	**0.525****

. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed), ***ww* = wastewater

Table 3 PCM between heavy metals within human blood and metals of surface soil

	Cd	*Cr*	*Cu*	*Mn*	*Ni*	*Pb*	*ssCd*	*ssCr*	*ssCu*	*ssMn*	*ssNi*	*ssPb*
Cd	1	**0.922****	**0.794****	**0.568****	**0.957****	**0.704****	0.219	0.197	**0.616****	**0.393***	0.33	0.232
Cr		1	**0.729****	**0.449***	**0.900****	**0.807****	0.102	0.076	**0.656****	**0.439***	0.223	0.13
Cu			1	**0.938****	**0.919****	**0.872****	**0.525****	**0.540****	**0.743****	-0.092	0.25	**0.548****
Mn				1	**0.745****	**0.739****	**0.645****	**0.677****	**0.648****	-0.352	0.198	**0.661****
Ni					1	**0.840****	**0.400***	**0.390***	**0.734****	0.173	0.242	**.420***
Pb						1	**0.494****	**0.490****	**0.809****	-0.104	-0.006	**0.528****

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed), ***ss***= surface soil

Table 4. PCM between heavy metals in human blood and metals of foodstuffs

	Cd Hbloo[a]	*Cr Hblood*	*Cu Hblood*	*MnHblood*	*Ni Hblood*	*PbHblood*	*Cd milk*[b]	*Cr milk*	*Cu milk*	*Mn milk*	*Ni milk*	*Pb milk*
Cd chicken	**0.673****	**0.668****	**0.926****	**0.879****	**0.820****	**0.846****	**0.890****	**0.646****	**0.729****	0.178	**0.773****	**0.684****
Cr chicken	**0.487****	**0.374***	**0.683****	**0.711****	**0.630****	**0.626****	**0.558****	**0.479****	**0.489****	-0.022	**0.675****	**0.445***
Cu chicken	**0.745****	**0.669****	**0.961****	**0.917****	**0.870****	**0.838****	**0.880****	**0.602****	**0.780****	0.196	**0.838****	**0.731****
Mn chicken	0.337	0.228	**0.659****	**0.755****	**0.526****	**0.608****	**0.512****	**0.629****	0.346	-0.235	**0.649****	**0.287**
Ni chicken	**0.366***	**0.383***	**0.699****	**0.716****	**0.487****	**0.536****	**0.640****	**0.621****	**0.446***	-0.015	**0.541****	**0.400***
Pb chicken	**0.736****	**0.756****	**0.928****	**0.837****	**0.867****	**0.901****	**0.938****	**0.600****	**0.790****	0.289	**0.756****	**0.753****
Cd Maize	**0.714****	**0.743****	**0.961****	**0.885****	**0.870****	**0.947****	**0.960****	**0.685****	**0.777****	0.234	**0.776****	**0.736****
Cr Maize	**0.659****	**0.837****	**0.615****	**0.392***	**0.726****	**0.878****	**0.826****	0.340	**0.703****	**0.558****	**0.394***	**0.713****
Cu Maize	**0.870****	**0.834****	**0.957****	**0.832****	**0.965****	**0.915****	**0.945****	**0.464****	**0.898****	**0.415***	**0.816****	**0.867****
Mn Maize	**0.624****	**0.619****	**0.905****	**0.878****	**0.804****	**0.911****	**0.869****	**0.712****	**0.671****	0.089	**0.767****	**0.622****
Ni Maize	**0.431***	**0.369***	**0.558****	**0.547****	**0.516****	**0.490****	**0.499****	**0.321**	**0.438***	0.044	**0.488****	**0.398***
Pb Maize	**0.605****	**0.562****	**0.917****	**0.920****	**0.787****	**0.866****	**0.838****	**0.732****	**0.648****	0.029	**0.800****	**0.593****
Cd water[c]	**0.549****	**0.527****	**0.556****	**0.459***	**0.594****	**0.530****	**0.536****	0.187	**0 .571****	0.315	**0.479****	**0.559****
Cr water	**0.481****	**0.447***	0.278	0.136	**0.442***	0.237	0.303	-0.174	**0.462***	**0.447***	0.235	**0.489****

Cu water	0.774**	0.614**	0.760**	0.676**	0.772**	0.568**	0.646**	0.172	0.766**	0.364*	0.723**	0.738**
Mn water	0.304	0.416*	0.641**	0.636**	0.467**	0.706**	0.682**	0.744**	0.384*	-0.023	0.448*	0.350
Ni water	0.341	0.230	0.591**	0.654**	0.439*	0.446*	0.438*	0.469**	0.364*	-0.108	0.548**	0.315
Cd Brassica	0.325	0.298	0.675**	0.744**	0.535**	0.714**	0.596**	0.720**	0.355	-0.202	0.613**	0.301
Cr Brassica	0.550**	0.791**	0.649**	0.458*	0.655**	0.895**	0.874**	0.533**	0.634**	0.437*	0.365*	0.633**
Cu Brassica	0.623**	0.810**	0.778**	0.615**	0.750**	0.954**	0.943**	0.615**	0.705**	0.390*	0.510**	0.692**
Mn Brassica	0.356	0.569**	0.571**	0.475**	0.528**	0.858**	0.742**	0.664**	0.429*	0.150	0.347	0.416*
Ni Brassica	-0.096	0.286	-0.082	-0.239	-0.042	0.366*	0.266	0.254	-0.016	0.225	-0.329	0.020
Pb Brassica	0.804**	0.804**	0.983**	0.880**	0.924**	0.930**	0.976**	0.590**	0.856**	0.336	0.806**	0.818**
Cd Hblood	1	0.922**	0.794**	0.568**	0.957**	0.704**	0.815**	0.005	0.991**	0.750**	0.691**	0.990**
Cr Hblood	0.922**	1	0.729**	0.449*	0.900**	0.807**	0.884**	0.095	0.948**	0.817**	0.533**	0.963**
Cu Hblood	0.794**	0.729**	1	0.938**	0.919**	0.872**	0.926**	0.585**	0.835**	0.251	0.867**	0.787**
MnHblood	0.568**	0.449*	0.938**	1	0.745**	0.739**	0.767**	0.725**	0.609**	-0.088	0.860**	0.540**
Ni Hblood	0.957**	0.900**	0.919**	0.745**	1	0.840**	0.917**	0.270	0.972**	0.580**	0.789**	0.953**
PbHblood	0.704**	0.807**	0.872**	0.739**	0.840**	1	0.962**	0.625**	0.765**	0.348	0.653**	0.743**
Cd milk	0.815**	0.884**	0.926**	0.767**	0.917**	0.962**	1	0.535**	0.874**	0.459*	0.711**	0.851**
Cr milk	0.005	0.095	0.585**	0.725**	0.270	0.625**	0.535**	1	0.097	-0.470**	0.429*	0.0354
Cu milk	0.991**	0.948**	0.835**	0.609**	0.972**	0.765**	0.874**	0.097	1	0.731**	0.703**	0.995**
Mn milk	0.750**	0.817**	0.251	-0.088	0.580**	0.348	0.459*	-0.470**	0.731**	1	0.134	0.786**
Ni milk	0.691**	0.533**	0.867**	0.860**	0.789**	0.653**	0.711**	0.429*	0.703**	0.134	1	0.650**
Pb milk	0.990**	0.963**	0.787**	0.540**	0.953**	0.743**	0.851**	0.035	0.995**	0.786**	0.650**	1

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed), [a] Human blood, [b] raw milk of buffaloes, [c] groundwater

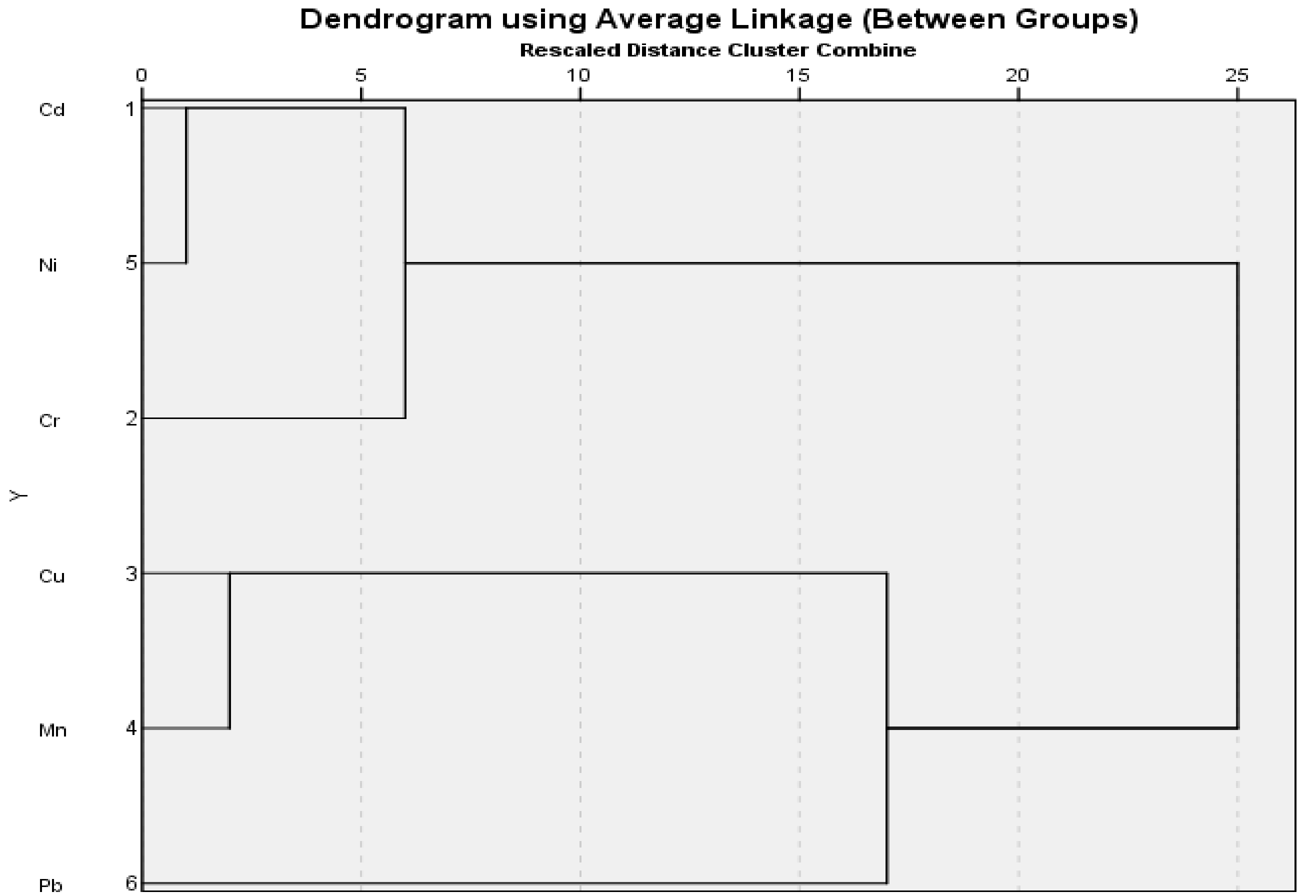

Figure 3 HCA of heavy metals in human blood across all irrigation farms

For PCA, one principal component having Eigenvalue higher than 1.0 was extracted (Table 5). The PC 1 (Eigenvalue 4.98) explained about 83 % of total variance in the analyzed data. PC 1 exhibited positive loadings of Cd, Cr, Cu, Mn, Ni and Pb showing anthropogenic source of dissemination of metals in human blood like industrial waste/sludge and sewage (Ma & Gui, 2017; Rinklebe *et al*. 2019). PCA biplot (Figure 4) showed that the metals framed two groups. Group 1retained Cr, Ni and Cd while group 2 retained Pb, Cu and Mn which is similar to HCA dendogram for human blood (Figure 3) and it confirmed the results of PCA.

MSA exhibited that wastewater/water used for irrigation containing heavy metal, contaminated soil, contaminated groundwater and contaminated food stuff like vegetables (Brassica), Maize plants, raw milk, chicken meat are common sources disseminating the heavy metals in human blood across all farms.

Table 5 Principal component loadings of heavy metals in human blood

	Human blood
	PC1
Eigenvalues	4.98
% Total Variance	83.0
% Cumulative Variance	83.0
Cd	0.408
Cr	0.396
Cu	0.43
Mn	0.363
Ni	0.441
Pb	0.407

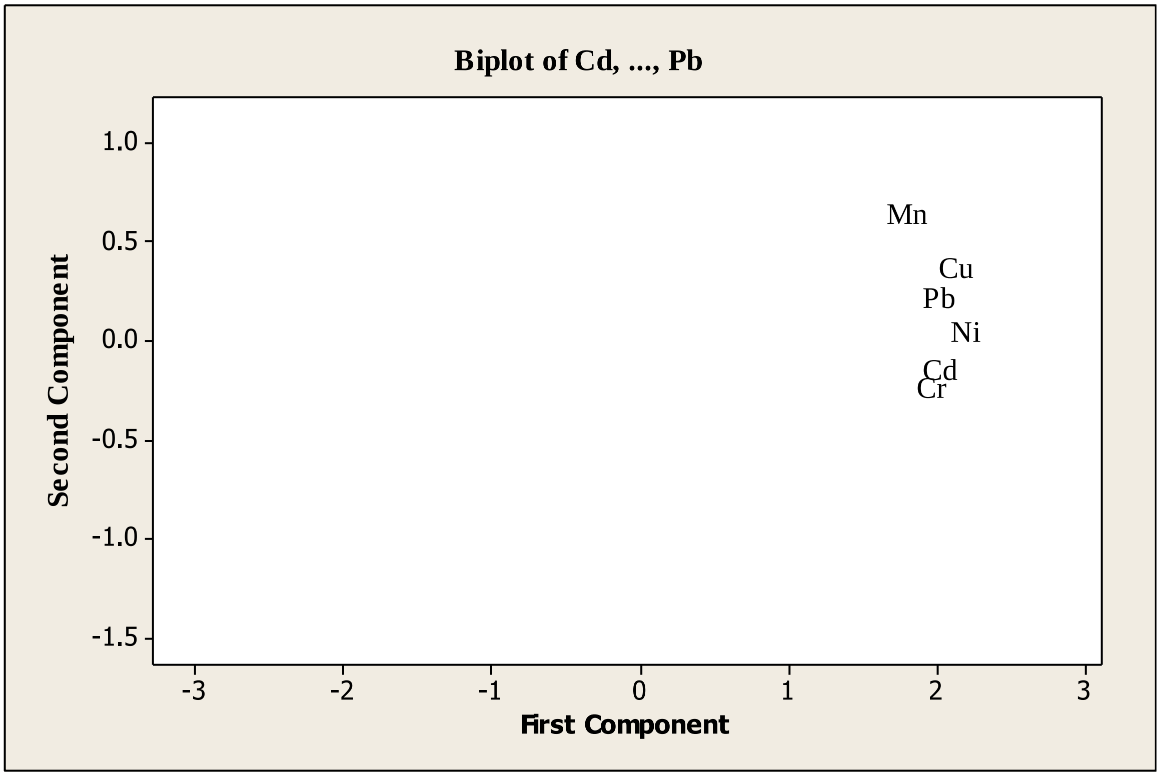

Figure 4 PCA biplot diagram of heavy metals in human blood across all agricultural farms

DISCUSSION

The results revealed that mean concentration of Ni, Mn, Cr and Cd exceeded WHO safe limit in the blood of human population exposed to wastewater irrigated agricultural fields and that all metals contents were within WHO safe limits at tube- well and canal water irrigation farms. The contamination level of human blood at industrial wastewater irrigated farm was highest and was lowest at tube- well water irrigated farm. Total contents of metals in human blood were 100 to 350 times higher at wastewater irrigated farms than that at canal water irrigated farm. It might be due to the availability of higher concentration of metals in wastewater, soil and other linked pathways. Multivariate statistical analysis indicated that wastewater and water irrigating the farms, contaminated soil, contaminated groundwater and contaminated food stuff like vegetables (Brassica), Maize plants, raw milk, chicken meat are common sources of metal contamination in human blood across all sites.

The mean contents of metals observed in this study in human blood are supported by the results of previous studies which indicated that blood of human population exposed to polluted environment and contaminated foodstuff was more contaminated with heavy metals than WHO safe limits (Akintujoye *et al*. 2013; Schultze *et al*. 2014; Schultze *et al*. 2013 ; Ahmad *et al*. 2014; Ahmad *et al*. 2013 ; Jan *et al*. 2011; Wasowicz *et al*. 2001; Nkolika and Benedict, 2009; Akan *et al*. 2014).

Novelty of this study is that a comparative analysis of human blood contamination of population was conducted with reference to their exposure to different agricultural farms irrigated by wastewater of different qualities, tube-well and canal water with source apportionment. Maximum mean concentration of selected metals were observed at farm-A irrigated by untreated industrial effluents of MIE. In MIE about 300 industrial units including pesticide formulation, tanneries, automobile parts manufacturing units, lead extraction from condemn batteries (smelting units), auto repair workshops, textile dyeing, edible oil, Phosphate fertilizer etc are operational and disseminate these metals into combined drainage system of MIE. Similar industrial clusters are operational in urban area of Multan city (Tariq *et al*. 2010; Iqbal *et al*. 2019). These metals are added in wastewater from smelting process, phosphate fertilizer, NiCd batteries, auto parts manufacturing and repairing process, plating, edible oil (ATSDR, 1999; Ali *et al*. 2019) functional in study area. Cr is used in leather process and originate from tanneries (Tariq *et al*. 2010; Ali *et al*. 2019) operational in study area.

Previous studies in study area revealed that application of untreated wastewater in irrigation has become source of food chain contamination and resulting in transmission of toxic heavy metals to human body exceeding WHO safe limits. Tariq *et al*. (2010) reported in study area that a large agricultural land became nonproductive due to very high contamination with heavy metals. Randhawa *et al*. (2014) reported that vegetables grown in study area were unfit for human consumption. Ismail *et al*. (2015) reported that vegetables grown with canal water were more contaminated than that with tube well water in study area. Iqbal (2018) reported in study area that the soils under wastewater irrigated farms exhibited very high ecological risk to moderate ecological risks and all vegetables grown with wastewater posed carcinogenic health risk to humans. Iqbal *et al*. (2019) reported in same study area that Maize plants grown with wastewater being used as fodder posed carcinogenic risk to animals due to intake of same heavy metals. Iqbal *et al*. (2020) reported that milk of buffaloes in study area exerted carcinogenic risk to humans. Therefore the intake of heavy metals and its buildup in human blood beyond WHO safe limits at wastewater irrigated farms may cause serious human health implications. There is variation among the contamination level of human blood according to respective contamination level of wastewater and water used for irrigation, soil and food stuff at respective agricultural farms. The contamination level of human blood at canal water irrigated farm was higher than that at Tube well water irrigated field. Pakistan has one of the largest canal irrigation systems in the world and it is indicator of canal water contamination and resulting food chain contamination. Saleemi (1993) elaborated this aspect of canal water contamination and reported that untreated industrial effluents are being discharged into rivers and canals in Pakistan and this practice is still continued. This study has provided basic information and data to policy makers, planners and decision makers and invites the attention of agricultural, environmental, food and health authorities to remediate the situation causing sever human health implications due to buildup of toxic elements in human bodies exposed to agricultural farming system in Pakistan.

Conclusions: The mean concentration of heavy metals Cd, Cr, Mn and Ni exceeded the WHO safe limits in human blood of population exposed to wastewater irrigated agricultural farms while were within limits in the blood of population exposed to tube well and canal water irrigated agricultural farms. Mean contents of Ni and Pb were within safe limits across all farms.

The total contents of metals in human blood of population exposed to wastewater irrigated agricultural farms were 100 to 350 times higher than that canal water irrigated farm. The contamination level of human blood of population at untreated industrial wastewater was highest and was lowest at tube well water irrigation farm having all metal contents within WHO safe limits. The human blood contamination beyond WHO safe limits may cause carcinogenic health risk to humans as elements like Cd, Cr, Ni and Pb are known carcinogenic elements.

Statistical analyses evidenced that the wastewater containing heavy metals used to irrigate land, contaminated soil, food stuffs like groundwater, vegetables, domestic chicken meat, and raw milk produced at respective farms are common sources of human blood contamination with heavy metals.

In Pakistan typically in Punjab, the canal irrigation system is largest in the world. The contamination level of blood of population at canal water irrigated fields higher than that at tube well water irrigated fields is indicator of canal water contamination with heavy metals and such contamination is being transmitted in human bodies via consumption of contaminated food stuff grown with canal water and other linked path ways. It invites immediate attention of the government to take mitigation measures to save quality of canal water and health of general public.

The long term application of wastewater containing heavy metals is source of soil and food stuff contamination at very high rate and the human population residing at wastewater irrigated agricultural fields having metal contents beyond WHO safe limits may suffer to carcinogenic effects. Therefore wastewater containing heavy metals may be properly treated before its use to irrigate agricultural farm producing human foodstuff to safeguard the health of humans and ecosystem.

Recommendations: By in house treatment of wastewater in industrial units, the concentration of highly toxic elements like Cd, Cr, Cu, Ni, Pb (having high toxic response factors) may be removed at source to minimize the toxicity of these metals in wastewater, soil, food stuff and ultimately in human bodies. The combined effluents treatment plant like SCAB method (Ahmad *et al*. 2019) may be useful to utilize the treated wastewater in agricultural farming system to save the health of public and ecosystem. There is urgent and dire need to frame policy and standards for proper treatment of wastewater to reuse in agriculture to protect public health.

REFERENCES

Abbas, Farhat, Ashfaq Ahmad, Mohammad Safeeq, Shafaqat Ali, Farhan Saleem, Hafiz Mohkum Hammad, and Wajid Farhad. 2014. "Changes in Precipitation Extremes over Arid to Semiarid and Subhumid Punjab, Pakistan." *Theoretical and Applied Climatology* 116 (3–4): 671–80.

Ahmad, Nisar, Muhammad Rahim, Mas Rosemal, and Hakim Mas. 2014. "Toxicological Impact Assessment of Heavy Metals in Human Blood and Milk Samples Collected in District" 26 (1): 223–26.

Ahmad M, Yousaf M, Nasir A, Bhatti IA, Mahmood A, Fang X, ... &Mahmood N (2019) Porous eleocharis@ MnPE layered hybrid for synergistic adsorption and catalytic biodegradation of toxic Azo dyes from industrial wastewater. Enviro Sci and Techno 53(4):2161–2170

Akan JC,Sodipo OA,Liman Y, Chellube ZM. 2014. “Determination of Heavy Metals in Blood, Urine and Water Samples by Inductively Coupled Plasma Atomic Emission Spectrophotometer and Fluoride Using Ion-Selective Electrode.” *Journal of Analytical & Bioanalytical Techniques* 5 (6).

Akesson, Agneta, Bettina Julin, and Alicja Wolk. 2008. “Long-Term Dietary Cadmium Intake and Postmenopausal Endometrial Cancer Incidence: A Population-Based Prospective Cohort Study.” *Cancer Research* 68 (15): 6435–41.

Akintujoye, J F, C I Anumudu, and H O Awobode. 2013. “Assessment of Heavy Metal Residues in Water, Fish Tissue and Human Blood from Ubeji, Warri, Delta State, Nigeria.” *Journal of Applied Sciences and Environmental Management*. http://search.ebscohost.com/login.aspx?direct=true&AuthType=cookie,ip,shib&db=awn&AN=jasem-92478&site=ehost-live%5Cnhttp://www.ajol.info/index.php/jasem/article/view/92478.

Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. Journal of chemistry, 2019.

AOAC (2000) Official Method 980.21, Aflatoxin M1 in milk and cheese. AOAC Methods of Analysis, 17th edn. Association of Official Analytical Chemists, Gaithersburg, MD

AOAC. (2012). AOAC INTERNATIONAL guidelines for validation of botanical identification methods. *Journal of AOAC International*, *95*(1), 268.

APHA. (2005). Standard methods for the examination of water and wastewater. American Public Health Association (APHA): Washington, DC, USA.

ASTM. (2007). Metals Test Methods and Analytical procedures. American Society for Testing and Materials. Washington, DC, USA.

ATSDR. 1999. Toxicological Profile for Cadmium Atlanta, GA Agency for Toxic Substances and Disease Registry Available: http://www.atsdr.cdc.gov/toxprofiles/tp5.html[accessed 29 May 2020]. Google Scholar

ATSDR. 2012. Toxicological Profile for Cadmium.” *Agency for Toxic Substances and Disease Registry, Public Health Service- U.S. Department of Health and Human Services*, September: 1–487. http://www.atsdr.cdc.gov/toxprofiles/tp5.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/books/NBK158845/.Accessed May 29, 2020

ATSDR. 2017. Health Effects of Exposure to Substances and Carcinogens. https://www.atsdr.cdc.gov/substances/ToxOrganSystems.asp.

ATSDR (Agency for Toxic Substances and Diseases Registry). 2000. Toxicological Profile for Chromium. *Public Health*, September: 421.

Cambra, Koldo, Teresa Martinez, Arantzazu Urzelai, and Eva Alonso. 1999. Risk Analysis of a Farm Area Near a Lead- and Cadmium-Contaminated Industrial Site. *Journal of Soil Contamination* 8 (5): 527–40.

Chen, T B, Y M Zheng, M Lei, Z C Huang, H T Wu, H Chen, K K Fan, K Yu, X Wu, and Q Z Tian. 2005. Assessment of Heavy Metal Pollution in Surface Soils of Urban Parks in Beijing, China. *Chemosphere* 60 (4): 542–51.

Ensink, Jeroen H J, Tariq Mahmood, Wim Van Der Hoek, Liqa Raschid-Sally, and Felix P. Amerasinghe. 2004. A Nationwide Assessment of Wastewater Use in Pakistan: An Obscure Activity or a Vitally Important One? *Water Policy* 6 (3): 197–206.

EC (European Commission). 2013. Soil Contamination : Impacts on Human Health. *Science for Environmental Policy*, no. 5: 1–29.

Gidikova, P., G. Sandeva, R. Deliradeva, G. Prakova, and M. Platikanova. 2015. Blood Concentration of Heavy Metals among Environmentally Exposed Residents of Stara Zagora Municipality (Bulgaria). *Trakia Journal of Science* 13 (4): 33–40.

Hernández, Teresa, Juan I. Moreno, and Francisco Costa. 1991. Influence of Sewage Sludge Application on Crop Yields and Heavy Metal Availability. Soil Science and Plant Nutrition 37 (2): 201–10.

Iqbal, Z, (2018) Environmental Impacts of urban sewage disposal in water bodies and sewage irrigation. Ph.D thesis. Government College University Faisalabad, Pakistan

Iqbal Z, Abbas F, Ibrahim M, Ayyaz M M, Ali S, Mahmood A (2019) SURVEILLANCE OF HEAVY METALS IN MAIZE GROWN WITH WASTEWATER AND THEIR IMPACTS ON ANIMAL HEALTH IN PERIURBAN AREAS OF MULTAN, PAKISTAN. Pak J Agri Sciences, 56(2) 321-328

Iqbal, Z., Abbas, F., Ibrahim, M., Qureshi, T. I., Gul, M., & Mahmood, A (2020). Human health risk assessment of heavy metals in raw milk of buffalo feeding at wastewater-irrigated agricultural farms in Pakistan. Environmental Science and Pollution Research, 1-13.

Irshad, Muhammad, Sajjad Ahmad, Arshid Pervez, and Mitsuhiro Inoue. 2015. Phytoaccumulation of Heavy Metals in Natural Plants Thriving on Wastewater Effluent at Hattar Industrial Estate, Pakistan. *International Journal of Phytoremediation* 17 (2): 154–58.

Jan, F. Akbar, M. Ishaq, S. Khan, M. Shakirullah, S. M. Asim, I. Ahmad, and Fazal Mabood. 2011. Bioaccumulation of Metals in Human Blood in Industrially Contaminated Area. *Journal of Environmental Sciences* 23 (12). The Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences: 2069–77.

Jiang,X., Lu, W. X., Zhao, H. Q., Yang,Q. C.,& Yang, Z. P. (2014).Potential Ecological Risk Assessment and Prediction of Soil Heavy-Metal Pollution around Coal Gangue Dump. Natural Hazards and Earth System Sciences 14 (6): 1599–1610.

Kabata-Pendias, A. 2010. Trace Elements in Soils and Plants, Fourth Edition. https://books.google.co.in/books?id=YQfMBQAAQBAJ.

Kabata-Pendias, Alina, and Henryk Pendias. 2001. *Trace Elements in Soils and Plants*. *New York*. Vol. 2nd.

Khan, S., Q. Cao, Y. M. Zheng, Y. Z. Huang, and Y. G. Zhu. 2008. Health Risks of Heavy Metals in Contaminated Soils and Food Crops Irrigated with Wastewater in Beijing, China. *Environmental Pollution* 152 (3): 686–92.

Kusin, F. M., Azani, N. N. M., Hasan, S. N. M. S., & Sulong, N. A. (2018). Distribution of heavy metals and metalloid in surface sediments of heavily-mined area for bauxite ore in Pengerang, Malaysia and associated risk assessment. Catena, 165, 454-464.

Ma, Li, and Herong Gui. 2017. Anthropogenic Impacts on Heavy Metal Concentrations in Surface Soils from the Typical Polluted Area of Bengbu, Anhui Province, Eastern China. *Human and Ecological Risk Assessment: An International Journal*, July. Taylor & Francis, 1–12.

Ministry of the Environment Finland (MEF). 2007. Government Decree on the Assessment of Soil Contamination and Remediation Needs (214/2007).

NESPAK. 2017. Master Planning of Water Supply, Sewerage and Drainage System of WASA Multan, Pakistan.

Nkolika, Ibeto Cynthia, and Okoye Chukwuma O Benedict. 2009. Elevated Cadmium Levels in Blood of the Urban Population in Enugu State Nigeria. *Applied Sciences* 7 (10): 1255–61.

Qadir, M., D. Wichelns, L. Raschid-Sally, P.G. McCornick, P. Drechsel, A. Bahri, and P.S. Minhas. 2010. The Challenges of Wastewater Irrigation in Developing Countries. *Agricultural Water Management* 97 (4): 561–68.

Qadir, M, A Ghafoor, G Murtaza, and T Mahmood. 1997. Metal Ion Contamination in Vegetables and Soils Irrigated with City Effluents. *Environmental Pollution*.

Randhawa, M. A., Ahmad,G., Anjum, F. M., Asghar, A., & Sajid, M. W. (2014). Heavy Metal Contents and Their Daily Intake in Vegetables under Peri-Urban Farming System of Multan, Pakistan. Pakistan Journal of Agricultural Sciences 51 (4): 1125–31.

Rinklebe, J., Antoniadis, V., Shaheen, S. M., Rosche, O., & Altermann, M. (2019). Health risk assessment of potentially toxic elements in soils along the Central Elbe River, Germany. Environment international, 126, 76-88.

Rodriguez, J. A., Nanos, N., Grau, J. M., Gil, L., & Lopez-Arias, M. (2008). Multiscale analysis of heavy metal contents in Spanish agricultural topsoils. Chemosphere, 70(6), 1085-1096.

Saleemi, M. A. (1993). Environmental assessment and management of irrigation and drainage scheme for sustainable agriculture growth. *EPA, Lahore, Pakistan*.

Schultze, Björn, P Monica Lind, Anders Larsson, and Lars Lind. 2014. Whole Blood and Serum Concentrations of Metals in a Swedish Population-Based Sample. *Scandinavian Journal of Clinical and Laboratory Investigation* 74 (2): 143–48.

Speight, James G. 2017. Industrial Organic Chemistry. In *Environmental Organic Chemistry for Engineers*, 87–151.

Tariq, S. R., Shaheen, N., Khalique, A., & Shah,M.H.(2010).Distribution, Correlation, and Source Apportionment of Selected Metals in Tannery Effluents, Related Soils, and Groundwater-a Case Study from Multan, Pakistan. Environmental Monitoring and Assessment 166 (1): 303–12.

Tedoldi, Damien, Ghassan Chebbo, Daniel Pierlot, Philippe Branchu, Yves Kovacs, and Marie Christine Gromaire. 2017. Spatial Distribution of Heavy Metals in the Surface Soil of Source-Control Stormwater Infiltration Devices ??? Inter-Site Comparison. *Science of the Total Environment* 579: 881–92.

Van Der Hoek, Wim, Mehmood Ul Hassan, Jeroen H J Ensink, Sabiena Feenstra, Liqa Raschid-Sally, Sarfraz Munir, Rizwan Aslam, Nazim Ali, Raheela Hussain, and Yutaka Matsuno. 2002. Urban Wastewater: A Valuable Resource for Agriculture. A Case Study from Haroonabad, Pakistan.

van Liedekerke, Marc, Gundula Prokop, Sabine Rabl-

Berger, Mark Kibblewhite, and Geertrui Louwagie. 2014. Contaminated Sites in Europe. https://scholar.googleusercontent.com/scholar.bib?q=info:aH6ptoA4zqoJ:scholar.google.com/&output=citation&scisig=AAGBfm0AAAAAWcS0Ecz0KJdYBz6gZXJ4mdIKgYBVwhTE&scisf=4&ct=citation&cd=-1&hl=en.

Wasowicz, W, J Gromadzińska, and K Rydzyński. 2001. Blood Concentration of Essential Trace Elements and Heavy Metals in Workers Exposed to Lead and Cadmium. *International Journal of Occupational Medicine and Environmental Health* 14 (3): 223–29.

Water, UN. 2015. Wastewater Management A UN-Water Analytical Brief.

WHO. 1996. Trace Elements in Human Nutrition and Health World Health Organization. *World Health Organization*, 1–360.

Yang, Q., Li, Z., Lu, X., Duan, Q., Huang, L., & Bi, J. (2018). A review of soil heavy metal pollution from industrial and agricultural regions in China: pollution and risk assessment. Science of the total environment, 642, 690-700.