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HEAVY METAL TOLERANCE AND BIOCHEMICAL ATTRIBUTES OF SELECTED WHEAT GENOTYPES ON IRRIGATION WITH INDUSTRIAL WASTEWATERS

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Present research was designed to investigate heavy metal (HM) accumulation, distribution, relative tolerance and biochemical attributes of diverse 30 wheat genotypes on irrigation with textile (T2) and iron-steel industrial wastewater (T3) in pots in natural environmental conditions. The T3 wastewater was more polluted in terms of HM i.e. Ni, Cr, Mn, Fe, Pb and Zn than T2 wastewater. Relatively acidic pH and low organic matter of T3 recipient soils facilitated higher HM accumulation in corresponding wheat genotypes than T2 and control (T1). Significant genotypic variations in metal accumulation were recorded and pattern of accumulation was i.e. roots>stem>>grain. Tolerant, sensitive and intermediate performing wheat genotypes from T2 and T3 were identified using multivariate techniques. Tolerant genotypes exhibited efficient biochemical mechanisms (antioxidant enzymes and proline) to overcome HM stresses as compared to sensitive genotypes. Cultivation of tolerant wheat genotypes in soils receiving similar HM wastes can minimize their hazardous effects on plant physiology and plant produce. Enzymatic antioxidants i.e. SOD, POD, CAT and proline were identified as important biomarkers of heavy metals toxicity in tolerant and sensitive wheat genotypes. Tolerant genotypes can potentially contribute in regional and global food safety programs on breeding.

Keywords: Heavy metals, wheat genotypes, wastewater, multivariate techniques, antioxidant system

INTRODUCTION

Global crop production targets are hampered due to rapidly progressing global climate change, escalating water shortage, deterioration in water quality and wastewater irrigation (Ali *et al.,* 2015). Water shortage problems have been prioritized globally and regionally, however deteriorating irrigation water quality has received little attention which has worsened agro-ecological conditions (Ma *et al.,* 2015). Decline in irrigation water quality is mainly attributable to untreated discharge of municipal and industrial wastes. In Pakistan like other developing countries wastewater generated from urban and industrial setups is directly discharged to the surface water channels i.e. canals, rivers etc. without any prior treatment leading to their considerable contamination. Nationally, less than 10% of municipal sewage from cities and 1% of industrial wastewater receive treatment (Ali *et al.,* 2015). Therefore, utilization of this low quality surface water in irrigation has revealed toxic effects on food crops in Pakistan (Khan *et al.,* 2014; Khan *et al.,* 2015). Heavy metals (HM) are the most important toxicants in surface irrigation water and their principal proportion is originated from the industrial sector. Textile and iron-steel industries are ranked amongst the major industries in Pakistan which contribute significant HM loads to the adjacent water channels and agricultural lands (Kabata-Pendias, 2011; Beh *et al.,* 2012; Ali *et al.,* 2015). Both industries are recognized as most important HM polluters of the aquatic and agricultural environments due to the unregulated and indiscriminate wastewater discharge. In rain-fed and canal end regions, where low quality surface water is not available, farmers exploit untreated textile and iron-steel industrial wastewaters to irrigate crops which exhibit serious ecotoxicological effects (Ali *et al.,* 2013).

Recently various studies have highlighted heavy metals (HM) in industrial wastewater, irrigated agricultural soils and potential health risks from the consumption of vegetables, pulses and cereal crops. However, very few studies have explored genotypic differences in HM accumulation and biochemical attributes of economically important crops to establish their sensitive or tolerant nature from wastewater irrigation. Wheat crop in this regard holds special place owing to its unique genotypic diversity, staple food for >35% of world population and 3rd most produced cereal (670.88 million metric tons) worldwide after maize and rice (Bermudez *et al.,* 2011; Iqbal *et al.,* 2015). It is also the staple food in Pakistan (5th most populous country with a population exceeding 207.77 million) and recognized conduit to food

security. Wheat crop faces serious water shortages and tempts farmers to use available industrial wastewater in irrigation when surface water is not available. Since varietal performance or genotypic variability of wheats cultivated with industrial wastewater is not known, therefore it is direly needed to explore HM accumulation in existing commonly cultivated wheat genotypes of Pakistan. The identification and selection of tolerant wheat genotypes with restricted metal absorption and translocation to edible grains can ensure safer crop production on irrigation with industrial wastewaters or low quality surface waters (receiving industrial wastes) and can also contribute to breeding HM tolerant wheat germplasm (Alybayeva *et al.*, 2014; Ali *et al.*, 2015). In this regard present study provides one of the first attempts to identify HM tolerant and sensitive wheat genotypes on irrigation with textile and iron-steel industries wastewater based on their HM accumulation potential and related biochemical changes.

MATERIALS AND METHODS

***Experimental site and soil preparation*:** Experiments were conducted at research facility of Wheat Wide Crosses and Cytogenetics Program (WWCCP), National Agricultural Research Center, Islamabad, Pakistan during the wheat growing season i.e. November 2013 to May 2014. NARC lies in the rain-fed agro-climatic conditions of the country. Soil for the present research experiments was collected in bulk from nearby agricultural fields with no wastewater irrigation history. Collected soil was air dried and passed through 2-mm sieve to remove foreign materials. Resultant soil was thoroughly mixed and introduced in to clean polyethylene pots (6.8 kg soil/pot). Recommended doses of nutrients were added in soil before the start of the experiment at the rate of 120:60:60 kg/hectare in the form of urea, diammonium phosphate and potassium sulfate respectively to ensure soil fertility and healthy plant growth (Pask *et al.*, 2012).

***Plant material, raising and study design*:** Plant material (30 wheat genotypes) was kindly provided by WWCCP, NARC (Pakistan) and detailed description is provided in the supplementary Table S1. Bread wheat genotypes commonly cultivated in irrigated [Aas-2011 (AAS), Millat-2011 (MIL), Lasani-2008 (LSN), Faisalabad-2008 (FSD), Fakhr-e-sarhad (FKH), Bakhtawar-92 (BKH), TJ83 (TJ), Kiran-95 (KRN), TD-1 (TD), Marvi-2000 (MRV)]and rain-fed [Chakwal-50 (CKW), Dharabi-2011 (DRB), Barsat-10 (BRS), NARC-2009 (NRC), Pirsabak-2005 (PIR), Tatara-96 (TTR)] agro-environments of Pakistan were selected. Durum wheat

Supplementary Table S1. Detailed description of studied plant material.

Sr.	Name	Abbr. used	Type (Species)	Pedigree
1	TJ83	TJ	Bread wheat (*Triticum aestivum* L.)	Bread wheat (Triticum aestivum L.)
2	Bakhtawar-92	BKH	Bread wheat (*Triticum aestivum* L.)	JUP/BJY//URES
3	Kiran-95	KRN	Bread wheat (*Triticum aestivum* L.)	WL 711/CROW"S"
4	Tatara-96	TTR	Bread wheat (*Triticum aestivum* L.)	JUP/ALD'S'//KLT'S'
5	Fakhr-e-sarhad	FKH	Bread wheat (*Triticum aestivum* L.)	PFAU'S'/SERI//BOW'S'
6	Marvi-2000	MRV	Bread wheat (*Triticum aestivum* L.)	CMH-77A917/PKV 1600//RL6010/6*SKA
7	TD-1	TD	Bread wheat (*Triticum aestivum* L.)	MAI'S'/NORTENO65/H68
8	Pirsabak-2005	PIR	Bread wheat (*Triticum aestivum* L.)	MUNIA/CHTO//AMSEL
9	LASANI-2008	LSN	Bread wheat (*Triticum aestivum* L.)	LUAN/KOH-97
10	Chakwal-50	CKW	Bread wheat (*Triticum aestivum* L.)	ATTILA/3/HUI/CARC//CHEN/CHTO/4/ATTILA
11	FAISALABAD-2008	FSD	Bread wheat (*Triticum aestivum* L.)	PBW65/2*Pastor
12	NARC-2009	NRC	Bread wheat (*Triticum aestivum* L.)	INQALAB 91*2/TUKURU
13	BARSAT-10	BRS	Bread wheat (*Triticum aestivum* L.)	FRET2
14	Aas-2011	AAS	Bread wheat (*Triticum aestivum* L.)	PRL/PASTOR//2236(V6550/SUTLEH-86)
15	Millat-2011	MIL	Bread wheat (*Triticum aestivum* L.)	CHENAB2000/INQ-91
16	Dharabi-2011	DRB	Bread wheat (*Triticum aestivum* L.)	HXL7573/2*BAU//PASTOR
17	Valnova	VLN	Durum wheat (*Triticum durum* Desf.)	GIORGIO-324//SENATORE-CAPELLI/YUMA
18	Adamello	ADM	Durum wheat (*Triticum durum* Desf.)	VALFORTE/(S)TURCHIA-7116
19	Gargano	GRG	Durum wheat (*Triticum durum* Desf.)	TRINAKRIA/VALFORTE//VALNOVA/APPULO
20	WC9	WC9	Bread wheat (*Triticum aestivum* L.)	ROLFO7/3/T.DICOCCON PI94625/AE. SQ (370)
21	WC11	WC11	Bread wheat (*Triticum aestivum* L.)	MAYOOR//TKSN 1081/AE. SQ. (222)/3/FLYCATCHER/4/IBWSN-225
22	EM13	EM13	Bread wheat (*Triticum aestivum* L.)	ATTILA/5/CHIR3/4/SIREN//ALTAR 84/AE. SQ (205)/3/3*BUC/6/FCT
23	N172	N172	Bread wheat (*Triticum aestivum* L.)	DVERD-2/AE. SQ (214)//2*ESDA/3/NS732/HER
24	EM	EM	Bread wheat (*Triticum aestivum* L.)	BW//SH/AE. SQ (305)
25	EBWYT510	EB10	Bread wheat (*Triticum aestivum* L.)	WBLL4/KUKUNA//WBLL1/3/WBLL1*2/BRAMBLING
26	EBWYT512	EB12	Bread wheat (*Triticum aestivum* L.)	ALTAR 84/AE. SQ (221)//3*BORL 95/3/URES/JUN//KAUZ/4 /WBLL1/5/KACHU/6/KIRITATI//PBW65/2*SERI.1B
27	EBWYT513	EB13	Bread wheat (*Triticum aestivum* L.)	FRNCLN*2/TECUE#1
28	EBWYT514	EB14	Bread wheat (*Triticum aestivum* L.)	MILAN/S87230//BAV92*2/3/AKURI
29	D7086	D86	Durum wheat (*Triticum durum* Desf.)	SOMAT_4/SILVER_1//POLARIS/5/NETTA_4/DUKEM_12//RASCON_ 19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//AIRON_1
30	D7093	D93	Durum wheat (*Triticum durum* Desf.)	SOMAT_4/INTER_8*2/5/NUS/SULA//5*NUS/4/SULA/RBCE_2/3/HUI/ /CIT71/CII

genotypes i.e. Adamello (ADM), Valnova (VLN) and Gargano (GRG) were used as checks in current experiments due to their known HM tolerance. Also, advanced selections from Pakistan (WC9, WC11, EM13), Nepal (N172), China (EM) and CIMMYT-Mexico [EBWYT510 (EB10), EBWYT512 (EB12), EBWYT513 (EB13), EBWYT514 (EB14), D7086 (D86), D7093 (D93)] were used to compare their HM tolerance with commonly grown Pakistani cultivars. Abbreviations used in current study against each genotype are provided in the parentheses. Approximately 70 healthy seeds of each genotype were surface sterilized for 10 minutes with 5% sodium hypochlorite (NaOCl) solution. Sterilized, healthy and uniform seeds were carefully rinsed with de-ionized water and germinated on moistened filter papers in petri plates in dark at 25°C for two days. After germination seedlings were transferred to jiffy trays containing peat moss for further growth at room temperature. After one week uniform seedlings of each genotype were selected and transplanted to polyethylene pots (five seedlings per pot). After another week's growth three uniform seedlings were retained/pot, allowed to grow to physiological maturity and thereafter harvested. Transplanted plant material (30 wheat genotypes) was subjected to three irrigation treatments i.e. 1-control (T1; groundwater), 2-textile industries (T2) wastewater, 3-iron-steel industries (T3) wastewater. Equal volume sub-samples were collected from three textile and iron-steel industrial units each (located in Rawalpindi and Hattar Industrial Estate, Khyber-Pakhtunkhwa) and composite used. Pot transplanted seedlings were initially irrigated with tap water for 2 weeks, afterwards subjected to selected wastewater irrigation treatments as per growing plant requirements (Pask *et al.,* 2012). All pots were equally/well-watered (via plastic cans) with selected irrigation sources, kept in natural environmental conditions at WWCCP research facility and protected from rain. Pots were rearranged every third day to ensure uniform growing conditions.

***Wastewater analyses*:** For analytical purposes duplicate wastewater samples were collected from selected irrigation sources in labeled 1L pre-cleaned plastic bottles at each time of wastewater collection that was used for irrigation experiments (APHA, 2005). In one of the duplicate bottles, 5 ml concentrated HNO_3 was added to reduce metal adsorption to the bottle walls. Wastewater sub samples from textile and iron-steel industries (three units of each) were combined to form a composite wastewater sample, kept in insulated cooler containing ice and delivered to the refrigerator in lab at 4°C the same day to prevent any change in the wastewater's chemistry until further processing. Wastewater parameters including pH, electrical conductivity (EC) and total dissolved solids (TDS) were determined in the field using portable combined meter (Lutron, WA-2015). Total hardness was calculated as amount of dissolved calcium and magnesium in water and expressed as mg/L of $CaCO_3$. Alkalinity was determined through acid-base and chlorides by silver-nitrate titrimetric methods. Nitrate-nitrogen (NO_3^{1-}-N; phenol-disulphonic acid), nitrite-nitrogen (NO_2^{1-}-N; Griess), Ortho-phosphate (PO_4^{3-}, ammonium molybdate), ammonia-nitrogen (NH_3–N; phenate), sulfates (SO_4^{2-}; barium chloride), chemical oxygen demand (COD; reactor digestion) and biological oxygen demand (BOD; Velp BOD Sensor System) were determined by respective methods (APHA, 2005).

***Soil and plant analyses*:** Thoroughly mixed triplicate soil samples per genotype from each treatment were combined to make a composite sample. Each composite soil sample was air-dried, crushed and passed through 2-mm sieve before further chemical processing. Soil pH, EC and TDS were determined by combined meter (Lutron, WA-2015) using 1:10 suspension of soil (w/v) in deionized water (Ali *et al.*, 2015). Soil alkalinity (acid-base) and chlorides (silver-nitrate) were determined by respective titrimetric procedures (Estefan *et al.,* 2014). Soil organic matter (OM%, Walkley-Black) and particle size distribution (Bouyoucos hydrometer) were determined by respective methods (Walkley, 1947). Soil nitrate-nitrogen (NO_3^{1-}-N) and phosphorus (P) were determined by AB-DTPA method (Soltanpour and Schwab, 1977).

Total chlorophyll contents were determined by incubating 0.05 g leaf tissue in 10 ml dimethyl sulfoxide at 65°C for 4 hours (Hiscox and Israelstam, 1979). Absorbance of the clear extract was recorded at 645 and 663 nm to determine chlorophyll contents as per standard method (Arnon, 1949). Carotenoids (Lichtenthaler and Wellburn, 1983), membrane stability index (Rady and Hemida, 2015), sugars (Dubois *et al.,* 1951) and proline contents (Bates *et al.,* 1973) were determined according to the methods by respective researchers. Protein contents were determined according to Bradford (1976) using bovine serum albumin (BSA) as standard. Superoxide dismutase (SOD; E.C. 1.15.1.1), peroxidase (POD; E.C. 1.11.1.7) and catalase (CAT; E.C. 1.11.1.6) enzymes were determined by the methods of Giannopolitis and Ries (1977), Gorin and Heidema (1976) and Aebi (1984), respectively. All biochemical parameters were analyzed on anthesis stage.

***Metal analyses*:** After harvesting; grains, stems and roots from each treatment were washed with tap water, followed by deionized water and dried in an oven at 70°C for 48 hours to attain constant weight. Finely ground triplicate grain, stem, root and composite soil samples (1 g each) were digested on a hot plate at 80°C using 15 ml of high purity tri-acid mixture (HNO_3, H_2SO_4 and $HClO_4$) in 5:1:1 ratio until a transparent solution was obtained (Allen *et al.,* 1986). Similarly wastewater samples (50 ml) containing 10 ml of highly pure HNO_3 were digested on a hot plate at 80°C to obtain a clear solution (APHA, 2005). Wastewater, soil and plant digests were filtered and volume was made up to 50 ml with deionized water followed by analyses of HM and macro-nutrients using fast sequential atomic absorption spectrophotometer. Metal contents in the digested samples

were determined in triplicate, averaged and reanalyzed if the relative standard deviation exceeded 5%. Procedural blanks were prepared using same acids, deionized water and digestion procedure to eliminate related contamination sources. Standard reference materials (SRM's) for wastewater (ERM CA-713 and BCR-715), soil (NIST 2711a-Montana II Soil) and plant (NIST 1573a, tomato leaves) were used to validate metal results in wastewater, soil and plant matrices.

***Statistical analyses*:** Experiments were conducted in factorial, randomized complete block design with three replications. Individual and combined effects of wheat genotypes and irrigation treatments on metal concentrations in plant organs (root, stem, grain) and biochemical attributes were examined by two-way analysis of variance (ANOVA). Wheat genotypes capable of retaining higher HM loads in roots with reduced translocation to aerial parts i.e. stems and grains are regarded as tolerant (Dong *et al.,* 2002; Ci *et al.,* 2009; Alybayeva *et al.,* 2014). Since grains are the edible part therefore grain HM contents are of pivotal importance in classifying genotypes in tolerant or sensitive categories. Worldwide wheat breeding programs consider grain HM contents as one of the most important selection criteria when soils contain or receive higher HM concentrations (Schnurbusch *et al.,* 2010; Alybayeva *et al.,* 2014). Identification and characterization of tolerant genotypes with low grain HM contents are also important in development of wheat germplasm resistant to the adverse effects of HM in agro-ecosystems (Rizwan *et al.,* 2016). Similarly, in current study tolerant, intermediate and sensitive genotypes were identified based on grain metal contents in T2 and T3 irrigation treatments using multivariate techniques i.e. principal component analysis (PCA) and hierarchical agglomerative cluster analysis (HACA). PCA was performed with varimax rotation to produce biplots to visualize genotypes against grain metals considered as variables (Nagar *et al.,* 2015).To confirm PCA results HACA was performed using Euclidean distance measure and Ward's linkage technique. To identify key biochemical attributes governing HM tolerance in studied genotypes heatmaps were generated showing two-way hierarchical clustering. Mentioned statistical computations were performed on Statistica *Ver.7* (Stat Soft Inc., Tulsa, OK, USA), XLSTAT *Ver.2017* (Addinsoft) and R *Ver.3.4.1*.

RESULTS

***Physico-chemical assessment of irrigating wastewaters and recipient soils*:** Mean pH of textile wastewater (8.16) was basic and slightly acidic (6.97) in iron-steel industrial wastewaters (Table 1). Mean EC (2.48 mS/cm), TDS (1613.09), COD (1117.65), BOD (609.48), Na (124.7), K

Table 1. Physico-chemical characteristics and heavy metal contents in control (groundwater), textile and iron-steel industrial wastewaters (n≥71).

Parameters[1]	Control	Textile	Iron-Steel	Irrigation Standards
	Mean ± SD	Mean ± SD	Mean ± SD	
pH[2]	7.2±0.22	8.16±1.39	6.97±0.34	6.5-8.4 [a]
EC (mS/cm)	0.53±0.16	2.48±0.33	1.8±0.45	3 [a]
TDS	340.55±103.104	1613.09±284.3	1188.38±293.5	2000 [a]
Alkalinity	271.14±36.43	339.86±64.95	442±146.08	613.1 [a]
Cl^{1-}	34.96±12.57	178.2±79.6	320.29±111.35	1065 [a]
Total Hardness	228.93±47.9131	331.71±116.87	687.67±237.75	-
PO_4^{3-}	0.19±0.13	4.88±1.45	5.78±2.52	2 [a]
NO_2^{1-}-N	ND[3]	0.06±0.03	0.28±0.36	-
NO_3^{1-}-N	0.81±0.62	11.34±7.39	24.82±14.76	10 [a]
NH_3-N	ND	7.94±3.8	18.5±7.77	5 [a]
SO_4^{2-}	28.26±5.74	293.34±107.66	554.77±264.4	960 [a]
COD	4.64±1.37	1117.65±186.4	798.91±165.72	150 [b]
BOD	0.9±0.62	609.48±119.49	357.95±93.29	100 [b]
Ca	45.1±10.5	89.16±42.19	166.12±62.89	400.8 [a]
Mg	28.24±5.27	23.25±7.59	43.75±18.92	60.8 [a]
Na	11.39±2.15	124.7±53.85	58.9±17.73	919.6 [a]
K	1.54±0.55	16.59±8.03	4.41±1.03	2 [a]
Fe	0.26±0.11	5.06±1.98	35.41±20.15	5 [c]
Co	0.02±0.008	0.37±0.12	0.15±0.08	0.05 [c]
Cu	0.035±0.015	7.49±1.83	2.08±1.25	0.2 [c]
Mn	0.023±0.013	4.73±1.92	18.29±5.54	0.2 [c]
Zn	0.053±0.023	5.25±2.1	21.27±8.59	2 [c]
Cd	ND	1.12±0.23	0.59±0.29	0.01 [c]
Pb	0.011±0.006	1.05±0.42	5.49±2.16	5 [c]
Cr	0.033±0.011	2.16±1.14	14.98±4.13	0.1 [c]
Ni	0.015±0.009	0.72±0.36	7.92±4.01	0.2 [c]

[1] All measurements in mg/L except where mentioned; [2] No Units; [3] Not Detected

[a] Ayers and Westcot, 1985; [b] Alberta Environment, 2000; [c] Rowe and Abdel-Magid, 1995

(16.59), Co (0.37), Cu (7.49) and Cd (1.12) levels were higher in textile wastewaters whereas alkalinity (442), Cl^{1-} (320.29), total hardness (687.67), PO_4^{3-} (5.78), NO_2^{1-}-N (0.28), NO_3^{1-}-N (24.82), NH_3-N (18.5), SO_4^{2-} (554.77), Ca (166.12), Mg (43.75), Fe (35.41), Mn (18.29), Zn (21.27), Pb (5.49), Cr (14.98) and Ni (7.92) in mg/L were higher in iron-steel industrial wastewaters. Textile irrigated soils showed alkaline pH (8.6) whereas soils irrigated with iron-steel wastewater showed comparatively acidic pH (6.73) (Table 2). Comparatively higher EC (3.68 mS/cm), TDS (2366.43 mg/L), OM% (6.05), Cu (80.26 mg/kg), Cd (23.19 mg/kg), Co (9.71 mg/kg), Na (3327.69 mg/kg) and K (4535.78 mg/kg) contents were recorded in textile treated soils. Soil parameters including alkalinity (572.95), P (11.44), Cl^{1-} (1648.92), NO_3^{1-}-N (539.7), Ni (116.78), Cr (231.41), Mn (556.90), Fe (11002.47), Pb (84.03), Zn (228.88), Ca (13610.76) and Mg (7284.03) in mg/kg were recorded higher in iron-steel irrigated soils.

***Heavy metal accumulation and distribution in wheat genotypes from three irrigation treatments*:** Basic descriptive statistics of HM and macro-nutrient accumulation in roots, stems and grains of studied genotypes from three irrigation treatments including a control and ANOVA results are provided in the supplementary Table S2. Among the HM, overall Fe contents were found highest in all treatments in mg/kg. After Fe; Zn and Mn were found in highest concentrations in roots>stems>grains of T3 and T2 irrigated genotypes than the rest of investigated HM. Similarly, Pb, Ni and Cr accumulated in higher concentrations in roots followed by stem and grains in all genotypes. Cobalt concentration was recorded marginally higher in T2 irrigated genotypes (root, 4.8-6.9; stem, 1.92-2.78 and grain, 0.012-0.052) than T3 (root, 3.87-5.88; stem, 1.55-2.37 and grain, 0.01-0.04) in mg/kg. Similarly, Cu and Cd levels in roots>stems>grains of T2 irrigated genotypes were marginally higher than T3 irrigated genotypes possibly due to elevated soil Cu and Cd levels. Among macro-nutrients, K was recorded in highest concentration and its pattern of accumulation was i.e. stem>roots>grains in three treatments. Na was recorded lowest among macro-nutrients and recorded accumulation pattern was i.e. T2>T3>T1. After K, maximum grain concentration was recorded for Mg in all treatments i.e., T3, 1119.28±138.42; T1, 1059.02±118.78 and T2, 1022.1±156.23.

Table 2. Effects of irrigation treatments on soil physico-chemical properties and heavy metal contents (n=30).

Parameters[1]	Irrigation Treatments			Uncontaminated Soil Standards
	Control	Textile	Iron-Steel	
	Mean ± SD	Mean ± SD	Mean ± SD	
pH[2]	7.31±0.18	8.6±0.28	6.73±0.19	6.6-8.4 [a]
EC (mS/cm)	0.42±0.08	3.68±0.26	2.96±0.3	4 [b]
TDS (mg/L)	271.83±49.39	2366.43±168.76	1901.32±190.26	-
Cl^{1-}	234.2±34.66	626.08±47.55	1648.92±67.63	3000 [a]
Alkalinity	458.15±59.94	533.61±64.97	572.95±55.77	-
P	4.8±1.4	8.35±0.96	11.44±1.45	>7 [b]
NO_3-N	3.37±1.53	285.86±1.28	539.7±28.86	1000 [a]
OM%	1.17±0.36	6.05±0.88	4.48±0.36	>0.86 [b]
Clay%	16.12±0.95	13.93±1.47	15.67±1.44	-
Silt%	35.08±1.51	36.2±2.96	35.87±2.64	-
Sand%	48.8±1.49	49.87±1.66	48.47±1.8	-
Ni	3.77±1.19	24.91±2.63	116.78±4.07	20 [c]
Cr	12.26±1.34	53.18±9.33	231.41±13.04	54 [c]
Cu	14.27±2.35	80.26±4.98	30.13±3.47	13-24 [c]
Cd	0.011±0.01	23.19±2.2	6.69±0.77	0.06-1.1 [c]
Mn	154.62±27.64	230.96±22.68	556.90±24.42	437 [c]
Co	0.41±0.22	9.71±0.85	7.38±1.45	7.9 [c]
Fe	9209.85±350.28	9328.75±104.7	11002.47±198.5	38000 [c]
Pb	18.45±1.95	27.24±3.36	84.03±7.91	32 [c]
Zn	56.45±5.85	73.55±5.75	228.88±10.88	64 [c]
Ca	10405.15±283.97	11128.9±222.96	13610.76±306.67	13700 [c]
Mg	6181.82±155.33	5979.83±150.85	7284.03±282.63	5000 [c]
Na	772.35±37.27	3327.69±87.45	1413.04±69.56	6300 [c]
K	3534.13±407.68	4535.78±198.27	3605.57±110.13	8300 [c]

[1] All measurements in mg/kg except where mentioned; [2] No Units; [a] Pedrero and Alarcon, 2009; [b]Alloway, 1995; [c] Alloway, 2013

***Identification of tolerant, intermediate and sensitive wheat genotypes*:** Principal component analyses (PCA) was performed on grain metal contents of T2 irrigated genotypes (Fig. 1a). HM were indicated by the vectors in the PCA biplot and their length indicated the extent of variation explained by each metal. The first two principal components i.e. PC1×PC2 explained 64.16% of variation. The position of the genotypes i.e. EM, PIR, VLN, NRC, TTR, EB10, ADM, WC11, GRG, EM13, WC9, AAS and MRV was opposite to the maximum HM vectors in the biplot. These genotypes were regarded as tolerant and their average HM contents i.e. Cr, Cu, Cd, Mn, Co and Pb were recorded lowest (Table 3). The genotypes i.e. FKH, BKH, TJ, KRN, EB12, EB14, D93, BRS, MIL, LSN and D86 were influenced by most of the HM vectors as seen in the PCA biplot. These genotypes were regarded as sensitive and showed highest mean accumulation of Cu, Cd, Mn, Co and Zn as shown in Table 3. However, intermediate performing genotypes (N172, FSD, DRB, TD, EB13 and CKW) showed high average accumulation of Cr and Pb only and therefore classified between sensitive and tolerant genotypes. Hierarchical agglomerative cluster analysis (HACA) largely confirmed PCA results and grouped T2

Supplementary Table S2. Effects of genotypes, irrigation treatments and their interactions on wheat (root, stem and grain) heavy metals and macro-nutrient contents in mg/kg estimated by 2-way ANOVA.

Metals	Control Irrigated Genotypes	Textile Wastewater Irrigated Genotypes	Iron-Steel Wastewater Irrigated Genotypes	Genotypic Effects	Treatment Effects	Interactions
	Mean ± SD	Mean ± SD	Mean ± SD			
Ni-Root	2.18±0.14	18.98±1.09	27.71±1.62	***	***	**
Ni-Stem	0.86±0.05	11.36±0.65	19.44±1.06	***	***	**
Ni-Grain	0.027±0.01	1.84±0.3	3.91±0.6	***	***	**
Cr-Root	3.57±0.22	10.27±0.49	39.12±2.02	**	***	*
Cr-Stem	0.99±0.07	2.59±0.14	13.25±0.74	***	***	***
Cr-Grain	0.07±0.01	0.85±0.08	5.04±0.63	*	***	*
Cu-Root	8.11±0.78	25.52±3.02	22.62±2.35	***	***	ns
Cu-Stem	4.01±0.56	13.09±1.54	11.41±1.24	***	***	ns
Cu-Grain	1.67±0.21	4.77±1.38	4.43±1.1	***	**	ns
Cd-Root	0.096±0.02	10.87±2.68	8.78±1.67	***	***	*
Cd-Stem	0.02±0.01	3.06±0.78	2.49±0.48	***	***	*
Cd-Grain	ND	0.13±0.02	0.09±0.01	*	***	ns
Mn-Root	36.93±3.32	115.23±11.4	199.87±43.32	***	***	***
Mn-Stem	11.03±1.11	37.15±3.39	59.41±14.23	***	***	***
Mn-Grain	7.22±0.98	24.46±6.19	43.98±14.14	***	**	***
Co-Root	0.28±0.03	5.67±0.51	4.67±0.43	***	***	ns
Co-Stem	0.11±0.01	2.28±0.21	1.88±0.17	***	***	ns
Co-Grain	ND	0.034±0.01	0.03±0.01	***	**	ns
Fe-Root	502.25±109.74	531.1±69.56	1640.56±274.21	***	***	***
Fe-Stem	131.19±30.03	138.19±19.88	395.79±69.87	***	***	***
Fe-Grain	20.95±7	22.93±2.16	67.18±19.57	**	**	***
Pb-Root	3.46±0.91	8.37±1.44	41.79±6.01	***	***	***
Pb-Stem	1.0±0.26	3.3±0.59	12.21±1.91	***	***	**
Pb-Grain	0.014±0.009	0.12±0.04	2.18±0.64	**	***	***
Zn-Root	55.68±4.54	111.99±9.64	266.3±29.98	**	***	ns
Zn-Stem	19.55±1.59	40.95±3.92	94.9±11.6	**	***	ns
Zn-Grain	11.38±1.86	20.47±3.72	52.37±13.79	**	**	**
Ca-Root	3492.33±202.68	4333.29±225.2	4698.77±422.03	***	**	***
Ca-Stem	2763.38±185.76	3479.87±205.41	3748.35±378.26	***	**	***
Ca-Grain	351.22±22.45	388.96±21.18	444.71±28.31	*	**	*
Mg-Root	3605.01±352.85	2726.3±253.76	3869.77±371.68	***	**	ns
Mg-Stem	2007.85±252.08	1315.51±142.91	2171.38±266.07	***	**	ns
Mg-Grain	1059.02±118.78	1022.1±156.23	1119.28±138.42	***	*	ns
K-Root	5575.06±529.61	6183.61±597.55	5089.89±515.07	***	**	ns
K-Stem	10962.61±1948.24	12529.1±1329.08	10347.62±1401.2	***	*	ns
K-Grain	2690.16±226.22	2886.48±507.1	2366.83±305.57	***	*	*
Na-Root	1614.7±571.98	2518.4±1101.01	1852.47±584.74	***	*	ns
Na-Stem	439.14±164.3	679.6±314.85	502.95±166.76	***	*	ns
Na-Grain	42.05±5.43	73.33±19.55	49.49±8.96	**	**	ns

* Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$; *** Significant at $p \leq 0.001$; ns' Not Significant; ND' Not Detected

Table 3. Basic statistical summary of grain metal contents in tolerant, intermediate and sensitive genotypes identified through multivariate techniques.

Metals	Control		T2 irrigated genotypes						T3 irrigated genotypes					
			Tolerant		Intermediate		Sensitive		Tolerant		Intermediate		Sensitive	
	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max
Ni	0.027	ND-0.06	1.91	1.74-2.38	1.70	1.3-1.95	1.82	1.7-1.98	4.03	3.52-4.99	3.95	3.57-4.38	3.79	2.85-4.2
Cr	0.07	0.05-0.08	0.81	0.73-0.89	0.91	0.84-0.96	0.86	0.83-0.92	4.84	4.27-5.31	4.95	4.61-5.3	5.25	4.92-6.2
Cu	1.67	1.4-2.39	4.33	3.66-5.91	4.47	4.04-4.97	5.46	4.53-7.33	4.08	3.71-4.7	4.52	4.08-4.98	4.65	3.73-6.37
Cd	ND	ND	0.13	0.12-0.15	0.13	0.12-0.14	0.14	0.12-0.16	0.085	0.077-0.099	0.087	0.081-0.101	0.086	0.078-0.093
Mn	7.22	5.45-10.81	22.09	17.68-28.21	25.36	19.11-28.74	26.76	21.65-38.47	35.59	25.47-56.63	42.51	34.7-51.0	51.22	38.09-64.42
Co	ND	ND	0.029	0.019-0.034	0.037	0.034-0.041	0.038	0.03-0.043	0.022	0.017-0.033	0.031	0.027-0.037	0.030	0.023-0.037
Fe	20.95	6.98-36.18	23.07	21.39-26.02	22.99	21.74-24.21	22.74	20.86-26.6	62.42	55.86-83.47	61.58	54.88-69.9	73.86	57.7-105.7
Pb	0.014	ND-0.05	0.11	0.07-0.15	0.15	0.13-0.17	0.13	0.11-0.19	2.07	1.35-2.75	1.76	1.54-1.98	2.50	2.01-2.87
Zn	11.38	4.68-16.14	20.44	17.31-23.95	19.25	16.11-21.62	21.18	17.85-28.27	49.63	40.28-68.18	51.76	46.2-59.9	54.80	44.62-84.48
Ca	351.2	302-403	393.0	375-423	386.2	373-397	385.6	363-408	439.4	432-451	430.7	411-444	456	424-482
Mg	1059	782-1260	1001	831-1160	1016	822-1207	1050	941-1201	1042	941-1239	1137	1052-1216	1168	1027-1259
K	2690	2228-3477	2778	2170-3252	2777	2363-3098	3074	2625-3608	2244	2049-2581	2469	2283-2681	2405	1955-2784
Na	42.05	31.33-62.1	76.64	59.2-106.0	71.12	60.67-80.9	70.61	60.27-83.8	51.64	43.59-67.96	47.03	40.9-51.4	49.16	44.78-57.2

ND' Not Detected; T2' Textile; T3' Iron-Steel

irrigated genotypes in three clusters (Fig. 1b). Cluster 1 included tolerant genotypes including GRG, EM13, WC9, NRC, TTR, ADM, EM, EB10, PIR, VLN, WC11, AAS and MRV which were 43.33% of the total genotypes. Cluster 2 included 9 sensitive genotypes i.e. TJ, D86, KRN, D93, BRS, EB14, EB12, MIL and FKH which were 30% of the studied genotypes. Cluster 3 included intermediate performing genotypes and included LSN, N172, FSD, DRB, TD, EB13, BKH and CKW. In this group BKH was classified as an outlier and with respect to HM accumulation it was more related to sensitive group.

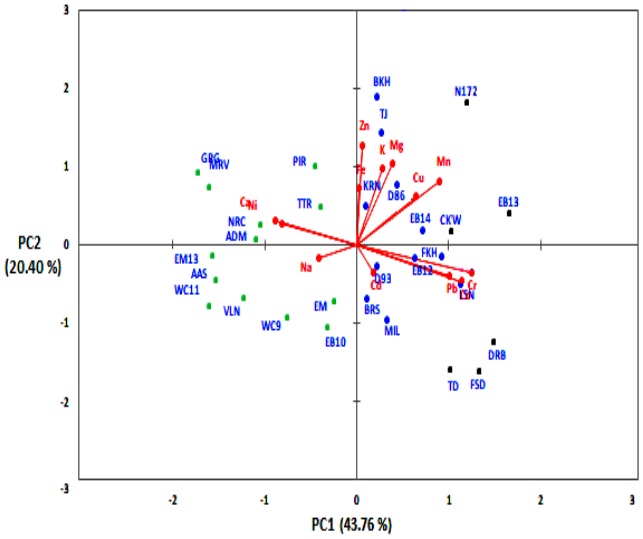

Figure 1a. Principal component analyses (PCA) performed on grain metal contents of textile irrigated genotypes. PCA biplot sorted genotypes in three groups represented by green (tolerant), black (intermediate) and blue (sensitive) colored symbols based on first two principal components (PC1×PC2).

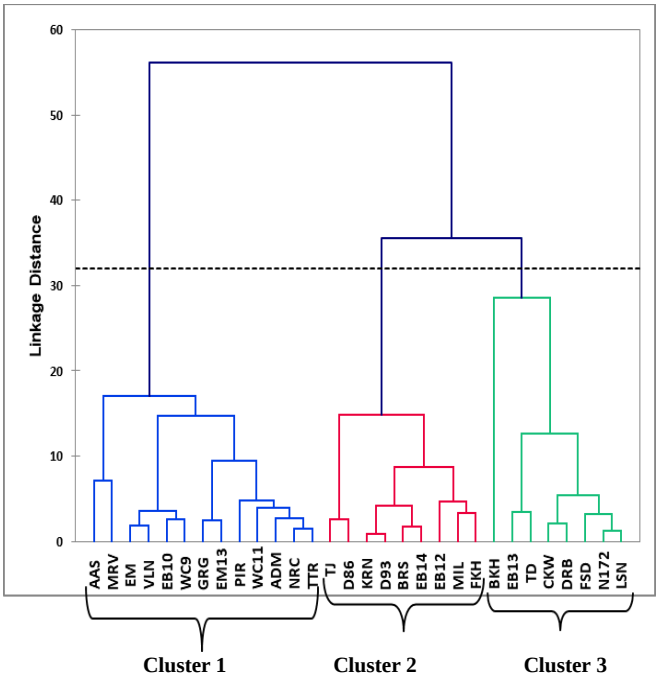

Figure 1b. Dendrogram displaying grouping of textile irrigated genotypes in three different clusters i.e. cluster 1 (tolerant), cluster 2 (sensitive) and cluster 3 (intermediate) based on grain metal contents.

Similarly, in PCA of T3 irrigated genotypes, the first two principal components explained 59.52% variation (Fig. 2a). The genotypes i.e. TJ, FKH, BKH, CKW, LSN, D86, N172, EB13, KRN, EB14, EB10, FSD and DRB were influenced by the maximum HM vectors in the biplot. Average HM contents i.e., Pb, Zn, Cu, Fe, Mn, Cr (Table 3) were recorded highest in these genotypes and classified in sensitive category. Tolerant genotypes i.e. GRG, ADM, TD, VLN, WC9, EM, AAS, WC11, MRV and EM13 were shown to be least

affected by HM vectors and their average Cr, Cu, Cd, Mn, Co and Zn levels were found lowest. Genotypes including NRC, BRS, PIR, D93, EB12, MIL and TTR were classified in the intermediate category and showed higher average Cd and Co levels only which were closely comparable to corresponding averages in sensitive genotypes (Table 3). HACA produced three clusters of T3 irrigated genotypes based on grain metal contents in agreement with PCA (Fig. 2b). Cluster 1 contained sensitive genotypes i.e. D86, FKH, TJ, FSD, EB14, N172, CKW, LSN, DRB, EB13, KRN and EB10 which were 40% of the studied genotypes. Cluster 2 included intermediate performing genotypes i.e. BRS, PIR, NRC, TTR, D93, EB12 with BKH and MIL being outliers to this group. The outlier status of BKH in the intermediate group was confirmed by PCA which classified it in sensitive genotypes; however, MIL was retained in the same group by PCA. Cluster 3 was comprised of ADM, VLN, GRG, AAS, MRV, WC11, EM, WC9, EM13 and TD which were 33.33% of total genotypes and corroborated with tolerant genotypes identified from PCA.

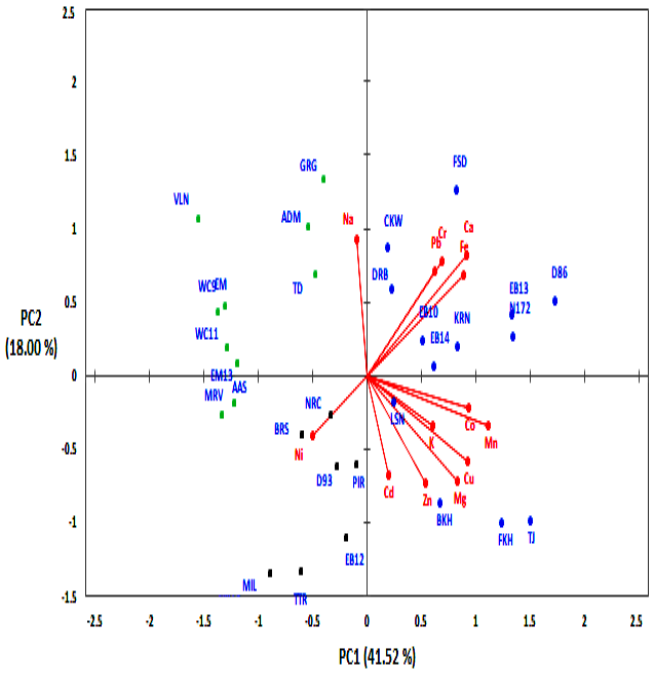

Figure 2a. Principal component analyses (PCA) performed on grain metal contents of iron-steel wastewater irrigated genotypes. PCA biplot sorted genotypes in three groups represented by green (tolerant), black (intermediate) and blue (sensitive) colored symbols based on first two principal components (PC1×PC2).

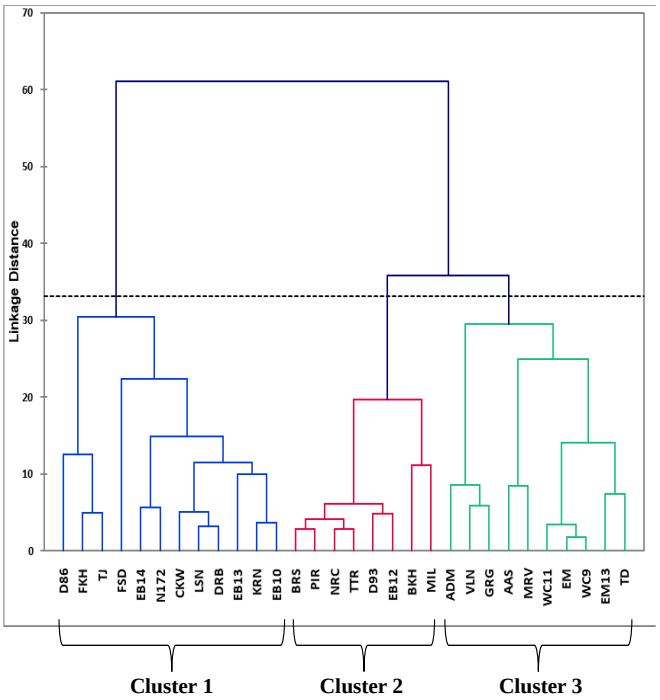

Figure 2b. Dendrogram displaying grouping of iron-steel wastewater irrigated genotypes in three different clusters i.e. cluster 1 (sensitive), cluster 2 (intermediate) and cluster 3 (tolerant) based on grain metal contents.

***Variations in biochemical characteristics and their association with tolerant, intermediate and sensitive genotypes*:** Basic statistical summary of biochemical attributes of wheat genotypes from three irrigation treatments and ANOVA results are provided in the supplementary Table S3. Interactive effects of irrigation treatments and studied genotypes were recorded significant for all biochemical attributes at $p \leq 0.05$. Among treatments, mean levels of chlorophyll, carotenoids, MSI%, proteins and sugars were found in the order T1>T2>T3 (Table S3). Mean antioxidant enzyme levels i.e. SOD (T2, 30.43>T3, 24.16>T1, 2.92 in units/mg.protein), POD (T2, 26.83>T3, 20.97>T1, 1.69; in units/min.mg.protein) and CAT (T2, 18.38>T3, 14.30>T1, 1.29; in units/min.mg.protein) were higher in T2 than corresponding values in T3 and T1 respectively. To associate biochemical attributes with T2 irrigated genotypes, heatmap was generated which classified wheat genotypes in to 3 clusters (Fig. 3a). Cluster 2 included genotypes i.e. EM, GRG, ADM, MRV, VLN, WC11, WC9 and EM13 which were identified as tolerant by multivariate techniques. These genotypes maintained relatively higher levels of i.e. photosynthetic pigments (chlorophyll and carotenoids), sugars, proline, MSI%, proteins and antioxidant enzymes (SOD, POD and CAT) than remaining genotypes.

Supplementary Table S3. Two-way ANOVA results describing effects of genotypes, irrigation treatments and their interactions on biochemical attributes of selected wheat genotypes.

Biochemical Parameters	Control Irrigated Genotypes		Textile Wastewater Irrigated Genotypes		Iron-Steel Wastewater Irrigated Genotypes		Genotypic Effects	Treat. Effect	Interaction
	Min-Max	Mean ± SD	Min-Max	Mean ± SD	Min-Max	Mean ± SD			
Chlorophyll (mg/g)	1.68-3.91	2.76±0.4	0.8-2.54	1.71±0.3	0.57-2.6	1.35±0.35	*	***	*
Carotenoids (mg/g)	0.5-1.18	0.8±0.1	0.26-0.86	0.54±0.1	0.15-0.74	0.37±0.1	*	***	*
MSI (%)	70.36-93.8	83.94±5.17	40.58-85.35	63.54±11.37	30.12-75	54.5±12.72	**	***	*
Protein (mg/g)	29.16-44.6	36.45±2.55	13.99-36.96	25.98±4.46	8.88-30.62	19.07±4.09	*	***	*
Sugar (mg/g)	0.78-2.81	1.9±0.42	0.76-2.75	1.57±0.46	0.51-2.12	1.18±0.37	**	**	*
Proline (mg/g)	0.01-0.32	0.17±0.04	0.78-2.95	1.78±0.36	1.65-4.5	2.9±0.72	**	***	*
SOD (Units/mg.Protein)	0.94-4.45	2.92±0.87	16.08-44.1	30.43±5.78	14.32-34.86	24.16±5.16	**	***	*
POD (Units/min.mg.Protein)	0.38-3.1	1.69±0.61	15.6-40.65	26.83±4.88	9.64-31.37	20.97±4.63	*	***	*
CAT (Units/min.mg.Protein)	0.52-2.9	1.29±0.47	7.1-29.77	18.38±3.97	6.28-25.97	14.3±3.69	*	***	*

* Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$; *** Significant at $p \leq 0.001$

Cluster 3 included sensitive genotypes i.e. EB14, D86, TJ, LSN, EB12, FKH, BKH and showed lowest levels of studied biochemical parameters among all T2 irrigated genotypes (Fig. 3a). Cluster 1 was comprised of genotypes which exhibited intermediate level of biochemical performance i.e. FSD, TTR, D93, EB13, EB10, N172, DRB, PIR, AAS, NRC, MIL, KRN, BRS, CKW and TD. Among these TTR, EB10, PIR, AAS and NRC were identified tolerant whereas D93, MIL, KRN and BRS were identified sensitive by PCA and HACA, however on biochemical basis these genotypes behaved like intermediate performing genotypes.

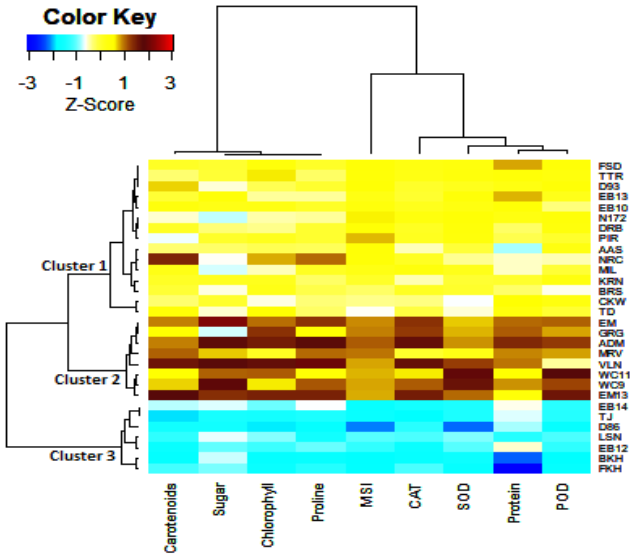

Figure 3a. Heatmap showing unsupervised two-way hierarchical clustering to associate textile irrigated genotypes with their corresponding biochemical attributes. Mean values of studied biochemical parameters of all genotypes were standardized by conversion to Z-scores before heatmap generation. The color scale (blue-cyan-white-yellow-brown-red) along with Z-score values reflect the relative levels of biochemical parameters: blue being lowest and red being highest. The rows represent the genotypes and the columns represent the biochemical parameters.

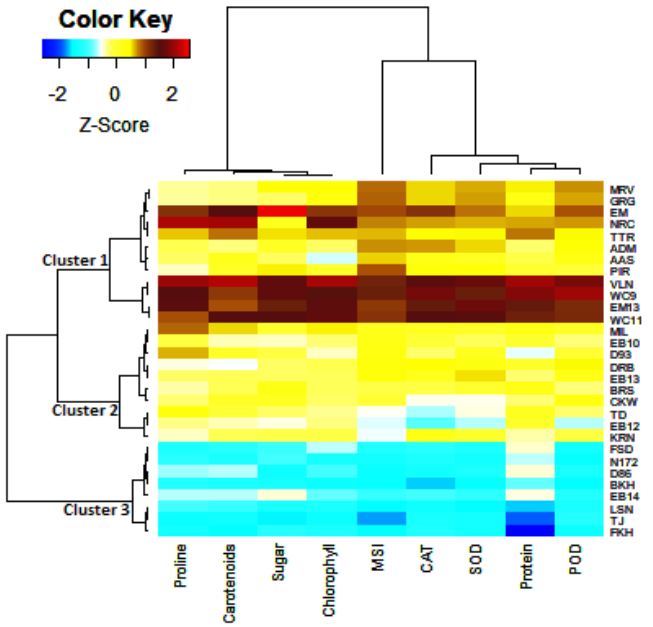

Figure 3b. Heatmap showing unsupervised two-way hierarchical clustering to associate iron-steel wastewater irrigated genotypes with their corresponding biochemical attributes. Mean values of studied biochemical parameters of all genotypes were standardized by conversion to Z-scores before heatmap generation. The color scale (blue-cyan-white-yellow-brown-red) along with Z-score values reflect the relative levels of biochemical parameters: blue being lowest and red being highest. The rows represent the genotypes and the columns represent the biochemical parameters.

Likewise, heatmap was generated to associate T3 irrigated genotypes with their corresponding biochemical traits which resulted in 3 clusters (Fig. 3b). Cluster 1 included genotypes which maintained relatively higher levels of i.e. photosynthetic pigments (chlorophyll and carotenoids), sugars, proline, MSI%, proteins and antioxidant enzymes (SOD, POD and CAT) than remaining genotypes and

included MRV, GRG, NRC, EM, TTR, ADM, AAS, PIR, VLN, WC9, WC11, EM13. These genotypes mostly matched with PCA and HACA identified tolerant genotypes. NRC, TTR and PIR were contained within intermediate performing genotypes in the previous section; however, with respect to their biochemical activity these were similar to tolerant genotypes. Cluster 3 included genotypes i.e. FSD, N172, D86, FKH, BKH, TJ, LSN, EB14 with lowest levels of biochemical constituents. These genotypes were same as sensitive genotypes identified through multivariate procedures. Cluster 2 included genotypes (MIL, D93, BRS, EB12, EB10, DRB, EB13, CKW, KRN) with intermediate levels of biochemical constituents (Fig. 3b). Among these genotypes i.e. EB10, DRB, EB13, CKW, KRN were grouped in the sensitive genotypes by PCA and HACA in previous section, anyhow their biochemical profiles were better than sensitive genotypes.

DISCUSSION

All measured physico-chemical parameters and HM contents in control (groundwater) were within irrigation standards (Ayers and Westcot, 1985; Rowe and Abdel-Magid, 1995; Alberta-Environment, 2000) which revealed its aptness for agricultural purposes. Textile and iron-steel industrial wastewater are characterized with high levels of TDS, EC, COD, BOD and heavy metals (Manzoor *et al.*, 2006; Bose and Bhattacharyya, 2008; Beh *et al.*, 2012). The pH, EC TDS, alkalinity, Cl^{1-} and SO_4^{2-} concentrations were within permissible irrigation standards whereas PO_4^{3-}, NO_3^{1-}-N, NH_3-N, COD, BOD, Fe, Co, Cu, Mn, Zn, Cd, Cr and Ni exceeded irrigation standards in both industrial wastewaters (Ayers and Westcot, 1985; Rowe and Abdel-Magid, 1995; Alberta-Environment, 2000). Iron-steel industrial wastewaters were found more polluted than textile wastewaters and showed higher metal loads. Wastewater irrigation treatments considerably increased soil metal contents, nutrients, EC and OM% as compared to the control (Ma *et al.*, 2015). Heavy metals i.e. Cu, Cd and Co in textile irrigated soils whereas Ni, Cr, Mn, Pb and Zn in iron-steel wastewater irrigated soils exceeded permissible soil standards (Alloway, 2013). Soil texture was predominantly loam and remained unaffected in irrigation treatments.

It was noted from supplementary Table S2 that genotypes, irrigation treatments and most of their interactions (except Cu, Co, Mg, Na, grain Cd, root and stem Zn & K) significantly affected the HM and macro-nutrient contents of wheat plants. Comparatively higher HM loads (i.e. Ni, Cr, Mn, Fe, Pb, Zn) in T3 wastewaters and recipient soils were recorded. Favorable soil conditions i.e. relatively acidic pH and low OM% resulted in the higher HM uptake and accumulation in T3 irrigated genotypes. Consequently, observed accumulation pattern for Ni, Cr, Mn, Fe, Pb and Zn was T3>T2>T1 in wheat genotypes. Among studied HM highest Fe accumulation was recorded in wheat roots, stem and grains which is consistent with the findings of Bose and Bhattacharyya (2008). Nickel alike Zn, Fe, Cu and Mn was fairly transported to the aerial parts in T2 and T3 irrigation treatments perhaps due to; a) its ability to cross root endodermis barrier and reach stellar tissues, b) facilitated translocation through metal chelators in xylem (Kabata-Pendias, 2011; Matraszek *et al.*, 2016). Higher accumulation of Cu, Fe, Mn and Zn among studied HM can be due to their importance as micro-nutrients besides their established toxic nature (Ficco *et al.*, 2009; Wang *et al.*, 2009). Among HM, Co was recorded in lowest concentration in grains of T2 and T3 possibly due to its strong affinities in roots coupled with low mobility restricting its aerial transport (Kabata-Pendias, 2011). Similarly, higher Pb contents in root tissues compared to aerial parts can be ascribed to its strong bonding with the carboxyl groups of glucouronic acid and galactouronic acid in carbohydrates of cell walls restricting apoplastic transport (Kabata-Pendias, 2011). It was worth noting that despite higher Cu, Cd and Co levels in T2 wastewater and corresponding soils, their uptake in respectively irrigated wheat genotypes was limited. This was possibly due to alkaline pH and relatively higher OM% (Bose and Bhattacharyya, 2008; Ali *et al.*, 2015; Ma *et al.*, 2015).

Stem HM contents in all genotypes from 3 irrigation treatments were 1.4 to many times less than corresponding HM levels in roots. Various researchers have shown similar HM accumulation pattern in wheat plants i.e. root>stem (Bose and Bhattacharyya, 2008; Kabata-Pendias, 2011; Bini *et al.*, 2013; Dalir and Khoshgoftarmanesh, 2014; Gramss and Voigt, 2016). Higher metal accumulation in wheat roots than other plant parts was due to large surface area in contact with irrigation wastewater and metal enriched soils (Wang *et al.*, 2009). Roots also acted as barrier and prevented metal transfer (either by retention or immobilization in the apoplast/symplast) to aerial parts (stems and grains) protecting them from hazardous HM effects (Dalir and Khoshgoftarmanesh, 2014; Shi *et al.*, 2015). Retention of higher HM contents in roots reflects wheat's internal detoxification mechanism (Liu *et al.*, 2009; Boussen *et al.*, 2013). Following roots, increased HM and macro-nutrient contents in stems were attributable to either; continuing root to stem translocation or remobilization from root reserves (Dalir and Khoshgoftarmanesh, 2014; Shi *et al.*, 2015).

Elevated soil metal supplies in T2 and T3 modified wheat grain metallome to lesser extent than the roots and stems due to disjointed xylem transport at the base of wheat grains (Gramss and Voigt, 2016). Therefore, grain HM levels were recorded far less than stem and roots in all genotypes from 3 irrigation treatments. All HM were mostly taken up in divalent form i.e. Cd^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Pb^{2+}, Ni^{2+} either through active (essential elements) or passive (non-essential elements) transport except Cr (Cr^{6+} and Cr^{3+}) (Kabata-Pendias, 2011; Matraszek *et al.*, 2016). Highest

concentration of K among macro-nutrients is due to the fact that it is a major inorganic essential univalent constituent in wheat cells responsible for osmotic adjustments and found higher in stems than roots (Rascio *et al.,* 2001; Ficco *et al.,* 2009). Our results indicating lowest Na levels among macro-nutrients were consistent with Subbarao et al. (2001). Calcium (Ca^{2+}) and magnesium (Mg^{2+}) were absorbed as divalent cations and together with K are regarded as important plant macro-nutrients, generally not considered harmful (Ali *et al.,* 2015). Increase in mean macro-nutrient levels (Ca^{2+}, Mg^{2+} in T3 and K^{1+}, Ca^{2+}, Na^{1+} in T2) coupled with increase in specific HM in studied genotypes in respective treatments exhibited macro-nutrient stabilizing and alleviating role for HM toxicity due to mutual competition for binding sites in soil particles, transporters/carriers in cell walls and cell membranes (Aziz *et al.,* 2015; Matraszek *et al.,* 2016).

Majority of the genotypes identified as tolerant, sensitive and intermediate performers by PCA in T2 and T3 treatments were confirmed by HACA results. Therefore, tolerant genotypes with reduced HM accumulation should be selected for cultivation in agricultural areas receiving textile and iron-steel industrial wastes and cultivation of sensitive genotypes must be discouraged. Tolerant genotypes showing minimum HM accumulation in their grains cannot only be used to improve HM resistance in wheat germplasm through breeding but also their cultivation in affected areas can reduce public health risks (Bermudez *et al.,* 2011; Alybayeva *et al.,* 2014).

It is well established that excess HM exposure decreases membrane stability, increases proteolytic activity, disrupts photosynthetic machinery and carbon metabolism (Ci *et al.,* 2009; Islam *et al.,* 2014; Aziz *et al.,* 2015; Hussain *et al.,* 2015). Proline mean levels were found highest in the T3 indicating elevated HM stress experienced by wheat genotypes in this treatment followed by T2 and T1. Higher proline accumulation in response to HM stress is via orinithine-ariginine or glutamate pathway. Decline in antioxidant enzyme activities in T3 could be linked to ROS (reactive oxygen species) induced damage to antioxidant system due to very high ROS levels and plant's inability to scavenge them (Ci *et al.,* 2009; Rady and Hemida, 2015). Lowered activities of antioxidant enzymes in T3 provided less protection against ROS leading to greater decline in photosynthetic pigments, membrane stability and osmolytes concentrations compared to T2. Among antioxidant enzymes, higher activities of SOD were recorded than POD and CAT which is regarded as first line of defense to ROS attack. SOD dismutates superoxide anion ($O_2^{\cdot-}$) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) whereas POD and CAT enzymes eliminate H_2O_2 (Gill and Tuteja, 2010; Li *et al.,* 2013).

Capability of T2 tolerant genotypes to accumulate higher levels of antioxidant enzymes (SOD, POD, CAT) reflected their ability to prevent oxidative damage induced by ROS ($O_2^{\cdot-}$, $RO^{\cdot}$, $OH^{\cdot}$, $HO_2^{\cdot}$, $^1O_2$, and H_2O_2) to membranes and macromolecules under HM stress (Fig. 3a) (Gill and Tuteja, 2010; Islam *et al.,* 2014). Similarly, higher proline accumulation in these genotypes played important role in their osmoregulation, metal chelation and ROS detoxification (Gill and Tuteja, 2010; Rady and Hemida, 2015). This resulted in relatively less decline in photosynthetic pigments, osmolytes concentrations and membrane stability in tolerant genotypes. Lower antioxidant enzyme activities and reduced proline levels exhibited by T2 sensitive genotypes were unable to provide them tolerance against deleterious effects of ROS on membranes and macromolecules (Fig. 3a) (Ci *et al.,* 2009; Hussain *et al.,* 2015). This led to chloroplast disorganization and inhibition of enzymes essential for biosynthesis of photosynthetic pigments (Aziz *et al.,* 2015; Matraszek *et al.,* 2016). Significant decrease in photosynthetic pigments reduced photosynthetic activity and lowered sugar contents. Also ROS negative impacts on carbon metabolism plays role in declining sugar contents of sensitive genotypes (Ci *et al.,* 2009; Rady and Hemida, 2015). Maximum decline in protein contents was probably due to oxidative damage induced by ROS which operated via increased breakup of peptide chains, site specific amino acid modifications, oxidation of susceptible residues, aggregation of cross-linked reaction products and changes in electrical charges etc. (Islam *et al.,* 2014). Cell membranes are the primary target of ROS induced HM damages which impair their function by altered permeability, higher solutes leakage and lipid peroxidation (Aziz *et al.,* 2015). Higher decrease in MSI% values in sensitive genotypes can be associated with increased ROS activity (Hussain *et al.,* 2015).

Capability to accumulate higher proline and antioxidant enzyme levels in T3 irrigated tolerant genotypes in response to HM stresses were correlated with their high degree of metal tolerance (Fig. 3b) (Li *et al.,* 2013). It is obvious form the results that tolerant genotypes showed tendency to accumulate higher levels of proline, sugars, proteins and antioxidant enzymes which enabled them to resist the toxic HM effects, maintain membrane integrity, protect photosynthetic activity centers and escape HM stresses. From biochemical perspective sensitive genotypes in T2 and T3 behaved contradictory to the tolerant ones (Nagar *et al.,* 2015). With few exceptions the levels and expression of biochemical constituents largely confirmed the tolerant, intermediate or sensitive nature of studied genotypes (Dong *et al.,* 2002; Ci *et al.,* 2009). Hence it is imperative to state that these biochemical constituents can be used as important biomarkers of HM stresses in wheat genotypes.

***Conclusions*:** In present study, tolerant, intermediate and sensitive wheat genotypes were identified from textile and iron-steel industrial wastewater irrigation treatments. Tolerant genotypes exhibited minimum HM accumulation compared to intermediate and sensitive genotypes in T2 and T3. Efficient enzymatic antioxidants (i.e. SOD, POD and

CAT) and elevated proline levels in tolerant genotypes swiftly removed ROS generated under HM stresses which reduced damage to photosynthetic pigments (chlorophyll and carotenoids), membranes (MSI%), proteins and sugars as per findings of this study. Therefore, the constituents of antioxidant system i.e. SOD, POD, CAT and proline, can be regarded as potential biomarkers of HM toxicity in studied wheat genotypes. Cultivation of identified tolerant genotypes in agricultural ecologies receiving textile and iron-steel industrial wastes can effectively minimize potential HM related health hazards to humans. Further these tolerant genotypes can be used in development of advanced metal tolerant wheat germplasm to combat metal stresses emanating from industrial wastewaters or soils receiving similar chemistry of wastes (Alybayeva *et al.,* 2014). Sensitive genotypes showed higher HM accumulation in T2 and T3 with lower levels of biochemical constituents. Hence, identified sensitive genotypes from T2 and T3 should not be cultivated in areas receiving similar type of HM wastes. On the contrary, higher mineral efficiency of sensitive genotypes can be exploited in meeting essential micro-nutrients requirements for humans in soils with low levels of soil mineral contents i.e. Cu, Zn, Mn and Fe.

REFERENCES

Aebi, H. 1984. Catalase *in vitro*. Method. Enzymol. 105:121-126.

Alberta-Environment. 2000. Guidelines for municipal wastewater irrigation, Municipal Program Development Branch, Environmental Sciences Division, Environmental Service. Alberta, Canada.

Ali, Z., B. Ali, A. Mohammad, M. Ahmad, I. Ahmad, A. Napar, A. Kazi, A. Ali, S. Shah and A. Mujeeb-Kazi. 2013. Combating water scarcity for global food security. In: Amir Raza (ed.), Agricultural Systems in the 21st Century. Nova Science Publishers, Hauppauge, NY, USA; pp.1-30.

Ali, Z., R.N. Malik, Z.K. Shinwari and A. Qadir. 2015. Enrichment, risk assessment, and statistical apportionment of heavy metals in tannery-affected areas. Int. J. Environ. Sci. Technol. 12:537-550.

Ali, Z., R.N. Malik, A. Gul and A. Mujeeb-Kazi. 2015. Taming food security through wastewater irrigation practices. In: Munir Ozturk (ed.), Plants, Pollutants and Remediation, Springer Publishing, Netherlands; pp.111-136.

Allen, S., H. Grimshaw and A. Rowland. 1986. Chemical analysis. In: P.D. Moore and S.B. Chapman (eds.), Methods in Plant Ecology. Blackwell Scientific Publications, Oxford, UK; pp.285-344.

Alloway, B.J. 2013. Bioavailability of elements in soil. In: Selinus O (ed.), Essentials of Medical Geology, Springer Publishing, Netherlands; pp.351-373.

Alybayeva, R., S. Kenzhebayeva and S. Atabayeva. 2014. Resistance of winter wheat genotypes to heavy metals. IERI Procedia 8:41-45.

APHA. 2005. Standard methods for the examination of water and wastewater, 21st Ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.

Ayers, R.S. and D.W. Westcot. 1985. Water quality for agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy.

Aziz, H., M. Sabir, H.R. Ahmad, T. Aziz, M. Zia-ur-Rehman, K.R. Hakeem and M. Ozturk. 2015. Alleviating effect of calcium on nickel toxicity in rice. CLEAN–Soil, Air, Water 43:901-909.

Bates, L., R. Waldren and I. Teare. 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39:205-207.

Beh, C., T. Chuah, M. Nourouzi and T. Choong. 2012. Removal of heavy metals from steel making waste water by using electric arc furnace slag. J. Chem. 9:2557-2564.

Bermudez, G.M., R. Jasan, R. Plá and M.L. Pignata. 2011. Heavy metal and trace element concentrations in wheat grains: Assessment of potential non-carcinogenic health hazard through their consumption. J. Hazard. Mater. 193:264-271.

Bini, C., S. Fontana and M. Spiandorello. 2013. Towards food safety. Potentially harmful elements (PHE's) fluxes from soil to food crops. EQA-Int. J. Environ. Qual. 10:23-36.

Bose, S. and A. Bhattacharyya. 2008. Heavy metal accumulation in wheat plant grown in soil amended with industrial sludge. Chemosphere 70:1264-1272.

Boussen, S., M. Soubrand, H. Bril, K. Ouerfelli and S. Abdeljaouad. 2013. Transfer of lead, zinc and cadmium from mine tailings to wheat (*Triticum aestivum*) in carbonated mediterranean (northern Tunisia) soils. Geoderma 192:227-236.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Ci, D., D. Jiang, T. Dai, Q. Jing and W. Cao. 2009. Effects of cadmium on plant growth and physiological traits in contrast wheat recombinant inbred lines differing in cadmium tolerance. Chemosphere 77:1620-1625.

Dalir, N. and A.H. Khoshgoftarmanesh. 2014. Symplastic and apoplastic uptake and root to shoot translocation of nickel in wheat as affected by exogenous amino acids. J. Plant Physiol. 171:531-536.

Dong, B., W. Sang, X. Jiang, J. Zhou, F. Kong, W. Hu and L. Wang. 2002. Effects of aluminum on physiological

metabolism and antioxidant system of wheat (*Triticum aestivum* L.). Chemosphere 47:87-92.

Dubois, M., K. Gilles, J. Hamilton, P. Rebers and F. Smith. 1951. A colorimetric method for the determination of sugars. Nature 168:167-167.

Estefan, G., R. Sommer and J. Ryan. 2014. Analytical methods for soil-plant and water in the dry areas. A manual of relevance to the west asia and north africa region, (3rd edition). International Center for Agricultural Research in the Dry Areas, Aleppo, Syria.

Ficco, D., C. Riefolo, G. Nicastro, V. De Simone, A. Di Gesu, R. Beleggia, C. Platani, L. Cattivelli and P.De Vita. 2009. Phytate and mineral elements concentration in a collection of italian durum wheat cultivars. Field Crops Res. 111:235-242.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases. I. Occurrence in higher plants. Plant Physiol. 59:309-314.

Gill, S.S. and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48:909-930.

Gorin, N. and F.T. Heidema. 1976. Peroxidase activity in golden delicious apples as a possible parameter of ripening and senescence. J. Agric. Food Chem. 24:200-201.

Gramss, G. and K.D. Voigt. 2016. Stability of the inherent target metallome in seed crops and a mushroom grown on soils of extreme mineral spans. Agronomy 6:14.

Hiscox, J.T. and G. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57:1332-1334.

Hussain, I., M.A. Ashraf, R. Rasheed, A. Asghar, M.A. Sajid and M. Iqbal. 2015. Exogenous application of silicon at the boot stage decreases accumulation of cadmium in wheat (*Triticum aestivum* L.) grains. Braz. J. Bot. 38:223-234.

Iqbal, M., I. Hussain, H. Liaqat, M.A. Ashraf, R. Rasheed and A.U. Rehman. 2015. Exogenously applied selenium reduces oxidative stress and induces heat tolerance in spring wheat. Plant Physiol. Biochem. 94:95-103.

Islam, F., T. Yasmeen, Q. Ali, S. Ali, M.S. Arif, S. Hussain and H. Rizvi. 2014. Influence of pseudomonas aeruginosa as pgpr on oxidative stress tolerance in wheat under Zn stress. Ecotox. Environ. Safe. 104:285-293.

Kabata-Pendias, A. 2011. Trace elements in soils and plants, 4t Ed. CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA.

Li, X., Y. Yang, L. Jia, H. Chen and X. Wei. 2013. Zinc-induced oxidative damage, antioxidant enzyme response and proline metabolism in roots and leaves of wheat plants. Ecotox. Environ. Safe. 89:150-157.

Lichtenthaler, H.K. and A.R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11:591-592.

Liu, W.X., J.W. Liu, M.Z. Wu, Y. Li, Y. Zhao and S.R. Li. 2009. Accumulation and translocation of toxic heavy metals in winter wheat (*Triticum aestivum* L.) growing in agricultural soil of Zhengzhou, China. Bull. Environ. Contam. Toxicol. 82:343-347.

Ma, S.C., H.B. Zhang, S.T. Ma, R. Wang, G.X. Wang, Y. Shao and C.X. Li. 2015. Effects of mine wastewater irrigation on activities of soil enzymes and physiological properties, heavy metal uptake and grain yield in winter wheat. Ecotox. Environ. Safe. 113:483-490.

Manzoor, S., M.H. Shah, N. Shaheen, A. Khalique and M. Jaffar. 2006. Multivariate analysis of trace metals in textile effluents in relation to soil and groundwater. J. Hazard. Mater. 137:31-37.

Matraszek, R., B. Hawrylak-Nowak, S. Chwil and M. Chwil. 2016. Macronutrient composition of nickel-treated wheat under different sulfur concentrations in the nutrient solution. Environ. Sci. Pollut. Res. 23:5902-5914.

Nagar, S., V. Singh, A. Arora, R. Dhakar and S. Ramakrishnan. 2015. Assessment of terminal heat tolerance ability of wheat genotypes based on physiological traits using multivariate analysis. Acta Physiol. Plant. 37:257.

Pask, A., J. Pietragalla, D. Mullan and M. Reynolds. 2012. Physiological breeding II: A field guide to wheat phenotyping. CIMMYT, Mexico.

Rady, M.M. and K.A. Hemida. 2015. Modulation of cadmium toxicity and enhancing cadmium-tolerance in wheat seedlings by exogenous application of polyamines. Ecotox. Environ. Safe. 119:178-185.

Rascio, A., M. Russo, L. Mazzucco, C. Platani, G. Nicastro and N. Di Fonzo. 2001. Enhanced osmotolerance of a wheat mutant selected for potassium accumulation. Plant Sci. 160:441-448.

Rizwan, M., S. Ali, T. Abbas, M. Zia-ur-Rehman, F. Hannan, C. Keller, M.I. Al-Wabel and Y.S. OK. 2016. Cadmium minimization in wheat: A critical review. Ecotox. Environ. Safe. 130:43-53.

Rowe, D.R. and I.M. Abdel-Magid. 1995. Handbook of wastewater reclamation and reuse. CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA.

Schnurbusch, T., J. Hayes and T. Sutton. 2010. Boron toxicity tolerance in wheat and barley: Australian perspectives. Breed Sci. 60:297-304.

Shi, G.L., S. Zhu, S.N. Bai, Y. Xia, L.Q. Lou and Q.S. Cai. 2015. The transportation and accumulation of arsenic, cadmium, and phosphorus in 12 wheat cultivars and their relationships with each other. J. Hazard. Mater. 299:94-102.

Soltanpour, P.A. and A. Schwab. 1977. A new soil test for simultaneous extraction of macro-and micro-nutrients in alkaline soils. Commun. Soil Sci. Plant Anal. 8:195-207.

Subbarao, G., G.W. Stutte, R.M. Wheeler and W.L. Berry. 2001. Sodium: A functional nutrient in plants. Handbook of plant and crop physiology. Marcel Dekker, New York; pp.363-384.

Walkley, A. 1947. A critical examination of a rapid method for determining organic carbon in soils-effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci. 63:251-264.

Wang, S., Z. Nan, X. Liu, Y. Li, S. Qin and H. Ding. 2009. Accumulation and bioavailability of copper and nickel in wheat plants grown in contaminated soils from the oasis, Northwest China. Geoderma 152:290-295.