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ORIGINAL RESEARCH ARTICLE

Polyamines in relation to metal concentration, distribution, relative water content and abscisic acid in wheat plants irrigated with waste water heavily polluted with heavy metals

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Abstract: Present study was carried out in order to investigate the effect of grain presoaking in spermine (0.15 mM), spermidine (0.3 mM) and their interaction on growth vigor, metal distribution as well as leaf turgidity and abscisic acid in wheat plants. Waste water at concentrations 25%, 50% and 100% caused noticeable decreases in growth vigor of root and shoot, leaf area, relative water content and water use efficiency. On the other hand, waste water stress caused remarkable increases in heavy metals and saturation water deficit as well as abscisic acid content of flag leaf. Exogenous application of Spm, Spd or their interaction could counteract the adverse effects of heavy metals in waste water by improving growth vigor of root and shoot, water use efficiency, retention of leaf turgidity and decreasing abscisic acid in leaves and grains. Furthermore, these polyamines reduced heavy metals translocation from root to leaves till reach to grains.

Key words: Abscisic Acid; Growth Vigor; Heavy Metal; Relative Water Content; Spermidine; Spermine; Wheat.

Introduction

The use of waste water for irrigation may serve as an additional source of water with fertilizing properties after appropriate dilution. Irrigation water quality not only affects the growth of crops, but also has long term effects on soil health, grain quality, fodder quality and health of consumers [1]. The waste waters of (paper, automobile, textile and food industry mills) were alkaline in nature with variable concentrations of different chemical species. Application of these untreated effluents altered the physicochemical properties of the soil and rate of seed germination in plants [2].

Waste water carry appreciable amount of toxic heavy metals [3] and concentrations of heavy metals in waste waters vary from city to city [4]. Important sources of heavy metals in waste water are urban and industrial effluents. Heavy metals are extremely persistent in the environment and accumulate to toxic levels [5]. High concentrations of heavy metals affect mobilization and balanced distribution of the elements in plant organs via the competitive uptake [6].

Heavy metals contamination of soil is one of the major environmental stresses that affect plant metabolism, and their toxic levels in soils are the result of heavy traffic, mining, industrial agricultural activity, smelting of metalliferous ores and electroplating [7].

Some heavy metals play a role in plant metabolism, and can be considered nutrients as in case of manganese (Mn), zinc (Zn) and nickel (Ni), which are involved in major functions [8]. Welch [9] reported that, manganese has been shown to play a role in enzyme activation, biological redox systems (e.g. electron transport reactions in photosynthesis) or detoxification of oxygen free radicals; while Zn is involved in membrane integrity, enzyme activation and gene expression. Nickel is needed for urea metabolism, iron absorption and nitrogen (N) fixation.

The primary distribution of heavy metals via the xylem, the re-translocation via the phloem, and the transfer from the xylem to the phloem must be considered as important processes for the distribution of elements within the plant. The transport in the xylem is directed from the roots to shoot, whereas the phloem transport takes place from sources to sinks and is more selective [10]. Xylem-to-phloem transfer can take place all along the pathway from the roots to the shoots [11]. Plants may play a role in the redistribution of heavy metals in the environment. Heavy metals present in the atmosphere may be deposited on the surface of the plants and enter the shoot. They may either remain in the leaves and be released on the surface of the soil when the leaves fall or transported from the shoot to the root system via the phloem. Additionally, heavy metals in the roots may be released from living roots into the soil or remain in the soil after the death of the roots. Moreover, Zn, Cd, Mn and Co was released from the root system of wheat and lupin plants into the rhizosphere and bulk soils [12].

Wheat is one of several crops that tend to accumulate relatively high concentrations of heavy metals specially cadmium in plant tissues when grown in soils that contain elevated levels of that toxic metal. Because cadmium (Cd) represents a potential health threat to consumers, international

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trade organizations have sought to limit the acceptable concentration of Cd in edible crops sold in international markets. In this respect, Jonathan *et al.,* [13] has proposed maximum levels of 0.2 mg Cd/kg for wheat grain. A high Cd level in the wheat grains may be caused by a high Cd uptake in the roots, a high translocation of Cd from roots to shoot, and/or a high translocation of Cd within the shoot to the grains [14]. After taken up, Cd is partly accumulated in the roots and partly translocated into the xylem and further to the shoot. Thus Cd that is not bound or incorporated into cell constituents may be stored in root-cell vacuoles [15], or transported to shoots in the transpiration stream of the xylem [16]. Movement of Cd into developing grains occurs via phloem, probably by re-translocation from leaves and stems [11].

High concentrations of heavy metals in waste water affect mobilization and balanced distribution of the fundamental elements in plant organs via the competitive uptake [6]. Generally, disturbance in plant nutrition may occur when absorption and accumulation of heavy metals increase in soil and plant [17]. The concentrations of Cu, Zn, Cd, Pb, Ni and Cr in root and shoots of *Beta vulgaris* plants showed significant and strong positive relationships with concentrations of fly ash that contains large amounts of heavy metals [18].

Polyamines regulate the voltage-dependent inward K^+ channel in the plasma membrane of guard cells and modulate stomatal movement [19]. In this connection, treatment of wheat plants with exogenous Spd alleviated the osmotic injury, as judged by increase in RWC of wheat leaves [20]. Polyamines may prevent the membrane system from denaturing under stress conditions and consequently improving ion balance [21]. Moreover, polyamines increased water uptake by root as well as the transpiration and consequently increases the uptake and translocation of K^+, Na^+ and Ca^{++} contents which were driven by transpiration [22].

This work was under taken to evaluate the effect of grain priming in spermine or spermidine or their interaction on improving growth vigor of root and shoot, leaf turgidity, ABA, water use efficiency as well as heavy metals distribution between root, shoot and grains.

Materials and Methods

Plant material and growth conditions

Homogeneous lot of wheat grains (*Triticum aestivum)* variety Sakha 94 were surface sterilized by soaking in 0.001M $HgCl_2$ solution for 3 minutes, then washed thoroughly with distilled water, and divided into four sets which soaked in distilled water to serve as control, spermine (0.15 mM), spermidine (0.3mM) or (spermine 0.15mM + spermidine 0.3mM) respectively for about six hours. After soaking, the thoroughly washed grains were planted on 15th November 2011 in plastic pots (25 cm width x 30 cm height) filled with 6 kg mixture of soil (clay and sand = 2:1, v/v). The pots were kept in greenhouse, where the plants subjected to natural day / night conditions (minimum / maximum temperature and relative humidity were: 29.2 / 33.2 ºC and 63 / 68 % respectively, at mid-day) during the experimental period. The plants in all sets were irrigated to field capacity by normal tap water. Fifteen days after planting, the plants were thinned to five / pot.

On day 21 from sowing, the pots of each set were subdivided into four groups. The pots of the first group (each group 20 pots) in each set still irrigated with tap water, while 2nd, 3rd and 4th groups in all sets were irrigated with 25%, 50% or 100% waste water respectively.

Analysis of irrigation water revealed that, the standard fresh water contained [chemical oxygen demand (COD) = 5.0, biochemical oxygen demand (BOD) = 2.0, total suspended solids (TSS) = 4.0, total hardness = 60.0, Cd^{++} = 0.05, Pb^{++} = 0.05, Cu^{++} = 0.04, Ni^{++} = 0.07, Zn^{++} = 0.08, Na^+ = 0.02, K^+ = 0.01, Ca^{++} = 0.01, total phosphorus = 0.07, Cl^- = 45.0, SO^{--}_4 = 00.0, NO_3^- = 0.01, NO_2^- = 0.002 ppm] while the untreated waste water contained [COD = 150.0, BOD = 60.0, total suspended solids (TSS) = 226.0, total hardness = 770.0, Cd^{++} = 0.12, Pb^{++} = 0.23, Cu^{++} = 0.12, Ni^{++} = 0.20, Zn^{++} = 0.93, Na^+ = 0.22, K^+ = 0.14, Ca^{++} = 0.19, total phosphorus = 0.38, Cl^- = 283.6, SO_4^- = 72.0, NO_3^- = 50.0, NO_2^- = 7.3 ppm]. These analyses were carried out according to Clescrei *et al.,* [23]. The main source of untreated waste water was the main Aga drain in Dakahliya Province, Egypt.

At tillering stage (i.e. 21 days from planting) and at heading (65 days from planting), the plants received 35 kg N / ha as urea and 35 kg P / ha as potassium dihydrogen phosphate as a fertilizer.

The resulting sixteen treatments were marked as follows: -

Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
WW%	0	25	50	100	0	25	50	100	0	25	50	100	0	25	50	100
Spm	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Spd	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Spm + Spd	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+

WW=waste water; Spm=spermine; Spd=spermidine

In this study, samples for growth and chemical analyses were taken at heading (65 days from planting) and anthesis stages (80 days from planting) of wheat plants. Ten replicates were used for measuring growth criteria and triplicates for measuring relative water content, water use efficiency, abscisic acid and heavy metals, (Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++} & Zn^{++}).

Determination of water use efficiency

Water use efficiency (WUE) was calculated by dividing the grain yield (t ha^{-1}) or the biomass yield (t ha^{-1}) by the amount of water added by (gallons). Therefore, water use efficiency for grain yield (WUE_G) was calculated from the grain yield and water use efficiency for biomass yield (WUE_B) was estimated from the biomass yield [24].

$$(WUE_G) = \text{Grain yield (t)} / \text{Total water used (gallon)}$$

$$(WUE_B) = \text{Biomass yield (t)} / \text{Total water used (gallon)}$$

Measurements of relative water content

In measuring relative water content, the method of Weatherly [25] and its modification by Weatherly and Barrs [26] was adopted.

Estimation of Saturation Water Deficit (SWD)

Saturation water deficit (SWD) was calculated from the following equation:

$$SWD = 100 - RWC$$

Measurement of abscisic acid (ABA)

The initial procedure involved preparing lyophilized plant tissues (0.3 g leaf) which were crushed in liquid nitrogen using a mortar and a pestle and extracted with 3 ml acetone- water-acetic acid (80:19:1, v/v). The extracts were transferred to 2-ml tubes and after – centrifugation at 1300 rpm for 2 min, the supernatant was collected and residues were re-extracted with 3ml extraction. The dried sample was reconstituted in 200 ml acetonitrile– water (15:85, v/v) containing 12mM acetic acid (pH3.3). A portion (110 ml) of the sample was loaded onto a HP 1100 Series HPLC system equipped with a 100 × 2.1 mm, 5 mm SB-C 18 LC--rate of 0.2ml/min and a binary solvent system comprising MS column using a flow 12mM acetic acid in water (A) and 12mM acetic acid in Acetonitrile-Water (90:10, v/v) (B). Typically, the solvent gradient was programmed to change linearly from 15% B to 33% B over the first 10min and then to 100% B over the next 6.7 min before returning to the initial composition at 22 min. For a 10-ml injection of sample prepared with 20 ng internal standard and reconstituted in 200 ml initial mobile phase, the limit of detection (LOD) and limit of quantification (LOQ) were calculated from calibration curves and samples using the Quantify module of Mass Lynx version 3.5 software [27].

Estimation of heavy metals

Cadmium, Pb^{++}, Cu^{++}, Ni^{++} and Zn^{++} cations were determined by the Atomic Absorption Spectrophotometry (BHF 80B biologie spectrophotometer). The samples were diluted with $LiCl_3$ to suppress the interference of Na^+, K^+ and Ca^{++}.

Table 1: Effect of spermine, spermidine and their interaction on growth vigor of root of wheat plants (at heading and anthesis stages) irrigated with different concentrations of waste water.

Parameters	Growth vigor of root							
	Root length (cm)		Root Fresh mass (g)		Root dry mass (g)		Root /shoot ratio	
Treatments	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis
Cont.	12.96	14.52	0.69	0.80	0.36	0.43	0.14	0.13
WW 25%	13.36	13.98	0.71	0.76	0.44	0.53	0.20	0.17
WW 50%	12.42	13.19	0.66	0.69	0.53	0.68	0.26	0.28
WW 100%	11.20	12.02	0.58	0.67	0.64	0.88	0.37	0.31
Spm	16.20	18.20	1.15	1.36	0.38	0.46	0.12	0.12
Spm+WW 25%	16.00	17.28	0.92	1.19	0.42	0.50	0.14	0.14
Spm+WW 50%	15.00	15.92	0.82	0.93	0.46	0.58	0.17	0.17
Spm+WW 100%	13.44	14.08	0.70	0.77	0.52	0.65	0.22	0.20
Spd	15.93	17.00	1.05	1.26	0.39	0.49	0.12	0.13
Spd+WW 25%	13.60	14.80	0.84	1.09	0.44	0.58	0.15	0.17
Spd+WW 50%	12.64	14.22	0.80	0.86	0.49	0.68	0.20	0.21
Spd+WW 100%	11.85	13.40	0.66	0.76	0.54	0.75	0.23	0.26
Spm+Spd	16.60	19.12	1.22	1.46	0.37	0.44	0.10	0.11
Spm+Spd+WW 25%	16.72	18.20	1.11	1.27	0.40	0.47	0.12	0.12
Spm+Spd +WW 50%	15.33	16.04	0.82	0.96	0.43	0.51	0.15	0.15
Spm+Spd+WW 100%	14.20	14.66	0.74	0.78	0.48	0.59	0.18	0.19
LSD at $P \leq 0.05$	2.56	1.82	0.08	0.15	0.06	0.06	0.01	0.04

Statistical analysis

The main effect of factors (heavy metals and both used polyamines), and the interaction (heavy metals X polyamines) were evaluated by general linear model (two ways ANOVA) using SPSS program. Tests for significant differences between means at P = 0.05 were given by LSD test [28].

Results

Changes in growth criteria

Changes in growth vigor of root: The pattern of results in table 1 showed that, all examined concentrations of waste water decreased both root length and root fresh mass at heading and anthesis stages as compared to the control value except at

25% of waste water during heading stage, where the two abovementioned parameters were non-significantly increased. The root length and root fresh mass of treated plants were in general, stimulated by Spm, Spd or Spm+Spd treatments. The magnitude of increase was more pronounced with Spm+Spd pretreatment.

All the examined concentrations of waste water (25%, 50% and 100%) induced massive increase ($P \leq 0.05$) in root dry mass at heading and anthesis stages comparing with the control plants. In relation to control values of wheat plants, generally, grain priming with Spm, Spd or their interaction led to marked increase in root dry mass of wheat plants at heading and anthesis stages. These increases were lower than that obtained with waste water treated plants alone (Table 1). Moreover, waste water at all the used levels increased ($P \leq 0.05$) root / shoot ratio during the heading and anthesis stages as compared to the untreated control plants (Table 2). In the majority of cases, the application of the two used chemicals and their interaction caused increases in this ratio particularly at higher concentrations of waste water during the heading and anthesis stages (Table 2).

Table 2: Effect of spermine, spermidine and their interaction on growth vigor of shoot of wheat plants (at heading and anthesis stages) irrigated with different concentrations of waste water.

Parameters / Treatments	Growth vigor of shoot							
	Plant height (cm)		Shoot fresh mass (g)		Shoot dry mass (g)		Flag leaf area (cm)^2	
	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis
Cont.	73.04	76.90	5.85	7.34	2.60	3.33	12.99	14.08
WW 25%	71.22	73.52	5.37	6.46	2.26	3.18	12.10	12.80
WW 50%	64.25	67.74	5.05	6.04	2.04	3.13	10.44	11.49
WW 100%	61.70	63.36	3.55	5.40	1.72	2.88	9.26	10.16
Spm	78.92	82.91	8.42	9.75	3.34	3.97	16.63	21.89
Spm+WW 25%	75.46	79.55	7.44	9.22	3.07	3.69	14.52	18.46
Spm+WW 50%	71.65	77.27	7.08	8.27	2.73	3.38	13.12	13.62
Spm+WW 100%	68.73	72.38	5.74	7.48	2.41	3.27	10.54	11.94
Spd	75.72	79.11	7.33	8.41	3.21	3.72	16.93	19.88
Spd+WW 25%	72.33	78.90	6.22	8.15	2.90	3.36	14.22	15.94
Spd+WW 50%	70.51	75.16	6.00	7.50	2.44	3.22	11.78	13.71
Spd+WW 100%	68.76	72.24	4.69	6.61	2.29	2.87	10.52	11.63
Spm+Spd	81.94	86.19	8.85	11.14	3.86	4.04	18.50	23.72
Spm+Spd+WW 25%	78.76	82.69	7.93	10.71	3.46	3.82	15.68	20.23
Spm+Spd +WW 50%	74.60	78.44	6.72	8.88	2.97	3.34	13.31	16.27
Spm+Spd+WW 100%	70.83	73.23	5.71	7.57	2.64	3.02	11.71	13.36
LSD at $P \leq 0.05$	2.21	6.58	0.25	0.27	0.08	0.14	2.72	7.83

Changes in growth vigor of shoot

The data presented in table 2 showed that, waste water at all examined concentrations caused progressive decreases in the growth vigor of shoot (i.e. plant height, shoot fresh and dry masses as well as flag leaf area) from heading to anthesis. In general, waste water caused noticeable decreases ($P \leq 0.05$) in the shoot vigor of wheat plants at heading and anthesis stages. On the other hand, application of spermine, spermidine or their interaction induced increases in the all shoot growth vigor of wheat plants at heading and anthesis.

Changes in relative water content (RWC %)

The data presented in table 3 clearly showed that, all examined concentrations of waste water led to significant decreases ($P \leq 0.05$) in RWC % of wheat flag leaf at heading stage as compared to the control ones. In the majority of cases, the application of Spm, Spd and their interaction increased ($P \leq 0.05$) RWC% particularly at lower concentrations (25% and 50%) of waste water during heading stage as compared to the value detected in control plants.

Table 3: Effect of spermine, spermidine and their interaction on relative water content and saturation water deficit in flag leaf of wheat plants (at heading stage) irrigated with different concentrations of waste water.

Parameters / Treatments	RWC%	SWD%
Cont.	84.66	15.34
WW 25%	80.49	19.51
WW 50%	77.45	22.55
WW 100%	72.13	27.87
Spm	89.24	10.76
Spm+WW 25%	86.52	13.48
Spm+WW 50%	81.69	18.31
Spm+WW 100%	76.85	23.15
Spd	85.96	14.04
Spd+WW 25%	81.22	18.78
Spd+WW 50%	78.68	21.32
Spd+WW 100%	74.81	25.19
Spm+Spd	93.11	6.89
Spm+Spd+WW 25%	88.75	11.25
Spm+Spd +WW 50%	83.47	16.53
Spm+Spd+WW 100%	79.76	20.24
LSD at $P \leq 0.05$	1.85	0.98

Changes in ABA level in flag leaf and grain

Changes in saturation water deficit (SWD %)

In general, all examined doses of waste water increased significantly saturation water deficit (SWD %) in flag leaves of wheat plants at heading stage. In the majority of cases, Spm or Spd and their interaction led to significant decreases in SWD% especially at lower concentrations (25% and 50%) of waste water during heading stage as compared to the control value (Table 3).

The results indicated that, waste water at all the examined concentrations (25%, 50% and 100%) caused a significant increase ($P \leq 0.05$) in ABA level in the flag leaves at heading stage comparing with the control ones (Table 4). As compared with waste water treated plants, treatment with Spm, Spd and their interaction caused a reduction ($P \leq 0.05$) in the ABA level of wheat flag leaf during this stage. The reduction was more pronounced with Spm+Spd pretreatment in wheat plants. Irrigation of wheat plants with waste water at all doses induced noticeable increases ($P \leq 0.05$) in ABA content of yielded grains (Table 4).

Table 4: Effect of spermine, spermidine and their interaction on ABA (µg g^{-1} fresh wt) level in flag leaf (at heading stage) and developing grains of wheat plants irrigated with different concentrations of waste water.

Parameters Treatments	ABA (Leaf)	ABA (Grains)
Cont.	1.07	1.44
WW 25%	1.47	2.02
WW 50%	2.08	2.72
WW 100%	2.77	4.51
Spm	0.82	1.16
Spm+WW 25%	0.98	1.42
Spm+WW 50%	1.33	2.16
Spm+WW 100%	1.54	2.57
Spd	0.83	1.21
Spd+WW 25%	1.09	1.5 7
Spd+WW 50%	1.36	2.42
Spd+WW 100%	1.74	2.85
Spm+Spd	0.79	0.8 7
Spm+Spd+WW 25%	0.88	1.25
Spm+Spd +WW 50%	1.14	1.70
Spm+Spd+WW 100%	1.43	2.10
LSD at $P \leq 0.05$	0.016	0.070

In general, Spm, Spd or their interaction seemed to alleviate the effect of waste water by decreasing the inhibitors (i.e. ABA) in yielded grains of wheat plants as compared to the values detected with those in waste water– treated wheat plants alone.

Changes in water use efficiency (WUE)

It is clear from the results in table 5 that, the values of WUE_G and WUE_B in the waste water-treated-wheat plants were significantly lower than that of the control ones. Application of Spm, Spd or their interaction clearly improved WUE_G and WUE_B values in stressed wheat plants. In addition, treatments with Spm+Spd gave highest WUE_G and WUE_B values than the other treatments.

Changes in the content of heavy metals in root, flag leaf and grain

In relation to control values, the content of heavy metals (Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++} and Zn^{++}) in the roots of wheat plants at heading and anthesis stages showed high increases ($P \leq 0.05$) with the increase in concentrations of waste water used (Table 6). In the majority of cases, grain priming with the used polyamines and their interaction caused drastic decrease in the heavy metals contents of wheat roots at all the used concentrations of waste water during heading and anthesis stages as compared with the corresponding values of waste water-treated plants alone.

Table 5: Effect of spermine, spermidine and their interaction on water use efficiency of wheat plants irrigated with different concentrations of waste water.

Parameters Treatments	WUE_G	WUE_B
Cont.	8.94	19.58
WW 25%	8.25	18.29
WW 50%	7.84	16.68
WW 100%	6.11	15.91
Spm	10.23	20.14
Spm+WW 25%	9.57	19.56
Spm+WW 50%	8.28	17.86
Spm+WW 100%	6.92	16.97
Spd	9.88	19.66
Spd+WW 25%	9.14	18.87
Spd+WW 50%	7.69	17.27
Spd+WW 100%	6.67	16.69
Spm+Spd	11.17	22.17
Spm+Spd+WW 25%	10.34	21.68
Spm+Spd +WW 50%	8.76	19.49
Spm+Spd+WW 100%	7.53	17.73
LSD at $P \leq 0.05$	0.54	0.42

As expected and in comparing to control values, the content of measured heavy metals (Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++} and Zn^{++}) in wheat flag leaf at heading and anthesis stages showed progressive increases ($P \leq 0.05$) with the increase in concentrations of waste water used (Table 7). In the majority of cases, grain presoaking in the used polyamines and their interaction caused decline in the heavy metals contents of flag leaf at all examined concentrations of waste water during heading and anthesis stages as compared with the corresponding values of waste water-treated plants alone.

Compared with control value, waste water at all examined concentrations caused significant increases ($P \leq 0.05$) in heavy metals (Cd^{++}, Zn^{++}, Cu^{++}, Pb^{++} and Ni^{++}) content in yielded grains with increase in concentrations of waste water (Table 8). In the majority of cases, grain presoaking in Spm, Spd or their interaction appeared to partially ameliorate the effect of waste water and decreased ($P \leq 0.05$) the heavy metals content of wheat grains as compared with the corresponding values of waste water-treated plants alone.

Table 6: Effect of spermine, spermidine and their interaction on heavy metals content (m mole g^{-1} dwt) in root of wheat plants (at heading and anthesis stages) irrigated with different concentrations of waste water.

Parameter / Treatments	Cd^{++}		Pb^{++}		Cu^{++}		Ni^{++}		Zn^{++-}	
	Heading	**Anthesis**	**Heading**	**Anthesis**	**Heading**	**Anthesis**	**Heading**	**Anthesis**	**Heading**	**Anthesis**
Cont.	1.43	1.50	0.21	0.28	0.08	0.09	0.09	0.10	0.07	0.09
WW 25%	8.75	7.10	4.89	5.05	5.34	5.43	6.32	6.14	15.77	16.29
WW 50%	12.23	10.44	9.61	10.19	8.61	9.00	12.14	11.85	24.80	23.05
WW 100%	18.24	16.34	17.20	18.22	12.80	11.64	19.63	16.72	32.66	30.27
Spm	0.90	0.92	0.12	0.15	0.08	0.10	0.09	0.09	0.08	0.08
Spm+WW 25%	4.62	3.76	3.02	3.17	3.79	3.85	4.05	4.20	8.22	6.83
Spm+WW 50%	7.88	6.93	5.51	5.90	4.91	5.18	6.11	5.94	10.75	9.13
Spm+WW 100%	9.80	9.18	8.69	9.51	6.80	6.69	9.55	8.41	14.72	12.52
Spd	1.15	1.14	0.17	0.23	0.08	0.11	0.09	0.10	0.08	0.09
Spd+WW 25%	4.76	3.93	3.75	4.21	4.08	4.14	4.79	4.85	9.37	7.50
Spd+WW 50%	8.53	7.71	6.87	7.32	5.21	5.88	6.65	6.27	12.50	9.77
Spd+WW 100%	11.10	10.22	10.09	10.68	8.11	7.02	10.17	9.14	16.67	14.47
Spm+Spd	0.65	0.78	0.11	0.17	0.08	0.08	0.07	0.09	0.09	0.09
Spm+Spd+WW 25%	3.45	3.36	3.12	3.33	2.59	3.06	3.30	3.59	5.63	4.99
Spm+Spd +WW 50%	5.69	4.91	5.94	6.14	3.65	3.71	5.18	5.22	8.32	7.68
Spm+Spd+WW 100%	7.51	6.42	7.24	7.50	4.75	4.63	8.59	7.78	12.43	10.75
LSD at $P \leq 0.05$	0.17	0.15	0.14	0.11	0.13	0.12	0.18	0.16	0.22	0.19

Table 7: Effect of spermine, spermidine and their interaction on heavy metals content (m mole g^{-1} d wt) in flag leaf of wheat plants (at heading and anthesis stages) irrigated with different concentrations of waste water.

Parameters / Treatments	Heavy metals content (mmole g^{-1} dwt)									
	Cd^{++}		Pb^{++}		Cu^{++}		Ni^{++}		Zn^{++}	
	Heading	**Anthesis**	**Heading**	**Anthesis**	**Heading**	**Anthesis**	**Heading**	**Anthesis**	**Heading**	**Anthesis**
Cont.	0.29	0.31	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.01
WW 25%	2.44	3.54	2.44	4.31	2.12	2.33	3.28	5.96	7.37	11.08
WW 50%	4.06	4.21	4.08	4.96	3.36	2.93	5.11	7.56	9.66	14.07
WW 100%	5.32	4.82	5.55	5.33	4.09	3.25	6.46	8.33	13.30	16.53
Spm	0.27	0.28	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.01
Spm+WW 25%	1.67	1.78	1.67	2.17	1.63	1.13	2.76	3.24	5.06	5.73
Spm+WW 50%	1.95	2.27	2.07	2.78	1.86	1.35	3.19	3.93	6.32	6.98
Spm+WW 100%	2.76	2.87	2.85	3.51	2.69	2.24	4.15	4.69	9.75	8.33
Spd	0.27	0.28	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.01
Spd+WW 25%	1.72	1.96	1.83	2.41	1.66	1.74	3.27	3.51	6.90	5.95
Spd+WW 50%	2.16	2.35	2.24	3.11	2.15	2.14	3.70	4.35	8.07	6.83
Spd+WW 100%	2.92	3.14	3.10	3.84	2.87	2.28	4.26	5.07	10.50	9.10
Spm+Spd	0.24	0.29	0.01	0.01	0.00	0.01	0.00	0.00	0.02	0.01
Spm+Spd+WW 25%	1.26	1.46	1.48	1.84	1.23	1.06	2.35	2.75	3.82	5.24
Spm+Spd +WW 50%	1.76	1.68	1.84	2.26	1.71	1.23	3.01	2.98	5.48	5.97
Spm+Spd+WW 100%	2.29	2.35	2.47	2.52	2.31	1.43	3.77	3.59	6.99	7.43
LSD at $P \leq 0.05$	0.09	0.11	0.07	0.09	0.05	0.08	0.07	0.08	1.08	1.24

Table 8: Effect of spermine, spermidine and their interaction on heavy metals content (m mole g^{-1} d wt) in yielded grains of wheat plants irrigated with different concentrations of waste water.

Parameter / Treatments	Heavy metal contents (m mole g^{-1} d wt)				
	Cd^{++}	Pb^{++}	Cu^{++}	Ni^{++}	Zn^{++}
Cont.	0.42	0.01	0.01	0.00	0.01
WW 25%	1.64	1.48	1.28	3.22	3.60
WW 50%	1.80	1.91	1.54	3.53	3.98
WW 100%	2.62	2.30	1.89	4.19	4.70
Spm	0.33	0.01	0.01	0.00	0.01
Spm+WW 25%	1.26	1.20	0.92	2.08	2.87
Spm+WW 50%	1.53	1.56	1.22	2.61	3.16
Spm+WW 100%	2.06	1.96	1.48	3.36	3.58
Spd	0.36	0.01	0.01	0.00	0.01
Spd+WW 25%	1.42	1.39	1.03	2.47	3.20
Spd+WW 50%	1.95	1.94	1.27	3.03	3.45
Spd+WW 100%	2.16	2.18	1.66	3.59	3.85
Spm+Spd	0.31	0.01	0.01	0.00	0.01
Spm+Spd+WW 25%	0.73	1.07	0.72	1.67	2.40
Spm+Spd +WW 50%	1.24	1.35	0.87	1.95	2.60
Spm+Spd+WW 100%	1.73	1.58	1.21	2.24	3.16
LSD at $P \leq 0.05$	0.06	0.02	0.04	0.04	0.02

Discussion

In all over the world, treatment and reuse of waste water take the attention to face the continuous water shortage. As the wheat has about 70 – 75% carbohydrates, it considers the main source of plant carbohydrates in the food for human consumption, so wheat has up-normal importance.

Thus, this work was undertaken to evaluate the possible role of polyamines (spermine, spermidine or their interaction) in mitigating the harmful effects of irrigation of wheat plants with untreated waste water polluted mainly with heavy metals. There was dramatic difference in analysis between fresh water and untreated waste water. This analysis revealed that, (Cd in waste water = 2.4 times in fresh water, Pb = 4.6 times, Cu = 3 times, Ni = 2.9 times, Zn = 11.6 times).

Plant growth is a function of cell wall extensibility, water conductivity, osmotic potential, and threshold turgor among other factors. When these factors are insufficient, growth does not occur [29]. Shoot growth and morphological characteristics were shown as important factors in controlling the yield of crop plant [30]. Thus, the present results revealed that, waste water particularly at higher concentrations reduced both root and shoot growth vigor of wheat plants during heading and anthesis stages. In this respect, the higher concentrations (50% and 100%) of waste water caused noticeable decrease in height of marigold plants. This may be due to excessive sodium or other cations in waste water which can substitute for other cations in the soil [31].

Application of industrial effluent decreased the budding and growth rate of vegetables [32]. Presumably the effect of Cd and other heavy metals could be due to inhibition of RNA and protein synthesis and consequently cell division or elongation as well as the extension of the cell cycle [33]. Cadmium decreased the cell viability, inhibited the cell division and the accumulation of total biomass. Cadmium at 1mM killed most of the cells and caused rapid destruction in internal architecture as well as degradation or loss of cytoplasmic strands with alteration in vacuolar system and malformation of nuclear shape and size [34].

The negative effect of waste water (mostly polluted with heavy metals) on root and shoot fresh weight may probably be due to that these metals decrease the root water uptake and relative water content as suggested by other workers using different plant species [33].

The decrease in shoot dry masses of wheat plants as a result of waste water treatments may probably be due to the effect of heavy metals in decreasing the ion contents (i.e. Na^+, K^+ and Ca^{++}) of wheat shoot [35]. In addition, the reduction in shoot growth (plant height, shoot fresh weight and leaf area) due to industrial effluents was because of the presence of metal ions like Fe, Cu, Mn and Zn as identified in effluents [36].

It is clear that waste water at all examined concentrations induced massive increases in root dry mass and root / shoot ratio at heading and anthesis stages. These increases may be due to the accumulation of heavy metals in root system [13]. In this regard, stated that lead concentration in comestible plant parts related to the dry mass has increased from 0.05 to 3.0 mg/g. Moreover, the dry weight per seedling increased at 100% of effluent in *Cicer arietinum* plants as compared with the control [37].

The importance of leaf area in controlling plant dry matter and growth has been appreciated [38]. Waste water at all examined levels caused noticeable decrease in flag leaf area of wheat plants. The delay in the flag area leaf production in wheat plants in response to waste water treatments could be due to the slow rate of movement of nutrients and hormones transported with transpiration stream from the root to shoot [39]. Moreover, Barcelo *et al.,* [40] related the negative effect of Cd and other heavy metals on specific leaf area (SLA) with disorders on water supply in *Phaseolus vulgaris* plants. These authors suggested that, reduced cell turgor potential and cell wall elasticity led to formation of small cells and intercellular space area in heavy metal-stressed plants. In addition, presence of Zn at higher concentrations retarded the growth and development of plants by interfering with certain important metabolic processes [41].

The application of spermine, spermidine or their interaction appeared to improve the growth vigor of wheat plants irrigated with different concentrations of waste water by increasing the plant height, fresh and dry masses (for root and shoot), flag leaf area and root length. Grain priming with Spm+Spd appears to be the most effective treatment in inducing faster growth of both root and shoot of wheat plants irrigated with different concentrations of waste water.

The protective role of polyamines under stress conditions on different plant species may be due to their action as a new category of plant growth regulators that are purported to be involved in a large spectrum of physiological processes, such as embryogenesis, cell division, cell elongation, morphogenesis and development [42]. The repairing effect of Spm, Spd or their interaction on growth of waste water-irrigated plants may be due to the increase in water uptake by the root system of wheat plants as reported in other studies [22]. In addition, the observed increase in growth vigor of wheat plants as a result of grain presoaking in polyamines may be explained on the fact that, PAs stimulate many physiological processes such as DNA replication, transcription and translation [42], nucleic acids and protein synthesis [43].

Tiburcio *et al.,* [44] concluded that, PAs are able to modulate plant growth and development through a fundamental mechanism common to all plants. The application of PAs in different plant species increased leaf area, fresh and dry weights of root and shoots and consequently improved growth [45]. Moreover, polyamines play a multiple essential functions in plant cell; they facilitated cell division and growth, increased in the cell number and fresh mass. Also, PAs play an important role in protective response of tobacco cells to Cd stress by the activation of stress-defending mechanisms which prevented the cells before programmed cell death (PCD) [34].

The inhibition in flag leaf area of wheat plants in response to examined concentrations of waste water was mitigated partially when the grains were treated with applied polyamines. This recovery may be due to PAs stimulate the rate of movement of nutrients and hormones from root toward shoot which can accelerate the rate of leaf expansion in developing leaves. The aforementioned pattern of results was in the same direction with many authors ([46] using sunflower and wheat plants; [47] using wheat plants).

The importance of the internal water balance in plant water relations is generally accepted because of the close relationship between the balance and turgidity, to the rates of physiological processes that control the quality and quantity of growth [48]. In the present work, irrigation of wheat plants with all examined concentrations of waste water decreased relative water content (RWC %) in flag leaves of wheat plants at heading stage. This decrease might be explained on the fact that, heavy metals especially Cd may decrease the water uptake by root in different plant species. These results were in accord with those obtained by many authors [49, 50]. Also, the aforementioned results were in close parallelism with those obtained by Valerie *et al.,* [33].

Grain presoaking in Spm, Spd or their interaction recovered the turgidity of flag leaf by increasing the RWC% of wheat plants irrigated with untreated waste water. The increase in RWC % of wheat flag leaf in Spm+Spd treatment were more pronounced than other treatments. These results were similar to those obtained by Kun-Liu *et al.,* [19] using *Vicia faba* plants. The beneficial effect of used polyamines might be due to the increased water uptake that led to powerful water supply to shoot. This mechanism may be due to diminishing the level of ABA which acts as antitranspirant. In addition, treatment of wheat plants with exogenous Spd alleviated the osmotic injury, as judged by increase in RWC of wheat leaves [20].

Water use efficiency (WUE) is the ability of the crop to produce biomass per unit of water transpired [51] or the efficiency for producing dry matter per unit absorbed water, and the ability to allocate an increased proportion of the biomass into grains. Water scarcity is a major limiting factor in agricultural production all over the world [52]. The values of WUE_G and WUE_B in the waste water-irrigated-wheat plants were significantly lower than that of the control ones. These decreases in WUE_G and WUE_B might probably be due to the decreases in grain yield and biomass yield of wheat plants [35].

Treatments with Spm, Spd or their interaction mitigated the harmful effect of waste water stress on WUE_G and WUE_B of wheat plants. The improvement of WUE in non-stressed or stressed wheat plants under polyamines treatment might be due to the increases in both grain and biomass yields of wheat plants [35]. Furthermore, the increases in WUE_G and WUE_B values were higher in Spm+Spd treatment than that of the others.

Waste water at all examined concentrations resulted in marked increases in the ABA content in flag leaves of wheat plants at heading stage. In this connection, ABA level was higher under heavy metals stress conditions in different plant species [53, 54]. In addition, such accumulation of ABA may be the reason of stomata closure on both upper and lower sides of the flag leaves. This result was in accord with those obtained by Omer *et al.,* [55] who reported that, the increase in endogenous ABA levels caused by heavy metal ions can be used in explanation of ceasing water uptake from roots to shoots. The amount of ABA is determined by the dynamic balance between biosynthesis and degradation, and these two processes are influenced by plant development and different environmental factors [56].

In fact, unfavorable environmental factors lead to sharp changes in the balance of growth hormones associated with not only the accumulation of abscisic acid (ABA), but also with a decline in the level of the growth activating hormones: indole acetic acid (IAA), gibberellic acid (GA_3) and cytokinins [57]. These changes would result in a new endogenous hormone balance that would be favorable to the plant's response to waste water. In the present investigation, waste water at all examined doses caused marked increases in ABA levels in yielded grains of wheat plants. This result was in accordance with those obtained by Aldesuquy [58] who proved that, Cd stress induced the increase in ABA content in yielded grains of sorghum plants. This increase in ABA content detected in grains may probably be due to its biosynthesis within the grains or may be possibly translocate from the leaves. From another point of view, different heavy metals may interfere with hormone metabolism by preventing the ABA catabolism in wheat grains. In this respect, the effects of mercury, cadmium and copper showed significant increases in ABA contents in grains of wheat plants exposed to heavy metal ions [55].

Polyamines have been proposed as a new category of plant growth regulators that are purported to be involved in a large spectrum of physiological processes and act as hormonal second-messengers [42]. Thus, PAs are synthesized in large amounts in young tissues (meristems and growing tissues) in different species of plants [59]. In addition, they have been shown to be an integral part of plant stress response [22]. The application of Spm, Spd or their interaction counteracts the stress induced by heavy metals in waste water on the internal growth regulators in wheat grains by reducing the ABA level and at the same time increases the production of growth stimulators within the developing grains.

Grain priming with Spm, Spd or their interaction may ameliorate the deleterious effects of waste water on wheat plants by decreasing the ABA content in wheat flag leaf. In this connection, exogenous application of Spd or Spm significantly inhibited the Cu-induced enhancement of H_2O_2 generation, decreased the levels of free radicals, decreased the accumulation of ABA and thereby mitigated the stress in *Nymphoides peltatum* leaves under Cu treatment [60].

Perusal of data revealed that, the contents of heavy metals (Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++} and Zn^{++}) in the roots of wheat plants at heading and anthesis stages were highly increased with increasing the examined concentrations of waste water. The massive accumulation of heavy metals in roots may be ascribed to formation of complex between heavy metals and sulfhydryl groups of polypeptides that results less transport of heavy metals to shoots [61]. Furthermore, cadmium accumulated in root treated wheat plants rather than in shoots may be result from reduction in transport from root to shoot due to formation of a high-molecular-weight complex with heavy metals (phytochelatins) in roots as suggested by Jonathan *et al.,* [13].

It is clear from the experimental results that, Spm, Spd or their interaction play an important role in increasing the tolerance of wheat plants to waste water treatment by decreasing the accumulation of Cd, Pb, Cu, Ni and Zn in root and consequently in shoot. This repairing effect induced by exogenous application of PAs may be due to PAs: (1) increase the production of phytochelatins (PCs) particularly in root; (2) increase the cell wall and vacuolar storage of these heavy metals; (3) increase the detoxification of heavy metals by increasing the accumulation of these metals in trichomes of leaves and peduncles of wheat plants; (4) acted as an efficient antioxidants and free radical scavengers under this stress [33]; (5) increase the root exudates into the soil (biosphere). In addition, exogenous Spd and Spm evidently decreased the accumulation of Cu and effectively restored the balance of nutrient elements in cells of *Nymphoides peltatum* plants [62].

Heavy metals (Cd^{++}, Pb^{++} Cu^{++}, Ni^{++} & Zn^{++}) in flag leaves of wheat plants at heading and anthesis stages showed dramatic increases with an increase in examined concentrations of waste water. These results are comparable to those data obtained by Jonathan *et al.,* (2005); using wheat plants. A large amount of the heavy metals moved from the root into the leaves in the transpiration stream via the xylem [33]. The negatively charged cell walls can attract mobile cations. Indeed, interactions between cations and cell walls may vary considerably depending on the plant species or genotype [63]. Heavy metals such as Zn, Mn and Cd were released from the roots to the shoot then redistributed from the oldest leaves to youngest leaves, mainly via the phloem [61].

The transport of Cd from roots to shoot in wheat plants may occur through xylem system, phloem cells or cadmium may be recognized as a toxic metal by the roots of wheat thus, it leads to the activation of adaptive mechanisms such as sequestration in the vacuole or in the cell walls [64] in order to avoid an accumulation of this metal in the shoot. There are differences between the wheat cultivars in their ability to accumulate Cd in the leaves and grains. These differences were due to variation in the translocation of Cd from root to shoot and within the shoot, rather than to Cd uptake in the root [65]. Moreover, [33] observed the presence of heavy metals at the edge of older leaves in wheat and plants. This observation might be explained by transport of these metals with the transpiration stream and excretion of these metals in excess with guttation. The guttation fluid may serve to excrete various elements, such as potassium, magnesium and calcium in different plant species [66].

Grain priming with Spm, Spd or their interaction play an important role in increasing the tolerance of wheat plants to waste water by decreasing the accumulation of free Cd^{++}, Pb^{++} Cu^{++}, Ni^{++} & Zn^{++} contents in flag leaves. This reducing effect induced by exogenous application of PAs may increase the accumulation of heavy metals in trichomes by increasing their number in leaves and peduncles of wheat plants [58]. The ameliorating effect of Spm, Spd or their interaction might be explained on the fact that, PAs act as an efficient antioxidants and free radical scavengers under this stress [12]. In addition, exogenous Spd and Spm evidently decreased the accumulation of Cu and effectively restored the balance of nutrient elements in cells of *Nymphoides peltatum* plants [62].

Irrigation of wheat plants with waste water at all examined concentrations resulted in marked increases in Cd^{++}, Zn^{++}, Cu^{++}, Pb^{++} and Ni^{++} contents of yielded grains. These results were in conformity with those of Aldesuquy [58] and Valerie *et al.,* (2006) by using different plant species. In addition, a high Cd level in the wheat grains may be caused by a high Cd uptake in the roots, a high translocation of Cd from roots to shoot, and/or a high translocation of Cd within the shoot to the grains [14].

Maturing grains represent phloem sinks in plant shoots. Thus, the heavy metals reach the grains either directly, via the xylem, or are firstly transfer from the xylem to the phloem and then reach the wheat grains within the phloem. This transfer from xylem to phloem might have happened in the leaf, in the peduncle, or in the glumes then to the grains via the phloem of wheat plants. Comparing with the other heavy metals, Ni showed the highest mobility in the phloem but Mn was the lowest. Although Zn and Cd are chemically similar, their phloem mobility was different. The Zn was exported more rapidly and in large amounts than was Cd. Moreover, Zn was more efficiently removed from the xylem sap compared with Cd, while a higher proportion of the latter was directed to organs with a high transpiration rate [67].

Cadmium is probably either translocated directly via xylem to the grains during maturity or is translocated as a result of the bulk stream of photosynthates from source to sink (i.e. from leaves to the grains via the phloem). According to Mengel and Kirkby [68], the flag leaf is the most important provider of photosynthates to the grain in the later stage of the grain-filling period, contributing to 70 to 80 % of the grain filling. The remainder of assimilate mainly comes from the ear itself. Furthermore, [11] suggested that, the xylem-to-phloem transfer is important for the Cd accumulation in the maturing grains of wheat. It is possible that Cd follows the photosynthate bulk stream from the flag leaf to the grains. Thus, from the root to the grain, there are many processes that may regulate the accumulation of Cd in wheat grains.

Conclusion

Overall, in the present study imposition of irrigation of wheat plants with untreated waste water mostly polluted with heavy metals at all examined doses has a negative effect on growth, metal concentration, distribution, relative water content and ABA of wheat plants. Furthermore, continuous application of waste water may lead to accumulation of plant nutrients and heavy metals to undesirable high levels in the crops and thereby reducing their quality and nutritional value as forage crops for animal feeding. On the other hand, grain presoaking in spermine, spermidine or their interaction displayed a positive role in improving the growth vigor of both root and shoot as well as the ability of wheat cultivar (Sakha 94) to tolerate heavy metals stress in waste water by hormonal regulation and improvement of leaf turgidity by decreasing saturation water deficit and increasing relative water content as well as water use efficiency for economic yield of wheat plants. Furthermore, polyamines decrease heavy metals concentrations and their distribution between root, shoot and grains.

References

1. Garg VK and Kaushik K. “Influence of textile mill waste water irrigation on the growth of sorghum cultivars.” *Applied Ecology and Environmental Reshearch* 6 (2007): 1-12.

2. Samoshekar RK, Gowda MTG, Shettigarh SLN and Srinath KP. “Effect of industrial effluents on crop plants.” *Indian Journal of Environmental Health* 26 (1984): 136-146.

3. Salehi AM and Tabari J. “Accumulation of Zn, Cu, Ni and Pb in soil and leaf of *Pinus eldarica* following irrigation with municipal effluent.” *Research Journal of Environmental Science* 2 (2008): 291 – 297.

4. Aghabarai A, Hosseini SM, Esmaili A. and Maralian H. “Growth and mineral accumulation in *Olea europaea* L. tree irrigated with municipal effluent.” *Research Environmental Science* 2 (2008): 281 – 290.

5. Sharma RK, Agrawal M and Marshall FM. “Heavy metals contamination of soil and vegetables in suburban areas of *Varanasi India.*” *Ecotoxicology Environmental Safety* 66(2007): 258–266.

6. Yantiang MH. “Uptake, distribution and binding of cadmium and nickel in different plant species.” Plant Nutrition 18(1995): 2691- 2700.

7. Bose S and Bhattacharyya AK. “Heavy metal accumulation in wheat plant grown in soil amended with industrial sludge.” *Chemosphere* 70 (2008):1264 – 1272.

8. Ximenez-Embun P, Rodriguez-Sanz B, Madrid-Allbarran Y and Camara C. “Uptake of heavy metals by lupin plants in artificially contaminateds and: preliminary results.” *Journal of Environmental Analytical Chemistery* 82(2002): 805 - 813.

9. Welch R. “Micronutrient nutrition of plants.” *Critical Reviews in Plant Science* 14(1995): 49 - 82.

10. Marschner H. “Mineral Nutrition of Higher Plants.” *Academic Press. New York* (1995).

11. Herren T and Feller U. “Transport of cadmium via xylem and phloem in maturing wheat shoots: comparison with the translocation of zinc, strontium and rubidium.” *Annual Botany (Lond.)* 80 (1997): 623-628.

12. Valeria S, Rita C., Bruno T, Valeriana M and Anna S. "Uptake and toxicity of Cr (III) in celery seedlings". *Chemosphere* 64 (2006): 1695 – 1703.

13. Jonathan J, Ross M, Wendell A, John M and Leon V. "Characterization of cadmium uptake, translocation and Storage in near-isogenic lines of durum wheat that differ in grain cadmium concentration." *New phytology* 172 (2006): 261-271.

14. Li YM, Chaney RL, Schneiter AA, Miller JF, Elias EM and Hammond JJ. "Screening for low grain cadmium phenotypes in sunflower, durum wheat and flax." *Euphytica* 94 (1997): 23 - 30.

15. Ortiz DF, Ruscitti T, Mc-Cue KF and Ow DW. "Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacular membrane protein." *Biological Chemistery* 270 (1995): 4721-4728.

16. Salt DE, Prince RC, Pickering IJ and Raskin I. "Mechanism of cadmium mobility and accumulation in Indian mustard." *Plant Physiology* 109 (1995): 1427-1433.

17. Toze S. "Reuse of effluent water – benefits and risks." *Agriculture Water Management* 80 (2006): 147 – 159.

18. Anurag S, Annamaria R, Antonella C, Francesca S, Mariam C, Rajesh KS and Shashi BA. "Effects of fly ash incorporation on heavy metal accumulation, growth and yield responses of *Beta vulgaris* plants." *Bioresource Technology* 99 (2008):7200–7207.

19. Kun-Lui L, Huihua F, Qixin B and Sheng L. "Inward Potassium Channel in Guard Cells as a Target for Polyamine Regulation of Stomatal Movements." *Plant Physiology* 124 (2000): 1315-1326.

20. Liu H, Yu BJ, Zhang W and Liu Y. "Effect of osmotic stress on the activity of H+ -ATPase and the levels of covalently and non-covalently conjugated polyamines in plasma membrane preparation from wheat seedling roots." *Plant Science* 168 (2005): 1599–1607.

21. Ji-Hong L, Hiroyasu K, Jing W, Yuzuke B and Takaya M. "Polyamines and their ability to provide environmental stress tolerance to plants." *Plant Biotechnology* 24(2007): 117–126.

22. Alcazar R, Marco FH, Cuevas JC, Parton M, Ferrando A, Carrasco P, Tiburcio A F and Altabellai T. "Involvement of polyamines in plant response to abiotic stress." *Biotechnology Letter* 28 (2006): 1867–1876.

23. Clescrei LS, Greenberg AE and Eaton AD. "Standard methods for the examination of water and waste water." 20th Edition, Amer (1998) *Public Health Association (APHA).*

24. Stanhill G. "Water use efficiency." *Adv. Agronomy*, 39 (1987): 53-85.

25. Weatherly PE. "Studied on the water relations of the cotton plants. I. The field measurement of water deficits in leaves." *New Phytology* 49 (1950): 81-97.

26. Weatherly PE and Barrs C. "Are-examination of relative turgidity technique for estimating water deficits in leaves." *Australian Journal of Biological Science* 15 (1962): 413 - 428.

27. Snedecor GW and Cocharan WG. "Statistical Methods." 6th Ed. Oxoford IBH Publishing Co. New. Delhi, 1967.

28. Zhao FJ, Lombi E, Mcgrath SP. "Assessing the potential for zinc and cadmium phytoremediation with the hyper accumulator Thlaspi." *Plant and soil* 249 (2003): 37- 43.

29. Barcelo J., Poschendieder C. "Plant water relations as affected by heavy metal stress: a review." *Plant Nutrition* 13(1990): 1- 37.

30. Aldesuquy HS. "Gibberellic acid-induced modification in yield quality, grain biomass and biochemical aspects in developed grains of seawater-treated wheat plants." *Acta Botanica Hungarica* 42 (1999): 1-13.

31. Mohmmad AA and Khan AU. "Effect of textile factory effluent on soil and crop plants." *Environmental Pollution (Series A)* 37(1985): 131-148.

32. Ihekeronye AI and Ngoddy PO. "Integral Food Science and Technology for the Tropics. 2nd Edn., Macmillian Education Ltd." *Oxford and London* (1985) pp: 293.

33. Valerie P, Renee-Clairei L and Urs F. "Partitioning of zinc,cadmium, manganese and cobalt in wheat (*Triticum aestivum*) and lupin (*Lupinus albus*) and further release into the soil." *Environmental Experiment Botany* 58 (2006): 269 - 278.

34. Andrea K, Lenka G, Sylva Z, Josef E, Ivana M, Denek OZ and Milena C. "Cytological changes and alterations in polyamine contents induced by cadmium in tobacco BY-2 cells." *Journal of Plant Physiology Biochemistery* 42 (2004): 149-156.

35. Aldesuquy HS, Haroun SA, Abo- Hamed SA and El-Said AA. "Physiological studies of some polyamines on wheat plants irrigated with waste water." I. Osmolytes in relation to osmotic adjustment and grain yield." *Phyton* 50 (2003): 263-268.

36. Arif MA, Amer HS, Tahir MS and Rashid AK. "Interaction of Salinity and Industrial Effluents on the Growth of *Dalbergia sissoo* (Shisham) Seedlings." *Journal of Agriculture Biology* 47 (2002): 1 – 12.

37. Nawaz S, Maria SA and Azra Y. "Effect of industrial effluents on seed germination and early growth of *Cicer arietinum*." *Journal of Biological Science* 6 (2006): 49 – 54.

38. Aldesuquy HS. "Effect of indol-3-acetic acid on photosynthetic characteristics of wheat flag leaf during grain filling." *Photosynthetica* 38 (2000): 135-141.

39. Abo-Hamed SA, Mansour FA and Aldesuquy HS. "Shoot growth and morphological characteristics of wheat as influenced by sodium salicylate, alar, asulam and kinetin." *Mansoura Sci. Bulletin* 14 (1987): 203-221.

40. Barcelo J, Vazquez M and Poschendier C. "Structural and utrastructural disorders in cadmium-treated bush bean plants (*Phaseolus vulgaris* L.)." New Phytology 108 (1988):37-49.

41. Alia KV, Prasad SK, and Pardha SP. "Effect of zinc on free radicals and proline in *Brassica juncea* and *Cajanus cajanas*." *Phytochemistry* 39 (1995): 45-47.

42. Bais HP and Ravishankar GA. "Role of polyamines in the ontogeny of plants and their biotechnological applications." *Plant Cell, Tissue and Organ Culture* 69 (2002): 1-34.

43. Galston AW and Tiburcio AF. (Ed). "Lecture Course on Polyamines as Modulators of Plant Development 257:" *Fundacion Jaun Madrid, March, 1991.*

44. Tiburcio AF, Altabella T, Borrell A, Masgrau A, Tiburcio AF, Campos JL, Figueras X and Besford RT. "Recent advances in the understanding of polyamine functions during plant development." *Plant Growth Regulation* 12 (1993):331- 340.

45. Krizek DT, Kramer GF and Mirecki RM. "Influence of UV-β radiation and putrescine on shoot and root growth of cucumber seedlings grown in nutrient solution." *Plant Nutrition* 6 (1997): 613- 623.

46. Groppa D, Benavides P and Tomaro L. "Polyamine metabolism in sunflower and wheat leaf discs under cadmium or copper stress." *Plant Science* 164 (2003): 293-299.

47. Liu H, Liu J, Zhang W and Liu Y. "Relationship between ATPase activity and conjugated polyamines in mitochondrial membrane from wheat seedling roots under osmotic stress." *Environmental Science* 16 (2004): 712- 716.

48. Aldesuquy HS and Ibrahim AH. "The role of shikimic acid in regulation of growth, transpiration, pigmentation, photosynthetic activity and productivity of *Vigna sinensis* plants." *Phyton* 40 (2000): 277 - 299.

49. David ES, Roger CP, Lngrid JP and Ilya R. "Mechanisms of cadmium mobility and accumulation in Indian mustard." *Plant Physiology* 109(1995): 1427-1433.

50. Haroun SA, Aldesuquy HS, Abo- Hamed SA and El-Said AA. "Kinetin-induced modification in growth criteria, ion contents and water relations of sorghum plants treated with cadmium chloride." *Acta Botanica Hungarica* 45 (2003): 113-126.

51. Liang ZS, Yyang JW, Shao HB and Han RL. "Investigation on water consumption characteristics and water use efficiency of Poplar under soil water-deficits on the *Loess Plateau*." *Biointerfaces* 53 (2006): 23–28.

52. Shao HB, Chu LY, Jaleel AC, Zhao CX. "Water-deficit stress-induced anatomical changes in higher plants." *C. R. Biologies* 331(2008):215–225.

53. Sharma S.S. & Kumar V. "Responses of wild type and abscisic acid mutants of *Arabidopsis thaliana* to cadmium." *Plant Physiology* 159(2002): 1323–1327.

54. Yanbo L, Helena and Chunyang L. "Physiological and biochemical responses to high Mn concentrations in two contrasting *Populus cathayana* populations." *Chemosphere* 68 (2007): 686 – 694.

55. Omer M, Fikriye K, Zengin ZY. "The abscisic acid levels of wheat (*Triticum aestivum* L. cv. Cakmak 79) Seeds that were germinated under Heavy Metal (Hg, Cd, Cu)." Stress Journal Science 21 (2008): 1 - 7.

56. Cutler AJ and Krochko JE. "Formation and breakdown of ABA." *Trends Plant Science* 12 (1999): 472 - 478.

57. Jackson M. "Hormones from roots as signals for the shoots of stressed plants." *Elsevier trends journals* 2 (1997): 22–28.

58. Aldesuquy HS. "Effect of spermine and spermidine on wheat plants irrigated with waste water: Conductive canals of flag leaf and peduncle in relation to grain yield." *Stress Physiology and Biochemistery* 10(2014): 145- 166.

59. Santanen A and Simola LK. "Metabolism of L [U-^{14}C] arginine and L [U-^{14}C]-ornithine in maturing and vernalised embryos and megagametophytes of (*Picea abies*)." *Physiologia Plantarum* 107 (1999): 433-440.

60. Alia P and Matysik J. "Effect of proline on the production of singlet oxygen." *Amino Acids* 21 (2001): 195–200.

61. Singh RP and Agrawal M. "Effect of sewage sludge amendment on heavy metal accumulation and consequent responses of *Beta vulgaris* plants." *Chemosphere* 67(2008): 2229–2240.

62. Wang X, Guoxin S, Qinsong XU and Joinzh H. "Exogenous polyamines enhance copper tolerance of *Nymphoides peltatum*." *Plant Physiol* 164 (2007): 1062-1070.

63. Wang J and Evangelou VP. "Metal tolerance aspects of plant cell wall and vacuole. In: Handbook of plant and crop physiology." Pessarakli, M.(Ed.), *Marcel Dekker, Inc., New York* (1995) pp. 695 - 717.

64. Sanita L and Gabbrielli R. “Response to cadmium in higher plants.” *Environmental Experimental Botany* 41 (1999): 105-130.

65. Greger M and Lofstedt M. “Comparison of uptake and distribution of cadmium in different cultivars of bread and durum wheat.” *Crop Science* 44 (2004): 501-507.

66. Tanner W and Beevers eevers H. Transpiration, a prerequisite for long-distance transport of minerals in plants. *Proceeding National Acadamic Science USA* 98 (2001): 9443 - 9447.

67. Olivier R and Urs F. “Redistribution of nickel, cobalt, manganese, zinc and cadmium via the phloem in young and maturing wheat.” *Plant Nutrition* 28 (2005): 421- 430.

68. Mengel K and Kirkby EA. “Principles of plant nutrition.” 3 rd edition Int. Potash Inst., 1982.

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