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Involvement of spermine and spermidine in the control of productivity and biochemical aspects of yielded grains of wheat plants irrigated with waste water

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ABSTRACT

A pot experiment was conducted to evaluate the beneficial effect of grain presoaking in spermine (0.15 mM), spermidine (0.3 mM) and their interaction with waste water (25%, 50%, 100%) polluted with heavy metals on yield and biochemical aspects of yielded grains of wheat plants (*Triticum aestivum* L.) variety Sakha 94. Irrigation of wheat plants with waste water decreased significantly all yield components (100 kernel weight, grain yield/plant, straw yield/plant, mobilization and crop indices) and water use efficiency. On the other hand, polyamines appeared to ameliorate the harmful disordered of heavy metals of waste water on yield components as well as water use efficiency. The effect was more pronounced with Spm + Spd treatment. In the majority of cases, carbohydrates, protein, phosphorus, ions content and growth promoters in yielded grains were decreased in response to waste water stress in wheat plants, meanwhile, chloride, heavy metals content and abscisic acid level were increased in yielded grains of wheat plants. Application of spermine, spermidine or their interaction appeared to mitigate the deleterious effects of waste water on the above biochemical aspects of yielded grains of wheat plants. Protein banding pattern in yielded grains showed induction of proteins with molecular weights 73, 70, 24, 20 and 15 kDa in response to waste water application. Furthermore, spermidine treatment caused appearance of new proteins with molecular weights 73, 70, 57, 24, 23 and 17 kDa in yielded grains. Grain yield of wheat plants was negatively correlated with chloride, heavy metals and ABA.

Abbreviations: ABA, abscisic acid; Spm, spermine; Spd, spermidine; WW, waste water.

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1. 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 have 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) are 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]. In suburban areas, the use of municipal and industrial waste water is common practice in many parts of the world [3]. Waste water carry appreciable amount of toxic heavy metals 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].

Extensive irrigation by the effluents released from a paper mill have led to the accumulation of heavy metals (Cu, Zn, Pb, Co, Cd, Cr, and Ni) in the soil and different parts of the paddy crops [7]. 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 trade organizations have sought to limit the acceptable concentration of Cd in edible crops sold in international markets. In this respect, Sutapa and Bhattacharyya [8] have proposed maximum levels of 0.2 mg Cd/kg for wheat grain.

The accumulation of heavy metals in plant tissues might cause reduction in physiological and biochemical activities of plants resulting lower biomass and yields. Yield thus had significant and negative relationship with the concentrations of Ni^{++}, Cd^{++}, Cu^{++}, Pb^{++}, Zn^{++} and Cr^{+3} in root and shoot [9]. Jonathan et al. [10] proved that, the application of Zn to wheat plant reduced grain biomass and weights. Furthermore, the application of Cd and Zn to young wheat plants affected negatively yield of treated plants [11].

Several investigations showed that, polamines played important role in cell elongation and cell division of different plant species [12]. Polyamines stimulated DNA replication, transcription and translation [13]. In addition to their function in plant development, polyamines may play a role in stress responses because their levels in plant cells increase under a number of environmental stress conditions [14]. Plants respond to pollutants such as lead, producing high levels of polyamines [15]. Putrescine (Put) has been shown to accumulate in the plant cells following many different types of stress (drought, deficient mineral nutrition, acid stress, phytotoxic metals), and therefore it can be considered as a stress marker [16]. In addition to exogenous application of both Spd and Spm effectively reversed the harmful effects of Cu stress in *Nymphoides peltatum* plants [17].

The continuous use of waste water mostly polluted with heavy metals by Egyptian farmers in irrigation of many crops resulted in accumulation of heavy metals in soil and consequently continuous uptake of heavy metals by roots causing toxicity to soil, plants and consumers. Thus, the present work was undertaken to ameliorate the toxicity of heavy metals on yield and biochemical aspects of yielded grains of wheat plants by application of either spd or spm in addition to their interaction.

2. Materials and methods

2.1. Plant material and growth conditions

Homogeneous lot of wheat grains (*Triticum aestivum)* variety Sakha 94 were surface sterilized by soaking in 0.001 M $HgCl_2$ solution for 3 min, then washed thoroughly with distilled water, and divided into four sets which were soaked in distilled water to serve as control, spermine (0.15 mM), spermidine (0.3 mM) or (spermine 0.15 mM + spermidine 0.3 mM) respectively for about 6 h. After soaking, the thoroughly washed grains were planted on 15th November 2005 in plastic pots (15 grains per pot; 25 cm width × 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 °C 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 each one contained 20 pots. The pots of the first group 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. The resulting sixteen treatments were marked as follows:

Treatments	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 (0.15 mM)	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−
Spd (0.30 mM)	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−
Spm + Spd	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+

Data in Table 1 showed analyses of physicochemical characters of standard fresh water and untreated waste water (ppm). These analyses were carried out according to Clescrei et al. [18]. 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^{-1} as urea and 35 kg P ha^{-1} as potassium dihydrogen phosphate as

Table 1 – Physicochemical characters of fresh water and untreated waste water (ppm).

Character	Fresh water	Untreated waste water
Color	Colorless	Brownish black
Turbidity	Clear	Turbid
COD	5.0	150.0
BOD	2.0	60.0
Total suspended solids	4.0	266.0
Total hardness	60.0	770.0
Cd^{++}	0.05	0.12
Pb^{++}	0.05	0.23
Cu^{++}	0.04	0.12
Ni^{++}	0.07	0.20
Zn^{++}	0.08	0.93
Na^{+}	0.02	0.22
K^{+}	0.01	0.14
Ca^{++}	0.01	0.19
Total phosphorus	0.07	0.38
Cl^{-}	45.0	283.6
$SO^{--}4$	00.0	72.0
$NO3^{-}$	0.01	50.0
$NO2^{-}$	0.002	7.3

fertilizers. Ten samples for yield and triplicates for chemical analyses were taken at harvest.

2.2. *Yield analyses*

2.2.1 Harvest index = Economic yield (grain yield)/straw yield (above ground dry matter) [19].
2.2.2 Crop index = grain yield/Biological yield (grain yield + straw yield) [19].
2.2.3 Mobilization index = crop yield/straw yield [20].
2.2.4 Relative grain yield = yield in treated soil/yield in untreated (normal) soil × 100 [19].

2.3. *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 [21].

(WUE_G) = Grain yield (t)/Total water used (gallon)

(WUE_B) = Biomass yield (t)/Total water used (gallon)

2.4. *Estimation of carbohydrates*

2.4.1. *Estimation of glucose*

Glucose content was estimated using O-toluidine procedure of Feteris [22].

2.4.2. *Estimation of sucrose*

Sucrose was determined using the modification of Handel [23].

2.4.3. *Estimation of total soluble sugars*

Total soluble sugars were analyzed according to the modification of Yemm and Willis [24].

2.4.4. *Estimation of polysaccharides*

The method used for estimation of polysaccharides was that of Thayermanavan and Sadasivam [25].

2.5. *Estimation of protein*

Protein content was determined according to the method adopted by Lowry et al. [26].

2.6. *Estimation of phosphorus*

The procedures adopted for extraction of different phosphorus compounds were essentially those described by Barker and Mapson [27]. The method described by Humphries [28] was adopted to estimate both inorganic and total phosphorus, and the difference between them was equivalent to organic phosphorus.

2.7. *Determination of minerals*

Sodium, K^+ and Ca^{++} cations were estimated by the flame photometer. Standard Na^+, K^+ and Ca^{++} solutions with known concentrations were used to draw a standard curve against its atomic absorption [29]. 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^{++} [30].

2.7.1. *Determination of Cl^-*

For chloride analysis about 25 ml of deionized water was added to 50 ml tubes with a known weight of dried material. The tubes were placed in a boiling water bath for 1 h, then cooled, filtered into 50 ml volumetric flask, and brought to volume [31].

Chloride levels were determined by volumetric titration using N/35.5 Ag $NO_3$and 5% $K_2Cr_2O_4$ as an indicator.

2.8. *Determination of growth hormones*

Extraction procedure for abscisic acid, indole-3-acetic acid, gibberellic acid and cytokinin was that originally described by Shindy and Smith [32]. Abscisic acid, gibberellic acid, indolyl acetic acid and cytokinins were determined using two-dimensional HPLC according to Crocier et al. [33].

2.9. *Separation of protein on basis of molecular weight (SDS gel electrophoresis)*

The method for discontinuous SDS-PAGE techniques was based on that of Laemmli [34].

2.10. *Polyamine analysis*

Putrescine, spermine and spermidine were extracted and determined in all tested samples according to Maijala and Eerola [35].

Table 2 – Effect of spermine, spermidine and their interaction on yield components and water use efficiency (WUE) of wheat plants irrigated with different concentrations of waste water.

Treatments	Parameters												
	Main spike length (cm)	Number of spiklets/main spike	100 kernel wt. (g)	Grain No/ main spike	Grain yield/plant (g)	Straw yield/plant (g)	Crop yield/ plant (g)	Harvest index	Mobilization index	Crop index	Relative grain yield %	WUE_G for grains	WUE_B for biomass yield
Cont.	13.37	16.06	5.37	43.10	2.93	4.51	7.44	0.65	0.61	0.39	100.00	8.94	19.58
WW 25%	12.08	14.50	4.79	37.44	2.70	4.23	6.94	0.64	0.55	0.38	80.78	8.25	18.29
WW 50%	10.95	12.43	4.33	34.17	2.38	4.15	6.53	0.57	0.43	0.36	65.99	7.84	16.68
WW 100%	9.86	10.62	4.02	28.63	1.92	3.94	5.86	0.48	0.34	0.32	54.46	6.11	15.91
Spm	15.03	17.09	6.00	49.11	3.59	5.83	9.42	0.62	0.63	0.38	157.40	10.23	20.14
Spm + WW 25%	13.13	15.32	5.44	42.35	3.21	5.40	8.62	0.60	0.57	0.37	120.55	9.57	19.56
Spm + WW 50%	11.47	13.78	4.91	37.30	2.82	5.17	7.98	0.54	0.50	0.35	88.07	8.28	17.86
Spm + WW 100%	10.54	12.54	4.69	32.76	2.38	4.58	6.97	0.52	0.40	0.34	65.55	6.92	16.97
Spd	14.20	16.10	5.60	47.14	3.34	5.57	8.91	0.60	0.60	0.38	134.01	9.88	19.66
Spd + WW 25%	12.62	14.79	5.16	40.72	2.92	5.16	8.08	0.56	0.53	0.36	96.65	9.14	18.87
Spd + WW 50%	11.28	13.24	4.66	35.69	2.56	4.62	7.19	0.55	0.48	0.35	77.38	7.69	17.27
Spd + WW 100%	10.23	11.31	4.39	31.76	2.23	4.38	6.61	0.51	0.38	0.33	59.19	6.67	16.69
Spm + Spd	15.58	17.70	6.45	55.63	4.16	6.19	10.36	0.67	0.66	0.40	179.45	11.17	22.17
Spm + Spd + WW 25%	14.07	16.22	5.81	50.49	3.55	5.35	8.90	0.66	0.62	0.39	115.72	10.34	21.68
Spm + Spd + WW 50%	12.80	14.91	5.28	45.74	2.98	4.91	7.91	0.60	0.59	0.37	106.85	8.76	19.49
Spm + Spd + WW 100%	11.34	12.83	4.84	38.44	2.49	4.62	7.11	0.54	0.45	0.35	73.53	7.53	17.73
LSD $P < 0.05$	0.18	1.66	0.07	2.52	0.08	0.13	0.12	0.04	0.06	0.02	7.52	0.54	0.42

Table 3 – Effect of spermine, spermidine and their interaction on biochemical aspects of yielded grains of wheat plants irrigated with different concentrations of waste water.

Treatments	Parameters														
	Grain biomass (mg)		Total protein (mg g^{-1} dwt)	Carbohydrates content (mg g^{-1} dwt)					Phosphorus content (mg g^{-1} d wt)			Ionic content (m M g^{-1} d wt)			
	Grain fresh mass	Grain dry mass		G	S	TSS	Polys	TC	Inorganic	Organic	Total	Na^{+}	K^{+}	Ca^{++}	Cl^{-}
Cont.	58.82	54.63	92.82	2.52	14.92	20.55	731.52	752.05	0.09	0.62	0.71	1.73	2.89	1.67	0.22
WW 25%	51.61	47.90	85.41	2.26	12.63	17.24	707.93	725.11	0.07	0.50	0.57	1.59	2.79	1.48	2.03
WW 50%	44.44	41.22	79.88	1.84	10.78	14.55	671.22	685.73	0.06	0.41	0.47	1.34	2.55	1.19	2.22
WW 100%	39.58	36.84	70.24	1.33	8.78	13.24	615.76	629.03	0.04	0.32	0.36	1.18	2.13	0.89	2.41
Spm	76.40	71.10	112.58	3.04	16.20	25.33	752.58	777.88	0.11	0.75	0.86	2.20	3.18	1.84	0.28
Spm + WW 25%	69.43	64.55	101.64	2.73	14.10	22.24	728.91	751.11	0.10	0.64	0.74	1.85	2.90	1.74	1.90
Spm + WW 50%	63.89	59.44	85.22	2.22	12.22	19.71	688.33	708.1	0.09	0.48	0.57	1.60	2.63	1.50	2.06
Spm + WW 100%	59.50	55.29	79.19	1.85	11.83	15.22	653.84	669.04	0.06	0.37	0.43	1.38	2.38	1.20	2.18
Spd	71.33	66.33	98.74	2.87	15.90	23.57	740.04	763.64	0.11	0.71	0.82	2.10	2.94	1.60	0.35
Spd + WW 25%	68.32	63.50	96.20	2.32	12.79	20.60	718.40	739.02	0.09	0.61	0.70	1.81	2.63	1.31	1.95
Spd + WW 50%	62.94	58.47	80.72	1.92	11.54	16.88	671.11	687.78	0.08	0.45	0.53	1.63	2.46	1.12	2.12
Spd + WW 100%	57.49	53.50	78.22	1.77	10.43	14.65	642.22	656.80	0.06	0.35	0.41	1.33	2.17	0.95	2.31
Spm + Spd	80.04	74.41	120.78	3.74	19.11	28.22	767.21	795.41	0.13	0.83	0.96	2.47	3.64	2.27	0.26
Spm + Spd + WW 25%	73.43	68.22	110.84	3.33	16.44	25.44	735.67	761.10	0.11	0.73	0.84	2.16	3.48	2.07	1.65
Spm + Spd + WW 50%	68.11	63.22	98.91	2.91	13.09	21.93	699.24	721.09	0.09	0.66	0.75	1.77	2.97	1.83	1.84
Spm + Spd + WW 100%	59.93	55.72	91.33	2.38	12.04	17.70	669.07	686.74	0.07	0.45	0.52	1.48	2.75	1.53	2.08
LSD $P < 0.05$	7.37	6.40	2.14	0.09	1.21	1.41	6.52	8.18	0.04	0.09	0.05	0.02	0.01	0.40	0.11

G = glucose, S = sucrose, TTS = total soluble sugars, Polys = polysaccharides, TC = total carbohydrates.

2.11. Statistical analysis

The main effect of factors (heavy metals and both used polyamines), and the interaction (heavy metals × 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 [36].

3. Results

In general, waste water decreased significantly ($P \leq 0.05$) all yield components of wheat plants as compared to the control plants (Table 2). In the majority of cases, the application of Spm, Spd or their interaction appeared to mitigate the stress imposed by waste water on all yield components of wheat plants. In consequence to the previous determinations, treatment with Spm + Spd improved all components more than that of Spm or Spd alone.

It is clear from the results in Table 2 that, the values of WUE_G and WUE_B in the waste water-treated-wheat plants were significantly lower ($P \leq 0.05$) 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.

In relation to the control value, waste water at all the examined concentrations (25%, 50% and 100%) decreased ($P \leq 0.05$) the grain fresh and dry masses of wheat plants. On the other hand, Spm, Spd or their interaction appeared to improve the grain fresh and dry masses of wheat grains. Treatments with Spm + Spd caused additional increases ($P \leq 0.05$) in the grain fresh and dry masses of wheat grains as compared to the corresponding values detected in waste water-treated plants alone (Table 3).

As compared to control values, the results indicated that, waste water at all examined levels caused noticeable decreases ($P \leq 0.05$) in soluble sugars (glucose, sucrose and total soluble sugars) in the developed grains of wheat (Table 3). On the other hand, the applied chemicals induced increases in these soluble sugars in the developed grains of wheat particularly in their controls and lower concentrations (25% and 50%) of waste water. This effect was more pronounced with Spm + Spd treatments.

Waste water at all examined levels led to marked decreases ($P \leq 0.05$) in polysaccharides and total carbohydrates content in the developed grains of wheat plants as compared to control value (Table 3). In general, application of spermine, spermidine or their interaction to the stressed or control plants induced marked increases ($P \leq 0.05$) in polysaccharides and total carbohydrates content in the yielded grains of wheat plants under stressed and controlled conditions. The magnitude of increases was more pronounced with Spm + Spd treatment.

In relation to control value, all the examined levels of waste water (25%, 50% and 100%) induced significant decrease ($P \leq 0.05$) in the protein content of wheat grains. In general, treatments with Spm, Spd or their interaction caused marked increases ($P \leq 0.05$) in the protein content in grains of both stressed and non-stressed plants (Table 3).

As compared to control values, all examined concentrations of waste water caused decreases ($P \leq 0.05$) in the phosphorus content (inorganic, organic and total phosphorus) in the developed grains of wheat plants. On the other hand, the applied chemicals (Spm, Spd or their interaction) appeared to alleviate the effect of waste water and caused increases in the phosphorus content in the developed grains of wheat plants (Table 3). It appeared from Table 3 that, waste water at all examined concentrations decreased sodium, potassium and calcium contents but increased significantly ($P \leq 0.05$) the chloride content in the developed grains of wheat grains. In general, application of Spm, Spd or their interaction seemed to induce significant increase ($P \leq 0.05$) in ionic content of the developed grains.

Compared with control value, waste water at all examined concentrations caused significant increases ($P \leq 0.05$) in heavy metals content (Cd^{++}, Zn^{++}, Cu^{++}, Pb^{++} and Ni^{++}) with increase in concentrations of waste water (Table 4). 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.

As regards the effect of waste water on growth bioregulators at all examined concentrations, there was a marked decrease ($P \leq 0.05$) in IAA, GA_3, zeatin riboside, kinetin and benzyl adenine and consequently total cytokinins and noticeable increases ($P \leq 0.05$) in ABA content of yielded grains (Table 5). In general, Spm, Spd or their interaction increased the growth stimulators (i.e. IAA, GA_3 & total cytokinins) and decreased the inhibitors (i.e. ABA) in yielded grains of wheat plants as compared to waste water-treated wheat plants alone.

Table 4 – Effect of spermine, spermidine and their interaction on heavy metals content (mmole g^{-1} d wt) in yielded grains of wheat plants irrigated with different concentrations of waste water.

Treatments	Parameter: Heavy metal contents (mmole 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 $P < 0.05$	0.06	0.02	0.04	0.04	0.02

Table 5 – Effect of spermine, spermidine and their interaction on growth bioregulators (μg g^{-1} fresh wt) and polyamines content (n mole g^{-1} fresh wt) in yielded grains of wheat plants irrigated with different concentrations of waste water.

Treatments	Parameters									
	Growth inhibitor	Growth promotors (μg g^{-1} fresh wt)						Polyamines content (n mole g^{-1} fresh wt)		
	ABA	IAA	GA_3	Zeatin riboside	Kinetin	Benzyl adenine	Total	Put	Spm	Spd
Cont.	1.94	12.29	9.30	0.79	3.22	2.06	6.07	0.10	0.24	0.40
WW 25%	2.52	9.63	8.22	0.63	2.57	1.56	4.77	0.17	0.27	0.44
WW 50%	3.22	8.77	6.48	0.44	1.71	1.14	3.28	0.43	0.43	0.68
WW 100%	5.01	6.24	4.16	0.31	1.27	0.74	2.32	0.50	0.44	0.67
Spm	1.56	14.05	11.73	1.12	4.12	2.68	7.91	–	–	–
Spm + WW 25%	1.92	11.93	10.68	0.77	2.98	2.11	5.86	–	–	–
Spm + WW 50%	2.66	10.14	8.94	0.52	2.08	1.40	4.00	0.18	0.30	0.51
Spm + WW 100%	3.07	8.39	7.10	0.39	1.57	1.06	3.02	0.20	0.31	0.61
Spd	1.71	12.83	10.11	0.96	3.55	2.33	6.84	–	–	–
Spd + WW 25%	2.17	11.29	9.73	0.66	2.62	1.76	5.05	N	–	–
Spd + WW 50%	2.92	9.66	7.60	0.45	1.80	1.21	3.46	0.17	0.28	0.56
Spd + WW 100%	3.35	7.77	5.75	0.33	1.33	0.89	2.56	0.32	0.35	0.66
Spm + Spd	1.37	15.34	13.60	1.22	4.53	2.96	8.72	–	–	–
Spm + Spd + WW 25%	1.75	13.52	11.95	0.88	3.42	2.28	6.59	–	–	–
Spm + Spd + WW 50%	2.20	10.72	9.69	0.69	2.66	1.76	5.11	0.21	0.39	0.75
Spm + Spd + WW 100%	2.60	9.24	7.59	0.53	1.74	1.08	3.35	0.28	0.59	0.80
LSD at $P < 0.05$	0.12	0.48	0.32	0.04	0.04	0.02	0.22	0.02	0.016	0.022

Put. = Putrecine, Spm = Spermine, Spd. = Spermidine, – = Not measured.

Regarding the determined polyamines, waste water at all examined concentrations increased the accumulation of endogenous polyamines content (i.e. spermine, spermidine and putrescine) in yielded grains of wheat plants as compared to control plants (Table 5). In general, grain presoaking in Spm, Spd or their interaction led to an increase in endogenous polyamines comparing with the control value. In the majority of cases, the magnitude of increases was more pronounced with Spm + Spd treatment.

Scanning of the gel indicated that, irrigation of wheat plants with waste water at all examined concentrations increased the number of protein bands (8–10) as compared to the control plants (6 bands) and caused the induction of new proteins at molecular weights 73, 70, 24, 20 and 15 kDa. In addition, de-novo synthesis of new set of protein especially 20 kDa in yielded grains of wheat plants irrigated with waste water only. Grain priming with Spm, Spd or their interaction led to appearance of new protein bands with molecular weights 57, 23, 18 and 17 K Da as compared to either control or waste water treatments alone in yielded grains of wheat plants (Fig. 1). In general, Spm, Spd or their interaction increased the protein bands (9–10) as compared to the control treatment in yielded grains of wheat plants (Table 6 and Fig. 1).

In response to the applied waste water and the used chemicals, the grain yield was strongly correlated with all the estimated yield criteria (spike length, number of spikelets per main spike, 100 kernel weight, grain number per spike, grain yield per plant, straw yield per plant, crop yield per plant, harvest, mobilization and crop indices as well as relative grain yield) for wheat plants.

For biochemical aspects of yielded grains, the grain yield was positively correlated with grain fresh mass, grain dry mass, total protein, glucose, sucrose, TSS, polysaccharides, total carbohydrates, (inorganic, organic & total phosphorus), ions (Na^+, K^+ & Ca^{++}), IAA, GA_3, zeatin, kinetin, benzyl adenine as well as total cytokinins. On the other hand, the grain yield appeared to be negatively correlated with chloride, Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++}, Zn^{++}, ABA and endogenous polyamines (spermine, spermidine and putrescine) for wheat plants.

4. Discussion

Irrigation of wheat plants with different concentrations of waste water caused marked decreases in yield components (i.e. spike length, grain yield/main spike, grain yield/plant, straw yield/plant, crop yield/plant, number of spiklets, number of grains/main spike, weights of 100 fresh and dry grains, harvest, crop and mobilization indices as well as relative grain yield %). The reduction in yield of waste water-treated wheat plants can be attributed to the decrease in total cumulative leaf area, photosynthetic pigments, carbohydrates accumulation (polysaccharides) and nitrogenous compounds (total nitrogen and protein) in leaves and consequently in wheat yielded grains [37]. These results were in a good agreement with those obtained by Mallan and Farrant [38]. Decreases in yield and yield components in different crops under similar conditions have also been reported by many workers [38,39].

The results clearly indicated that application of Spm, Spd or their interaction was significant in alleviating the adverse effects of waste water on yield and yield components of wheat plants. The increase in yield production may be due to increase in longevity of leaves which perhaps contributed to grain filling by enhancing the duration of photosynthates

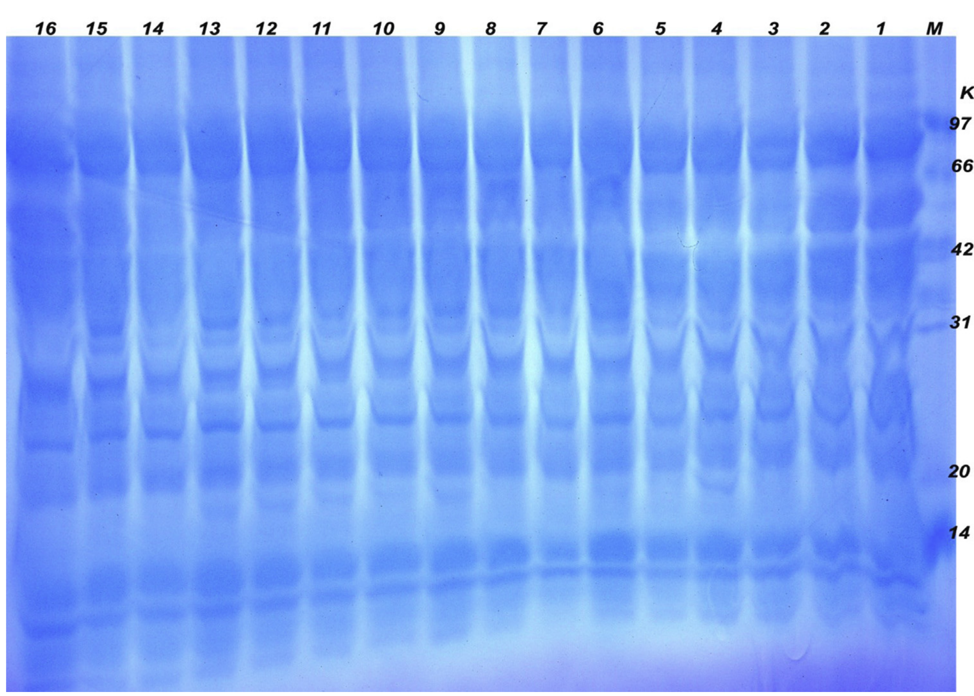

Fig. 1 – Effect of spermine, spermidine and their interaction on protein banding pattern in yielded grains of wheat plants irrigated with different concentrations of waste water.

Table 6 – Effect of spermine, spermidine and their interaction on molecular weight of different types of protein bands in yielded grains of wheat plants irrigated with different concentrations of waste water. (*) present, (–) absent.

Band No.	M wt. (KDa)	Cont.	WW 25%	WW 50%	WW 100%	Cont. Spm	Spm + WW 25%	Spm + WW 50%	Spm + WW 100%	Cont. Spd	Spd + WW 25%	Spd + WW 50%	Spd + WW 100%	Cont. Spm + Spd	Spm + Spd + WW 25%	Spm + Spd + WW 50%	Spm + Spd + WW 100%
1	148	*	*	–	–	*	*	–	–	–	*	–	–	*	–	*	*
2	100	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
3	86	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
4	73	–	–	*	–	–	*	*	*	–	*	–	–	–	*	*	*
5	70	–	*	–	*	*	–	–	–	*	–	*	*	*	–	–	–
6	57	–	–	–	–	–	–	*	*	*	–	*	–	–	–	–	–
7	49	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*
8	41	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
9	30	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
10	24	–	*	–	*	*	*	*	*	*	–	*	–	–	–	–	–
11	23	–	–	–	–	–	–	–	–	–	–	–	*	*	*	*	*
12	20	–	*	*	*	*	–	–	–	–	–	–	–	–	–	–	–
13	18	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*	*
14	17	–	–	–	–	–	–	*	*	*	*	*	*	*	–	–	–
15	15	–	*	*	*	*	*	–	–	–	–	–	–	–	–	–	–
16	13	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Total number of bands		6	10	8	9	10	9	9	9	9	8	9	8	9	8	10	10

supply to grains [40]. This phenomenon was manifested particularly when we found that, there was a positive correlation between phloem area in both flag leaf and peduncle of main shoot of wheat plants which accelerate rapid translocation of photo-assimilates from source (i.e. flag leaf) to sink (i.e. grain in spike) [41]. In this respect, PAs play very important role in many physiological processes (related to yield quality) such as reproductive organ development, tuberization, floral initiation and fruit development and ripening [42].

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. 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 treatment of polyamines might be due to the increases in both grain and biomass yields of wheat plants. Furthermore, the increases in WUE_G and WUE_B values were higher in Spm + Spd treatment than that of the others.

Generally, grain biomass (i.e. fresh and dry), polysaccharides, total carbohydrates and total protein were decreased in response to waste water stress in wheat plants. In this case we can suggest that, waste water might induce the massive production of ABA in flag leaves which in turn led to stomatal closure and consequently may decrease photosynthetic activity in those leaves [37]. Furthermore, waste water stress may stimulate the early senescence in wheat leaves which affects the translocation of photo-assimilates from leaves (particularly flag leaf) that represents the main export source towards the main import sink (developing grain). Bearing in mind the conclusion of Egeli et al. [43] that the accumulation of dry matter by grains requires the production of assimilates in the leaves, their translocation to the fruit, movement into the storage organs of seed, and the synthesis of materials to be stored .The above-mentioned results are in accord with those obtained by Poschendieder et al. [44] who proved that, seed number and size were reduced in Cd-treated *Phaseolus vulgaris* plants. The ameliorating effect induced by PAs may be attributed to the increase in leaf turgidity, which could possibly lead to the accumulation of excessive water, thus resulting consequently in an increase in fresh mass of grain [37].

The decrease in polysaccharide content of yielded grains of wheat plants in response to waste water treatments might be explained on the fact that, Cd^{++} stimulated the degradation of polysaccharide and at the same time increased the rate of dark respiration (Aldesuquy et al. [39] during which total soluble sugars were consumed as respiratory substrate. Furthermore, the noticed decrease in polysaccharides of wheat grains as a result of waste water stress could be attributed to impaired effect on the utilization of carbohydrates during the vegetative growth and reduced the area of conductive canals (mainly phloem), so reduction in the translocation of the assimilates towards the developed grains might have occurred [41]. Furthermore, the reduction in phosphorus contents of yielded grains as a result of waste water stress may probably be due to the accumulation of aluminum and iron in the irrigated soil with waste water under adverse acidity conditions leading to the production of unavailable forms of phosphate compounds for wheat plants. Application of Spm, Spd or their interaction caused an increase in phosphorus contents. This increase may suggest that, PAs increased water uptake by root and consequently increased the passive uptake and translocation of phosphate ions from the soil which were driven by transpiration stream [45].

The recorded decrease in Na^+, K^+ and Ca^{++} contents of wheat grains as a result of waste water treatment reveals that Cd may inhibit the accumulation of the mentioned elements in root and shoot and consequently inhibits the transport of these ions from shoot towards the developed grains [46]. On the hand, 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 are in good conformity with those of Valérie and Urs [47]; Valérie et al. [48] by using different plant species. In addition, the high Cd level in the wheat grains may be brought about rapid rate of 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 [49,50].

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 [51], the flag leaf is the most important donor of photosynthates to the grain in the later stage of the grain-filling period, contributing to 70–80% of the grain filling .The remainder of assimilate mainly comes from the ear itself. Furthermore, Herren and Feller [52] suggested that, the xylem-to-phloem transfer is important for the Cd accumulation in the maturing grains of wheat.

The observed suppression in Na^+, K^+ and Ca^{++} contents of yielded grains in response to waste water stress was relieved when grains were presoaked in Spm, Spd or their interaction. This ameliorating effect of PAs on mineral contents of yielded grains in response to waste water stress may presumably due to that these compounds are able to increase the uptake and transport of Na^+, K^+ and Ca^{++} increasing the rate of water uptake by roots. On the other hand, PAs appeared to suppress the accumulation of heavy metals in wheat grains and this may result in an increase in the tolerance of wheat plants against heavy metals in waste water.

Irrigation of wheat plants with all examined concentrations of waste water significantly decreased the protein content of the developed grains. In this respect, cadmium negatively affected soluble protein content as well as some enzyme activities such as nitrate and nitrite reductase or glutamine synthetase in *Corchorus olitorius* plants [53]. In addition, the decrease in protein content of wheat grains may be explained as follows, (1) cadmium increased the content of free amino acids due to the inhibition of protein synthesis in Cd-treated plants (Vassilev et al. [54], (2) cadmium bound with three families of peptides forming high-molecular-weight Cd-binding complexes, so the free peptides decreased and consequently protein synthesis inhibited [55]. Degradation of soluble protein is one of most obvious signs in plants exposed to heavy metal; therefore, Cu stress significantly decreased soluble protein content in *Nymphoides peltatum* plants [17]. Moreover, cadmium, lead and zinc treatments led to a decrease in assimilation of nitrogen and biosynthesis of amino acids and proteins in the aquatic moss *Fontinalis antipyretica* [56]. The decrease in protein content in yielded grains as a result of waste water application was alleviated by the application of Spm, Spd or their interaction. In connection with these results, Ye et al. [57] stated that, PAs increased the protein content in tobacco, cucumber and *Arabidopsis thaliana* plants.

In fact, unfavorable environmental factors lead to sharp changes in the balance of growth hormones associated with the accumulation of abscisic acid (ABA), and a decline in the level of the growth activating hormones: indole acetic acid (IAA), gibberellic acid (GA_3) and cytokinins [58]. 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 et al. [39] 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 translocated 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 [59]. The decrease in IAA in yielded grains of wheat as a result of waste water mostly polluted by heavy metals might be due to the formation of IAA-oxidase and peroxidase leading to destruction of IAA in resting grains and/or due to decrease in IAA biosynthesis in developed grains. Furthermore, the noticeable decline in gibberellins of wheat grains caused by waste water application may result from conversion of free active gibberellins into bound inactive ones. Another explanation may came from the fact that, heavy metals particularly cadmium may interfere with the metabolism of gibberellins; thus causing deactivation of gibberellins or inhibiting their biosynthesis. Waste water at all examined concentrations caused significant reduction in the cytokinin levels (i.e. zeatin riboside, kinetin, benzyl adenine and total cytokinins) of yielded grains of wheat plants. This is probably may be due to waste water inhibit the rate of translocation of cytokinins (CKs) from root towards the yielded grains.

The application of Spm, Spd or their interaction counteracts the stress induced by heavy metals of waste water on the internal growth bioregulators of wheat grains by reducing the ABA level and at the same time increases the production of growth stimulators within the developing grains. The increase in IAA level in wheat grains by the treatment of Spm, Spd or their interaction may probably be due to the stimulation of IAA biosynthesis or the inhibition of IAA-oxidase. Grain priming with polyamines resulted in an increase in gibberellins in the grains of wheat plants treated with waste water. Such increase led to the induction of influx of certain metabolites particularly sugars into the grains, resulting in an increase in the osmotic uptake of water resulted in sharp rise in fresh weight of the grains [40].

The noticeable increase in cytokinins content of yielded grains of wheat plants treated with waste water due to application with the used PAs may come from the fact that, after grain maturation, the cell division of the endosperm ceased and consequently there is a high amount of cytokinins in yielded grains, hence the produced cytokinin within the developed grains may be utilized during the developmental process [60].

Irrigation of wheat plants with waste water at different concentrations induced marked increases in polyamines content (i.e. spermine, spermidine and putrescine) in yielded grains of wheat plants. In this connection, accumulation of Put has been shown to occur in response to a large variety of stress factors, especially in cereals [61]. The accumulation of Put under heavy metals stress (Cd, Cu and Co) in wheat plants might be explained on the fact that; (1) these heavy metals enhanced both ADC and ODC activities; (2) these metals decreased DAO activity; (3) cadmium caused ethylene release that diverted S-adenosyl methionine (SAM) precursor a way from polyamines biosynthesis and inhibited the conversion of Put into Spm and Spd. However, Spm and Spd contents were reduced by these metals (mainly Cu^{2+}) in sunflower and wheat leaves [62]. The pronounced accumulation of Put soluble conjugates in Cd-treated tobacco cells coincided with the decline in the activity of diamine oxidase, an enzyme catalyzing Put oxidative deamination. This fact points to the important role of PA conjugation in controlling of free PA levels in cells under oxidative stress [63]. Furthermore, Yanbao et al. [64] showed that, Spm, Spd and Put increased in *Populus cathayana* populations under Mn stress. Copper at 0.05 mM increased the Put level and markedly decreased Spd and Spm levels, and these changes were accompanied by the substantial generation of ROS [17].

Grain presoaking in Spm, Spd or their interaction led to an increases in endogenous polyamines comparing with the control value. The magnitude of increases was more pronounced with Spm + Spd treatment. Similar results were obtained by Kubis et al. [65] who found that the exogenous application of putrescine and/or spermidine and/or spermine with concentrations ranged between (0.1–1 Mm) led to the accumulation of endogenous polyamines content (putrescine, spermidine and spermine) of wheat plants.

Irrigation of wheat plants with waste water mostly polluted with heavy metals increased the number of protein bands (8–10) as compared to the control plants (6 bands) and caused the induction of new proteins at molecular weights 73, 70, 24, 20 and 15 kDa in yielded grains of wheat plants. In this regard, the appearance of new protein bands with molecular weight 33 and 5 kDa in response to the three different whey concentrations could be considered as treatment-specific protein [66]. Moreover, the application of Al, Cu, Cd, and Co induced the accumulation of polypeptides with molecular mass of 14 and 16 kDa and a group of polypeptides around 27 kDa in the cell wall of barley plants. More pronounced accumulation and earlier induction of individual cell wall polypeptides in barley plants; might indicate some possible role of these polypeptides in plant resistance to heavy metals stress [67].

The appearance or disappearance of new bands was attributed either to alternation in the structural genes or changes in the expression of regulatory genes involved in regulating these genes due to mutagenic effect of heavy metals present in waste water. Mutational events occurring in the regulatory genes may led to inhibition or constitutive expression of concerned genes and this result in the disappearance of some bands or changes in some band intensities i.e. heavy metals present in sewage water result in an increase in the transcription of a number of stress-induced genes and lead to the accumulation of their polypeptides [68]. Giordani et al. [69] observed an accumulation of transcripts after exposure to trace metals such as copper and cadmium. Under waste water treatments, grain presoaking in Spm, Spd or their interaction led to appearance of new protein bands with molecular weights 57, 23, 18 and 17 KDa as compared to either control or waste water treatments alone in yielded grains of wheat plants. The ameliorating effect of PAs on heavy metals of waste water by inducing the synthesis of specific proteins may be attributed to the role of PAs in increasing the tolerance of wheat plants to heavy metals of waste water [70].

5. Conclusions

It is clear from this investigation that, irrigation of wheat plants with untreated waste water mostly polluted with heavy metals had negative effects on productivity and biochemical aspects of yielded grain. On the other hand, grain presoaking in spermine, spermidine or their interaction displayed a positive role in increasing yield and yield components and improving yield quality of yielded grains of cultivar (Sakha 94). Furthermore, on the basis of the results obtained, we concluded that when it is necessary to cultivate wheat cultivars Sakha 94 in waste water-irrigated soil, presoaking in spermine + spermidine is required to increase the tolerance ability of wheat plants to waste water stress conditions. The ameliorating effect of polyamines resulted in production of good quality and quantity grains.

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