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Physiological Studies of Some Polyamines on Wheat Plants Irrigated with Waste Water. I. Osmolytes in Relation to Osmotic Adjustment and Grain Yield

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With 1 Figure

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Summary

ALDESUQUY H. S., HAROUN S. A., ABO-HAMED S. A. & AL-SAIED A.A. 2011. Physiological studies of some polyamines on wheat plants irrigated with waste water. I. Osmolytes in relation to osmotic adjustment and grain yield. – Phyton (Horn, Austria) 50 (2): 263–286, with 1 figure.

A pot experiment was conducted to evaluate the beneficial effect of grain presoaking in spermine (0.15 mM), spermidine (0.30 mM) and their interaction on tolerance of wheat (*Triticum aestivum* L. cv Sakha 94) plants irrigated with waste water mostly polluted by heavy metals. Osmotic pressure (OP), some osmolytes concentration and grain yield were determined. Waste water at all examined concentrations caused marked increases in OP, osmolytes [proline, organic acids, chloride and heavy metals (Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++} & Zn^{++})] content in flag leaves of wheat plants at heading and anthesis stages. On the other hand, waste water stress induced marked decreases in total soluble nitrogen (TSN), total soluble sugars (TSS) and ions (Na^{+}, K^{+}, & Ca^{++}) as well as grain yield.

Exogenous application of polyamines either spermine, spermidine or their interaction mitigated the deleterious effects of waste water on wheat plants. The effect

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was more pronounced with spermine + spermidine treatment. The applied polyamines increased the osmotic pressure, TSN, TSS, proline, organic acids and ions (Na^+, K^+, & Ca^{++}) concentration as well as grain yield.

The osmotic pressure appeared to depend mainly on proline, organic acids, chloride and heavy metals content, where there is positive correlations between OP and proline, organic acids, and heavy metals. The economic yield (grain yield) was positively correlated with TSN, TSS and ion contents but negatively correlated with proline, organic acids, chloride, heavy metals and OP.

Zusammenfassung

ALDESUQUY H. S., HAROUN S. A., ABO-HAMED S. A. & AL-SAIED A.A. 2011. Physiological studies of some polyamines on wheat plants irrigated with waste water. I. Osmolytes in relation to osmotic adjustment and grain yield. [Physiologische Untersuchungen einiger Polyamine an Weizenpflanzen unter dem Einfluss von Abwasser I. Osmolyte im Verhältnis zu osmotischer Anpassung und Ertrag]. – Phyton (Horn, Austria) 50 (2): 263–286, 1 Abbildung.

In der vorliegenden Arbeit wurde untersucht, ob eine Vorbehandlung von Weizenkörnern mit Spermin (0,15 mM), Spermidin (0,30 mM), oder Spermin und Spermidin in Kombination die Toleranz von in Töpfen gezogenen Weizenpflanzen (*Triticum aestivum* L. cv Sakha 94) gegenüber Abwasser (hauptsächlich durch Schwermetalle verschmutzt) erhöht. Der osmotische Druck (OP), die Konzentration einiger Osmolyte und der Kornertrag wurden bestimmt. Abwasser erhöhte die Konzentration des OP, den Osmolytgehalt [Prolin, organische Säuren, Chloid und Schwermetalle (Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++} & Zn^{++})] in den Fahnenblättern im Blühstadium markant. Andererseits sank der Gehalt an löslichem Gesamtstickstoff (TSN), gesamtlöslichen Kohlenhydraten (TSS), Ionen (Na^+, K^+, & Ca^{++}) und der Ertrag beträchtlich.

Die externe Applikation der Polyamine Spermin und Spermidin einzeln verabreicht oder in Kombination, verringerte die schädliche Wirkung des Abwassers für Weizenpflanzen, wobei die Wirkung der Spermine + Spermidin Kombination größer war. Die verwendeten Polyamine erhöhten den OP, den Gehalt an TSN, TSS, Prolin, organischen Säuren und Ionen (Na^+, K^+, & Ca^{++}) und auch den Ertrag.

Der OP scheint hauptsächlich von Prolin, organischen Säuren, Chlorid und dem Gehalt an Schwermetallen abzuhängen. Aus ökonomischer Sicht war der Ertrag mit TSN, TSS und dem Ionengehalt positiv, mit Prolin, organischen Säuren, Chlorid, Schwermetallen und dem OP jedoch negativ korreliert.

Introduction

In suburban areas, the use of municipal and industrial waste water is common practice in many parts of the world (SINGH & AGRAWAL 2007). Waste water carry appreciable amount of toxic heavy metals (SALEHI & TABARI 2008) and concentrations of heavy metals in waste waters vary from city to city (AGHABARATI & al. 2008). 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 (Sharma & al. 2007). High concentrations of heavy metals affect mobilization and balanced distribution of the elements in plant organs via the competitive uptake (SCHAT & TEN BOOKUM 1992). 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 (SUTAPA & BHATTACHARYYA 2008).

In general, osmotic adjustment (OA) is achieved by absorbing ions (e.g., K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^-) or by accumulating organic solutes (e.g., free amino acids, quaternary ammonium compounds, and sugars) (MOINUDDIN & al. 2005). Many plant species naturally accumulate glycine betaine (GB) and proline as a major organic osmolytes when subjected to different abiotic stresses. These compounds are thought to play adaptive roles in mediating osmotic adjustment and protecting subcellular structures in stressed plants (ASHRAF & FOOLAD 2007). The accumulation of some functional substances, such as compatible solute and protective proteins, is an important element of the physiological and biochemical response to the stressful conditions (JI-HONG & al. 2007).

Excessive amounts of Cd, Zn, Cu, Pb and Ni in the soil decreased plant yields or degraded quality of food or fiber products in different plant species (SUTAPA & BHATTACHARYYA 2008). Furthermore, treatment of Cd and Zn to young wheat plants affected negatively yield of treated plants (SHARIF & SUZELLE 2006).

Polyamines (PAs), mainly the triamine spermidine (Spd), and the tetra-amine spermine (Spm), are polycationic compounds of low molecular weight that are present in all living organisms (LIU & al. 2005). Many studies indicate that, they might play an important role in enhancing plant tolerance to some of environmental stress conditions (ALCÁZAR & al. 2006, JI-HONG & al. 2007).

The present work was undertaken to investigate the effect of grain presoaking in spermine, spermidine and their interaction on osmolytes in relation to osmotic adjustment and grain yield of waste water-irrigated wheat plants.

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 were 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 15^{th} November 2006 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 irri-gated to field capacity by normal tap water. After fifteen days from planting, the plants were thinned to five / pot.

Table 1. The resulting sixteen treatments.

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 (0.15mM)	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
Spd (0.30 mM)	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
Spm + Spd	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+

WW=waste water
Spm=spermine
Spd=spermidine

On day 21 from sowing, the pots of each set were subdivided into four groups. The pots of the 1^{st} group (each group 20 pots) in each set still irrigated with tap water, while 2^{nd}, 3^{rd} and 4^{th} groups in all sets were irrigated with 25%, 50% or 100% waste water respectively. The resulting sixteen treatments were summarized as follows (Table 1).

Physicochemical analyses of irrigation water (the standard fresh water and the untreated waste water) were summarized in table 2. These analyses were carried out according to Clescrei & al. 1998.

The osmotic pressure of flag leaf sap was measured by the cryoscopic method (Walter 1949) and described by El-Sharkawi and Abdel-Rahman 1974.

Table 2. Physicochemical analyses 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
NO_3^{-}	0.01	50.0
NO_2^{-}	0.002	7.3

Estimation of proline:

The method adopted for Determination of proline was essentially that described by SNELL & SNELL 1954.

Determination of the total soluble nitrogen:

The total soluble nitrogen was determined by the conventional semi- micro-modification of Kjeldahl method (PINE 1955).

Determination of total soluble sugars:

Total soluble sugars was extracted and determined by anthrone method of RIAZI & al. 1985.

Determination of Keto-acids:

Keto-acids were determined according to the method adopted by FRIEDEMANN & HAUGEN 1943.

Determination of citric acid:

The method adapted for estimation of citric acid was essentially that described by SNELL & SNELL 1949.

Determination of Na^{+}, K^{+}, Ca^{++}, Cl^{-}, Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++} and Zn^{++}:

Sodium and K^{+} cations were estimated by the flame photometer (YOUNIS & al. 1994) whereas anions Cl^{-} chlorides were determined by the $AgNO_3$ titration method as described by HANSEN & MUNNS 1988. Calcium, Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++} and Zn^{++} cations were determined by the Atomic Absorption Spectrophotometry (BHF 80B biologie spectrophotometer).

Statistical analysis

The results were first subjected to an analysis of variance (ANOVA). If ANOVA showed a significant ($P \le 0.05$) effect, the least significant difference was used to compare between treatments (SNEDECOR & COCHRAN 1976).

Results

Changes in Osmotic Pressure

Waste water at all examined concentrations (25%, 50% and 100%) induced noticeable increases ($P \le 0.05$) in osmotic pressure, as compared to the control plants, of wheat flag leaf at heading and anthesis stages. Treatments with spermine or spermidine caused additional increases ($P \le 0.05$) in osmotic pressure during the heading and anthesis stages (Table 3). Furthermore, spermine+ spermidine treatment increased osmotic pressure additionally more than the other treatments under controlled and waste water treatment at heading and anthesis stages of wheat plants.

Changes in Proline

As compared to the control values, waste water at all examined doses caused significant increase in proline concentration in wheat flag leaf at heading and anthesis stages (Table 3). Comparing with control plants, grain priming with spermine or spermidine caused more significant increase ($P \leq 0.05$) in proline content in comparing with the corresponding treatments of waste water alone during both stages. In addition, plants treated with Spm+Spd had the highest proline concentrations compared with those treated with Spm or Spd only at heading and anthesis stages in flag leaves of wheat plants under controlled and waste water stress conditions.

Changes in Total Soluble Nitrogen (TSN)

In relation to control values, waste water at all concentrations (25%, 50% and 100%) induced noticeable decreases ($P \leq 0.05$) in total soluble nitrogen of wheat flag leaf at heading and anthesis stages (Table 3). In general, grain presoaking in spermine or spermidine caused significant increases in TSN of their controls and at lower concentrations of waste water during heading and anthesis stages. In the majority of cases, the interaction between spermine and spermidine caused increases ($P \leq 0.05$) in total soluble nitrogen content in control and stressed wheat plants at heading and anthesis stages.

Changes in Total Soluble Sugars (TSS)

Waste water at all the examined concentrations (25%, 50% and 100%) induced noticeable decreases ($P \leq 0.05$) in total soluble sugars of wheat flag leaf at heading and anthesis stages as compared to control plants (Table 3). In general, Spm or Spd and their interaction increased the TSS content in wheat flag leaf particularly at lower concentrations (25% and 50%) of waste water during heading and anthesis stages under control and waste water treatment.

Changes in Organic Acids

Changes in Keto-Acids

In relation to control values, keto-acids accumulated in response to waste water stress (induced by heavy metals) in wheat flag leaf at heading and anthesis stages (Table 3). In general, Spm or Spd induced more accumulation ($P \leq 0.05$) in the keto-acids level in flag leaf of wheat plants at heading and anthesis under control and heavy metals stress conditions. The effect was more pronounced with Spm+Spd treatment.

Changes in Citric Acid

Waste water at all examined concentrations caused increases in the citric acid in wheat flag leaf at both stages comparing with the control ones. In the majority of cases, Spm, Spd and their interaction induced more noticeable increases ($P \leq 0.05$) in the citric acid concentrations under control and waste water treatment (Table 3). In comparing all treatments, the effect of Spm and Spd on citric acid contents of wheat flag leaf was more effective.

Changes in Ions Content

Changes in Na^+, K^+ and Ca^{++}

In relation to control values, all the examined concentrations of waste water (25%, 50% and 100%) caused significant decreases ($P \leq 0.05$) in Na^+, K^+ and Ca^{++} contents in wheat flag leaf at heading and anthesis stages as compared to the control sample (Table 4). In general, the application of Spm or Spd and their interaction reverse this situation i.e. increased these contents particularly in controls and lower concentrations (25% and 50%) of waste water during heading and anthesis stages as compared to the untreated control plants.

On the other hand, waste water at all concentrations increased Na^+ / K^+ ratio and chloride content of wheat flag leaf at heading and anthesis stages as compared to the control plants. Also, treatments of Spm or Spd increased Na^+ / K^+ ratio particularly at higher concentrations of waste water. Meanwhile, the combination between the two chemicals significantly decreased Na^+ / K^+ ratio comparing with the control ones at heading and anthesis stages (Table 4).

In relation to the control value, waste water at all examined concentrations increased chloride content in wheat flag leaf at heading and anthesis stages. In the majority of cases, Spm or Spd and their interaction reduced the increase in chloride content at heading and anthesis stages; these increases were lower than those of the corresponding values of waste water alone (Table 4).

Changes in Heavy Metals Content

In comparison 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 5). 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.

Changes in Grain Yield

Irrigation of wheat plants with all examined concentrations of waste water caused marked decrease in grain yield of wheat plants (Fig. 1). Ap-

Table 3. Effect of spermine, spermidine and their interaction on osmotic pressure (atm.) and some osmolytes (proline, total soluble nitrogen, total soluble sugars and organic acids) (mg g^{-1} d wt) in flag leaf of wheat plants (at heading and anthesis stages) irrigated with different concentrations of waste water. The least significant difference (LSD at $P \leq 0.05$) was used to compare between treatments.

Parameters									Organic acids (mg g^{-1} d wt)			
	OP (atm.)		Proline (mg g^{-1} d wt)		Total soluble nitrogen (mg g^{-1} d wt)		Total soluble sugars (mg g^{-1} d wt)		Keto-acids		Citric acid	
Treatments	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis
Cont.	0.69	0.77	0.27	0.39	4.25	7.18	39.66	37.28	0.47	0.43	5.92	6.54
WW 25%	0.72	0.88	0.35	0.51	3.87	6.83	36.20	31.45	0.52	0.48	7.11	7.89
WW 50%	0.81	1.06	0.44	0.65	3.67	5.61	28.50	25.30	0.58	0.57	8.75	9.23
WW 100%	0.88	1.29	0.54	0.97	3.29	5.15	23.62	18.47	0.63	0.61	10.79	11.26
Spm	0.75	0.86	0.47	0.57	4.88	8.26	51.23	43.40	0.54	0.48	7.78	7.83
Spm+WW 25%	0.83	0.93	0.57	0.68	4.51	7.28	43.55	37.91	0.58	0.52	9.58	8.85
Spm+WW 50%	0.90	1.17	0.66	0.92	4.23	6.87	36.50	31.77	0.64	0.63	10.25	10.13
Spm+WW 100%	1.01	1.42	0.78	1.25	3.83	5.27	26.41	22.43	0.68	0.71	11.21	11.56
Spd	0.74	0.81	0.41	0.51	4.38	7.77	49.43	42.55	0.51	0.45	6.70	7.23
Spd+WW 25%	0.79	0.90	0.48	0.64	4.18	6.68	41.43	35.22	0.58	0.50	7.93	8.72
Spd+WW 50%	0.85	1.10	0.58	0.84	3.88	6.31	34.30	27.40	0.61	0.59	9.35	9.81
Spd+WW 100%	0.88	1.25	0.72	1.19	3.69	4.97	27.40	23.22	0.64	0.68	10.72	10.86
Spm+Spd	0.82	1.03	0.52	0.63	5.33	9.44	55.46	48.78	0.58	0.49	8.02	8.09
Spm+Spd+WW25%	0.93	1.16	0.62	0.88	4.71	8.39	48.33	41.23	0.65	0.54	8.77	9.23
Spm+Spd +WW 50%	1.09	1.32	0.87	1.24	4.35	6.79	38.67	33.85	0.71	0.66	9.29	11.18
Spm+Spd+WW 100%	1.12	1.63	1.09	1.74	4.09	6.00	30.48	26.60	0.82	0.73	11.82	13.27
LSD at $P \leq 0.05$	0.01	0.03	0.03	0.03	0.32	0.12	1.63	4.89	0.09	0.05	1.11	1.74

Table 4. Effect of spermine, spermidine and their interaction on some mineral ions (mmole g^{-1} d wt) in flag leaf of wheat plants (at heading and anthesis stages) irrigated with different concentrations of waste water. The least significant difference (LSD at $P \leq 0.05$) was used to compare between treatments.

Parameters	Mineral ions (mmole g^{-1} d wt)									
	Na^+		K^+		Na^+/K^+ ratio		Ca^{++}		Cl^-	
Treatments	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis
Cont.	2.93	3.19	4.04	4.47	0.73	0.71	2.70	3.13	0.26	0.33
WW 25%	2.62	2.93	3.51	4.08	0.75	0.72	2.27	2.92	0.29	0.37
WW 50%	2.47	2.63	3.02	3.54	0.82	0.74	1.84	2.67	0.36	0.44
WW 100%	2.11	2.36	2.59	2.99	0.82	0.79	1.43	2.29	0.40	0.53
Spm	3.14	3.32	4.70	4.99	0.67	0.67	3.22	3.58	0.26	0.33
Spm+WW 25%	2.81	3.12	4.03	4.54	0.70	0.69	2.93	3.31	0.26	0.34
Spm+WW 50%	2.54	2.91	3.42	3.94	0.74	0.74	2.67	3.14	0.30	0.39
Spm+WW 100%	2.30	2.63	2.95	3.65	0.78	0.72	2.23	2.76	0.32	0.43
Spd	3.08	3.23	4.17	4.72	0.74	0.68	3.11	3.26	0.25	0.33
Spd+WW 25%	2.64	3.08	3.82	4.49	0.69	0.69	2.88	2.95	0.30	0.37
Spd+WW 50%	2.46	2.80	3.33	3.87	0.74	0.72	2.48	2.70	0.32	0.41
Spd+WW 100%	2.12	2.43	2.89	3.49	0.73	0.70	2.18	2.47	0.37	0.45
Spm+Spd	3.31	3.44	5.17	5.38	0.64	0.64	3.44	3.70	0.25	0.32
Spm+Spd+WW 25%	3.06	3.24	4.61	5.05	0.66	0.64	3.15	3.47	0.26	0.35
Spm+Spd +WW 50%	2.78	2.89	4.17	4.55	0.66	0.64	2.73	3.19	0.29	0.39
Spm+Spd+WW 100%	2.37	2.73	3.69	3.99	0.64	0.68	2.39	2.79	0.31	0.40
LSD at $P \leq 0.05$	0.01	0.03	0.03	0.03	0.05	0.06	0.31	0.24	0.03	0.05

plication 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 economic yield (grain yield) was positively correlated with TSN (R = 0.98), TSS (r = 0.98), Na^+ (r = 0.95), K^+ (r = 0.77), Na^+/K^+ – (r = –0.77) and Ca^{++} (r = 0.98) negatively correlated with osmotic pressure (r = –0.48), proline (r = –0.45), keto-acids (r = –0.60), citric acid (r = –0.59) and heavy metals content of leaf; Cd^{++} (r = –0.84), Pb^{++} (r = –0.85) Cu^{++} (r = –0.83), Ni^{++} (r = –0.85) & Zn^{++} (r = –0.82) for wheat plants.

Discussion

In order to understand the physiological adaptation of *Triticum aestivum* to stress induced by heavy metals in waste water, osmotic pressure, proline, total soluble nitrogen (TSN), total soluble sugars (TSS), organic acids and ions content as well as heavy metals content in wheat flag leaf were measured particularly at ear emergence (at heading) and at the beginning of the grain set (at anthesis). The results in table 3 showed that waste water at all examined concentrations induced a marked increase in osmotic pressure. This is probably due to the increases in proline, organic acids, chlorides and heavy metals content particularly, when we found that osmotic pressure appeared to be positively correlated with above mentioned osmolytes.

Solutes accumulation in plant cells creates an intracellular osmotic potential which in the presence of rigid cell wall generates turgor pressure. Maintaince of turgor is necessary for maintance of growth through cell elongation (YANCEY & al. 1982). Moreover, the accumulation of these solutes may not be important for osmotic stress tolerance but the metabolic pathways may have adaptive value (HASEGAWA & al. 2000). A further hypothesis is that compatible solutes are also involved in scavenging of reactive oxygen species (CHEN & MURATA 2002). The accumulation of some functional substances, such as compatible solute and protective proteins, is an important element of the physiological and biochemical response to the stressful conditions (JI-HONG & al. 2007).

In the majority of cases, grain priming with spermine, spermidine or their interaction caused an additional increase in the measured osmotic pressure due to the increases in concentration of some osmolytes (proline, TSN, TSS, organic acids and minerals) in wheat plants subjected to waste water polluted with heavy metals. Furthermore, spermine+spermidine treatment induced additional increase in osmotic pressure of wheat plants more than the other treatments of control and waste water stress (see Table 3). In accordance to these results, WRIGHT & al. 1983 concluded that osmolytes accumulation at sensitive reproductive stages of crops has been reported to play a constructive role against floral abortion. Additionally, osmolytes accumulation has also been claimed to facilitate a better translocation of pre-anthesis carbohydrate reserves to the grain during the grain-filling period (SUBBARAO & al. 2000).

Proline content of the flag leaves increased at heading and anthesis stages in wheat plants irrigated with waste water at different doses (Table 3). In accord with these results, GARDEY & al. 2003 reported that proline accumulated in response to several environmental types of stress, such as exposure to heavy metals in soybean plants. Futhermore, proline accumulation in soybean plants could be a protective response, not only due to the osmoprotectant role of proline, but also for the radical scavenging and protein stabilization properties (KUZNETSOV & SHEVYAKOVA 1997).

The accumulation of proline, primarily in the cytosol, often occurs in plants under stress with strong correlation between stress tolerance and proline accumulation, but the relationship is not universal and may be species dependent (ASHRAF & FOOLAD 2007). There are other roles proposed for proline besides osmotic adjustment in stressed plants which include acting as a sink of carbon and nitrogen for stress recovery and buffering cellular redox potential under stress (LEE & al. 2008). Moreover, high levels of proline may enable the plant to maintain low water potentials (SANKAR & al. 2007).

SHANTI & al. 1998 reported that proline can protect enzymes (glucose-6-phosphate dehydrogenase and nitrate reductase) from heavy metal attack in vitro. Many plant species naturally accumulate glycine betaine (GB) and proline as a major organic osmolytes when subjected to different abiotic stresses. These compounds are thought to play adaptive roles in mediating osmotic adjustment and protecting sub-cellular structures in stressed plants (ASHRAF & FOOLAD 2007).

It is clear from our results that, Spm, Spd or their interaction play an important role in protecting the wheat plants from the harmful effect of waste water by increasing the accumulation of proline. Therefore, exogenous PAs application might be a useful method to improve growth and productivity of wheat plants and retard the aging process under waste water stress conditions. In this regard, a mixture of Put, Spd and Spm increased proline concentration in bean plants (JIMENEZ-BREMONT & al. 2006).

In the present investigation, waste water at all examined concentrations induced noticeable decrease in total soluble nitrogen (TSN) in wheat flag leaf at both heading and anthesis stages. The decrease in the content of TSN might be explained on the fact that heavy metals in waste water (Cd, Ni and Mo) inhibited the uptake of nitrate ions by the root as well as their translocation by the xylem to the site of their reduction in pea seedlings (HERNANDEZ & al. 1996). Also, the inhibition in TSN by waste water stress may probably be due to the inhibition of nitrate reductase (NR) in cytoplasm and nitrite reductase in plastids (MAZEN 2004, LIN & al. 2008).

In addition, cadmium inhibited both ATPase and nitrate reductase activities in the roots and shoots of wheat seedlings (NASER & AAL 2001). Cadmium, lead and zinc treatments led to a decrease in assimilation of nitrogen in the aquatic moss *Fontinalis antipyretica* (SUTTER & al. 2001). Furthermore, copper treatment induced changes in nitrogen metabolism with a reduction of total nitrogen (ZHITING & CHAO LIU 2006).

The application of spermine, spermidine or their interaction increased TSN content in flag leaf of wheat plants irrigated with waste water at heading and anthesis stages (Table 3). These data are comparable to those obtained by CHEN & CHING 1996 using rice plants and by IBRAHEEM 1999 using french bean plants. This increase in TSN may probably be due to the increase of biosynthesis of nitrate reductase with inhibition of their degradation in different plant species (PANDY & SRIVASTAVA 1995, IBRAHEEM 1999). Furthermore, PAs are considered as one of the reserves of carbon and nitrogen in plant tissues (KAKKAR & al. 2000). In addition, the application of putrescine on comice pear (*Pyrus communis* L.) flowers increased the nitrogen contents as compared to untreated flowers (CRISOSTO & al. 1992). SUNG & al. 1995 demonstrated the increase in different nitrogenous com-pounds such as ammonia, amino acids and total nitrogen in rice plant as a result of Spm and Spd treatment.

In comparing with control plants, soil drench with waste water at all examined concentrations caused marked decrease in total soluble sugars (TSS) in flag leaves of wheat plants at heading and anthesis stages. It is clear that the change in TSS during growth and development of wheat leaves is consistent with the changes in leaf area and photosynthetic pigments due to waste water stress. Thus the observed decrease in flag leaf area and photosynthetic pigments (unpublished data) in waste water-treated plants was accompanied by a simultaneous decrease in TSS in wheat leaves. The massive reduction in TSS of waste water-treated plants was probably due to the inhibition of chlorophyll biosynthesis leading to a decrease in TSS as recommended by ALDESUQUY & al. 2004 and VALERIA & al. 2006, or to the retardation of photosynthetic activity PSI and PSII as well as its specific enzymes (RAU & al. 2007). Moreover, the application of Zn and Cd caused structural changes in leaves including shrinkage of palisade and spongy parenchyma cells in stressed-mustard plants (MARUTHI & al. 2005). The decrease in TSS under waste water stress might be explained on the fact that heavy metals in waste water induced the inhibition of α-amylase activity (HIRAM 2005).

Grain priming with Spm, Spd or their interaction increased the content of total soluble sugars in flag leaf of waste water-stressed and non-stressed wheat plants (see table 3). This increase in TSS might be explained on the fact that PAs increases the cumulative leaf area (GROPPA & al. 2003, LIU & al. 2004), production of photosynthetic pigments as well as its biogenesis (NASSER 1997) and consequently stimulated the photosynthetic activity. Moreover, polyamines, particularly spermine, improved the efficiency of thylakoid membranes and increased the activity of PSII to five times (NIKOLOAS & KIRIAKOS 2007). The pronounced increase in total soluble sugars by PAs treatments in waste water-treated plants may probably be due to an increase in α-amylase activity in wheat flag leaves. Exogenously applied spermine, spermidine and putrescine during germination of barley grains induced an increase in á-amylase activity of such seedlings as compared with control untreated seedlings (TIPIRDAMAZ & al. 1995). These authors proved that the adverse effect of salt stress on germination can be partially rectified by polyamines. There is a good relationship between the level of carbohydrate and the components of leaf system relating to nitrogen compounds. For example, when carbohydrate levels were high, the reduction of nitrate, ammonia and amino acids were also relatively high (ASLAM & HUFFER 1984). Furthermore, the contents of starch, maltose and sucrose were higher in response to treatments with polyamine compounds in relation to control levels (LEE & al. 1994). KAKKAR & NAGGAR 1996 reported that treatment of *Camella sinensis* leaves with different concentrations of spermine, spermidine and putrescine increased the contents of starch and sucrose.

Generally, when the leaves of wheat plants started to senesce, there was a gradual loss of chlorophylls and carbohydrate fractions (ALDESUQUY & al. 2009). Soil drench with waste water appeared to induce clear senescence particularly if compared with control plants. On the other hand, the application of polyamines played an important role in delaying the senescence of wheat leaves by retaining its chlorophyll and enhancing the formation of carbohydrate fractions. As a result of such changes caused by PAs, there may be rapid movement of the photoassimilates from the leaves (source) to the developing grains (sink) resulting in improved yield quality of the wheat plants exposed to waste water stress.

Irrigation of wheat plants with all examined concentrations of waste water induced noticeable increases in the content of organic acids (citric acid and keto acids) of wheat flag leaves at both heading and anthesis stages. These results were in harmony with those obtained by VENEKAMP & al. 1989 stated that the increase in organic acids content may be due to their role for plant osmotic adjustment and regulation of pH in plant cells under water stress. The increase in citric acid content might be explained on the fact that the role of citric acid decarboxylation to increase CO_2 concentration in mesophyll during the day, while night-time citric acid accumulation increases the buffer capacity of the vacuoles (FRANCO & al. 1992).

In the majority of cases, the application of Spm, Spd or their interaction caused additional increases in organic acids during heading and anthesis stages in flag leaves of wheat plants (Table 3). These increases in the organic acids may probably be due to the importance of these organic acids in osmotic adjustment of wheat plants under waste water stress. In this connection, treatment with exogenous Spd alleviated the osmotic injury of wheat seedlings (LIU & al. 2005).

The plasma membrane is the biomembrane that encloses the cellular content. It regulates the passage of solutes between the cell and the external environment by selectively absorbing nutrients into the cell against a concentration gradient and preventing the entry of certain solutes present in the environment (WANG & al. 2007) showed that, copper at concentration (0.05 mM) disturbed the selective uptake and balance of nutrient elements in *Nymphoides peltatum* leaves.

Generally, waste water at all the examined concentrations (25%, 50% and 100%) induced significant decreases ($P \leq 0.05$) in Na^+, K^+ and Ca^{++} contents in wheat flag leaves (Table 4). The decrease in ion contents in waste water-treated wheat plants may be due to heavy metals inhibited ATPase activity of the plasma membrane, lowered ATP content and inhibited K^+ carriers which are bound to the plasma membrane (LIU & al. 2004). Also subsequent reduction of water uptake and translocation might have occurred (DAVID & al. 1995). Furthermore, the decrease in Ca^{++} content in root under waste water stress may probably be due to the competition between Ca^{++} and divalent cations of heavy metals (Cd^{++}, Zn^{++}, Pb, etc.) on the active sites of specific carriers and / or the antagonism between them in the soil solution (HAROUN & al. 2003).

Chromium toxicity may result from the displacement of other cations, including Ca^{++} from binding sites in the plasma membranes and cell walls, leading to cell malfunction (RYAN & al. 1997). Moreover, chromium can interfere with up-take of other ionically similar elements like Fe and S (SKEFFINGTON & al. 1976). In addition, cadmium inhibited uptake and accumulation of K^+, Ca^{+2} and Fe^{+3} in different plant species but at higher concentrations of Cd^{++}, it caused efflux of K^+,but not of Mg^{++} (HAROUN & al. 2003). ABO-KASSEM & al. 1997 demonstrated that cadmium inhibited the transport of Ca^{++}, Na^+ and K^+ in Cd-treated wheat plants at concentrations of 1, 5 and 10 μM of cadmium sulfate. Moreover, the main basis of cadmium toxicity in biological systems lies in the competition for the adsorption sites between Cd and several mineral nutrients sharing similar chemical properties (SANITA & GABBRIELLI 1999), the reduction of plasma membrane H^+-ATPase activity (OBATA & al. 1996), inhibition of enzyme activities (VANASSCHE & CLIJSTERS 1990), and alteration of nutrient levels (SANDALIO & al. 2001). On the other hand, SMOLDERS & MCLAUGHLIN 1996 found that increased Cl^- concentration in the exposure solution resulted in enhanced accumulation of Cd (in relation to the solution of Cd concentration).

Generally, grain presoaking in spermine, spermidine or their interaction induced marked increases in ion contents. In this respect, the increases in ion contents might be explained on the fact that exogenous spermidine treatment activated ATPase in mitochondrial membranes, increased ATP content which used in the uptake of minerals by carriers in the plasma membranes and also to pump H^+ across the inner mitochondrial membrane in wheat seedlings (LIU & al. 2004). Moreover, polyamines increase water uptake by roots and consequently increase the uptake and translocation of K^+, Na^+ and Ca^{++} ions driven by the transpiration stream (ALCÁZAR & al. 2006).

Polyamines may modulate the activities of membrane-associated enzymes indirectly (SLOCUM & al. 1984). Furthermore, Spd treatment increased greatly PM-H^+-ATPase activity in roots of wheat plants subjected to osmotic stress (LIU & al. 2005). This positive effect of polyamines may presumably be due to the cationic nature of PAs which enable them to interact more freely with anionic molecules, such as proteins and membrane lipids (ALBERTS & al. 2002) and prevent denaturation of the membrane system under stress conditions and consequently improve the ion balance (JI-HONG & al. 2007).

The content of heavy metals (Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++}, and Zn^{++}) in flag leaves of wheat plants at heading and anthesis stages showed significant increases ($P \leq 0.05$) with an increase in applied concentrations of waste water (see Table 5). These results are comparable to those data obtained by JONATHAN & al. 2006 using wheat plants and VALERIE & al. 2006 using white lupin and wheat plants.

A large amount of the heavy metals was translocated from the root into the leaves in the transpiration stream via the xylem (VALERIE & al. 2006). The negatively charged cell walls can attract mobile cations and interactions between cations and cell walls may vary considerably depending on the plant species or genotype (WANG & EVANGELOU 1995). This fact may explain the different retention of Cd and Co in the roots of wheat and lupin. Heavy metals such as Zn, Mn and Cd were released from the roots to the shoot and redistributed from the oldest leaves to youngest leaves, mainly via the phloem (SINGH & AGRAWAL 2007, SUTAPA & BHATTACHARYYA 2008).

Translocation of different heavy metals from root to shoot was demonstrated in maize plants (YANTIANG 1995), in corn, soybean and wheat plants (RAUL & al. 2001), in wheat plants (VALERIE & URS 2005) and in wheat and lupin plants (VALERIE & al. 2006). Furthermore, 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 (SANITA & GABBRIELLI 1999)

Table 5. Effect of spermine, spermidine and their interaction on heavy metals content (mmole g^{-1} d wt) in flag leaf of wheat plants (at heading and anthesis stages) irrigated with different concentrations of waste water. The least significant difference (LSD at $P \leq 0.05$) was used to compare between treatments.

Parameters	Heavy metals content (mmole g^{-1} d wt)									
	Cd^{++}		Pb^{++}		Cu^{++}		Ni^{++}		Zn^{++}	
Treatments	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

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 (GREGER & LOFSTEDT 2004). Moreover, COSIO & al. 2005 and VALERIE & al. 2006 observed the presence of heavy metals at the edge of older leaves in wheat and lupin plants. This observation might be explained by transport of these metals with the transpiration stream and excretion of these metals in excess by guttation. The guttation fluid may serve to excrete various elements, such as potassium, magnesium and calcium in different plant species (TANNER & BEEVERS 2001).

Grain priming with Spm, Spd or their interaction play an important role in increasing the tolerance of wheat plants to waste water stress by decreasing the accumulation of free Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++}, and Zn^{++} contents in flag leaves. This repairing effect induced by exogenous application of PAs could be due to different mechanisms: (1) PAs may increase the production of phytochelatins particularly in root; (2) PAs may increase the cell wall and vacuolar storage of these heavy metals in roots; (3) PAs may increase the accumulation of heavy metals in trichomes by increasing their number in leaves and peduncles of wheat plants; (4) the ameliorating effect of Spm, Spd or their interaction might be explained on the fact that PAs act as efficient antioxidants and free radical scavengers under this stress (FERREIRA & al. 2002, VALERIA & al. 2006). 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 (WANG & al. 2007).

Yield is a result of the integration of metabolic reactions in plants; consequently any factor that influences this metabolic activity at any period of plant growth can affect the yield (IBRAHIM & ALDESUQUY 2003). Thus, in the present work irrigation of wheat plants with all examined concentrations of waste water caused marked decrease in grain yield of wheat plants (Fig. 1).

The reduction in yield of stressed wheat plants can be attributed to the decrease in photosynthetic pigments, carbohydrates accumulation (polysaccharides) and nitrogenous compounds (total nitrogen and protein) in grains of wheat plants. These results were in a good agreement with those obtained by GALSTON & TIBURCIO 1991 and MALAN & FARRANT 1998 in different plant species. The decreases in yield and yield components in different crops under similar conditions has also been reported by many workers (MALAN & FARRANT 1998, ALDESUQUY & al. 2004). The application of excessive amounts of Cd, Zn, Cu, Pb and Ni in the soil decreased plant yield or degraded quality of food or fiber products in different plant species (TANI & BARRINGTON 2005, SUTAPA & BHATTACHARYYA 2008). Furthermore, treatment of Cd and Zn to young wheat plants affected negatively the yield of treated plants (Sharif & Suzelle 2006). Moreover, cadmium reduced grain yield and straw yield in wheat (Stadelmann & al. 1986) and in barley (Juwarker & Shende 1986). Application of Spm, Spd or their interaction was significant in alleviating the adverse effects of waste water on yield and yield component of wheat plants. The increase in yield pro-

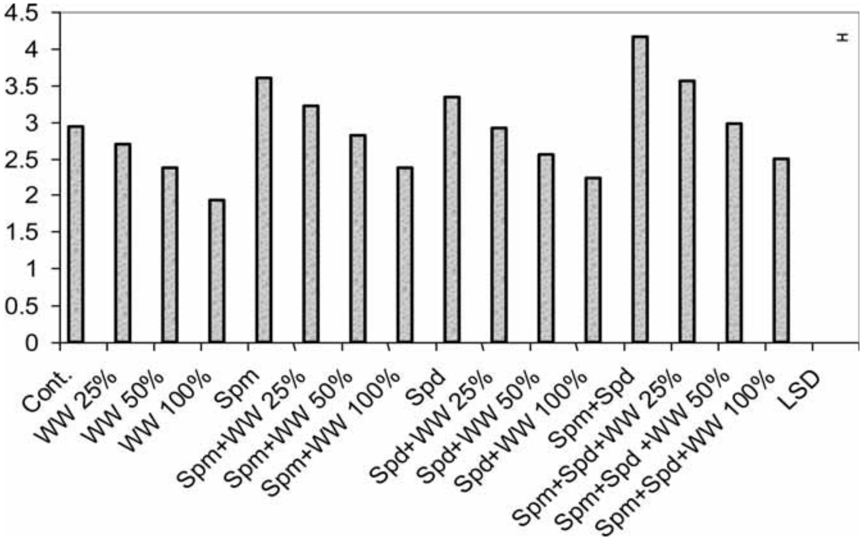

Fig. 1. Effect of spermine, spermidine and their interaction on grain yield / plant (g) of wheat plants irrigated with different concentrations of waste water. Vertical bars represent LSD at $P \leq 0.05$.

duction may be due to increase in longevity of leaves which perhaps contributed to grain filling by enhancing the duration of photosynthate supply to grains (Kaur-Sawhney & al. 1982).

Finally, we can conclude that the interactive effect of spermine + spermidine represent an effective treatment in improving the tolerance of wheat plants to waste water stress by increasing the osmolytes and consequently increase the osmotic adjustment and grain yield.

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