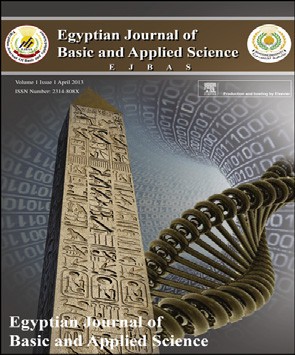
[e gypti an j o ur nal o f b a sic and a pp l i ed sci e n c e s 1 ( 2014) 16](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) e[28](http://dx.doi.org/10.1016/j.ejbas.2013.12.001)

Available online at [www.sciencedirect.com](http://www.sciencedirect.com/science/journal/2314808X)

ScienceDirect

journal homepage: <http://ees.elsevier.com/ejbas/default.asp>

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

*Heshmat Aldesuquy*[*\**](#_bookmark0)*, Samia Haroun, Samy Abo-Hamed, Abdel-Whab El-Saied*



*Department of Botany, Faculty of Science, Mansoura University, P.O. Box 35516, Mansoura, Egypt*

## a r t i c l e i n f o

*Article history:*

Received 27 September 2013 Received in revised form

26 December 2013

Accepted 27 December 2013

Available online 5 February 2014

*Keywords:* Heavy metals Spermine Spermidine Waste water Wheat

Yield

## a b s t r a c t

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 pro-

nounced 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.

Copyright ª 2013, Mansoura University. Production and hosting by Elsevier B.V. All rights

reserved.

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

\* *Corresponding author*. Tel.: +20 1006573700; fax: +20 50 2246254.

E-mail addresses: [heshmat-aldesuquy@hotmail.com](mailto:heshmat-aldesuquy@hotmail.com), [Aboneel@yahoo.com](mailto:Aboneel@yahoo.com) (H. Aldesuquy).

Peer review under responsibility of Mansoura University

**Production and hosting by Elsevier**

2314-808X/$ e see front matter Copyright ª 2013, Mansoura University. Production and hosting by Elsevier B.V. All rights reserved. <http://dx.doi.org/10.1016/j.ejbas.2013.12.001>

[e g ypti an j o ur nal o f b a sic and a pp l i e d sci en c e s 1 ( 2014) 16](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) e[28](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) 17

# Introduction

The use of waste water for irrigation may serve as an addi- tional source of water with fertilizing properties after appro- priate 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]](#_bookmark8). 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]](#_bookmark9). In suburban areas, the use of municipal and industrial waste water is common practice in many parts of the world [[3]](#_bookmark10). Waste water carry appreciable amount of toxic heavy metals and concentrations of heavy metals in waste waters vary from city to city [[4]](#_bookmark11). Important sources of heavy metals in waste water are urban and industrial effluents. Heavy metals are extremely persis- tent in the environment and accumulate to toxic levels [[5]](#_bookmark12). High concentrations of heavy metals affect mobilization and balanced distribution of the elements in plant organs via the competitive uptake [[6]](#_bookmark13).

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]](#_bookmark14). Wheat is one of several crops that tend to accumu- late 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, interna- tional 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]](#_bookmark15) 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]](#_bookmark16).

Jonathan et al. [[10]](#_bookmark17) 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]](#_bookmark18).

Several investigations showed that, polamines played important role in cell elongation and cell division of different plant species [[12]](#_bookmark19). Polyamines stimulated DNA replication, transcription and translation [[13]](#_bookmark20). 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]](#_bookmark21). Plants respond to pollutants such as lead, producing high levels of

polyamines [[15]](#_bookmark22). Putrescine (Put) has been shown to accu- mulate 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]](#_bookmark23). In addition to exogenous application of both Spd and Spm effectively reversed the harmful effects of Cu stress in *Nymphoides peltatum* plants [[17]](#_bookmark24).

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 conse- quently 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 ofwheat plants by application of either spd or spm in addition to their interaction.

# Materials and methods

### *Plant material and growth conditions*

Homogeneous lot of wheat grains (*Triticum aestivum)* variety Sakha 94 were surface sterilized by soaking in 0.001 M HgCl2 solution for 3 min, then washed thoroughly with distilled water, and divided into four sets which were soaked in

midine (0.3 mM) or (spermine 0.15 mM + spermidine 0.3 mM) distilled water to serve as control, spermine (0.15 mM), sper- respectively for about 6 h. After soaking, the thoroughly

pots (15 grains per pot; 25 cm width × 30 cm height) filled with washed grains were planted on 15th November 2005 in plastic 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/

relative humidity were: 29.2/33.2 ◦C and 63/68% respectively, night conditions (minimum/maximum temperature and 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 sub- divided 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) | e | e | e | e | + | + | + | + | e | e | e | e | e | e | e | e |
| Spd (0.30 mM) | e | e | e | e | e | e | e | e | + | + | + | + | e | e | e | e |
| Spm + Spd | e | e | e | e | e | e | e | e | e | e | e | e | + | + | + | + |

Data in [Table 1](#_bookmark1) 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]](#_bookmark25). The main source of untreated waste water was the main Aga drain in Dakahliya Province, Egypt.

(65 days from planting), the plants received 35 kg N ha—1 as At tillering stage (i.e. 21 days from planting) and at heading urea and 35 kg P ha—1 as potassium dihydrogen phosphate as

18 [e gypti an j o ur nal o f b a sic and a pp l i ed sci e n c e s 1 ( 2014) 16](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) e[28](http://dx.doi.org/10.1016/j.ejbas.2013.12.001)

### *2.4.4. Estimation of polysaccharides*

|  |  |  |
| --- | --- | --- |
| Table 1 e 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 |

The method used for estimation of polysaccharides was that of Thayermanavan and Sadasivam [[25]](#_bookmark32).

### *Estimation of protein*

Protein content was determined according to the method adopted by Lowry et al. [[26]](#_bookmark33).

### *Estimation of phosphorus*

The procedures adopted for extraction of different phos- phorus compounds were essentially those described by Barker and Mapson [[27]](#_bookmark34). The method described by Humphries [[28]](#_bookmark35) was adopted to estimate both inorganic and total phos- phorus, and the difference between them was equivalent to organic phosphorus.

### *Determination of minerals*

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

### *Yield analyses*

* + 1. Harvest index = Economic yield (grain yield)/straw yield (above ground dry matter) [[19]](#_bookmark26).
    2. Crop index = grain yield/Biological yield (grain yield + straw yield) [[19]](#_bookmark26).
    3. Mobilization index = crop yield/straw yield [[20]](#_bookmark27).
    4. Relative grain yield = yield in treated soil/yield in un- treated (normal) soil × 100 [[19]](#_bookmark26).

### *Determination of water use efficiency*

grain yield (t ha—1) or the biomass yield (t ha—1) by the amount Water use efficiency (WUE) was calculated by dividing the of water added by (gallons). Therefore water use efficiency for

grain yield (WUEG) was calculated from the grain yield and water use efficiency for biomass yield (WUEB) was estimated from the biomass yield [[21]](#_bookmark28).

(WUEG) = Grain yield (t)/Total water used (gallon) (WUEB) = Biomass yield (t)/Total water used (gallon)

### *Estimation of carbohydrates*

* + 1. *Estimation of glucose* Glucose content was estimated using O-toluidine procedure of Feteris [[22]](#_bookmark29).

### *Estimation of sucrose*

Sucrose was determined using the modification of Handel [[23]](#_bookmark30).

### *Estimation of total soluble sugars*

Total soluble sugars were analyzed according to the modifi- cation of Yemm and Willis [[24]](#_bookmark31).

Sodium, KD and Ca++ cations were estimated by the flame photometer. Standard NaD, KD and Ca++ solutions with

against its atomic absorption [[29]](#_bookmark36). Cadmium, Pb++, Cu++, Ni++ known concentrations were used to draw a standard curve and Zn++ cations were determined by the Atomic Absorption

Spectrophotometry (BHF 80B biologie spectrophotometer).

ference of Na+, K+ and Ca++ [[30]](#_bookmark37). The samples were diluted with LiCl3 to suppress the inter-

### *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]](#_bookmark38).

Chloride levels were determined by volumetric titration using N/35.5 Ag NO3and 5% K2Cr2O4 as an indicator.

### *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]](#_bookmark39). Abscisic acid, gibberellic acid, indolyl acetic acid and cytokinins were determined using two- dimensional HPLC according to Crocier et al. [[33]](#_bookmark40).

### *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]](#_bookmark41).

### *Polyamine analysis*

Putrescine, spermine and spermidine were extracted and determined in all tested samples according to Maijala and Eerola [[35]](#_bookmark42).

19

e g ypti an j o ur nal o f b a sic and a pp l i e d sci en c e s 1 ( 2014) 16 e28

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 2 e 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 | Main spike length (cm) | Number of spiklets/main spike | 100  kernel wt. (g) | Parameters  Grain No/ Grain Straw Crop Harvest Mobilization Crop Relative main spike yield/plant yield/plant yield/ index index index grain yield  (g) (g) plant (g) % | | | | | | | | WUEG  for grains | WUEB 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 | 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 |
| 25% |  |  |  |  | | | | | | | |  |  |
| Spm + Spd + WW | 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 |
| 50% |  |  |  |  | | | | | | | |  |  |
| Spm + Spd + WW | 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 |
| 100% |  |  |  |  | | | | | | | |  |  |
| 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 |

20

e gypti an j o ur nal o f b a sic and a pp l i ed sci e n c e s 1 ( 2014) 16 e28

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 3 e Effect of spermine, spermidine and their interaction on biochemical aspects of yielded grains of wheat plants irrigated with different concentrations of waste water. | | | | | | | | | | | | | | | | |
| Treatments |  | Grain biomass (mg)  Grain fresh mass Grain dry mass | | Total protein (mg g—1 dwt) | G | Parameters  (mg g—1 dwt) Carbohydrates content  S TSS Polys TC | | | | (mg g—1 d wt) Phosphorus content  Inorganic Organic Total | | | (m M g—1 d wt) Ionic content  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. | | | | | | | | | | | | | | | | |

[e g ypti an j o ur nal o f b a sic and a pp l i e d sci en c e s 1 ( 2014) 16](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) e[28](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) 21

### *Statistical analysis*

amines), and the interaction (heavy metals × polyamines) The main effect of factors (heavy metals and both used poly- were evaluated by general linear model (two ways ANOVA)

means at *P* = 0.05 were given by LSD test [[36]](#_bookmark43). using SPSS program. Tests for significant differences between

# Results

In general, waste water decreased significantly (*P* ≤ 0.05) all yield components of wheat plants as compared to the control

plants ([Table 2](#_bookmark2)). 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](#_bookmark2) that, the values of

were significantly lower (*P* ≤ 0.05) than that of the control WUEG and WUEB in the waste water-treated-wheat plants ones. Application of Spm, Spd or their interaction clearly

addition, treatments with Spm + Spd gave highest WUEG and improved WUEG and WUEB values in stressed wheat plants. In WUEB values than the other treatments.

In relation to the control value, waste water at all the examined concentrations (25%, 50% and 100%) decreased

(*P* ≤ 0.05) the grain fresh and dry masses of wheat plants. On

the other hand, Spm, Spd or their interaction appeared to

Treatments with Spm + Spd caused additional increases improve the grain fresh and dry masses of wheat grains. (*P* ≤ 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](#_bookmark3)).

As compared to control values, the results indicated that, waste water at all examined levels caused noticeable de-

creases (*P* ≤ 0.05) in soluble sugars (glucose, sucrose and total

soluble sugars) in the developed grains of wheat ([Table 3](#_bookmark3)). On

the other hand, the applied chemicals induced increases in these soluble sugars in the developed grains of wheat partic- ularly in their controls and lower concentrations (25% and

Spm + Spd treatments. 50%) of waste water. This effect was more pronounced with

Waste water at all examined levels led to marked decreases

(*P* ≤ 0.05) in polysaccharides and total carbohydrates content in the developed grains of wheat plants as compared to con-

trol value ([Table 3](#_bookmark3)). In general, application of spermine, sper- midine or their interaction to the stressed or control plants

induced marked increases (*P* ≤ 0.05) in polysaccharides and

total carbohydrates content in the yielded grains of wheat

tude of increases was more pronounced with Spm + Spd plants under stressed and controlled conditions. The magni- treatment.

In relation to control value, all the examined levels of waste water (25%, 50% and 100%) induced significant decrease

(*P* ≤ 0.05) in the protein content of wheat grains. In general,

increases (*P* ≤ 0.05) in the protein content in grains of both treatments with Spm, Spd or their interaction caused marked stressed and non-stressed plants ([Table 3](#_bookmark3)).

tions of waste water caused decreases (*P* ≤ 0.05) in the As compared to control values, all examined concentra- phosphorus content (inorganic, organic and total phos-

phorus) 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](#_bookmark3)). It appeared from [Table 3](#_bookmark3) that, waste water at all examined concentrations

increased significantly (*P* ≤ 0.05) the chloride content in the decreased sodium, potassium and calcium contents but developed grains of wheat grains. In general, application

cant increase (*P* ≤ 0.05) in ionic content of the developed of Spm, Spd or their interaction seemed to induce signifi- grains.

concentrations caused significant increases (*P* ≤ 0.05) in heavy Compared with control value, waste water at all examined metals content (Cd++, Zn++, Cu++, Pb++ and Ni++) with in-

crease in concentrations of waste water ([Table 4](#_bookmark4)). In the ma-

jority of cases, grain presoaking in Spm, Spd or their interaction appeared to partially ameliorate the effect of

waste water and decreased (*P* ≤ 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 bio-

marked decrease (*P* ≤ 0.05) in IAA, GA3, zeatin riboside, kinetin regulators at all examined concentrations, there was a noticeable increases (*P* ≤ 0.05) in ABA content of yielded grains and benzyl adenine and consequently total cytokinins and ([Table 5](#_bookmark5)). In general, Spm, Spd or their interaction increased

the growth stimulators (i.e. IAA, GA3 & 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 e Effect of spermine, spermidine and their interaction on heavy metals content (mmole gL1 d wt) in yielded grains of wheat plants irrigated with different concentrations of waste water. | | | | | |
| Treatments | Cd++ | Parameter  (mmole g—1 d wt) Heavy metal contents 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 |

22

e gypti an j o ur nal o f b a sic and a pp l i ed sci e n c e s 1 ( 2014) 16 e28

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 5 e Effect of spermine, spermidine and their interaction on growth bioregulators (mg gL1 fresh wt) and polyamines content (n mole gL1 fresh wt) in yielded grains of wheat plants irrigated with different concentrations of waste water. | | | | | | | | | | |
| Treatments | Growth inhibitor ABA | IAA | GA3 | Growth promotors (mg g—1 fresh wt) Parameters  Zeatin riboside Kinetin Benzyl adenine | | | Total | (n mole g—1 fresh wt) Polyamines content  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 | e | e | e |
| Spm + WW 25% | 1.92 | 11.93 | 10.68 | 0.77 | 2.98 | 2.11 | 5.86 | e | e | e |
| 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 | e | e | e |
| Spd + WW 25% | 2.17 | 11.29 | 9.73 | 0.66 | 2.62 | 1.76 | 5.05 | N | e | e |
| 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 | e | e | e |
| Spm + Spd + WW 25% | 1.75 | 13.52 | 11.95 | 0.88 | 3.42 | 2.28 | 6.59 | e | e | e |
| 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, e = Not measured. | | | | | | | | | | |

[e g ypti an j o ur nal o f b a sic and a pp l i e d sci en c e s 1 ( 2014) 16](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) e[28](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) 23

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](#_bookmark5)). 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

with Spm + Spd treatment. of cases, the magnitude of increases was more pronounced

Scanning of the gel indicated that, irrigation of wheat

plants with waste water at all examined concentrations increased the number of protein bands (8e10) 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](#_bookmark6)). In general, Spm, Spd or their interaction increased the protein bands (9e10) as compared to the control treatment in yielded grains of wheat plants ([Table 6](#_bookmark7) and [Fig. 1](#_bookmark6)). 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,

ions (Na+, K+ & Ca++), IAA, GA3, zeatin, kinetin, benzyl total carbohydrates, (inorganic, organic & total phosphorus), adenine as well as total cytokinins. On the other hand, the

Cd++, Pb++, Cu++, Ni++, Zn++, ABA and endogenous poly- grain yield appeared to be negatively correlated with chloride, amines (spermine, spermidine and putrescine) for wheat

plants.

# 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, carbo- hydrates accumulation (polysaccharides) and nitrogenous compounds (total nitrogen and protein) in leaves and consequently in wheat yielded grains [[37]](#_bookmark44). These results were in a good agreement with those obtained by Mallan and Farrant [[38]](#_bookmark45). Decreases in yield and yield components in different crops under similar conditions have also been re- ported by many workers [[38,39]](#_bookmark45).

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 in- crease in longevity of leaves which perhaps contributed to grain filling by enhancing the duration of photosynthates

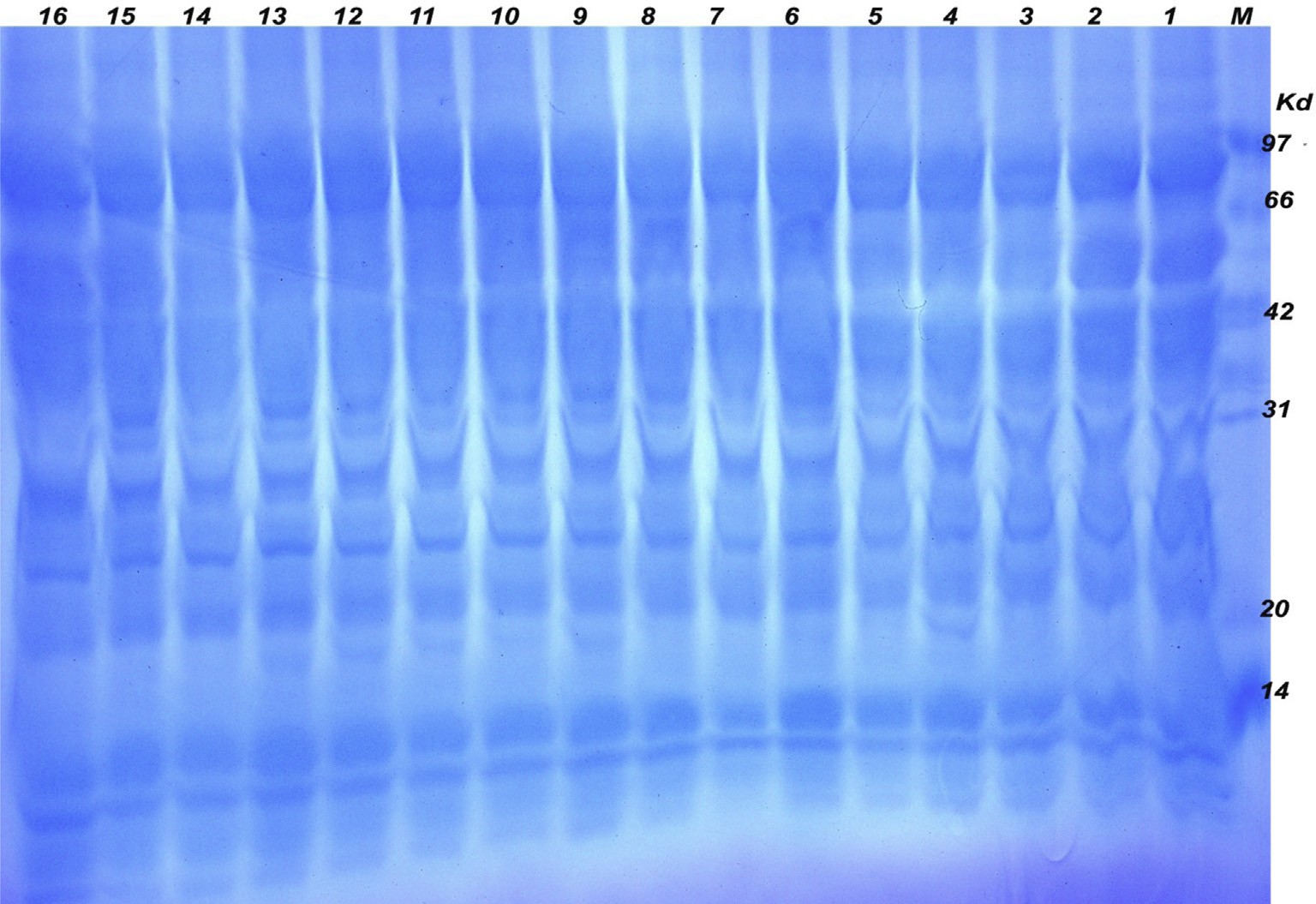


Fig. 1 e Effect of spermine, spermidine and their interaction on protein banding pattern in yielded grains of wheat plants irrigated with different concentrations of waste water.

24

e gypti an j o ur nal o f b a sic and a pp l i ed sci e n c e s 1 ( 2014) 16 e28

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

[e g ypti an j o ur nal o f b a sic and a pp l i e d sci en c e s 1 ( 2014) 16](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) e[28](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) 25

supply to grains [[40]](#_bookmark46). This phenomenon was manifested particularly when we found that, there was a positive corre- lation between phloem area in both flag leaf and peduncle of main shoot of wheat plants which accelerate rapid trans- location of photo-assimilates from source (i.e. flag leaf) to sink (i.e. grain in spike) [[41]](#_bookmark48). 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]](#_bookmark49).

The values of WUEG and WUEB in the waste water- irrigated-wheat plants were significantly lower than that of the control ones. These decreases in WUEG and WUEB 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 WUEG and WUEB of wheat plants. The improvement of WUE in non-stressed or stressed wheat plants under treat- ment of polyamines might be due to the increases in both grain and biomass yields of wheat plants. Furthermore, the

increases in WUEG and WUEB values were higher in Spm + Spd

treatment than that of the others.

Generally, grain biomass(i.e. fresh anddry), 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 pro- duction of ABA in flag leaves which in turn led to stomatal closure and consequently may decrease photosynthetic activity in those leaves [[37]](#_bookmark44). Furthermore, waste water stress may stimulatetheearlysenescenceinwheatleaveswhichaffectsthe 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]](#_bookmark50) 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 Pos- chendieder et al. [[44]](#_bookmark51) who proved that, seed number and size were reduced in Cd-treated *Phaseolu*s *vulgaris* plants. The ameliorating effect induced by PAs may be attributed to the in- crease in leaf turgidity, which could possibly lead to the accu- mulation of excessive water, thus resulting consequently in an increase in fresh mass of grain [[37]](#_bookmark44).

The decrease in polysaccharide content of yielded grains

of wheat plants in response to waste water treatments might be explained on the fact that, CdDD stimulated the degra- dation of polysaccharide and at the same time increased the rate of dark respiration (Aldesuquy et al. [[39]](#_bookmark47) 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 carbohy- drates 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]](#_bookmark48). 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 trans- location of phosphate ions from the soil which were driven by transpiration stream [[45]](#_bookmark52).

The recorded decrease in NaD, KD and CaDD 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]](#_bookmark53). On the hand, irrigation of wheat plants with waste water at all examined concentrations resulted in marked increases in CdDD, ZnDD, CuDD, PbDD and NiDD contents of yielded grains. These results are in good conformity with those of Vale´rie and Urs [[47]](#_bookmark54); Vale´rie et al. [[48]](#_bookmark55) 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 trans- location of Cd within the shoot to the grains [[49,50]](#_bookmark56).

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]](#_bookmark57), the flag leaf is the most important donor of photosynthates to the grain in the later stage of the grain- filling period, contributing to 70e80% of the grain filling .The remainder of assimilate mainly comes from the ear itself. Furthermore, Herren and Feller [[52]](#_bookmark58) suggested that, the xylem- to-phloem transfer is important for the Cd accumulation in the maturing grains of wheat.

The observed suppression in NaD, KD and CaDD 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 con- tents 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 NaD, KD and CaDD 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 ac- tivities such as nitrate and nitrite reductase or glutamine synthetase in *Corchorus olitorius* plants [[53]](#_bookmark59). 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]](#_bookmark60), (2) cadmium bound with three families of peptides forming high-molecular-weight Cd-bind- ing complexes, so the free peptides decreased and conse- quently protein synthesis inhibited [[55]](#_bookmark61). 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]](#_bookmark24). Moreover, cadmium, lead and zinc treatments led to a decrease in

26 [e gypti an j o ur nal o f b a sic and a pp l i ed sci e n c e s 1 ( 2014) 16](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) e[28](http://dx.doi.org/10.1016/j.ejbas.2013.12.001)

assimilation of nitrogen and biosynthesis of amino acids and proteins in the aquatic moss *Fontinalis antipyretica* [[56]](#_bookmark62). 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]](#_bookmark63) 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 (GA3) and cytokinins [[58]](#_bookmark64). 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 ob- tained by Aldesuquy et al. [[39]](#_bookmark47) who proved that, Cd stress induced the increase in ABA content in yielded grains of sor- ghum 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]](#_bookmark65). 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 destruc- tion 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 gibber- ellins 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 counter-

acts 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 gibberel- lins in the grains of wheat plants treated with waste water. Such increase led to the induction of influx of certain me- tabolites 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]](#_bookmark46).

The noticeable increase in cytokinins content of yielded

grains of wheat plants treated with waste water due to

application with theused PAs may come from thefact 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]](#_bookmark66).

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]](#_bookmark67). 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 Cu2+) in sunflower and

wheat leaves [[62]](#_bookmark68). The pronounced accumulation of Put sol-

uble conjugates in Cd-treated tobacco cells coincided with the decline in the activity of diamine oxidase, an enzyme cata- lyzing 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]](#_bookmark69). Furthermore, Yan- bao et al. [[64]](#_bookmark70) 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]](#_bookmark24).

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 pro-

nounced with Spm + Spd treatment. Similar results were

obtained by Kubis et al. [[65]](#_bookmark71) who found that the exogenous

application of putrescine and/or spermidine and/or spermine with concentrations ranged between (0.1e1 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 (8e10) 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 concentra- tions could be considered as treatment-specific protein [[66]](#_bookmark72). 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]](#_bookmark73).

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

[e g ypti an j o ur nal o f b a sic and a pp l i e d sci en c e s 1 ( 2014) 16](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) e[28](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) 27

expression of concerned genes and this result in the disap- pearance 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]](#_bookmark74). Giordani et al. [[69]](#_bookmark75) 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 mo- lecular 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]](#_bookmark76).

# 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 pos- itive 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 culti-

spermine + spermidine is required to increase the tolerance vars Sakha 94 in waste water-irrigated soil, presoaking in ability of wheat plants to waste water stress conditions. The

ameliorating effect of polyamines resulted in production of good quality and quantity grains.

## referen c es

1. [Garg VK, Kaushik P. Influence of textile mill wastewater](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref1) [irrigation on the growth of sorghum cultivars. Appl Ecol](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref1) [Environ Res 2007;6:1](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref1)e[12](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref1).
2. [Saomashekar RK, Gowda MTG, Shettigar SLN, Srinath KP.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref2) [Effect of industrial effluents on crop plants. Indian J Environ](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref2) [Hlth 1984;26:136](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref2)e[46](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref2).
3. [Singh RP, Agrawal M. Effect of sewage sludge amendment on](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref3) [heavy metal accumulation and consequent responses of *Beta*](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref3)[*vulgaris* plants. Chemosphere 2008;67:2229](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref3)e[40](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref3).
4. [Aghabarati A, Hosseini SM, Esmaili A, Maralian H. Growth](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref4) [and mineral accumulation in *Olea europaea* L. tree irrigated](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref4) [with municipal effluent Res. J Environ Sci 2008;2:281](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref4)e[90](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref4).
5. [Sharma RK, Agrawal M, Marshall FM. Heavy metals](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref5) [contamination of soil and vegetables in suburban areas of](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref5) [Varanasi India. Ecotoxicol Environ Saf 2007;66:258](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref5)e[66](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref5).
6. [Schat H, Ten Bookum W. Metal specificity of metal tolerance](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref6) [syndromes in higher plants. In: Proter JA, Baker JM,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref6)

[Reeves RD, editors. The ecology of ultramafic (serpentine)](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref6) [oils. MA: Intercept Andover; 1992. pp. 337](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref6)e[52](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref6).

1. [Fazeli M, SKhosravan F, Hossini M, Sathyanarayan S,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref7) [Satish PN. Enrichment of heavy metals in paddy crops](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref7) [irrigated by paper mill effluents near Nanjangud, Mysore](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref7) [District, Karnatake, India. Environ Geol 1998;34:201](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref7)e[9](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref7).
2. [Sutapa B, Bhattacharyya AK. Heavy metal accumulation in](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref8) [wheat plant grown in soil amended with industrial sludge.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref8) [Chemosphere 2008;70:1264](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref8)e[72](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref8).
3. [Sharma RK, Agrawal M, Agrawal SB. Interactive effects of Cd](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref9) [and Zn on carrots: growth and biomass accumulation. J Plant](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref9) [Nutr 2008;31:1](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref9)e[17](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref9).
4. [Jonathan J, Ross M, Wendell A, John M, Leon V. Zinc effects](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref10) [on cadmium accumulation and partitioning in near-isogenic](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref10) [lines of durum wheat that differ in grain cadmium](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref10) [concentration. New Phytol 2005;167:391](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref10)e[401](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref10).
5. [Sharif AS, Suzelle FB. Effect of soil fertility and transpiration](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref11) [rate on young wheat plants (*Triticum aestivum*) Cd/Zn uptake](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref11) [and yield. Agric Water Manag 2006;82:177](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref11)e[92](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref11).
6. [Gorecka K, Cvikrova M, Kowalska U, Josef Eder J,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref12) [Szafranska K, Gorecki R, et al. The impact of Cu treatment on](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref12) [phenolic and polyamine levels in plant material regenerated](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref12) [from embryos obtained in anther culture of carrot. Plant](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref12) [Physiol Biochem 2007;45:54](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref12)e[61](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref12).
7. [Bais HP, Ravishankar GA. Role of polyamines in the ontogeny](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref13) [of plants and their biotechnological applications. Plant Cell](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref13) [Tissue Organ Cult 2002;69:1](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref13)e[34](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref13).
8. [Alca´zar R, Marco F, Cuevas JC, Patron M, Ferrando A,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref14) [Carrasco P, et al. Involvement of polyamines in plant](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref14) [response to abiotic stress. Biotechnol Lett 2006;28:1867](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref14)e[76](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref14).
9. [Wellburn FAM, Wellburn AR. Variable patterns of](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref15) [antioxidant protection but similar ethene emission](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref15) [differences in several ozone-sensitive and ozone-tolerant](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref15) [plant selections. Plant Cell Environ 1996;19:754](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref15)e[60](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref15).
10. [Hauschild MZ, Jacobsen S. Putrescine as a marker of](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref16) [pollution induced stress in higher plants. In: Flores HE,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref16) [Arteca RN, Shannon JC, editors. Polyamines and ethylene.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref16) [Biochemistry, physiology and interactions. Rockville: Amer.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref16) [Soc. Plant. Physiol.; 1990. pp. 405](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref16)e[8](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref16).
11. [Wang X, Guoxin S, Qinsong Xu, Jinzhao H. Exogenous](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref17) [polyamines enhance copper tolerance of *Nymphoides*](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref17)[*peltatum*. Plant Physiol 2007;164:1062](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref17)e[70](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref17).
12. [Clescrei LS, Greenberg AE, Eaton AD. Standard methods for](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref18) [the examination of water and waste water. 20th ed. Amer.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref18) [Public Health Association (APHA); 1998](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref18).
13. [Beadle CL. Growth analysis. In: Hall DC, Scurlock JMO,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref19) [Bolhar-Nordenkampf HR, Leegod RC, Long SP, editors.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref19) [Photosynthesis and production in a changing environment.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref19) [A field and laboratory manual. London: Chapman and Hall;](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref19) [1993. pp. 36](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref19)e[46](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref19).
14. [Ray S, Choudhuri MA. Regulation of flag leaf senescence in](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref20) [rice by nutrients and its impact on yield. Physiol Plant](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref20) [1980;59:343](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref20)e[6](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref20).
15. [Stanhill G. Water use efficiency. Adv Agron 1987;39:53](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref21)e[85](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref21).
16. [Feteris AW. A serum glucose method without protein](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref22) [precipitation. Am J Med Technol 1965;31:17](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref22)e[21](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref22).
17. [Handel EV. Direct microdeterminations of sucrose. Anal](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref23) [Biochem 1968;22:280](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref23)e[3](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref23).
18. [Yemm EW, Willis AJ. The respiration of barley plants. IX. The](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref24) [metabolism of roots during assimilation of nitrogen. New](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref24) [Phytol 1956;55:229](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref24)e[34](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref24).
19. Thayermanavan V, Sadasivam S. Qual Plant Foods Hum Nutr 34:253e7. Quoted from Biochemical Methods. Sadasivam S, A Manickam, editor; 1984; 2nd ed., 11e12. New ag. inter. Limit. Publ. New Delhi, India.
20. [Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref25) [measurement with the Folin phenol reagent. Biol Chem](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref25) [1951;193:265](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref25)e[75](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref25).
21. [Barker J, Mapson LW. Studies in the respiratory and](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref26) [carbohydrate metabolism of plant tissues. J Exp Bot](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref26) [1964;15:272](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref26)e[83](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref26).
22. [Humphries EC. Mineral components and ash analysis. In:](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref27) [Pesch K, Tracey MV, editors. Modern method of plant](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref27) [analysis, vol. 1. Berlin: Springer](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref27)e[Verlag; 1956. p. 468](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref27).
23. [Younis ME, Abbas MA, Shukry WM. Salinity and hormone](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref28) [interaction in affecting growth, transpiration and ionic](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref28) [relations of *Phaseolus vulgaris*. Biol Plant 1994;36:83](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref28)e[9](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref28).

28 [e gypti an j o ur nal o f b a sic and a pp l i ed sci e n c e s 1 ( 2014) 16](http://dx.doi.org/10.1016/j.ejbas.2013.12.001) e[28](http://dx.doi.org/10.1016/j.ejbas.2013.12.001)

1. [Haroun SA, Aldesuquy HS, Abo-Hamed SA, El-Said AA.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref29) [Kinetin-induced modification in growth criteria, ion contents](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref29) [and water relations of sorghum plants treated with cadmium](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref29) [chloride. Acta Bot Hungar 2003;45:113](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref29)e[26](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref29).
2. [Hansen EM, Munns DN. Effect of CaSO4 and NaCl on](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref30) [mineral content of *Leucaena leucocephala*. Plant Soil](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref30) [1988;6:101](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref30)e[7](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref30).
3. [Shindy WW, Smith OE. Identification of plant hormones](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref31) [from cotton ovules. Plant Physiol 1975;55:550](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref31)e[4](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref31).
4. [Crocier A, Moritz T. Physico-chemical methods of plant](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref32) [hormone analysis. In: Hooykaas PJJ, Hall MA, Libbenga KR,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref32) [editors. Biochemistry and molecular biology of plant](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref32) [hormones. Amsterdam: Elsevier; 1999. pp. 23](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref32)e[60](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref32).
5. [Laemmli UK. Cleavage of structural proteins during the](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref33) [assembly of the head of bacteriophage T4. Nat Lond](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref33) [1970;227:280](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref33)e[5](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref33).
6. [Maijala RL, Eerola SH. Contaminant lactic acid bacteria](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref34) [histamine and tyramine. Meat Sci 1993;35:387](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref34)e[95](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref34).
7. [Snedecor GW, Cochran WG. Statistical methods. 6th ed. New](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref35) [Delhi: Oxoford IBH Publishing Co; 1976](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref35).
8. [Aldesuquy HS, Haroun SA, Abo-Hamed SA, Al-Saied AA.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref36) [Physiological studies of some polyamines on wheat plants](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref36) [irrigated with waste water. I. Osmolytes in relation to](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref36) [osmotic adjustment and grain yield. Phyton 2011;50:263](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref36)e[8](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref36).
9. [Mallan HI, Farrant JM. Effect of metal pollutants cadmium](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref37) [and nickel on soybean seed development. Seed Sci Res](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref37) [1998;8:445](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref37)e[53](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref37).
10. [Aldesuquy HS, Haroun SA, Abo-Hamed SA, El-Said AA.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref38) [Ameliorating effect of kinetin on pigments, photosynthetic](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref38) [characteristics, carbohydrate contents and productivity of](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref38) [cadmium treated *Sorghum bicolor* plants. Acta Bot Hungar](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref38) [2004;46:1](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref38)e[21](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref38).
11. [Kaur-Sawhney R, Shih-Flores HE, Galston AW. Relation of](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref39) [polyamine synthesis and titer to aging and senescence in oat](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref39) [leaves. Plant Physiol 1982;69:405](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref39)e[10](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref39).
12. [Aldesuquy HS. Effect of spermine and spermidine on wheat](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref69) [plants irrigated with waste water: conductive canals of flag](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref69) [leaf and peduncle in relation to grain yield. J Stress Physiol](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref69) [Biochem 2014;10:145](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref69)e[66](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref69).
13. [Tiburcio AF, Altabella T, Masgrau C. Polyamines. In:](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref40) [Bisseling T, Schell J, editors. New developments in plant](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref40) [hormone research. New York: Springer-Verlag; 2002](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref40).
14. [Egeli DB, Guffy RD, Meckel LW, Leggett JE. The effect of source-](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref41) [sink alterations on soybean seed growth. Ann Bot](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref41) [1985;55:395](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref41)e[9](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref41).
15. [Poschendieder C, Cabot C, Barcelo J. Influence of high](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref42) [concentrations of cadmium on the growth, development and](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref42) [photosynthetic pigment of *Phaseolus vulgaris*. Anales de](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref42) [Edaflogia Agrobiologia 1983;42:315](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref42)e[27](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref42).
16. [Jalil A, Selles F, Clarke JM. Effect of cadmium on growth and](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref43) [the uptake of cadmium and other elements by durum wheat.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref43) [Plant Nutr 1994;17:1839](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref43)e[58](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref43).
17. [Abo-Kassem EM, El-Din AS, Mohamed YAH, Foda EA. Effect](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref44) [of different cadmium concentrations on growth,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref44) [photosynthesis and ion relation of wheat plants. Egypt J](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref44) [Physiol Sci 1997;21:41](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref44)e[51](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref44).
18. [Vale´rie P, Urs F. Selective transport of zinc, manganese,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref45) [nickel, cobalt and cadmium in the root system and transfer](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref45) [to the leaves in young wheat plants. Ann Bot 2005;96:425](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref45)e[34](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref45).
19. [Vale´rie P, Renee-Claire L, Urs F. Partitioning of zinc,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref46) [cadmium, manganese and cobalt in wheat (*Triticum aestivum*)](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref46) [and lupin (*Lupinus albus*) and further release into the soil.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref46) [Environ Exp Bot 2006;58:269](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref46)e[78](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref46).
20. [Li YM, Chaney RL, Schneiter AA, Miller JF, Elias EM,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref47) [Hammond JJ. Screening for low grain cadmium phenotypes](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref47) [in sunflower, durum wheat and flax. Euphytica](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref47) [1997;94:23](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref47)e[30](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref47).
21. [Olivier R, F Urs. Redistribution of nickel, cobalt, manganese,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref48) [zinc and cadmium via the phloem in young and maturing](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref48) [wheat. Plant Nutr 2005;28:421](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref48)e[30](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref48).
22. [Mengel K, Kirkby EA. Principles of plant nutrition. 3rd ed. Int.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref49) [Potash Inst; 1982](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref49).
23. [Herren T, Feller U. Transport of cadmium via xylem and](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref50) [phloem in maturing wheat shoots: comparison with the](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref50) [translocation of zinc, strontium and rubidium. Ann Bot](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref50) [(Lond.) 1997;80:623](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref50)e[8](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref50).
24. [Mazen AMA. Accumulation of four metals in tissues of](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref51) [*Corchorus olitorius* and possible mechanisms of their](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref51) [tolerance. Biol Plant 2004;48:267](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref51)e[72](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref51).
25. [Vassilev A, Berova M, Zelatev Z. Influence of Cd on growth,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref52) [chlorophyll content and water relations in young barley](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref52) [plants. Biol Plant 1998;41:601](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref52)e[6](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref52).
26. [Wilfried ER, Philippe M. Retention of cadmium in roots of](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref53) [maize seedlings. Plant Physiol 1995;109:195](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref53)e[202](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref53).
27. [Sutter K, Jung K, Krauss GJ. Effects of heavy metals on the](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref54) [nitrogen metabolism of the aquatic moss *Fontinalis*](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref54)[*antipyretica* L.ex Hedw. AN Tracer study. Environ Sci Pollut](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref54) [Res 2001;9:417](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref54)e[21](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref54).
28. [Ye XS, Sergei AA, Joseph K. Effect of polyamines on in vitro](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref55) [phosphorylation of soluble and plasma membrane proteins](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref55) [in tobacco, cucumber and *Arabidopsis thaliana*. Plant Sci](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref55) [(Limerick) 1994;97:109](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref55)e[18](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref55).
29. [Jackson M. Hormones from roots as signals for the shoots of](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref56) [stressed plants. Elsevier Trends J 1997;2:22](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref56)e[8](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref56).
30. [Omer M, Fikriye K, Zengin ZY. The abscisic acid levels of wheat](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref57) [(*Triticum aestivum* L. cv. Cakmak 79) seeds that were germinated](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref57) [under heavy metal (Hg, Cd, Cu ) stress. J Sci 2008;21:1](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref57)e[7](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref57).
31. [Van Staden J. Cytokinins, seed development and](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref58) [germination. South Afr J Bot 1983;2:257](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref58)e[61](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref58).
32. [Martin-Tanguy J. Metabolism and function of polyamines in](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref59) [plants: recent development (new approaches). Plant Growth](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref59) [Reg 2001;34:135](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref59)e[48](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref59).
33. [Groppa D, Benavides P, Tomaro L. Polyamine metabolism in](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref60) [sunflower and wheat leaf discs under cadmium or copper](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref60) [stress. Plant Sci 2003;164:293](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref60)e[9. Bradford MM (1976) A rapid](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref60) [and sensitive method for the quantitation of microgram](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref60) [quantities of protein utilizing the principle of protein dye-](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref60) [binding. Analytical Biochem.72: 248](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref60)e[251](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref60).
34. [Lovaas E. Antioxidant and metal-chelating effects of](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref61) [polyamines. In: Sies H, editor. Advances in pharmacology,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref61) [antioxidants in disease mechanisms and therapy, 38. New](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref61) [York: Academic Press; 1996. pp. 119](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref61)e[49](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref61).
35. [YanbaoL, HelenaK, ChunyangL. Physiological andbiochemical](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref62) [responses to highMn concentrationsintwo contrasting *Populus*](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref62)[*cathayana* populations. Chemosphere 2007;68:686](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref62)e[94](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref62).
36. [Kubis J, Skoczek H, Krzywanski Z. Exogenous polyamines](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref63) [alter the activity of protease, RNAase and membrane](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref63) [permeability in wheat leaves under water stress conditions.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref63) [Acta Physiol Plant 1991;13:139](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref63)e[46](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref63).
37. [King J. The genetic basis of plant physiological processes.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref64) [New York: Oxford Univ. press.pub; 1991. pp. 304](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref64)e[31](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref64).
38. [Ladislav T, Jana H, Lenka H, Igor M. The effect of aluminium](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref65) [on polypeptide pattern of cell wall proteins isolated from the](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref65) [roots of Al-sensitive and Al-resistant barley cultivars. Acta](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref65) [Physiol Plant 2001;23:161](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref65)e[8](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref65).
39. [Zeid IM, Abou ElGhate HM. Effect of sewage water on growth,](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref66) [metabolism and yield of bean. J Biol Sci 2007;7:34](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref66)e[40](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref66).
40. [Giordani T, Natal L, Maserti BE, Taddeinand S, Cavallini A.](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref67) [Characterization and expression of DNA sequences encoding](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref67) [putative type-II metallothionine in the sea-grass *Posidonia*](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref67)[*oceanica*. Plant Physiol 2007;123:1571](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref67)e[82](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref67).
41. [Walters DR. Polyamines and plant disease. Phytochem](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref68) [2003;64:97](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref68)e[107](http://refhub.elsevier.com/S2314-808X(14)00005-0/sref68).