ARTICLE IN PRESS

Journal of the Saudi Society of Agricultural Sciences (2015) **xxx**, xxx–xxx

King Saud University

Journal of the Saudi Society of Agricultural Sciences

www.ksu.edu.sa
www.sciencedirect.com

FULL LENGTH ARTICLE

Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats

Khalil M. Saad-Allah [*], **M.A. Elhaak**

Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt

Received 11 June 2015; revised 14 August 2015; accepted 18 August 2015

KEYWORDS

Solanum nigrum;
Phytoremediation;
Sewage water;
Glycine betaine;
Alkaloids;
Proline

Abstract The present study was conducted to evaluate the pollution phytoremediation capacity and pollution tolerance of *Solanum nigrum* in two habitats varied in their pollution intensity with heavy metals. We investigated the relative contributions of the hyperaccumulator *S. nigrum* in eliminating some pollutants from agricultural soils such as Zn, Pb, Ni and Cd in Tanta and in the sewage water irrigated area at El-Gabal El-Asfar (GA) region. The study also concluded that heavy metals pollution resulted in a significant amendment in the primary and secondary metabolic pathways of the plant. The exposure of *S. nigrum* to heavy metals pollution in GA region resulted in a relative accumulation in soluble sugars, soluble proteins and free amino acids in the plant root, stem and leaves, but their levels in berry were to some extent inferior to those of Tanta region. In addition, the exposure of *S. nigrum* plants to sewage water effluents in GA region improved the accumulation of osmoprotectant and antioxidant molecules such as proline, glycine betaine, flavonoids and phenolic compounds. Moreover, the medicinally active alkaloids have accumulated in response to sewage irrigation in various organs of GA region plants comparable with those of Tanta region.

1. Introduction

Soil pollution by heavy metals has become a severe environmental disaster, causing considerable health problems to humans as well as to ecosystems. Heavy metals in contaminated soils cannot be mineralized or degraded to less toxic forms and accumulate through the food chain. Thus, it requires suitable methods for their elimination (Chen et al., 2014). These heavy metals are released into the environment by a large number of human activities such as electroplating, dyes and pigment manufacturing, wood preservation, leather

---

[*] Corresponding author. Tel.: +20 1004260648; fax: +20 403344512.
E-mail addresses: khalil.saadallah@science.tanta.edu.eg, khalilmahfouz@yahoo.com (K.M. Saad-Allah).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jssas.2015.08.001
1658-077X

Please cite this article in press as: Saad-Allah, K.M., Elhaak, M.A. Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.08.001

tanning industry, manufacture of alloys (Nayak et al., 2015), mining wastewater, solid wastes, soil additives, fertilizers, pesticides and air pollutants (Rhoads et al., 1989).

The phytoremediation could be defined as using plants (including trees and grasses) to remove toxic ions from soil and water to mitigate contaminated areas. Phytoremediation is considered as low-cost technology to remediate the polluted regions (Chen et al., 2014). Hyperaccumulator plants can remediate pollutants through several processes such as adsorption, transport and translocation, hyperaccumulation or transformation and mineralization (Meagher, 2000) without suffering metal toxicity or cell damage. The characteristics that make any species useful for phytoremediation include fast-growth with capability to accumulate large biomass, easier and rapid propagation, abundant root system, high metal accumulation ability, tolerance to harsh local soil condition, and inedibility by livestock (Pandey et al., 2012). About 400 species of natural metal hyperaccumulators belonging to 45 families have been documented in the world. These have an innate capability to absorb metal at levels 100 times greater than average plants (Zhou and Song, 2004). Compared with crops, weed plants demonstrate strong stamina to adverse environmental conditions, and high capacity to absorb water and fertilizers (Wei and Zhou, 2006).

Solanum nigrum (black nightshade) is a relatively fast-growing annual medicinal plant belonging to family Solanaceae (Jain et al., 2011) with an erect angular stem, normal tap root, and ovate leaves with dentate margin. The fruits are dull black and globose in extra-axillary umbel inflorescences (Chauhan et al., 2012). *S. nigrum* was found to be a high-biomass hyperaccumulator (Wei et al., 2006) compared with some well-known hyperaccumulators, and it could withstand high heavy metal concentration in contaminated soils (Luo et al., 2011). It is tolerant to adverse environment, so it could fill the gap of known hyperaccumulating plants (Zhou and Song, 2004). For the preceding reason, we decided studying and contrasting the potential of root to shoot then to berry translocation and the superior ability of *S. nigrum* to detoxify and sequester heavy metals in various organs to insure its possible use in phytoremediation and cleaning up of heavily polluted soils paving the way for the cultivation of such areas. Another possibility for our research targets is to explore the disparity in the medicinal components such as alkaloids, flavonoids and phenolics in sites which are polycontaminated with several metals rather than only one metal.

So, the objectives of the present study were to investigate the hyperaccumulation capacity, distribution, bioaccumulation and biotranslocation of various pollutants in *S. nigrum* organs, including the berry, in two habitats differing in their pollution intensity and contrasting the metabolic response of the plant in the two habitats.

2. Materials and methods

2.1. Sampling

S. nigrum plants were harvested during the summer season of 2014 at the maturity stage (before berry turning black) from two different localities in Egypt. The first one was El-Gabal El-Asfar farm at the northern-east of the Egyptian Delta (30°13′N, 31°23′E) and subjected to sewage effluent irrigation for more than 60 years. However, the second one was Tanta city at the middle of Egyptian Delta (30°47′N, 31°00′E), which is an agricultural field irrigated with water supplied from El-Qassed canal, which is a mixture of Nile water and sewage effluent. In addition to the plant samples, soil samples were collected from the rhizosphere of the growing plants.

The harvested plants were separated into root, stem, leaf and berries and washed with tap water several times, and after that with deionized water. The samples were dried at 60 °C in an air-forced oven until dry to constant weight. The dried plant samples were grounded to powders using an electrical mixer and passed through a 2 mm sieve and stored in paper bags at 4 °C for further analysis. However, soil samples were air-dried, grounded and passed through a 2 mm sieve, and then stored in paper bags at room temperature for further analysis.

2.2. Mineral analysis of plant and soil samples

Plant and soil samples were digested with a mixture of 69% HNO_3 and 30% H_2O_2 (5:2 v/v). The concentrations of heavy metals in digested solutions were determined using inductively coupled plasma-optical emission spectroscopy (Polyscan 61E, Thermo Jarrell-Ash Corp., Franklin, MA, USA) at the Central Lab of Tanta University. The used wavelengths for Cd, Zn, Ni, Pb, Fe, Co, As, Cr and Al were 214.4, 206.2, 231.6, 220.3, 283.2, 238.9, 197.2, 267.7 and 394.4 nm, respectively.

2.3. Phytochemical analysis

2.3.1. Preparation of methanolic extract

The fine powdered plant tissues (5 g for each sample) were extracted with 50 ml of 95% methanol for 12 h at room temperature in an orbital shaker (Panasonic, MIR-S100, Japan). The extracts were filtered through Whatman No. 1 filter paper. The residues were extracted twice again as previously and extracts were combined. The combined extracts were concentrated under reduced pressure at 40 °C using rotary evaporator (Heidolph) and adjusted to 50 ml by methanol. Extracts were stored in glass vials at 4 °C until the time of analysis. All the spectrophotometric measurements were carried out using JENWAY 6315 UV/Visible Spectrophotometer (Japan).

2.3.2. Determination of soluble sugars

The total soluble sugar content in the methanolic extract of different plant parts was measured according to the phenol–sulfuric acid method of Dubios et al. (1956) using glucose as a standard and expressed as mg/g d.wt.

2.3.3. Determination of soluble proteins

Total soluble protein content of the extracts was measured according to Bradford (1976) using bovine serum albumin (BSA) as a protein standard. The extract or the standard protein was mixed with Coomassie brilliant blue G250 reagent, the absorbance was measured at 595 nm and the results were expressed as mg/g d.wt.

2.3.4. Determination of free amino acids

Amino acid content was analyzed by ninhydrin assays in the methanolic extract using glycine as a standard amino acid according to Lee and Takahashi (1966). Samples or standard

Please cite this article in press as: Saad-Allah, K.M., Elhaak, M.A. Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.08.001

glycine was mixed with ninhydrin-citrate buffer-glycerol mixture (0.5 ml 1% ninhydrin solution in 0.5 M citrate buffer pH 5.5, 1.2 ml of 55% (v/v) glycerol solution and 0.2 ml of 0.5 M citrate buffer). The mixture was then shaken and boiled in a water bath for 12 min, cooled, and shaken well, the absorbance was measured at 570 nm and the result was calculated as mg/g d.wt.

2.3.5. *Determination of proline*

The proline contents were estimated by the method of Bates et al. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate mixture was centrifuged at 50,000 rpm. Supernatant was used for estimation of proline contents. The reaction mixture consisted of 2 ml acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100 °C for 1 h, then the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml of toluene and absorbance was read at 520 nm. Concentration of proline was quantified from the standard graph plotted using proline and expressed as mg/g d.wt.

2.3.6. *Determination of glycine betaine contents*

Glycine betaine (GB) content was estimated in various plant parts by the method designed by Grieve and Grattan (1983). The plant tissues were mechanically shaken with 20 ml of deionized water for 24 h at room temperature. The samples were then filtered and the filtrates were diluted (1:1) with 2 N HCl. Aliquots were cooled in ice water for 1 h. Cold KI-I_2 reagents were added and the reactants were gently stirred with a vortex mixture. The tubes were stored at 4 °C for 16 h and then centrifuged at 10,000 rpm for 15 min, while the supernatant was carefully recovered with a fine glass tube. The periodide crystals were dissolved in 9 ml of 1,2- dichloroethane. After 2 h, the absorbance was measured at 365 nm. Glycine betaine content was estimated by using a standard curve and expressed in μg/g d.wt.

2.3.7. *Determination of phenolic compounds*

The total phenolic content in the methanolic extract was determined using the method described by Jindal and Singh (1975). Aliquot from this extract was mixed with 0.1 ml Folin-Ciocalteu's reagent and 1 ml 20% Na_2CO_3, then completed up to a known volume with distilled water. Thereafter, the absorbance was measured at 650 nm after 30 min. A standard graph was prepared using gallic acid as the previous procedures and used for the determination of the total phenolic compound content (mg/g d.wt).

2.3.8. *Determination of flavonoids*

Colorimetric aluminum chloride method was used for flavonoid determination (Chang et al., 2002). Aliquot of 0.5 m1 methanolic extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2 ml of distilled water and left at room temperature for 30 min, then the absorbance of the reaction mixture was measured at 417 nm. Total flavonoid contents were calculated as quercetin from a calibration curve and expressed as mg/g d.wt.

2.3.9. *Determination of alkaloids*

Alkaloids were determined using the method of Harbone (1973). 2.0 g of the dry powders was extracted by 50 ml of 10% acetic acid in ethanol for 4 h at 200 rpm using an orbital shaker (Panasonic, MIR-S100, Japan). This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.3.10. *Statistical analysis*

Data were processed with the Microsoft Excel software. All the values expressed are mean ± standard deviation (SD) based on three replicates. Data were analyzed by one-way ANOVA with the Duncan's multiple range test (DMRT) to separate means with significance levels at $P \leqslant 0.05\%$. All statistical analyses were carried out using SPSS 19.0 software.

3. Results and discussion

The concentrations of various heavy metals (Cd, Zn, Ni, Pb, Fe, Co, As, Cr and Al) in the soils and roots, stems, leaves and berries of *S. nigrum* plants collected from the two differentially polluted habitats, El-Gabal El-Asfar (GA) and Tanta, in Egypt significantly varied (Table 1). Difference was remarkable in heavy metals accumulation and transport from soils to the different organs of *S. nigrum*. The soil of GA, although it has a sandy nature, is heavily polluted with verity of heavy metals due to the sewage water effluent. The most pronounced concentration of metals was recorded for Cr, Pb, Fe, Al, and Zn (50.0, 39.0, 18.8, 8.7 and 4.0 μg/g d. wt, respectively). Also, the soil of Tanta with clayey nature showed to some extent high levels of some heavy metals such as Fe, Cr, As, Al and Co (22.3, 7.5, 6.8, 3.3 and 2.6 μg/g d.wt, respectively). The listed data, identified sewage water irrigation as the apparent cause of the heavily polluted toxic metals in the soil of GA region compared with soil collected from Tanta region. Arsenate, as a very toxic element, is mainly derived from sediments erosion, and is believed to enter in the solution following reductive release from solid phases under anaerobic conditions (Polizzotto et al., 2008). The highest accumulation of heavy metals in GA was achieved by Zn in *S. nigrum* roots and leaves (90 and 46 μg/g d.wt, respectively) and by Pb in stems and berries (71 and 89 μg/g d.wt, respectively). Cobalt, though not detected in GA soil, was detected in *S. nigrum* organs with concentrations ranged from 0.2 mg/g d.wt in the root to 1.6 mg/g d.wt in the berry. Hence, this proves the high hyperaccumulation activity of *S. nigrum* toward some heavy metals such as cobalt and it could be used as the best solution for cleaning the soil from such elements. However, in the plants growing in Tanta region Pb was the principal metal accumulated in roots, stems and leaves (28, 30 and 84 μg/g d.wt, respectively), but in berries it was not appreciable (6.6 μg/g d.wt). This showed responsive role of traffic pollution in the Tanta area as compared with GA area which subjected to industrial or domestic effluents which are known to supply soil as well as crop plants with heavy metals (Chopra et al., 2013). These heavy metals are extremely hazardous due to their non-biodegradable nature, long biological half-lives and their potential to accumulate in different plant parts (Tandi et al., 2005). In *S. nigrum* in the two habitats, it was apparent that

Table 1 Heavy metal concentrations (μg/g d.wt) of soil and organs of *S. nigrum* in GA and Tanta regions.

Metals (μg/g d.wt)	El-Gabal El-Asfar					Tanta				
	Soil	Root	Stem	Leaf	Berry	Soil	Root	Stem	Leaf	Berry
Cd	2.6 ± 0.0^{d}	ND	0.8 ± 0.1^{b}	1.0 ± 0.1^{c}	0.6 ± 0.0^{a}	0.2 ± 0.0^{a}	0.4 ± 0.0^{b}	0.6 ± 0.1^{c}	0.6 ± 0.0^{c}	0.2 ± 0.0^{a}
Zn	4 ± 0.2^{a}	90 ± 1.3^{d}	62 ± 0.9^{c}	46 ± 1.1^{b}	45 ± 2.2^{b}	1.5 ± 0.7^{a}	ND	ND	11 ± 3.1^{c}	4.4 ± 1.0^{b}
Ni	3.4 ± 0.4^{d}	4.8 ± 0.2^{e}	0.8 ± 0.1^{a}	2.4 ± 0.2^{c}	1.2 ± 0.2^{b}	1.2 ± 0.3^{b}	2.6 ± 0.2^{d}	0.8 ± 0.0^{a}	1.4 ± 0.2^{c}	1.2 ± 0.1^{b}
Pb	39 ± 1.9^{a}	ND	71 ± 3.5^{b}	110 ± 5.4^{d}	89 ± 2.7^{c}	ND	28 ± 1.7^{a}	30 ± 3.3^{a}	84 ± 4.2^{b}	ND
Fe	18.8 ± 2.5^{d}	24.8 ± 2.2^{e}	8.0 ± 0.9^{b}	2.4 ± 0.5^{a}	9.5 ± 1.2^{c}	22.3 ± 2.3^{e}	10.5 ± 1.3^{d}	2.2 ± 0.4^{a}	4.6 ± 0.2^{b}	6.4 ± 0.9^{c}
Co	ND	0.2 ± 0.0^{a}	1.2 ± 0.2^{c}	0.4 ± 0.0^{b}	1.6 ± 0.2^{d}	2.6 ± 0.1^{c}	1.2 ± 0.1^{a}	1.6 ± 0.2^{b}	1.2 ± 0.0^{a}	1.6 ± 0.3^{b}
As	2.2 ± 0.2^{a}	ND	ND	4.2 ± 0.3^{b}	ND	6.8 ± 1.1^{c}	ND	ND	5.8 ± 0.6^{a}	6.6 ± 0.3^{b}
Cr	50.0 ± 1.6^{e}	12.0 ± 1.0^{c}	7.6 ± 0.6^{b}	13.0 ± 0.2^{d}	4.2 ± 0.3^{a}	7.5 ± 1.1^{c}	3.6 ± 0.4^{b}	2.8 ± 0.3^{b}	3.2 ± 0.2^{b}	1.8 ± 0.0^{a}
Al	8.7 ± 2.4^{d}	6.1 ± 1.2^{c}	1.7 ± 0.6^{b}	8.0 ± 2.2^{a}	0.9 ± 0.6^{a}	3.8 ± 5.3^{e}	2.0 ± 1.1^{c}	1.3 ± 0.5^{b}	2.6 ± 2.2^{d}	0.5 ± 0.4^{a}

[a] The lowest value.

[e] The highest value.

Different letters indicate significant differences, where the two columns with the same letter are non-significant, while columns with different letters are statistically significant.

the plant has high bioaccumulation activity toward some metals such as Zn, Pb, Ni and Cd in its various tissues to levels exceeding the level of these metals in the soil, supporting the previous research done before by many workers concerning the hyper accumulation activity of *S. nigrum* (Wang et al., 2008; Dwivedi et al., 2014). Also, the structure of the soil has also been found to have a significant consequence on the extent of the metals taken up by plants. Ghosh and Singh (2005) reported that metal solubility in soils is principally controlled by pH, amount of metals, cation exchange capacity, organic carbon content and oxidation state of the system.

Metal hyperaccumulation of *S. nigrum* without showing toxicity symptoms may be attributed to well developed detoxification mechanism based on sequestration of heavy metal ions in vacuoles, by binding them on suitable ligands such as organic acids, proteins and peptides in the presence of enzymes that can function at high level of toxic metals (Cui et al., 2007).

Data represented in Fig. 1 show the total soluble sugar content of *S. nigrum* various organs in two differentially polluted regions; El-Gabal El-Asfar (GA) and Tanta. The sugar content showed progression from root to the berry, where the berry in the two habitats possessed the highest sugar content, nevertheless the root possessed the lowest one. Moreover, the sugar content of root, stem and leaves in GA region is higher than that of the plant organs in Tanta region, although the sugar content of the berry is converse to that. The increase in carbohydrate content in plants irrigated with wastewater is reported by Rong Guo et al. (2007) and Bamniya et al. (2010). Mechri et al. (2011) reported that irrigation of olive trees with olive mill wastewater increased concentration of soluble carbohydrate in the olive leaves, mostly due to decreased sink demand for carbon by the root. However, Rija et al. (2005) reported that the increase in free sugars and protein following wastewater irrigation could be due to the action of some microorganisms found in wastewater which can convert organic substances into by-products such as CO_2, NO_3, PO_4, SO_4, NH_3, H_2S and CH_4. Also, occurrence of elevated amounts of macronutrients in the wastewater provides cofactors to enzymes, essential for the synthesis of proteins and sugars. We suggest that the significant accumulation of soluble carbohydrates in plants of GA region higher than that of Tanta region, especially in the vegetative parts, can be related to

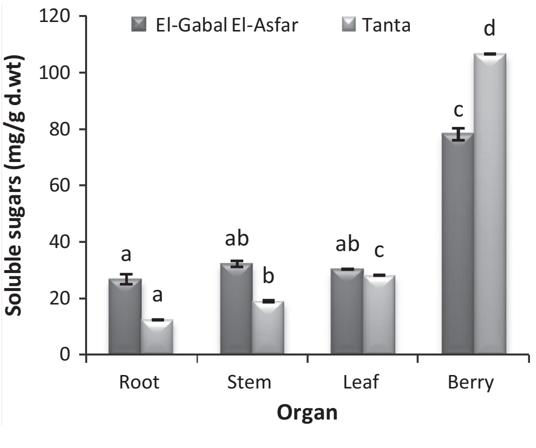

Figure 1 Soluble sugar content of *S. nigrum* various organs growing at GA and Tanta regions.

Please cite this article in press as: Saad-Allah, K.M., Elhaak, M.A. Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.08.001

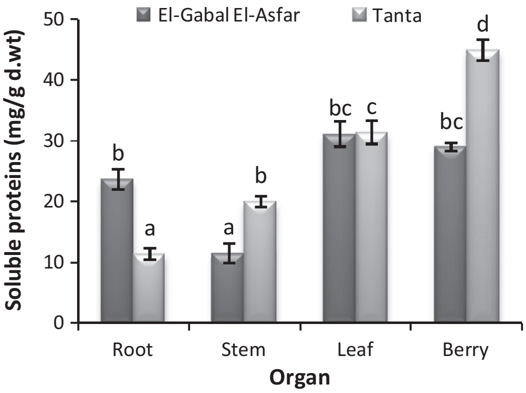

Figure 2 Total soluble protein content of *S. nigrum* various organs growing at GA and Tanta regions.

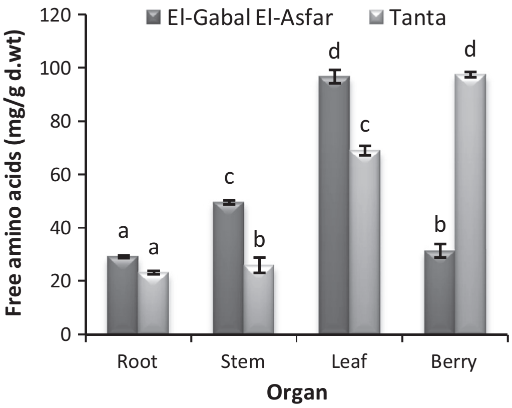

Figure 3 Total free amino acid content of *S. nigrum* various organs growing at GA and Tanta regions.

reduction of the water potential in the vegetative parts, due to osmotic stress by soil pollutants, and the accumulated soluble carbohydrates serve as osmoregulators to improve water uptake.

The data corresponding to soluble protein content of *S. nigrum* growing in El-Gabal El-Asfar region and Tanta region are reported in Fig. 2. Total soluble protein content of the stem and the berry of plants growing in Tanta region are significantly higher than those of GA region; however, that of root followed the reverse trend, although the plant leaves of both regions have the same content of soluble proteins. In general, the uppermost protein content was recorded in the berry of Tanta region and the lowest one was recorded in the root of the same region.

The recorded data illustrated that wastewater irrigation in GA region raised the level of protein content in the root of *S. nigrum*, but reduced that of stem and berry and has no effect on protein content of leaves, in comparison with those of Tanta region. As roots are in direct contact with the soil solution, they are foremost to face excess heavy metals pollution and are potentially the first line of defense. So the induction of the stress response in roots leads to expression of a group of proteins known as stress proteins, which are thought to protect the cell (Gabara et al., 2003). Ozyazici (2013) suggested that such increase in protein content after the sludge treatment might be on the other hand the result of the greater available soil nitrogen levels.

Nevertheless, the reduction in stem and berry protein content in wastewater irrigated *S. nigrum* in GA region is compatible with the results of Ismaiel et al. (2014), who concluded that the decrease in protein content in faba bean plants irrigated with wastewater may be interpreted on the basis of enhanced protein degradation process as a result of increased protease activity. Moreover, John et al. (2009) reported that heavy metals in sewage effluent may result in lipid peroxidation and breakdown of proteins due to toxic effects of ROS which led to reduced protein content. Also, the reduction in total soluble protein content in the stem and berry might be due to its chelating role with heavy metals through the covalent interaction of the sulfhydryl groups of some proteins with the existed root heavy metals to prevent its distribution within the plant.

Amino acids play a key central role in plant metabolism as they connect carbon assimilation with nitrogen metabolism. The data of nitrogen metabolism in the present study indicated that the irrigation of *S. nigrum* with wastewater in El-Gabal El-Asfar region resulted in a significant accumulation of total free amino acids in root, shoot and leaves comparable to plants grown in Tanta region, although the free amino acid content of the berry showed a reverse leaning (Fig. 3). In general context, the highest total free amino acids pool was recorded in the leaves of the two habitats. These results are in accordance with those obtained by Abdel Latef and Sallam (2015), who reported that wastewater irrigation improved the accumulation of total free amino acids and proline contents in maize. Also, Homer et al. (1997) observed the accumulation of amino acids in three hyperaccumulator species, *Walsura monophylla*, *Phyllanthus palwanensis*, and *Dechampetalum geloniodes* in response to heavy metals pollution.

The accumulated free amino acids in plants under stress have important roles in metal toxicity tolerance via detoxifying heavy metals, regulating ion transport, regulating intracellular pH and ion transport, modifying stomatal conductance, and scavenging the ROS (Rai, 2002). To cope with stress, plants evolved some enzymes that convert amino acids, amides, and keto-acids to be used as carbon source when carbon deficiency becomes a limiting factor for growth and development (Miflin and Habash, 2002).

Proline analysis results of *S. nigrum* various vegetative parts in GA and Tanta regions reflected that the uppermost accumulation of proline was in the plant leaves at the two locations, nevertheless the lowest accumulation in GA region was in the berry and in Tanta was in root system. However, plants irrigated with swage water effluent in GA region showed greater accumulation of proline in all studied parts compared to those growing in Tanta region (Fig. 4).

Proline is the most common organic compatible solute that accumulates in higher plants under abiotic stresses and considered as the main mediator of stress tolerance as well as a source of nitrogen and osmoprotectant under stress conditions. The increased proline accumulation under different stresses might be due to either *de novo* synthesis or decreased degradation or both (Kasai et al., 1998). Accumulation of proline after wastewater irrigation was reported in many plant species such as *Beta vulgaris* (Singh and Agrawal, 2010), *Triticum aestivum* (Kumar et al., 2012), *Cicer arietinum* and *Vigna radiata* (Iti and Angoorbala, 2014). It has established that heavy metals

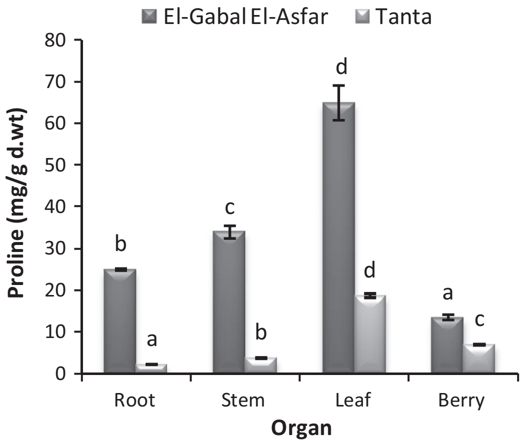

Figure 4 Free proline content of *S. nigrum* various organs growing at GA and Tanta regions.

in wastewater affect permeability of plant membranes, which possibly result in water stress like condition stimulating the production of proline (Basak et al., 2001). From this study, it could be suggested that proline accumulation might provide osmotic adjustment, enzyme protection, maintenance of membranes fluidity and scavenging stress mediated ROS promoting well growth under sewage effluent irrigation.

Highly significant variations were observed among *S. nigrum* organs in glycine betaine (GB) content with respect to the growth habitat (Fig. 5). Heavy metals-highly polluted soil in GA region caused a greater accumulation of GB in *S. nigrum* organs comparable to those of Tanta region. The elevation in GB level in GA region was about three-fold in all organs, except in the stem it was about half-fold, than that of Tanta region.

Accumulation of specific metabolites such as proline, glutathione and glycine betaine is a good marker for the intensity of the stress possessed by plants. GB is an important quaternary ammonium compound and is considered as the most efficient osmoprotectant (Burnet et al., 1995). GB was reported to play a significant role in improving plant tolerance to many environmental stresses such as salt, drought and high temperature (Quan et al., 2004). Raza et al. (2007) concluded that GB promotes plant growth and yield under normal or stress conditions as it has osmoprotective effect on photosynthetic machinery and has an important role in regulating ion homeostasis. Also, Taiz and Zeiger (2006) showed that GB might be involved in the transport of photoassimilates due to its role in biosynthesis and transport of some hormones such as cytokinins. Bharwana et al. (2014) in their study on cotton showed that GB alleviated Pb toxicity via enhancing the chlorophyll synthesis, photosynthetic activities, antioxidant enzyme activities and membrane stability and via reducing lipid peroxidation and hydrogen peroxide levels.

Data shown in Fig. 6 summarize the results of flavonoids quantification in *S. nigrum* organs at two different growth habitats varying in their heavy metals pollution intensity as a result of variation in the source of irrigation water. The data cleared out that heavy metals pollution in wastewater at GA region resulted in a significant increase in flavonoid content of root, stem and leaves comparable with their contents in plants of Tanta region. However, the berry showed the superiority of flavonoid content in Tanta region grown plants over those of GA region.

The increase in flavonoid content, after wastewater irrigation reported in this study, is in accordance with the results obtained by Hassanein et al. (2013), who reported that irrigation with industrial wastewater resulted in a significant increase in total phenolics and flavonoid content in lettuce and turnip. They attributed this increase in flavonoids to their powerful chelating properties which enable them to bind with transition metals in chelate complexes. Also, Bai et al. (2004) confirmed that flavonoids are natural metal chelators through their interaction with heavy metals to produce complexes that prevent the metal ions from contribution in free-radical generation. In addition to their metal-chelating properties, flavonoids are reported as antioxidant agents by scavenging ROS. Their potential as antioxidants depends on the reduction potentials of their radicals and accessibility of the radicals (Heim et al., 2002). Furthermore, modification of flavonoids structure by glycosylation, prenylation and methylation might influence their antioxidant properties enabling them inhibit lipid peroxidation and biomolecules damage in stress imposed plants (Caturla et al., 2003).

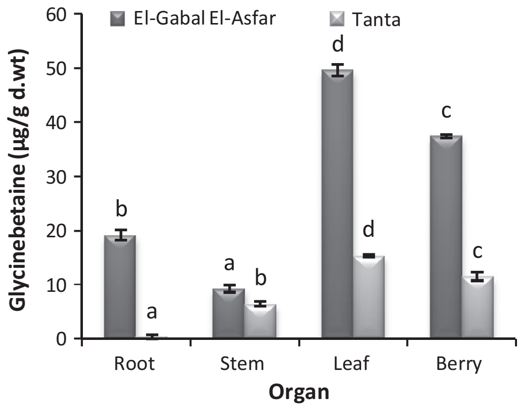

Figure 5 Glycine betaine content of *S. nigrum* various organs growing at GA and Tanta regions.

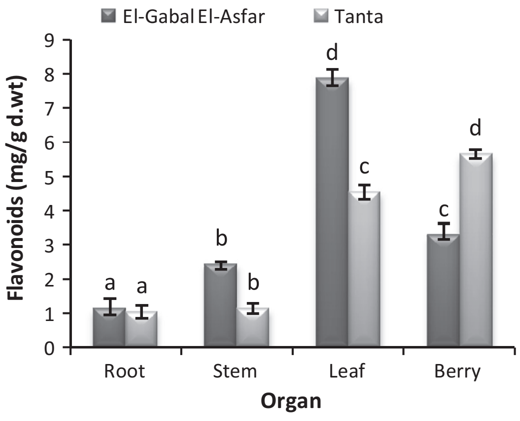

Figure 6 Total flavonoid content of *S. nigrum* various organs growing at GA and Tanta regions.

Please cite this article in press as: Saad-Allah, K.M., Elhaak, M.A. Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.08.001

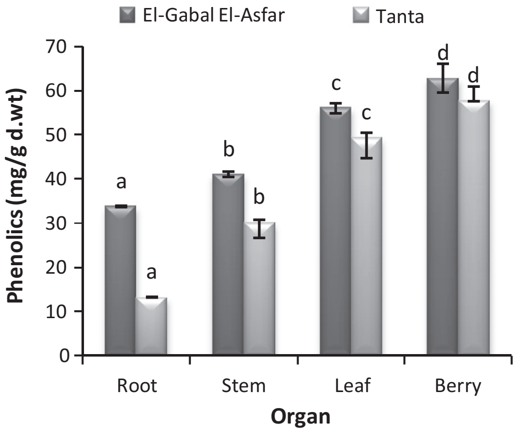

Figure 7 Total phenolic content of *S. nigrum* various organs growing at GA and Tanta regions.

Fig. 7 presents data of total phenolic compounds of *S. nigrum* collected from GA and Tanta regions at the level of root, stem, leaves and berries. The data showed a significant gradual increase in phenolics from the root to the direction of berries, with the superiority of phenolics in GA regions over those of Tanta region in all studied organs. So, the present data establish conclusive evidence that pollution of irrigation water with heavy metals is combined with accumulation of protective antioxidant compounds such as phenolics and flavonoids.

Accumulation of phenolic compounds in response to soil pollution seems to be a general protective mechanism followed by many plant species. These compounds have strong antioxidant activity and are involved in the defense mechanism of plants in response to biotic and abiotic stresses (Dudjak et al., 2004). This increase interrelated to the increase in activity of some enzymes responsible for phenolic compounds *de novo* synthesis especially under heavy metal stress (Michalak, 2006). The antioxidative properties of phenolic components have been attributed to their potential to chelating transition metal ions, the inhibition of superoxide-driven Fenton reaction, hindering the diffusion of free radicals and modifying lipid peroxidation kinetics in membranes by altering lipid packing order (Arora et al., 2000).

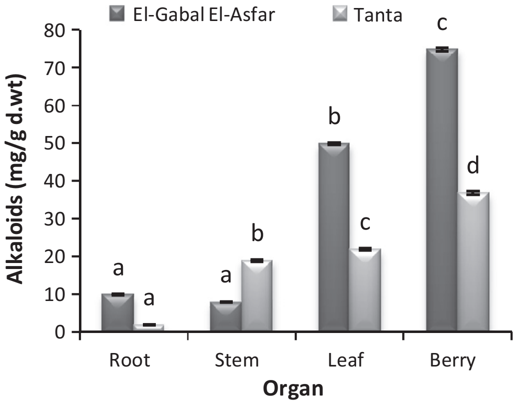

Figure 8 Total alkaloid content of *S. nigrum* various organs growing at GA and Tanta regions.

Response of total alkaloid content to the intensity of heavy metals pollution in *S. nigrum* different organs is shown in Fig. 8. The pattern of alkaloid content has followed the same one possessed by phenolic compounds, where alkaloid content showed a significant gradual enhancement from the root to the berry with the superiority of its content in GA region over that in Tanta region. The recorded data revealed that the pollution of wastewater with heavy metals resulted in at least two-fold increase in total alkaloid content in *S. nigrum* grown in GA region at the level of the studied organs in comparison with those of Tanta region.

S. nigrum is a valuable medicinal plant and its medicinal significance is mainly accredited to the alkaloidal contents especially solanine, which is a neurotoxic glycoalkaloid (Abbas et al., 1998). Solanine is found naturally in all the plant parts and is considered as the plant's major natural defenses as it is toxic even in small quantities (Lin et al., 2007). Recently, Ravi et al. (2009) reported that the berry of *S. nigrum* contains four steroidal alkaloid glycosides namely, solamargine, solasonine, α and β solanigrine. A number of previous studies focused on the response of alkaloidal content in plants to heavy metals pollution in the growth medium. Abd-El-Kareem et al. (2011) in their study on *Catharanthus roseus* concluded that the highest total alkaloids accumulation was recorded when the shoot and root calli were supplemented with 0.8 mM $HgCl_2$. Abo-Hamad et al. (2013) reported that exposure of *Brassica rapa* to cadmium stress resulted in a progressive accumulation of total alkaloids in root and shoot system with the increase of cadmium concentration. It is well established that stress exposure results in a major shift in metabolic activities toward protective secondary metabolite accumulation. Moons et al. (1997) explained the possible increase in alkaloids under abiotic stress as a consequence of increment in the level of endogenous methyl jasmonate, which can catalyze the enzymes involved in alkaloids biosynthesis. The accumulated alkaloids are involved in defensive mechanism against stress in the plant (Gogoi and Islam, 2012).

4. Conclusion

Pollution of irrigation water with heavy metals is one of the most important problems facing the civil societies in the modern age. The risk here is not only represented in reduced agricultural production and efficiency, but the health problems facing human through the entrance of these pollutants into the food chain, resulting in a lot of chronic diseases. Thus it has become a necessity to explore scientific solutions to the problem of water pollution, either using water treatment technology or using non-edible hyperaccumulator plants that can get rid of the heavy metals found in agricultural soil. The results presented in this paper highlighted and quantified the response of the hyperaccumulator *S. nigrum* to heavy metals pollution in terms of phytoremediation capacity and metabolic activity. Elemental analysis of the soil and various plant organs revealed that *S. nigrum* could accumulate heavy metals in its organs to an extent higher than that of the polluted soil. Furthermore, presence of high concentrations of metal pollutants in the growth medium altered several physiological and biochemical processes in the plant organs assisting the plant to withstand such drastic environment. These mechanisms are represented in accumulating higher quantities of soluble

Please cite this article in press as: Saad-Allah, K.M., Elhaak, M.A. Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.08.001

sugars, proteins, proline, glycine betaine, flavonoids, phenolics and alkaloids, in order to minimize the deleterious impact of the persistent heavy metals. Although the plant in the polluted area acquired a marked accumulation of important medicinal compounds, its utilization could not be saved due to the accumulated heavy metals in its organs especially roots. The plant fruits acquire the least pollutant accumulation.

Conflict of interest

Authors confirm that there are no known conflicts of interest associated with this publication.

References

Abbas, K.H., Paul, R.N., Riley, R.T., Tanaka, T., Shier, W.T., 1998. Ultrastructural effects of AAL-toxin TA from the fungus *Alternaria alternaria* on black nightshade (*Solanum nigrum* L.) leaf disc and correlation with biochemical measures of toxicity. Toxicon. 36 (12), 1821–1832.

Abdel Latef, A.A., Sallam, M.M., 2015. Changes in growth and some biochemical parameters of maize plants irrigated with sewage water. Austin J. Plant Biol. 1 (1), 1004.

Abd-El-Kareem, F.M., Abd-El Kawy, A.M., Taha, H.S., 2011. Effect of heavy metal (HgCl2) on accumulation and production of total indole alkaloids, vinblastine and/or vincristine from Egyptian *Catharanthus Roseus* (L.) G. Don. Calli Cultures. J. App. Sci. Res. 7 (4).

Abo-Hamad, S., Saad-Allah, K., Abo-Kassem, E., 2013. Effect of gamma irradiation or potassium on some primary and secondary metabolites of *Brassica rapa* (L.) root under cadmium stress. Int. Res. J. Agri. Sci. Soil Sci. 3 (12), 408–415.

Arora, A., Byrem, T.M., Nari, M.G., Strasburg, G.M., 2000. Modulation of liposomal membranes fluidity by flavonoids and isoflavonoids. Arch. Biochem. Biophys. 373, 102–109.

Bai, Y., Song, F., Chen, M., Xing, J., Liu, Z., Liu, S., 2004. Characterization of the rutin-metal complex by electrospray ionisation tandem mass spectrometry. Anal. Sci. 20, 1147–1151.

Bamniya, B.R., Kapasya, V., Kapoor, C.S., 2010. Physiological and biochemical studies on the effect of waste water on selected crop plants. Biol. Forum Int. J. 2 (2), 1–3.

Basak, M., Sharma, M., Chakraborty, U., 2001. Biochemical responses of *Camellia sinensis* (L.) O. Kuntze to heavy metal stress. J. Environ. Biol. 22, 37–41.

Bates, L.S., Waldern, R.P., Teare, I.D., 1973. Rapid assay for determination of free proline for water stress studies. Plant Soil. 39, 205–207.

Bharwana, S.A., Ali, S., Farooq, M.A., Iqbal, N., Hameed, A., Abbas, F.M., Ahmad, S., 2014. Glycine betaine-induced lead toxicity tolerance related to elevated photosynthesis, antioxidant enzymes suppressed lead uptake and oxidative stress in cotton. Turk. J. Bot. 38, 281–292.

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

Burnet, M., Lafontaine, P.J., Hanson, A.D., 1995. Assay, purification and partial characterization of Choline monoxygenase from spinach. Plant Physiol. 168, 581–588.

Caturla, N., Vera-Samper, E., Villalain, J., Reyes Mateo, C., Micol, V., 2003. The relationship between the antioxidant and antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. Free Rad. Biol. Med. 34, 648–662.

Chang, C., Yang, M., Wen, H., Chern, J., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 10, 178–182.

Chauhan, R., Ruby, K., Shori, A., Dwivedi, J., 2012. *Solanum nigrum* with dynamic therapeutic role: a review. Int. J. Pharm. Sci. Rev. Res. 15 (1), 65–71.

Chen, L., Luo, S., Li, X., Wan, Y., Chen, J., Liu, C., 2014. Interaction of Cd-hyperaccumulator *Solanum nigrum* L. and functional endophyte *Pseudomonas* sp. Lk9 on soil heavy metals uptake. Soil Biol. Biochem. 68, 300–308.

Chopra, A.K., Srivastava, S., Kumar, V., Pathak, C., 2013. Agro-potentiality of distillery effluent on soil and agronomical characteristics of *Abelmoschus esculentus* L. (okra). Environ. Monit. Assess. 185 (8), 6635–6644.

Cui, S., Zhou, Q., Chao, L., 2007. Potential hyper-accumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China. Environ. Geol. 51, 1043–1048.

Dubios, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Dudjak, J., Lachman, J., Miholova, D., Kolihova, D., Pivec, V., 2004. Effect of cadmium on polyphenol content in young barley plants (*Hordeum vulgare* L.). Plant Soil Environ. 50, 471–477.

Dwivedi, G.K., Upadhyay, S.K., Mishra, A.K., Singh, K.A., 2014. Hyper accumulation of cadmium in *Solanum nigrum* L. and their effects on phyto-chemicals and antioxidant enzymatic activities. Int. J. Pharm. Sci. Res. 5, 1424–1430.

Gabara, B., Sklodowska, M., Wyrwicka, A., Glinska, S., Gapinska, M., 2003. Changes in the ultrastructure of chloroplasts and mitochondria and antioxidant enzyme activity in *Lycopersicon esculentum* Mill. leaves sprayed with acid rain. Plant Sci. 164 (4), 507–516.

Ghosh, M., Singh, S.P., 2005. A review on phytoremediation of heavy metals and utilization of its byproducts. App. Eco. Environ. Res. 3, 1–18.

Gogoi, P., Islam, M., 2012. Phytochemical screening of *Solanum nigrum* L. and *S. myriacanthus* Dunal from districts of upper Assam, India. IOSR J. Pharm. 2, 455–459.

Grieve, C.M., Grattan, S.R., 1983. Rapid assay for determination of water soluble quanternary ammonium compounds. Plant Soil. 70, 303–307.

Harbone, J.B., 1973. Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, pp. 185–186.

Hassanein, R.A., Hashem, H.A., El-Deep, M.H., Shouman, A., 2013. Soil contamination with heavy metals and its effect on growth, yield and physiological responses of vegetable crop plants (Turnip and Lettuce). J. Stress Physio. Biochem. 9 (4), 145–162.

Heim, K.E., Tahliaferro, A.R., Bobilya, D.J., 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13, 572–584.

Homer, F.A., Reeves, R.D., Brooks, R.R., 1997. The possible involvement of amino acids in nickel chelation in some nickel accumulating plants. Curr. Top. Phytochem. 14, 31–33.

Ismaiel, A.A., Hegazy, H.S., Azb, M.A., 2014. Physiological response of *Vicia faba* L. to inoculation with *Rhizobium* and arbuscular mycorrhizal fungi: comparative study for irrigation with Nile water and wastewater. Aust. J. Crop Sci. 8 (5), 781–790.

Iti, R., Angoorbala, B., 2014. Study the stress of treated pharmaceutical effluent on peroxidase, MDA and proline content of brown gram (*Cicer arietinum*) and Moong (*Vigna radiata*). Int. Res. J. Environ. Sci. 3 (6), 1–4.

Jain, R., Sharma, A., Gupta, S., Sarethy, I.P., Gabrani, R., 2011. *Solanum nigrum*: current perspectives on therapeutic properties. Altern. Med. Rev. 16 (1), 78–85.

Jindal, K.K., Singh, R.N., 1975. Phenolic content in male and female *Carica papaya*: a possible physiological marker for sex identification of vegetable seedlings. Physiol. Plant. 33, 104–107.

John, R., Ahmad, P.J., Gadgil, K., Sharma, S., 2009. Heavy metal toxicity: effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. Int. J. Plant Prod. 3, 65–75.

Please cite this article in press as: Saad-Allah, K.M., Elhaak, M.A. Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.08.001

Kasai, Y., Kato, M., Aoyama, J., Hyodo, H., 1998. Ethylene production and increase in 1-amino- cyclopropane-1-carboxylate oxidase activity during senescence of broccoli florets. Acta Horti. 464, 153–157.

Kumar, C.S., Singh, A., Sagar, R.K., Negi, M.P.S., Maurya, J.N., 2012. Study of indole acetic acid and antioxidant defense system of wheat grown under sewage water. Int. J. Environ. Sci. 3 (2), 821–832.

Lee, Y.P., Takahashi, T., 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. Anal. Biochem. 14 (1), 71–73.

Lin, H.M., Tseng, H.C., Wang, C.J., Chyau, C.C., Liao, K.K., Peng, P.L., Chou, F.P., 2007. Induction of autophagy and apoptosis by the extract of *Solanum nigrum* Linn in HepG2 cells. J. Agric. Food Chem. 55, 3620–3628.

Luo, S.L., Chen, L., Chen, J.L., Xiao, X., Xu, T.Y., Wan, Y., Rao, C., Liu, C.B., Lai, C., Zeng, G.M., 2011. Analysis and characterization of cultivable heavy metal resistant bacterial endophytes isolated from Cd-hyperaccumulator *Solanum nigrum* L. and their potential use for phytoremediation. Chemosphere 85, 1130–1138.

Meagher, R.B.P., 2000. Phytoremediation of toxic elemental and organic pollutants. Curr. Opin. Plant Biol. 3, 153–162.

Mechri, B., Cheheb, H., Boussadia, O., Attia, F., Mariem, F., Braham, M., Hammami, M., 2011. Effects of agronomic application of olive mill wastewater in a field of olive trees on carbohydrate profiles, chlorophyll a fluorescence and mineral nutrient content. Environ. Exp. Bot. 71, 184–191.

Michalak, A., 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish J. Environ. Stud. 15 (4), 523–530.

Miflin, B.J., Habash, D.Z., 2002. The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. J. Exp. Bot. 53 (370), 979–987.

Moons, A., Prinsen, E., Bauw, G., Montagu, M.V., 1997. Antagonistic effects of abscisis acid and jasmonates on salt stress induced transcripts in rice roots. Plant Cell. 9, 2243–2259.

Nayak, A.K., Jena, R.C., Jena, S., Bhol, R., Patra, H.K., 2015. Phytoremediation of hexavalent chromium by *Triticum aestivum* L. Sci. Agri. 9 (1), 16–22.

Ozyazici, M.A., 2013. Effects of sewage sludge on the yield of plants in the rotation system of wheat-white head cabbage-tomato. Eurasian J. Soil Sci. 2, 35–44.

Pandey, V.C., Singh, K., Singh, J.S., Kumar, A., Singh, B., Singh, R.P., 2012. *Jatropha curcas*: a potential biofuel plant for sustainable environmental development. Renew. Sust. Energy Rev. 16, 2870–2883.

Polizzotto, M.L., Kocar, B.D., Benner, S.G., Sampson, M., Fendorf, S., 2008. Near-surface wetland sediments as a source of arsenic release to ground water in Asia. Nature 454, 505–508.

Quan, R.D., Shang, M., Zhang, H., 2004. Improved chilling tolerance by transformation with betA gene for the enhancement of glycinebetaine synthesis in maize. Plant Sci. 166, 141–149.

Rai, V., 2002. Role of amino acids in plant responses to stresses. Biol. Plantarum. 45 (4), 481–487.

Ravi, V., Saleem, T.S.M., Patel, S.S., Ramamurthy, J., Gauthaman, K., 2009. Anti-inflammatory effect of methanolic extract of *Solanum nigrum* linn berries. Int. J. Appl. Res. Nat. Prod. 2 (2), 33–36.

Raza, S.H., Athar, H.R., Ashraf, M., Hameed, A., 2007. Glycinebetaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. Environ. Exp. Bot. 60, 368–376.

Rhoads, F.M., Olson, S.M., Manning, A., 1989. Copper toxicity in tomato plants. J. Environ. Qual. 18, 195–197.

Rija, H., Siddigui, Z.S., Zaman, A.U., 2005. Changes in chlorophyll, protein and carbohydrate contents of *Vigna radiate*, *Cicer arietinum* and *Lens culinaris* after irrigation with sewage water. Int. J. Biol. Biotech. 2, 981–984.

Rong Guo, T., Guo Ping, Z., Yan Hua, Z., 2007. Physiological changes in barley plants under combined toxicity of aluminum, copper and cadmium. Colloids Surf. B. Biointerf. 57, 182–188.

Singh, A., Agrawal, M., 2010. Effects of municipal waste water irrigation on availability of heavy metals and morpho-physiological characteristics of *Beta vulgaris* L. J. Environ. Biol. 31 (5), 727–736.

Taize, L., Zeiger, E., 2006. Plant Physiology, fourth ed. Sinauer Associates, Inc., Sunderland, MA, USA.

Tandi, N.K., Nyamangara, J., Bangira, C., 2005. Environmental and potential health effects of growing vegetables on soil irrigated using sewage sludge and effluent: a case of Zn and Cu. J. Environ. Sci. Health. 39 (3), 461–471.

Wang, L., Zhou, Q., Ding, L., Sun, Y., 2008. Effect of cadmium toxicity on nitrogen metabolism in leaves of *Solanum nigrum* L. as a newly found cadmium hyperaccumulator. J Hazard. Mater. 15, 818–825.

Wei, S.H., Zhou, Q.X., 2006. Phytoremediation of cadmium-contaminated soils by *Rorippa globosa* using two-phase planting. Environ. Sci. Pollut. Res. 13, 151–155.

Wei, S.H., Zhou, Q.X., Koval, P.V., 2006. Flowering stage characteristics of cadmium hyperaccumulator *Solanum nigrum* L. and their significance to phytoremediation. Sci. Total Environ. 369, 441–446.

Zhou, Q.X., Song, Y.F., 2004. Principles and Methods of Contaminated Soil Remediation. Science Press, Beijing, China.

Please cite this article in press as: Saad-Allah, K.M., Elhaak, M.A. Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.08.001