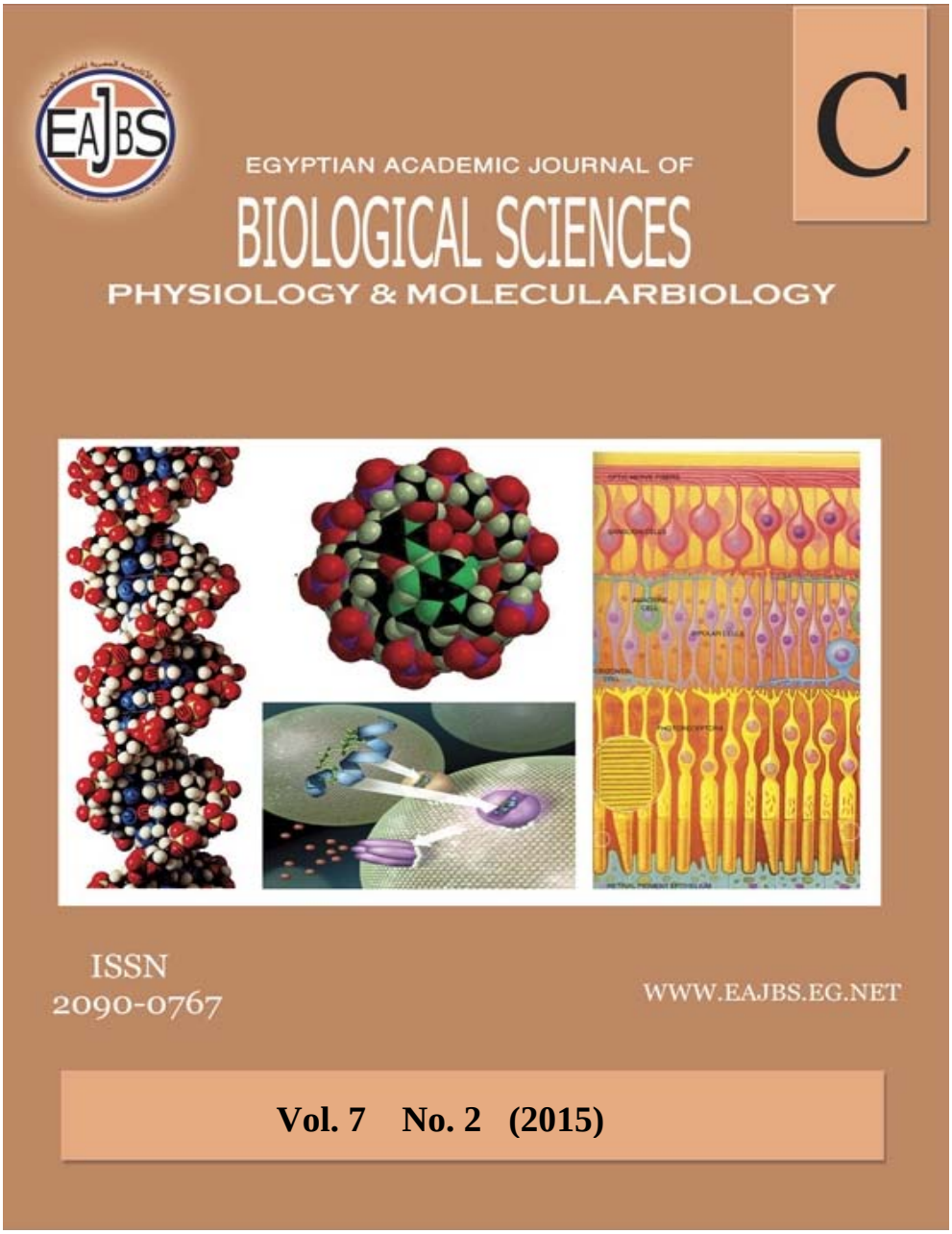

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Growth and Physiological Responses of Wheat Seedlings to Cadmium Alone and in Combination with SiO_2 Nanoparticles

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ABSTRACT

Heavy metals are the major environmental pollutants, mainly in areas with high anthropogenic activities. In this study, the effects of three cadmium (Cd) concentrations (30, 60, and 120 $mg^{-1}l$) alone and in combination with two concentrations of SiO_2 nanoparticles (10 and 100 $mg^{-1}l$) on growth and some physiological parameters of wheat (*Triticum aestivum*) seedlings were investigated. Cadmium treatments decreased the fresh and dry weight of roots, shoots, chlorophyll, carotenoid and total protein contents of the leaf tissues significantly. It also increased the amount of proline, lipid peroxidation and catalase activity of wheat seedling. The toxic effects of cadmium ions on growth and physiological activities of wheat seedlings were reduced in the presence of SiO_2 nanoparticles.

INTRODUCTION

Environmental pollution caused by heavy metals as the side effects of advanced technology, is one of the great concerns in industrial and semi-industrial countries. Unlike organic pollutants, heavy metals are not biologically degradable and as a result, their accumulation in the environment will have grave consequences for plants, animal and man lives. In general, heavy metals or metalloids refer to elements with atomic density of greater than 5 g/cm^3 which are mostly toxic to biological systems at low concentrations.

Heavy metals adversely affect various plants physiological and biochemical processes such as growth and biomass production, transpiration, photosynthesis, biosynthesis of important macromolecules, cell membranes integrity and functions (Wahid *et al*. 2008).

By binding to SH groups of amino acids at the enzymes active site, heavy metals inhibit enzymes activity (Steffens 1990), inhibit protein synthesis (Assche and Clijsters 1990) and cause the production of various reactive oxygen species (ROS) (Dietz *et al*. 1999). The extent of heavy metals damages depends up on plants species,

their age and developmental stage, pH, specific metals, concentrations and chemical forms (Keller *et al*. 2003). One of the criteria to evaluate the toxicity of heavy metals on plants growth and development is to treat plants with these metals at the early stages of growth, especially during seed germination and early seedlings growth (Li *et al*. 2005).

Cadmium is highly soluble in water and it is not an essential element for plants. Its effects on higher plants by several ways: blocking signaling receptors (Beyersmann and Hechtenberg 1997; Monroe and Halvorsen 2006), inhibiting enzymes action e.g. kinase, phosphatase, nitrate reductase, and ATP synthatase. The inhibiting enzymes, involved in photosynthetic CO_2 fixation (Herbette *et al*. 2006; Sharma and Dubey 2005), inducing oxidative stresses (Schützendübel and Polle 2002), disturb the metabolic activities including leaves chlorosis, growth inhibition, roots tips necrosis, cells membrane permeability, roots ions uptake and transport to shoots (Påhlsson 1989; Sanita di Toppi and Gabbrielli 1999).

According to Arnon and Stout (Arnon and Stout 1939), silicon is not classified as an essential element for all higher plants, but it is one of the beneficial element for number of plants species, that improves their growth and resistance to biotic and abiotic stresses (Ma *et al*. 2001; Ma 2004; Okuda and Takahashi 1965). In most plants especially monocots, it acts as physic mechanical barrier, by depositing on the walls of epidermal cells, vascular tissues, of the stem, leaf sheath and controlling plants physiological activities (Ma 2004; Reynolds *et al*. 2009).

In the present study, we evaluated some of the physiological parameters of young wheat seedlings affected by cadmium alone and in combination with SiO_2 nanoparticles.

MATERIAL AND METHODS

Plant materials and growth conditions

Seeds of wheat (*Triticum aestivum* L. var. Chamran) were obtained from Zarghan Agricultural Research Center, Iran. They were surface sterilized by soaking in 5% (w/v) sodium hypochlorite for 10 minutes and washed three times with distilled water then air-dried on filter papers. Seeds were allowed to germinate in the dark at 25°C on moist filter papers. Twenty of five-day old seedlings were transferred into small plastic containers filled with perlite and Hoagland nutrient solution (pH 6.2). Wheat seedlings were grown in growth chamber set at 16 h/8 h light-dark periods. Three replicates were used for each treatment.

Cadmium chloride and SiO_2 nanoparticles treatments

SiO_2 nanoparticles with average sizes of 20 nm and 99.5% purity were purchased from US Research Nanomaterials, lnc. (USA). Using Hoagland nutrient solution as solvent, two concentrations (10 and 100 mg/L) of SiO_2 nanoparticles were prepared. The dissolved particles were dispersed by a high-power probe-type Sonicator (Misonix, Q Sonica LLC, Newton, USA) for 30 minutes. Cadmium was used as $CdCl_2$ (Sigma-Aldrich). Wheat seedlings (21-day old) were collected one week after the beginning of treatments, washed with double-distilled water and used for analyses. Roots and shoots fresh and dry weight, chlorophyll and carotenoid pigments, catalase activity, lipid peroxidation, proline and leaves total protein contents were the analyzed parameters.

Seedlings fresh and dry weight

After washing with distilled water, wheat seedlings were blotted dry on tissue papers, their fresh weights, were measured and dried at 70 C for 48 h for dry weight analysis.

Photosynthetic Pigment Measurement

The contents of photosynthetic pigments were determined using Lichtenthaler and Wellburn methods (Wellburn and Lichtenthaler 1984). Fresh leaf tissue (200 mg) was weighed and powdered using liquid nitrogen. After adding 80% acetone, the volume was brought to 25 ml. This solution was centrifuged at 4800 rpm for 20 min. The supernatant were used for measuring the chlorophyll *a*, *b*, and carotenoid. Absorbance of the clear supernatant was read at 645 nm (chlorophyll *b*), 663 nm (chlorophyll *a*), and 470 nm (carotenoid).

Protein determination

Soluble protein was quantified according to Bradford (Bradford 1976). Samples were homogenized in 0.1 M Na-phosphate buffer (pH 7; 1:5 w/v). After adding the reagent, absorbance was recorded at 595 nm and the concentration was calculated using a calibration curve made with bovine serum albumin. Protein concentrations were determined after realizing a standard curve.

Proline determination

Free proline content was measured by the method of Bates (Bates *et al*. 1973). Fresh leaf tissue (100 mg) was homogenized in 3% (w/v) sulphosalicylic acid and proline was estimated by ninhydrin reagent (0.125 g of ninhydrin in 2 ml orthophosphoric acid 6 M, and 3 ml of acetic acid). The earned chromophore was extracted from liquid phase by toluene and remarking the organic layer at 520 nm. Proline concentrations were determined after realizing a standard curve.

Catalase determination

Catalase (CAT) activity was determined by decomposition of H_2O_2 and measured by a decrease in absorbance at 240 nm (Aebi 1984). The reaction mixture contained 200 mM KPO_4 buffer (pH 7.0), 30 mM H_2O_2 and enzyme extract. Catalase activity was calculated using Aebi formula and H_2O_2 decomposed g^{-1} FW min^{-1} was defined as a unit of CAT.

Lipid peroxidation

The lipid peroxidation in the leaf tissue was measured by malondialdehyde (MDA). Malondialdehyde was assayed by Thiobarbituric acid reactive substances contents (Heath and Packer 1968).

Statistical analysis

The experimental designs were randomized in a complete block and each reporte value corresponds the average of three repeats. The raw data were imported into Microsoft Excel 2007 program for calculations and graphic representation. SPSS (version 16.0) software was used for analysis of variance. Quantitative changes of parameters were analyzed by analysis of variance (one-way ANOVA, corresponds), using Duncan's multiple range tests at $P \leq 0.05$ to find out significant differences among treatments. The results are presented as the means ± standard deviation (SD).

RESULTS AND DISCUSSION

Plant growth

After one-week of exposure, three Cd concentrations alone and in combination with two concentrations of SiO_2 nanoparticles of fresh and dry weight of root and shoot of *T. aestivum* L. were measured (Table 1 & 2 and, Figures 1 & 2). A clear and significant growth inhibition was observed.

Based on the current results almost in all treatments, roots and shoot fresh and dry weight of wheat seedlings decreased significantly with increase in Cd concentrations, comparing with control. The amounts of root plus shoot fresh weight, in cadmium treated plants decreased approximately 28, 40 and, 54%, and, root plus shoot dry weight, decreased 31, 48 and, 64%, at 30, 60, and, 120 $mg^{-1}l$ $CdCl_2$ concentrations comparing with the control, respectively. Based on our findings, concentration of SiO_2 nanoparticles at 50 and 100 $mg^{-1}l$ in combination with Cd treatment, partially

reduced the adverse effects of cadmium ions on wheat seedlings. Alleviated effects of SiO_2 nanoparticles in 50 $mg^{-1}l$ concentrations were fairly more than 100 $mg^{-1}l$. In general, Cd in combination with SiO_2 nanoparticles increased root plus shoot fresh weight, approximately 36 and 28%, in 50 mg-^{l}l concentration and, 45 and 39%, in 100 mg-^{l}l concentration compared to the Cd alone. Reduction in the biomass of wheat in all Cd concentrations, supports the outcomes of previous studies regarding the effect of heavy metals on other plants.

Reduction in the biomass of wheat in all concentration of Cd as a result of our study supported the outcomes of found in the study of the effect of heavy metals on other plant (Glick 2003; de Albuquerque Lima *et al*. 2011; Påhlsson 1989; Peralta *et al*. 2001).

There is also evidence for alleviation effects of silicon and silicon oxide in some biotic and abiotic stresses as it has been reported earlier (Eraslan *et al*. 2008; Li *et al*. 2004; Masarovič *et al*. 2012; Mohaghegh *et al*. 2011; Nwugo and Huerta 2008; Savvas *et al*. 2007).

Table 1: Shoot, root and shoot + root fresh weights (mg) in *T. aestivum* plants subjected to three Cd concentrations alone and in combination with two concentrations of SiO_2 nanoparticles treatments for 7 days period.

Concentration (mg L^{-1})	**Shoot fresh weight**	**Root fresh weight**	**Shoot + Root fresh weight**
Control	0.435 ± 0.0213a	0.109 ± 0.0058b	0.545 ± 0.0155a
$CdCl_2$ 30	0.297 ± 0.0084d	0.090 ± 0.0037c	0.388 ± 0.0089d
$CdCl_2$ 60	0.233 ± 0.0101e	0.090 ± 0.0045c	0.324 ± 0.0134f
$CdCl_2$ 120	0.175 ± 0.0046f	0.070 ± 0.0023d	0.246 ± 0.0069g
$CdCl_2$ 30 + SiO_2 nanoparticles 50	0.408 ± 0.0140ab	0.117 ± 0.0062a	0.525 ± 0.0195a
$CdCl_2$ 60 + SiO_2 nanoparticles 50	0.348 ± 0.0098c	0.097 ± 0.0030b	0.446 ± 0.0129c
$CdCl_2$ 120 + SiO_2 nanoparticles 50	0.254± 0.0100e	0.085 ± 0.0032c	0.339 ± 0.0087e
$CdCl_2$ 30 + SiO_2 nanoparticles 100	0.379 ± 0.0135b	0.099 ± 0.0072b	0.479 ± 0.0064b
$CdCl_2$ 60 + SiO_2 nanoparticles 100	0.343 ± 0.0138c	0.090 ± 0.0028b	0.433 ± 0.0162c
$CdCl_2$ 120 + SiO_2 nanoparticles 100	0.241 ± 0.0120e	0.080 ± 0.0024c	0.321 ± 0.0096f

Values are means of three replicates ± SD per treatment. Means in each column followed by different letters are significantly different ($p \leq 0.05$).

Table 2: Shoot, root and shoot + root dry weights (mg) *in T. aestivum* plants subjected to Cd concentrations alone and in combination with two concentrations of SiO_2 nanoparticles treatments for 7 days period.

Concentration (mg L^{-1})	**Shoot dry weight**	**Root dry weight**	**Shoot + Root dry weight**
Control	0.053 ± 0.0025a	0.011± 0.0003b	0.064 ± 0.0027a
$CdCl_2$ 30	0.034 ± 0.0013d	0.010 ± 0.0002bc	0.044 ± 0.0016d
$CdCl_2$ 60	0.023 ± 0.0006d	0.009 ± 0.0003bc	0.033 ± 0.0002d
$CdCl_2$ 120	0.015 ± 0.0004e	0.008 ± 0.0001c	0.023 ± 0.0004e
$CdCl_2$ 30 + SiO_2 nanoparticles 50	0.049 ± 0.0019ab	0.011 ± 0.0004a	0.061 ± 0.0017ab
$CdCl_2$ 60 + SiO_2 nanoparticles 50	0.039 ± 0.0015c	0.009 ± 0.0003b	0.049 ± 0.0012c
$CdCl_2$ 120 + SiO_2 nanoparticles 50	0.025 ± 0.0008d	0.009 ± 0.0003b	0.034 ± 0.0007d
$CdCl_2$ 30 + SiO_2 nanoparticles 100	0.045 ± 0.0018b	0.011 ± 0.0004a	0.057 ± 0.0020b
$CdCl_2$ 60 + SiO_2 nanoparticles 100	0.038 ± 0.0014c	0.009 ± 0.0003b	0.048 ± 0.0016c
$CdCl_2$ 120 + SiO_2 nanoparticles 100	0.024 ± 0.0006d	0.009 ± 0.0003bc	0.033 ± 0.0009d

Values are means of three replicates ± SD per treatment. Means in each column followed by different letters are significantly different ($p \leq 0.05$).

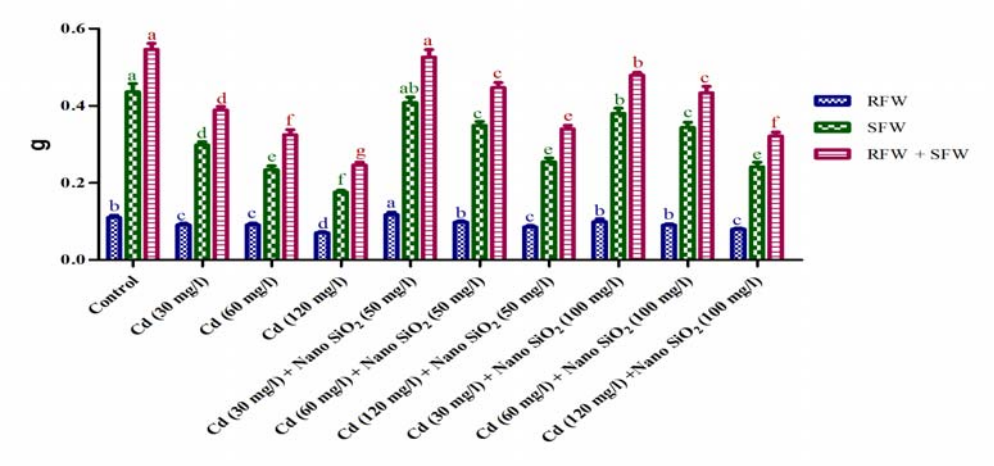

Fig. 1. Shoot, root and shoot + root fresh weights (mg) in *T. aestivum* plants treatment with Cd alone and in combination with SiO_2 nanoparticles.

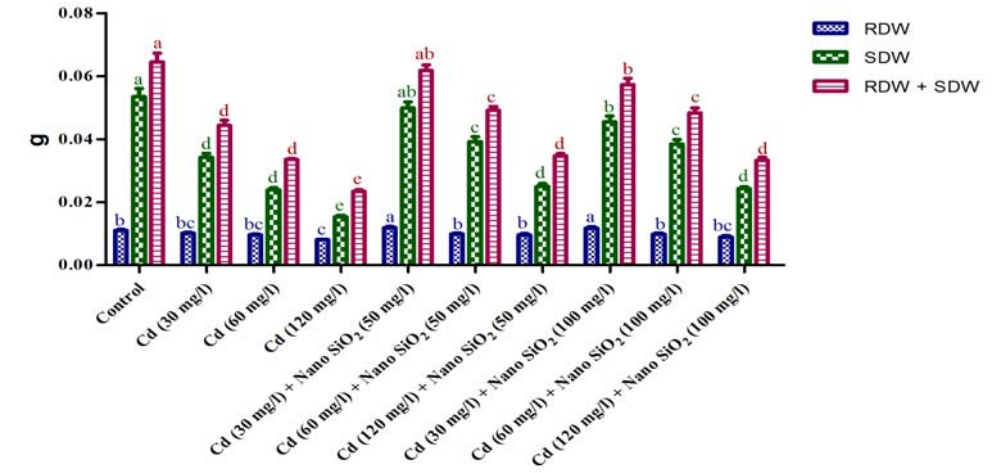

Fig. 2: Shoot, root and shoot + root dry weights (mg) in *T. aestivum* plants treatment with Cd alone and in combination with SiO_2 nanoparticles.

Content of photosynthetic pigments

The responses of photosynthetic pigments (chlorophyll *a*, *b* and carotenoid) in wheat are presented in table 3 and figures 3-4. Significant decrease in chlorophyll *a*, *b* and carotenoid contents in wheat seedlings (one-week exposed to Cd treatments) was observed in comparison with control. Chlorophyll *a* decreased approximately 34, 40 and 54%, chlorophyll *b*, 47, 58 and, 72%, chlorophyll *a* plus *b*, 38, 45 and, 59%, carotenoids, 31, 37 and, 50%, at 30, 60, and, 120 $mg^{-1}l$ $CdCl_2$ concentrations, compared to the control, respectively. Combination of Cd and SiO_2 nanoparticles 50 and 100 $mg^{-1}l$ concentrations, partially reduced the effects of cadmium ions on photosynthetic pigments. In general, Cd and SiO_2 nanoparticles combination at 50 and 100 $mg^{-1}l$ concentrations increased chlorophyll *a*, approximately 37 and 15%, respectively compared to Cd alone. Cadmium SiO_2 combination nanoparticles at 50 $mg^{-1}l$ increased chlorophyll *b*, approximately 29%, but at 100 $mg^{-1}l$ decreased chlorophyll *b*, approximately 13%, compared to Cd alone. This combination at 50 and 100 $mg^{-1}l$ concentrations increased of chlorophyll *a* and *b*, approximately 34

and 7%, respectively compared to Cd alone.

Finally, Cadmium and SiO_2 nanoparticles combination, at 50 $mg^{-1}l$ increased carotenoid approximately 22%, and with SiO_2 nanoparticles at 100 $mg^{-1}l$ decreased carotenoid approximately 3%, compared Cd alone. The declines in total chlorophyll and carotenoid contents can be regarded as general responses to metal toxicity (Chandra *et al*. 2009; MacFarlane and Burchett 2001; Radic *et al*. 2010; Ralph and Burchett 1998). Decrease in chlorophyll content depend up on several factors e.g. disturbance in the synthesis of pigments (Shweta and Agrawal 2006), pigments degradation (Prasad *et al*. 2001; Somashekaraiah *et al*. 1992), direct inhibition of enzymatic steps coupled with chlorophyll biosynthesis, protein composition of photosynthetic membranes (Mysliwa-Kurdziel *et al*. 2004; Prasad and Strzałka 1999) arrangement of photoactive protochlorophyll reductase enzyme complex and aminolevulinic acid (ALA) synthesis (Oncel *et al*. 2000; Stobart *et al*. 1985). The curent results support the previous researchers (Lagriffoul *et al*. 1998; Oukarroum *et al*. 2012; Ralph and Burchett 1998; Saison *et al*. 2010; Wei *et al*. 2010).

Table 3: Chlorophyll a, b, total chlorophylls and carotenoids (mg g^{-1} FW) in *T. aestivum* plants subjected to Cd concentrations alone and in combination with two concentrations of SiO_2 nanoparticles treatments for 7 days period.

Concentration (mg L^{-1})	Chlorophyll a	Chlorophyll b	Chlorophylls a + b	Carotenoids
Control	0.601± 0.0133a	0.244 ± 0.0054a	0.845 ± 0.0166a	0.204 ± 0.0163a
$CdCl_2$ 30	0.395 ± 0.0121e	0.128 ± 0.0034e	0.523 ± 0.0092e	0.140 ± 0.0089c
$CdCl_2$ 60	0.355 ± 0.0088g	0.102 ± 0.0023g	0.457 ± 0.0072g	0.128 ± 0.0073cd
$CdCl_2$ 120	0.271± 0.0064h	0.068 ± 0.0011h	0.339 ± 0.0075h	0.101 ± 0.0034e
$CdCl_2$ 30 + SiO_2 nanoparticles 50	0.525 ± 0.0166b	0.141 ± 0.0032b	0.667 ± 0.0134b	0.162 ± 0.0109b
$CdCl_2$ 60 + SiO_2 nanoparticles 50	0.505 ± 0.0140c	0.128 ± 0.0032c	0.634 ± 0.0120c	0.157 ± 0.0101bc
$CdCl_2$ 120 + SiO_2 nanoparticles 50	0.373 ± 0.0118f	0.117 ± 0.0039f	0.490 ± 0.0102f	0.136 ± 0.0082c
$CdCl_2$ 30 + SiO_2 nanoparticles 100	0.471 ± 0.0141d	0.113 ± 0.0039d	0.584 ± 0.0126d	0.126 ± 0.0081cd
$CdCl_2$ 60 + SiO_2 nanoparticles 100	0.402 ± 0.0117g	0.079 ± 0.0021g	0.481 ± 0.010g	0.121 ± 0.0087cd
$CdCl_2$ 120 + SiO_2 nanoparticles 100	0.300 ± 0.0099h	0.066 ± 0.0035h	0.366 ± 0.0122h	0.113 ± 0.0060d

Values are means of three replicates ± SD per treatment. Means in each column followed by different letters are significantly different ($p \leq 0.05$).

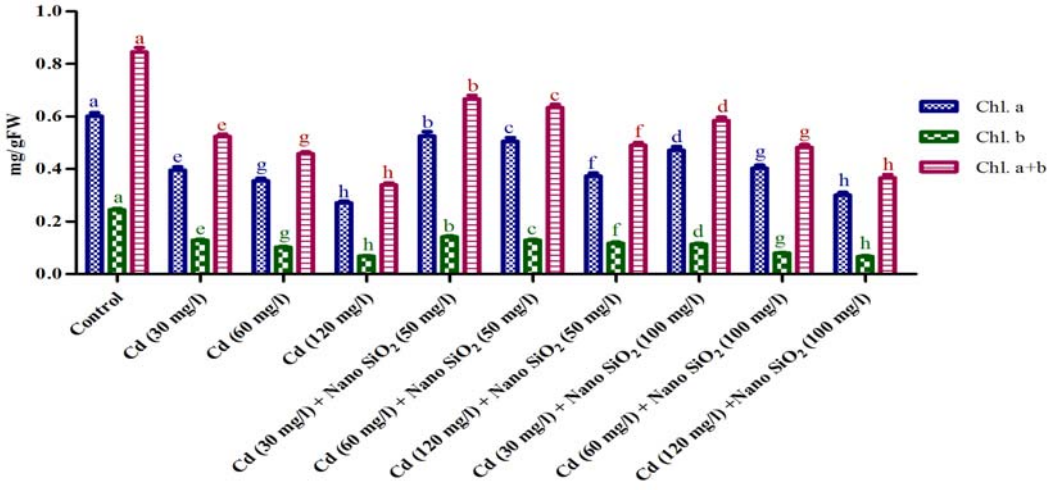

Fig. 3: Chlorophyll a, b, and chlorophyll a+b (mg g^{-1} FW) in *T. aestivum* plants treatment with Cd alone and in combination with SiO_2 nanoparticles.

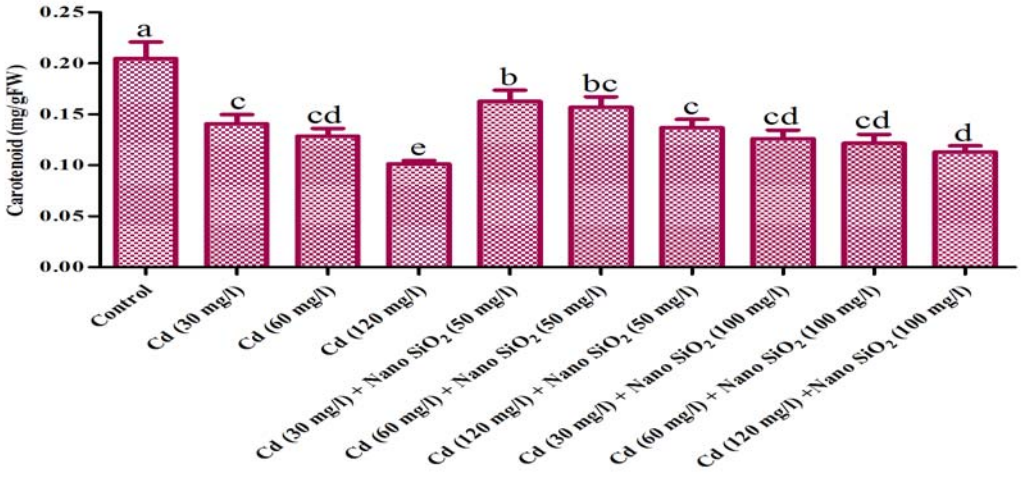

Fig. 4: Carotenoids (mg g^{-}1 FW) in *T. aestivum* plants treatment with Cd alone and in combination with SiO_2 nanoparticles.

Contents of proline

Proline contents of treated and untreated wheat are shown in Table 4 and Figure 5. Increase in cadmium concentration, caused the significant raise of the proline content of leaf, compared to the control. The maximum increase in proline content was observed at 120 mg^{-1}l of cadmium concntration. Proline contents of cadmium treated plants increased approximately 15, 18 and, 21%, at 30, 60, and, 120 mg^{-1}l $CdCl_2$ concentrations respectively compared to the control.

Also, proline contents in combination with SiO_2 nanoparticles at concentrations of 50 and 100 mg^{-1}l, partially reduced than Cd alone. In general, Cd in combination with SiO_2 nanoparticles at 50 and 100 mg^{-1}l concentration lead to decrease of MDA approximately 5% and 9% respectively, compared to Cd alone. Proline as an amino acid is an important osmolyte which accumulates in a broad range of organisms from bacteria to higher plants, after exposure to abiotic stress, for adapting to divers environmental stresses especially drought, cold, salinity, high temperature, nutrient lack, and exposure to heavy metals (Ashraf and Foolad 2007b).

Table 4: Proline, lipid peroxidation, catalase and total protein in *T. aestivum* plants subjected to Cd concentrations alone and in combination with two concentrations of SiO_2 nanoparticles treatments for 7 days period.

Concentration (mg L^{-1})	Proline	Lipid peroxidation	Catalase	Total protein
Control	25.9 ± 0.59c	31.2 ± 1.35e	0.010 ± 0.0010d	5.4 ± 0.70a
$CdCl_2$ 30	29.9 ± 9.17ab	40.2 ± 2.58c	0.016 ± 0.0014c	3.9 ± 0.53b
$CdCl_2$ 60	30.6 ± 1.32ab	49.9 ± 3.24ab	0.020 ± 0.0011b	2.5 ± 0.34c
$CdCl_2$ 120	31.5 ± 1.10a	52.6 ± 1.78a	0.022 ± 0.0014a	2.2 ± 0.29c
$CdCl_2$ 30 + SiO_2 nanoparticles 50	26.6 ± 0.80bc	39.0 ± 1.06c	0.018 ± 0.0011bc	4.8 ± 0.63ab
$CdCl_2$ 60 + SiO_2 nanoparticles 50	27.9 ± 0.85b	40.5 ± 0.66c	0.019 ± 0.0012b	3.4 ± 0.39bc
$CdCl_2$ 120 + SiO_2 nanoparticles 50	26.9 ± 1.15bc	45.1 ± 2.06b	0.019 ± 0.0011b	3.3 ± 0.47bc
$CdCl_2$ 30 + SiO_2 nanoparticles 100	28.3 ± 0.91b	34.2 ± 1.63d	0.015 ± 0.0013c	4.3 ± 0.63ab
$CdCl_2$ 60 + SiO_2 nanoparticles 100	24.5 ± 0.69d	38.5 ± 1.47c	0.017 ± 0.0014bc	2.8 ± 0.34c
$CdCl_2$ 120 + SiO_2 nanoparticles 100	25.2 ± 0.82cd	40.3 ± 1.53c	0.019 ±0.0010b	2.5 ± 0.32c

Values are means of three replicates ± SD per treatment. Means in each column followed by different letters are significantly different ($p \leq 0.05$).

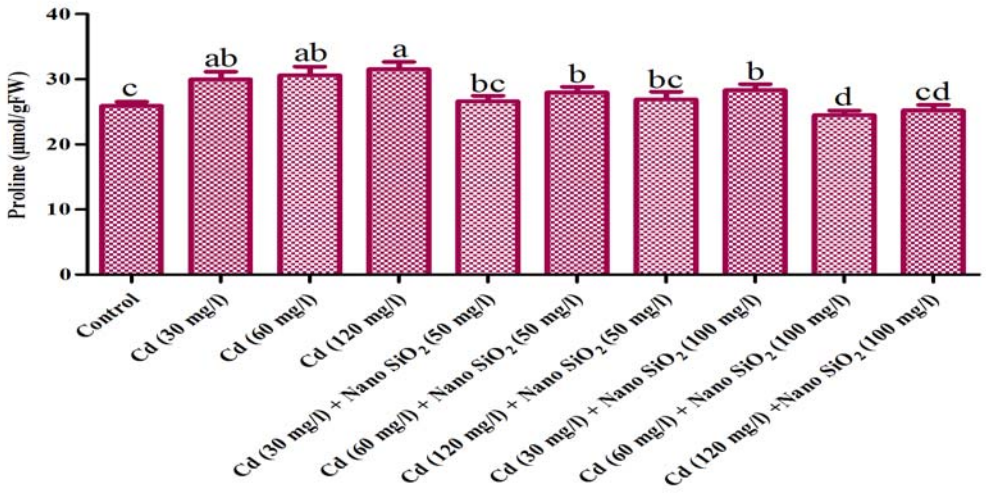

Fig. 5: Proline in *T. aestivum* plants treatment with Cd alone and in combination with SiO_2 nanoparticles.

Proline alleviates metal toxicity by acting as a metal chelator (Sharma and Dubey 2005), functioning as detoxification of reactive oxygen species (ROS) such as hydroxyl radical, singlet oxygen (Szabados and Savoure 2010) and osmoprotectant (Ashraf and Foolad 2007a; Tamayo and Bonjoch 2001), acting as a protection of the enzymes against denaturation and stabilization of protein synthesis (Sanchez-Partida *et al*. 1992; Shah and Dubey 1997). In addition, proline supports mitochondrial oxidative phosphorylation for protecting natural generation of ATP (Ashraf and Foolad 2007b; Siripornadulsil *et al*. 2002) and acts as an inhibitor of lipid peroxidation (Hara *et al*. 2003; Mehta and Gaur 1999). Our results were congruent with the previous investigators (Jiang *et al*. 2012; John *et al*. 2009; Kastori *et al*. 1992; Mehta and Gaur 1999).

Lipid peroxidation

The effect Cd alone and in combination with SiO_2 amounts of lipid peroxidation is meaningful (Table 4 and Figure 6). The amounts of MDA formation indicate the level of free radical production and lipid peroxidation (Dexter *et al*. 1989; Mak and Weglicki 1988). We realize the smallest amounts of lipid peroxidation was on control and the most of it was at 120 $mg^{-1}l$ concentration of $CdCl_2$. Amounts of MDA formation of cadmium treated plants increased approximately 28, 59 and, 68%, at 30, 60, and, 120 $mg^{-1}l$ $CdCl_2$ concentration compared to control, respectively. Increase of lipid peroxidation in Cd alone treatments is significantly more than that of combined with SiO_2 nanoparticles. Apart from the control sample, the lowest amounts of MDA were showed in 120 $mg^{-1}l$ concentration of Cd in combination with SiO_2 nanoparticles at 50 $mg^{-1}l$ concentration. In general, Cd in combination with SiO_2 nanoparticles at 50 and 100 $mg^{-1}l$ concentration leads to decrease of MDA approximately 13% and 23% respectively, compared to Cd alone. MDA contents is a product of lipid peroxidation and has been considered as an indicator of oxidative damage and peroxidation of membrane lipids in plants (Nacif de Abreu and Mazzafera 2005; Xu *et al*. 2006). The cell membrane is usually the main site of attack by any heavy metal in a plant cell. In the current experiments, we observed significant increase in MDA concentration with increasing the $CdCl_2$ concentration that indicates the negative

effect of heavy metals on membrane integrity and permeability. The free radicals produced by heavy metals, can attack the unsaturated fatty acid side chains of membrane lipids, and cause formation of lipid hydroperoxides (Halliwell and Chirico 1993). The similar result was obtained by previous investigators (Gallego *et al*. 1996; Ghosh *et al*. 2010; Panda *et al*. 2003; Sayes *et al*. 2005; Zhang *et al*. 2007).

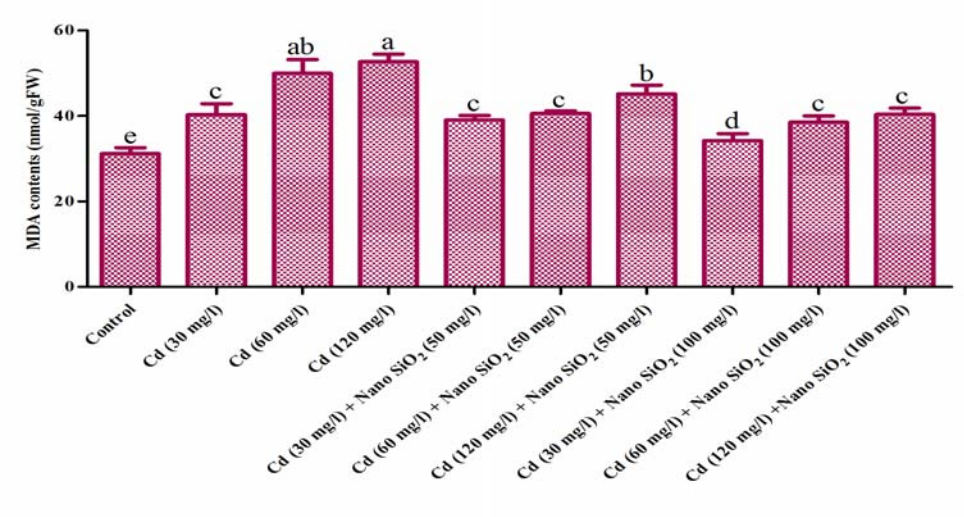

Fig. 6: Lipid peroxidation in *T. aestivum* plants treatment with Cd alone and in combination with SiO_2 nanoparticles.

Catalase activity

Significant increasing of catalase activity was observed in response to the increase of Cd concentrations (Table 4 and Figure 7). The highest value of catalase activity was recorded at 120 $mg^{-1}l$ $CdCl_2$. Increase of cadmium concentration, significantly enhance the catalase activity of wheat leaf compared with the control. Amounts of catalase activity of cadmium treated plants increased about 60, 100 and, 120%, at 30, 60, and, 120 $mg^{-1}l$ $CdCl_2$ concentration compared with the control, respectively.

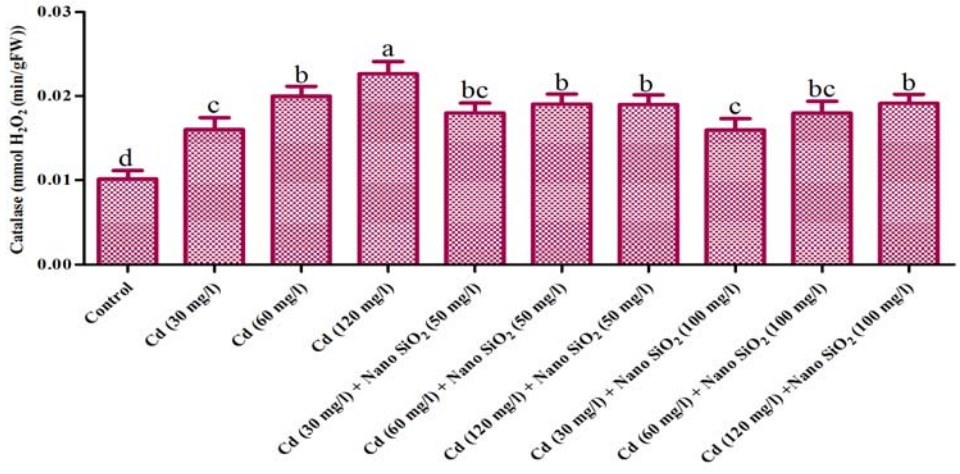

Fig. 7: Catalase activity in *T. aestivum* plants treatment with Cd alone and in combination with SiO_2 nanoparticles.

Also, catalase activity in all concentrations of cadmium in combination with SiO_2 nanoparticles at 50 and 100 $mg^{-1}l$ concentrations, moderately reduced than Cd alone. In general, Cd in combination with SiO_2 nanoparticles at 50 and 100 $mg^{-1}l$ concentration decreased of MDA approximately 3% and 12% respectively, compared to Cd alone. The activities of antioxidant enzyme have generally increased during abiotic stress, e.g. chilling, drought, high temperature, salt, and heavy metal stress (Baker and Orlandi 1995; Mittler 2006) and correlated with enhanced cellular protection of reactive oxygen species. Catalase is an important antioxidant which protects plants by suppressing oxidative injury and assist as a reactive species scavenger. This result were in agreement with that of the previous researchers (Du *et al*. 2011; Gallego *et al*. 1996; Krishnaraj *et al*. 2012; Zhang *et al*. 2007).

Contents of total protein

Increase in $CdCl_2$ concentrations led to the reduction of total protein contents, compared to control sample (Table 4 and Figure 8). Total protein contents of cadmium treated plants decreased approximately 22, 50 and, 56%, at 30, 60, and, 120 $mg^{-1}l$ $CdCl_2$ concentration compared to the control, respectively.

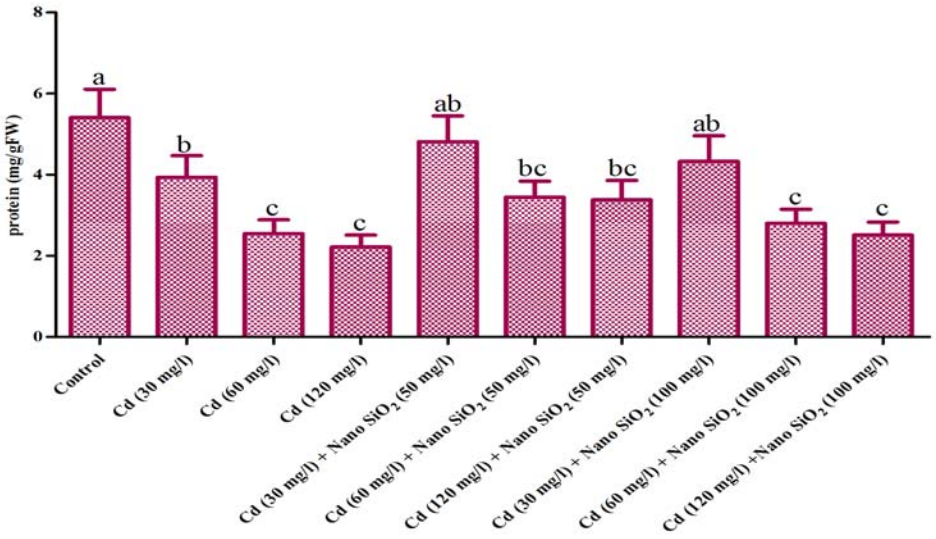

Fig. 8: Protein in *T. aestivum* plants treatment with Cd alone and in combination with SiO_2 nanoparticles.

Also, total protein contents in samples which treatment by in combination with SiO_2 nanoparticles moderately increased than Cd alone. In general, Cd in combination with SiO_2 nanoparticles at 50 and 100 $mg^{-1}l$ concentrations lead to increase in protein contents about 33% and 11% respectively compared Cd alone. Under heavy metals and nanoparticles, oxidative stresses results the generation of reactive oxygen species and degeneration of protein (Choi and Hu 2008; Rana 2008; Wan *et al*. 2012; Xia *et al*. 2008). The results showed that $AgNO_3$ has more negative effects than AgNPs, and in some cases, no significant difference was found between AgNPs at low concentration and control. This result confirms the previous studies. Both dissolved silver and AgNPs lead to the production of reactive oxygen species,

However, the later have direct toxic effects without dissolution (Yin *et al*. 2011). The toxicity of AgNPs to plants is obvious, while their negative effects and mechanisms on higher plants have not been completely characterized (Jiang *et al*. 2012).

CONCLUSION

The inhibitory effect of Cd on plant physiology and growth has been reported by several authors. Our study was focused on the potential effect of Cd alone and in combination with SiO_2 nanoparticles on wheat. Exposure of wheat plants to Cd on the whole, caused a significant decrease in fresh and dry weight of root and shoot, photosynthetic pigments and, protein of leaf and a significant increasing of proline, lipid peroxidation and, catalase activity. Almost in all cases, Cd in combination with SiO_2 nanoparticles improves the negative effects. The results of the previous and the present studies, revealed the negative aspects and toxicity problems in plants when they exposed to cadmium ions. Studies about SiO_2 nanoparticles on plants is infrequent, therefore for better understanding the effects of silicon oxide nanoparticles on plant exposed to heavy metal stress, further experiments should be performed.

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Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121-126

Arnon D, Stout P (1939) The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant physiology 14 (2):371

Ashraf M, Foolad M (2007a) Improving plant abiotic-stress resistance by exogenous application of osmoprotectants glycine betaine and proline. Environ Exp Bot 59:206-216

Ashraf M, Foolad M (2007b) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59 (2):206-216

Assche FV, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant, Cell & Environment 13 (3):195-206

Baker CJ, Orlandi EW (1995) Active oxygen in plant pathogenesis. Annual review of phytopathology 33 (1):299-321

Bates L, Waldren R, Teare I (1973) Rapid determination of free proline for water-stress studies. Plant and soil 39 (1):205-207

Beyersmann D, Hechtenberg S (1997) Cadmium, gene regulation, and cellular signalling in mammalian cells. Toxicology and applied pharmacology 144 (2):247-261

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 72 (1):248-254

Chandra R, Bharagava R, Yadav S, Mohan D (2009) Accumulation and distribution of toxic metals in wheat (Triticum aestivum L.) and Indian mustard (Brassica campestris L.) irrigated with distillery and tannery effluents. Journal of hazardous materials 162 (2):1514-1521

Choi O, Hu Z (2008) Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environmental science & technology 42 (12):4583-4588

de Albuquerque Lima M, de Castro VF, Vidal JB, Eneas-Filho J (2011) Aplicacao de silício em milho e feijao-de-corda sob estresse salino. Revista Ciência Agronomica 42 (2):398-403

Dexter D, Carter C, Wells F, Javoy-Agid F, Agid Y, Lees A, Jenner P, Marsden CD (1989) Basal lipid peroxidation in substantia nigra is increased in

Parkinson's disease. Journal of neurochemistry 52 (2):381-389

Dietz K-J, Baier M, Kramer U (1999) Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. In: Heavy metal stress in plants. Springer, pp 73-97

Du W, Sun Y, Ji R, Zhu J, Wu J, Guo H (2011) TiO2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. Journal of Environmental Monitoring 13 (4):822-828

Eraslan F, Inal A, Pilbeam DJ, Gunes A (2008) Interactive effects of salicylic acid and silicon on oxidative damage and antioxidant activity in spinach (Spinacia oleracea L. cv. Matador) grown under boron toxicity and salinity. Plant growth regulation 55 (3):207-219

Gallego SM, Benavídes MP, Tomaro ML (1996) Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. Plant Science 121 (2):151-159

Ghosh M, Bandyopadhyay M, Mukherjee A (2010) Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels: Plant and human lymphocytes. Chemosphere 81 (10):1253-1262

Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnology advances 21 (5):383-393

Halliwell B, Chirico S (1993) Lipid peroxidation: its mechanism, measurement, and significance. The American journal of clinical nutrition 57 (5):715S-724S

Hara M, Terashima S, Fukaya T, Kuboi T (2003) Enhancement of cold tolerance and inhibition of lipid peroxidation by citrus dehydrin in transgenic tobacco. Planta 217 (2):290-298

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of biochemistry and biophysics 125 (1):189-198

Herbette S, Taconnat L, Hugouvieux V, Piette L, Magniette M-L, Cuine S, Auroy P, Richaud P, Forestier C, Bourguignon J (2006) Genome-wide transcriptome profiling of the early cadmium response of Arabidopsis roots and shoots. Biochimie 88 (11):1751-1765

Jiang HS, Li M, Chang FY, Li W, Yin LY (2012) Physiological analysis of silver nanoparticles and AgNO3 toxicity to Spirodela polyrhiza. Environmental Toxicology and Chemistry 31 (8):1880-1886

John R, Ahmad P, 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 (3):65-75

Kastori R, Petrović M, Petrovic N (1992) Effect of excess lead, cadmium, copper, and zinc on water relations in sunflower. Journal of Plant Nutrition 15 (11):2427-2439

Keller C, Hammer D, Kayser A, Richner W, Brodbeck M, Sennhauser M (2003) Root development and heavy metal phytoextraction efficiency: comparison of different plant species in the field. Plant and Soil 249 (1):67-81

Krishnaraj C, Jagan E, Ramachandran R, Abirami S, Mohan N, Kalaichelvan P (2012) Effect of biologically synthesized silver nanoparticles on Bacopa monnieri(Linn.) Wettst. plant growth metabolism. Process Biochemistry 47 (4):651-658

Lagriffoul A, Mocquot B, Mench M, Vangronsveld J (1998) Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.). Plant and soil 200 (2):241-250

Li W-B, Shi X-H, Wang H, Zhang F-S (2004) Effects of silicon on rice leaves resistance to ultraviolet-B. ACTA BOTANICA SINICA-ENGLISH EDITION- 46 (6):691-697

Li W, Khan MA, Yamaguchi S, Kamiya Y (2005) Effects of heavy metals on seed germination and early seedling

growth of Arabidopsis thaliana. Plant Growth Regulation 46 (1):45-50

Ma J, Miyake Y, Takahashi E (2001) Silicon as a beneficial element for crop plants. Studies in Plant Science 8:17-39

Ma JF (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Science and Plant Nutrition 50 (1):11-18

MacFarlane G, Burchett M (2001) Photosynthetic Pigments and Peroxidase Activity as Indicators of Heavy Metal Stress in the Grey Mangrove, Avicennia marina (Forsk.) Vierh. Marine Pollution Bulletin 42 (3):233-240

Mak IT, Weglicki WB (1988) Protection by beta-blocking agents against free radical-mediated sarcolemmal lipid peroxidation. Circulation research 63 (1):262-266

Masarovič D, Slováková Ľ, Bokor B, Bujdoš M, Lux A (2012) Effect of silicon application on Sorghum bicolor exposed to toxic concentration of zinc. Biologia 67 (4):706-712

Mehta S, Gaur J (1999) Heavy-metal-induced proline accumulation and its role in ameliorating metal toxicity in Chlorella vulgaris. New Phytologist 143 (2):253-259

Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends in plant science 11 (1):15-19

Mohaghegh P, Khoshgoftarmanesh A, Shirvani M, Sharifnabi B, Nili N (2011) Effect of silicon nutrition on oxidative stress induced by Phytophthora melonis infection in cucumber. Plant Disease 95 (4):455-460

Monroe RK, Halvorsen SW (2006) Cadmium blocks receptor-mediated Jak/STAT signaling in neurons by oxidative stress. Free Radical Biology and Medicine 41 (3):493-502

Mysliwa-Kurdziel B, Prasad M, Strzałtka K (2004) Photosynthesis in heavy metal stressed plants. In: Heavy Metal Stress in Plants. Springer, pp 146-181

Nacif de Abreu I, Mazzafera P (2005) Effect of water and temperature stress on the content of active constituents of Hypericum brasiliense Choisy. Plant Physiology and Biochemistry 43 (3):241-248

Nwugo CC, Huerta AJ (2008) Effects of silicon nutrition on cadmium uptake, growth and photosynthesis of rice plants exposed to low-level cadmium. Plant and soil 311 (1-2):73-86

Okuda A, Takahashi E (1965) The role of silicon. The mineral nutrition of the rice plant 146

Oncel I, Keles Y, Ustun A (2000) Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environmental Pollution 107 (3):315-320

Oukarroum A, Bras S, Perreault F, Popovic R (2012) Inhibitory effects of silver nanoparticles in two green algae Chlorella vulgaris and Dunaliella tertiolecta. Ecotoxicology and environmental safety 78:80-85

Påhlsson A-MB (1989) Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. Water, Air, and Soil Pollution 47 (3-4):287-319

Panda S, Chaudhury I, Khan M (2003) Heavy metals induce lipid peroxidation and affect antioxidants in wheat leaves. Biologia Plantarum 46 (2):289-294

Peralta J, Gardea-Torresdey J, Tiemann K, Gomez E, Arteaga S, Rascon E, Parsons J (2001) Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). Bulletin of Environmental Contamination and toxicology 66 (6):727-734

Prasad M, Malec P, Waloszek A, Bojko M, Strzałka K (2001) Physiological responses of Lemna trisulca L.(duckweed) to cadmium and copper bioaccumulation. Plant Science 161 (5):881-889

Prasad M, Strzałka K (1999) Impact of heavy metals on photosynthesis. In: Heavy metal stress in plants. Springer, pp 117-138

Radic S, Babic M, Skobic D, Roje V, Pevalek-Kozlina B (2010) Ecotoxicological effects of aluminum and zinc on growth and antioxidants in *Lemna minor* L. Ecotoxicology and environmental safety 73 (3):336-342

Ralph P, Burchett M (1998) Photosynthetic response of Halophila ovalis to heavy metal stress. Environmental Pollution 103 (1):91-101

Rana SVS (2008) Metals and apoptosis: recent developments. Journal of trace elements in medicine and biology 22 (4):262-284

Reynolds OL, Keeping MG, Meyer JH (2009) Silicon-augmented resistance of plants to herbivorous insects: a review. Annals of Applied Biology 155 (2):171-186

Saison C, Perreault F, Daigle J-C, Fortin C, Claverie J, Morin M, Popovic R (2010) Effect of core–shell copper oxide nanoparticles on cell culture morphology and photosynthesis (photosystem II energy distribution) in the green alga Chlamydomonas reinhardtii. Aquatic toxicology 96 (2):109-114

Sanchez-Partida L, Maxwell W, Paleg L, Setchell B (1992) Proline and glycine betaine in cryoprotective diluents for ram spermatozoa. Reproduction, fertility and development 4 (1):113-118

Sanita di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environmental and Experimental Botany 41 (2):105-130

Savvas D, Gizas G, Karras G, Lydakis-Simantiris N, Salahas G, Papadimitriou M, Tsouka N (2007) Interactions between silicon and NaCl-salinity in a soilless culture of roses in greenhouse. European Journal of Horticultural Science 72 (2):73

Sayes CM, Gobin AM, Ausman KD, Mendez J, West JL, Colvin VL (2005) Nano-C 60 cytotoxicity is due to lipid peroxidation. Biomaterials 26 (36):7587-7595

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. Journal of experimental botany 53 (372):1351-1365

Shah K, Dubey R (1997) Effect of cadmium on proline accumulation and ribonuclease activity in rice seedlings: role of proline as a possible enzyme protectant. Biologia Plantarum 40 (1):121-130

Sharma P, Dubey RS (2005) Lead toxicity in plants. Brazilian Journal of Plant Physiology 17 (1):35-52

Shweta M, Agrawal S (2006) Interactive effects between supplemental ultraviolet-B radiation and heavy metals on the growth and biochemical characteristics of Spinacia oleracea L. Brazilian Journal of Plant Physiology 18 (2):307-314

Siripornadulsil S, Traina S, Verma DPS, Sayre RT (2002) Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. The Plant Cell Online 14 (11):2837-2847

Somashekaraiah B, Padmaja K, Prasad A (1992) Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorphyll degradation. Physiologia Plantarum 85 (1):85-89

Steffens J (1990) The heavy metal-binding peptides of plants. Annual review of plant biology 41 (1):553-575

Stobart AK, Griffiths WT, Ameen-Bukhari I, Sherwood RP (1985) The effect of Cd2+ on the biosynthesis of chlorophyll in leaves of barley. Physiologia Plantarum 63 (3):293-298

Szabados L, Savouré A (2010) Proline: a multifunctional amino acid. Trends in plant science 15 (2):89-97

Tamayo PR, Bonjoch NP (2001) Free proline quantification. In: Handbook of Plant Ecophysiology Techniques. Springer, pp 365-382

Wahid A, Ghani A, Javed F (2008) Effect of cadmium on photosynthesis, nutrition and growth of mungbean. Agronomy for sustainable development 28 (2):273-280

Wan R, Mo Y, Feng L, Chien S, Tollerud DJ, Zhang Q (2012) DNA Damage Caused by Metal Nanoparticles: Involvement of Oxidative Stress and Activation of ATM. Chemical research in toxicology 25 (7):1402-1411

Wei C, Zhang Y, Guo J, Han B, Yang X, Yuan J (2010) Effects of silica nanoparticles on growth and photosynthetic pigment contents of Scenedesmus obliquus. Journal of Environmental Sciences 22 (1):155-160

Wellburn A, Lichtenthaler H (1984) Formulae and program to determine total carotenoids and chlorophylls a and b of leaf extracts in different solvents. In: Advances in photosynthesis research. Springer, pp. 9-12

Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, Yeh JI, Zink JI, Nel AE (2008) Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS nano 2 (10):2121-2134

Xu S, Li J, Zhang X, Wei H, Cui L (2006) Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. Environmental and Experimental Botany 56 (3):274-285

Yin L, Cheng Y, Espinasse B, Colman BP, Auffan M, Wiesner M, Rose J, Liu J, Bernhardt ES (2011) More than the ions: the effects of silver nanoparticles on Lolium multiflorum. Environmental science & technology 45 (6):2360-2367

Zhang F-Q, Wang Y-S, Lou Z-P, Dong J-D (2007) Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (Kandelia candel and Bruguiera gymnorrhiza). Chemosphere 67 (1):44-50.