Changes in Cd/Pb Accumulation and Growth and Physiological Indices on *Sorghum bicolor* sp. Seedlings Exposed to Carbon Nano Tubes

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ABSTRACT

The effect of multiwall carbon nanotube (CNT) exposure (10 and 50 mg L^{-1}), was studied on sorghum (*Sorghum bicolor*) seedlings sown into petri dishes containing 500 μM $Pb(NO3)_2$ or 200 μM $CdCl_2$. Plant growth parameters, chlorophyll (Chl) content, lipid peroxidation and Cd/Pb accumulation recorded on 10–day old seedlings. Lead and cadmium treatment decreased the root and shoot length in sorghum seedlings. Our results showed diminished Chl content and enhanced lipid peroxidation in seedlings under Cd and Pb treatment which accumulated plenty amount of Pb/Cd. CNTs exposure, even under control conditions or heavy metals treatment, significantly increased shoots and root growth parameters. CNTs application also had significant positive impacts on total chlorophyll content in Pb-stressed seedlings. MDA content on control plants which did not received any heavy metals decreased by CNTs. On the other way, CNTs exposure increased lipid peroxidation in Pb or Cd treated seedlings. Pb absorption significantly increased by CNTs application while no significant changes was obvious on Cd accumulation by CNTs. Based on the results, it seems that CNTs application could alleviate the reduced growth parameters and Chl content induced by heavy metals–stressed sorghum seedlings. These findings reveal possible regulatory roles of CNTs on heavy metal resistance in plants.

Keywords: Carbon nanotubes, MDA content, chlorophyll content, seedling growth

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INTRODUCTION

Over the last century, lead and cadmium; two most important environmental contaminants have been widespread into the environment in increasing concentrations (Koeppe, 1977; Li *et al.*, 2005; Sun *et al.*, 2005; Smirjdkova *et al.*, 2005). Both Pb and Cd are toxic, non-essential metals of without any known function in biological systems (Singh *et al.*, 2013). Plants often accumulate heavy metals at higher concentrations than their levels in the soil, consequently they may easily enter food chains from plants (Koeppe, 1977). Increased Pb and Cd even at low concentrations may affect soil productivity and metabolic processes retardation in plants (Singh *et al.*, 2013).

Excessive amounts of Cd in soil caused elicitation of stress symptoms in plant growth and metabolic processes such as reduction of root and shoot growth and biomass production, perturbation of carbohydrate metabolism and mineral nutrition (Mohan and Hosetti, 1997). In plants, Cd and

Pb ions bound on the cell surface and within the cells; thereafter they interact with the functional groups of metabolites and induce various metabolic disorders. Cd^{2+} and Pb^{2+} disrupt photosynthesis in different ways including decrease in chlorophyll content. Photosynthesis processes is also inhibited in isolated chloroplasts by Pb and Cd. The effect heavy metals concentration on chlorophyll content depends on the plant species (Huang *et al.*, 1974).

Nowadays there is an extensive interest in applying nanoparticles (including carbon nanotubes; CNTs) to plants for agricultural and horticultural use (Bhatt and Tripathi, 2011). Carbon nanomaterials for their unique physical and chemical properties have been the focus of much interest (De La Torre-Roche *et al.*, 2013; Upadhyayula *et al.*, 2009). Studies have clearly demonstrated that CNTs have a high adsorption capacity for several divalent metal ions (Hu *et al.*, 2009; Kandah and Meunier, 2007; Li *et al.*, 2003; Lu and Chiu, 2006; Rao *et al.*, 2007; Stafiej and Pyrzynska, 2007) including lead (Xu *et al.*, 2008), nickel (Yang *et al.*, 2009), and cadmium (Gao *et al.*, 2008).

Multi-walled carbon nanotubes (MWCNT) which are consisted of multiple concentric rolled tubes of graphite, is taken up by plant roots and translocated to the leaves (Larue *et al.*, 2012). The positive effects of carbon nanotubes on plant growth and development have been described by number of research groups (Khodakovskaya *et al.*, 2013, Khodakovskaya *et al.*, 2011). Increase of root growth in response to carbon nanotubes was reported on onion, cucumber (Cañas *et al.*, 2008) and ryegrass (Lin and Xing, 2007). Liu *et al.* (2009) demonstrated that single walled nanotubes (SWCNTs) are capable to penetrate the walls and membranes of tobacco cells (Liu *et al.*, 2009). Miralles *et al.* (2012) demonstrated that MWCNTs increase seed germination and root elongation in alfalfa and wheat, but MWCNTs particles uptake and translocation was insignificant (Miralles *et al.*, 2012). In tomato plants, carbon nanotubes penetrate tomato seeds and affect their germination and growth rates (Khodakovskaya *et al.*, 2009). Khodakovskaya et al, argued that CNTs can be regarded as plant growth regulators which affects tomato growth, reproductive system, and soil microbial community (Khodakovskaya *et al.*, 2013).

CNTs application as adsorbent for removal of organic and inorganic contaminants has been proposed (Rao *et al.*, 2007). Based on the plant ability to accumulate heavy metals, plant–derived decontamination technologies like phytoremediation (the uptake of pollutants using plants) have been introduced. There are many studies have been conducted to improve the efficiency of phytoremediation techniques to enhance heavy metals accumulation in plants (Wu *et al.*, 2009; Sarma, 2011). Though many studies have shown that carbon-based nanomaterials may influence accumulation of pollutants and their impacts on plants (De La Torre-Roche *et al.*, 2012); but there is little information regarding CNTs application on plants under heavy metal stress. Based on our information, in a project Chai et al studied the interactive effects of cadmium and carbon nanotubes on the growth and metal accumulation in *Spartina alterniflora* (Poaceae) and concluded that under higher Cd stress (200 mg kg^{-1}), CNTs restore the reduced plant growth, and reduce Cd accumulation in shoots and roots (Chai *et al.*, 2013).

Selection of a plant species are able to tolerant excess metal, having high biomass production is also of essential requirement in an effective phytoremediation. Sorghum (*Sorghum bicolor*) has been reported matches with the above mentioned criteria (Soudek *et al.*, 2014). Sorghum bicolor is C4 grass widely used as a forage crop (Barbanti *et al.*, 2006). The crop is resistant to drought, temperature, and highly tolerant to metal pollution (Soudek *et al.*, 2014). Sorghum plants are able to accumulate large quantities of heavy metals such as Cd, Cu, Pb and Zn in shoots (Epelde *et al.*, 2009). In this study, the role of CNTs on some growth and physiological parameters and metal accumulation of Cd/Pb by *S. bicolor* has been investigated. Main hypothesis to perform this project is that CNTs may reinforce Cd/Pb accumulation in *S. bicolor* seedlings while concomitantly relieving the negative effects of Cd/Pb ions on sorghum seedlings.

MATERIAL AND METHODS

Seeds of *Sorghum bicolor* were obtained from Pakanbazr Inst., Isfahan (Iran). Multi-walled carbon nanotubes (MWCNTs: purity 90%, ID = 10 nm, OD = 30 nm and length = 100 nm) were provided by Chemistry Department, Tehran University. Before use, the CNTs were sonicated for 2 hrs. in an ultrasonic bath. The suspension was stirred at 55°C for 7 hrs. and cooled to room temperature.

The experiment was carried out based on completely randomized design, consisted of 9 treatments replicated three times. Seeds sterilized for 10 min with 0.01% (w/v) sodium hypochlorite solution were thoroughly rinsed with distilled water. 15 Germinated seeds sowed on to the filter paper in Petri dishes (12 mm diameter) imbibed with 10 ml 0, 10 or 50 mg/L MWCNTs solution containing 500 µM $Pb(NO3)_2$ or 200 µM $CdCl_2$. Treatments without Cd/Pb or CNTs addition were controls. Petri-dishes kept in a growth chamber under controlled conditions; 16/8 hrs. day/night photoperiod, 24/20 ± 1°C day/night temperature for 10 days.

Thereafter, seedlings roots and shoots length recorded. A portion of the fresh samples were taken to measure the physiological indices such as MDA and chlorophyll content of leaflets. The remainder of samples washed several times for removal of the contaminants from the surface and then they were dried at 70°C for 72 hrs. until a constant weight achieved. Dried samples were used for trace metal analysis.

Atomic Absorption Spectroscopy:

Atomic absorption spectroscopy was used to detect heavy metals accumulation into the plant seedlings. The whole-seedling mass was used for this purpose. To remove debris from the surface, seedlings cleaned up with filter paper, and then rinsed several times with distilled water. The plant samples were dried in an oven at 70°C for 48 hours and dried mass were considered for analysis of Cd or Pb concentration. The desiccated samples were finely milled to uniform size; 100 mg of them were taken to determine the content of Cd/Pb using atomic absorption spectrophotometer (GFA spectra-220 atomic adsorption spectrometer). Dried material of seedlings was digested with repeatedly additions of concentrated 1:1 nitric acid (HNO_3): perchloric acid (3:1, v/v) and heating at 95°C ± 3°C without boiling. After 2 hours, 5 mL digestion solution was again added to the reaction mixture and heating was continued for another hour. The solution was allowed to evaporate in watch glass until reaching 5 ml. The filtered digestate was then diluted to a final volume of 100 mL in a volumetric flask using double distilled water and used directly for determination of Cd/Pb ion content by GFA spectra-220 atomic adsorption spectrometer.

GTA-110 graphite tube atomizer and sample dispenser were used in this study. Deuterium background correction was used. All equipment's were from Varian Techron Pty. Limited, Mulgrave, Victoria, Australia.

Biochemical Parameters Measurements

Fresh leaves were taken to determine the content of chlorophyll (Chl) and lipid peroxidation (MDA content). Total chlorophyll content in fresh leaves (100 mg) of control and treated plants was determined following the extraction by grinding fresh leaf in 80% acetone. The extractor volume in all samples equalized to 15 ml using acetone 80%. Samples then centrifuged at 11,000 g for 10 min. Supernatants were used for estimation of chlorophyll content. The optical density of aliquots was measured at 645 and 663 nm by spectrophotometer (Turner, 1981). Total chlorophyll content estimated using following equation. After unit conversions, total chlorophyll content was expressed as mg/g fresh weight of leaflets.

$$\text{Total chlorophyll (mg/L)} = (0.0202 \times OD_{645}) + (0.0082 \times OD_{663})$$

To determine MDA content, TBARs were extracted by grinding 0.1 g fresh leaves in 0.1% trichloroacetic acid. Extracts then incubated for 30 mins at 95°C with 0.5% thiobarbituric acid prepared in 20% trichloroacetic acid, then absorbance at 532 nm and 600 nm were measured. For malondealdehyde (MDA) measurement, the non-specific absorbance of supernatant at 600 nm was subtracted from the maximum absorbance at 532 nm. An extinction coefficient of 1.55×10^5 $M^{-1}cm^{-1}$ was used for determination of MDA concentration (Heath and Packer, 1968).

Statistical Analysis

Treatments were compared by analysis of variance (ANOVA) and the significance of mean difference within and between the treatments was done by Duncan multiple range test (DMRT). All data were expressed as mean ± S.E.

RESULTS AND DISCUSSION

Growth Parameters

The experiment compared the growth performance of sorghum seedlings upon Cd/Pb exposure with or without treatment with MWCNTs. Figure 1 (A, B) shows the shoot and root growth of seedlings after 10 days treatment. In order to evaluate the effects of CNTs on the growth of Pb or Cd stressed plants, the concentrations of 0, 10 and 50 mg/L of the MWCNTs were adopted in this investigation. Either 200 μM of Cd or 500 μM of Pb suppressed shoot length compared to the control. Both CNT10 and 50 mg/L increased shoot length under control growth conditions (without Cd or Pb). In heavy metal stressed seedlings, even upon Cd or Pb treatment, CNT 50 resulted in a higher growth of shoots treated seedlings. Root growth test is a rapid and widely used acute phyto-toxicity test for its sensitivity, simplicity and low cost. Similar to shoot growth, root length was strongly retarded by cadmium and lead treatment. The result showed that the root growth was promoted in the presence of both CNT10 and 50 mg/L CNTs compared with the controls. CNTs application also raised root growth in seedlings under control conditions (figure 1B).

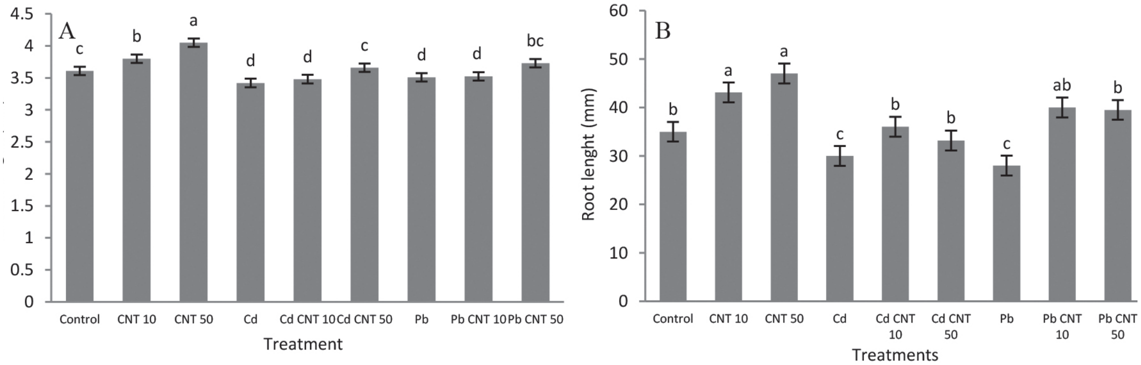

Figure 1 The effect of MWCNT on shoot growth (A), root growth (B) of *Sorghum bicolor* seedlings under Cd or Pb treatment CNT 10 or 50: carbon nanotubes 10 or 50 mg/L, Pb and Cd concentration: 500 and 200 μM, respectively. Bar on the each column shows ± SEM on three replicates

Growth inhibition, leaf chlorosis and changes the activity of many metabolic pathways enzymes are the most common effect of Cd toxicity in plants (Scebba *et al.*, 2006). Parameters such as fresh weight of shoot as well as root length are used as useful indicators of metal toxicity in plants. In our experiment, sorghum seedlings showed at 200 μM of Cd and 500 μM of Pb in term of plant growth parameters. Similarly, John *et al.* (2008) found decline in growth parameters of *Lemna polyrrhiza* under Pb and Cd stress. It was proposed that the reduction in the growth in the presence of heavy metals could be due to the suppression of the cells elongation by Cd, due to an irreversible inhibition on the proton pump responsible for the elongation process (Aidid and Okamoto, 1993). Root growth is more sensitive to heavy metals than shoot growth (Obroucheva *et al.,* 1998; Titov *et al.*, 1996). This evidence correlates with the data that heavy metals accumulate predominantly in roots (Wong *et al.*, 1988; Seregin and Ivanov, 1997). Our results showed a strong growth inhibition by even Cd or Pb treatments on sorghum seedlings.

In order to alleviate toxicity induced by heavy metal stress, various compounds have been tested for their potential use in alleviation of stress and remediation of polluted media. Recently, the development of high-efficient and environment-friendly amendments (such as CNTs application) and their application in decontaminating polluted soils and waters have attracted considerable attention. (Wang *et al.*, 2012). In this project, two different concentrations of MWCNTs (10 and 50 mg/L) were applied to evaluate their impacts on cadmium and lead stressed sorghum seedling. Shoot growth was promoted in the presence of the CNTs 50 mg/l compared with the controls. Both CNT 10 and 50 mg/l were positively effective in improving root growth in sorghum seedlings. In roots, the seedling length was positively related to the exposure concentration of CNTs. The results obviously support the beneficial effect of multi-walled carbon nano tubes in 10 and 50 mg/L on growth parameter of sorghum seedlings exposed to toxic Cd/ Pb concentrations. Similar effects were described earlier on tobacco and tomato (Khodakovskaya *et al.*, 2009; Khodakovskaya *et al.*, 2012; Khodakovskaya *et al.*, 2013). It was shown that on tomato plants receiving CNTs the height was significantly higher compared with control plants (Khodakovskaya *et al.*, 2009; Khodakovskaya *et al.*, 2013).

It was reported that the activated process of water uptake could be responsible for the significantly higher biomass production for the plants that were exposed to carbon nanotubes (Khodakovskaya *et al.*, 2009). Molecular mechanisms of CNT-induced water uptake inside plants seeds are not clear and measuring water content seems to be of the main factors should be evaluated in further experiments. Khodakovskaya *et al.*, reported that CNTs can stimulate growth and activate gene and protein expression of aquaporin in tobacco cells (Khodakovskaya *et al.*, 2012). It has been found that overexpression of Arabidopsis aquaporin by CNTs in tobacco plants lead to increase in plant growth. Additionally, marker genes for cell division and cell extension were up–regulated in tobacco cells exposed to CNTs compared to control samples (Khodakovskaya *et al.*, 2012). The gene expression studies support this presumption of carbon nanotubes ability to affect plants physiology through up-regulation genes essential for plant development. However, positive effect of CNTs on plant growth could have significant economic importance on various aspects including agriculture, horticulture, and the energy sectors (Khodakovskaya *et al.*, 2009).

Chlorophyll Content

Chlorophyll pigments, as the main component of photosynthetic apparatus, play a pivotal role in plant metabolism and energy supply. In our study, Cd and Pb treatments significantly reduced the amount of chlorophyll. The exposure to 200 μM $CdCl_2$ decreased total Chl to about 82% of the control. 500 μM $Pb(NO_3)_2$ showed the decline of 77% of the control after 10 days (Figure 2). Various abiotic stresses including heavy metals decrease the chlorophyll content in plants (Ahmad *et al.*, 2007). In this experiment, both Pb and Cd treatment decreased total chlorophyll content in a remarkable manner. There are many researches corroborated with our findings on decrease in chlorophyll content with heavy metals (John *et al.*, 2008; Fodor *et al.*, 1996; Singh *et al.*, 2013; Prasad and Prasad, 1987; Muradoglu *et al.*, 2015). Chlorophyll biosynthesis inhibited by metals stress in higher plants (Prasad and Prasad, 1987). Inhibition of important enzymes in Chl biosynthesis, impairment in the supply of Mg^{2+} and Fe^{2+} (required for the synthesis of chlorophylls), Zn^{2+} deficiency (resulting in inhibition of enzymes) and the replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecule are supposed to be main interferences of Cd^{2+}

and Pb^{2+} on chlorophyll biosynthesis in plants exposed to stress (Assche and Clijsters, 1990). The loss in chlorophyll content can consequently lead to disruption of photosynthetic machinery (John *et al.*, 2008) which may cause to the retardation of normal root growth (Ouzounidou, 1994).

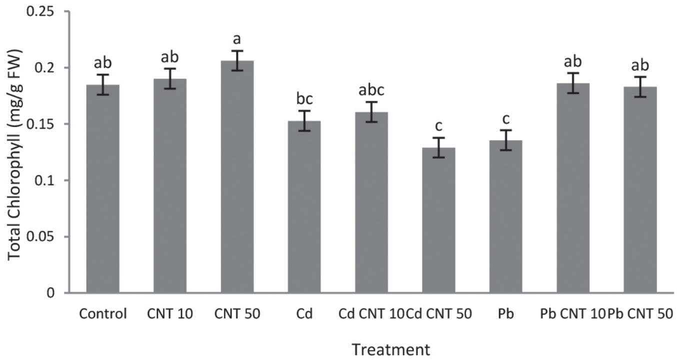

Figure 2 The effect of MWCNT on chlorophyll content of *Sorghum bicolor* seedlings under Cd or Pb treatment CNT 10 or 50: carbon nanotubes 10 or 50 mg/L, Pb and Cd concentration: 500 and 200 µM, respectively. Bar on each column shows ± SEM on three replicates

In the presence of MWCNTs, the inhibitory effects of Cd and Pb on chlorophyll content was considerably reduced. 50 mg/L CNTs increase total chlorophyll in sorghum seedlings under control conditions. MWCNTs application also improved Chl content heavy metal treated seedlings in some extent. MWCNT 50 mg/L was more effective on control plants. MWCNTs had a greater impact on alleviation of Chl decline in Pb stressed seedlings, rather to Cd stressed seedlings. Under Cd treatment, Chl content was improved by MWCNT 10 mg/L. Both MWCNT 10 and 50 mg/L were effective in improving Chl content of seedlings under Pb stress. It was previously demonstrated that photosynthetic efficiency may influenced by aquaporin overexpression on tobacco plants (Khodakovskaya *et al.*, 2012). Unfortunately, there are not comprehensive studies on the action mechanism of CNTs on photosynthetic apparatus and chlorophyll content. However our experiment showed that under heavy metal stress, CNTs protects seedlings from growth retardation and toxic effects of heavy metals on chlorophyll content.

MDA Content

Cd and Pb are highly toxic by inducing oxidative stress and ROS production in plant cells. Oxidative stress is indicated by the increased MDA contents of the cells. The present work investigated whether or not the MWCNTs interfere with the response of sorghum seedlings to heavy metals, since they have been reported to target membranes. Figure 3, compares malondialdehyde (MDA) contents of leaflets from sorghum plants subjected to Cd/Pb with or without presence of MWCNTs. MDA contents indicate lipid peroxidation and increased by about 66% upon Cd exposure and 72% upon Pb exposure in CNTs-free seedlings. MDA content decreased in seedlings exposed to MWCNT 50 mg/L under control condition (Pb0-Cd0). Cd-stressed seedlings treated by MWCNTs did not show significant changes in MDA content. Both MWCNT 10 and 50 mg/L increased lipid peroxidation in seedlings under Pb stress. A similar result was observed in a previous study about phyto-toxicity of Cd and Pb on strawberry (Muradoglu *et al.*, 2015; Sai Kachout *et al.*, 2015). Our findings suggest that decreasing growth might be the result of an MWCNT-induced oxidative stress and disturbance of photosynthesis pigments. It was reported that Taunit; an industrial material containing multi-walled carbon nanotubes, stimulates the growth of roots and stems and cause an increase in peroxidase activity in *Onobrychis arenaria* seedlings (Smirnova *et al.*, 2011). In this study, results on growth, chlorophyll content and MDA production confirmed the positive effects of MWCNTs under control condition. It seems that MWCNTs application alone reinforces plant growth by raising photosynthetic pigments and alleviation of lipid peroxidation under control condition. In Cd and Pb stressed seedlings, MWCNTs application had improving effects on growth but MWCNTs did not change chlorophyll and MDA content in a remarkable manner. It was also proposed that increase in peroxidase activity is associated with the oxidative stress caused by carbon nanomaterials (Smirnova *et al.*, 2011).

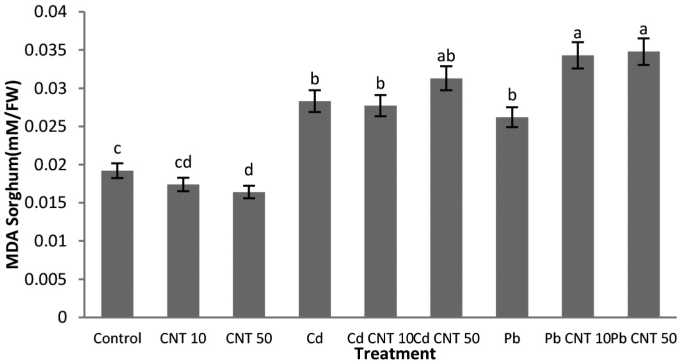

Figure 3 The effect of MWCNT on MDA content of *Sorghum bicolor* seedlings under Cd or Pb treatment CNT 10 or 50: carbon nanotubes 10 or 50 mg/L, Pb and Cd concentration: 500 and 200 μM, respectively. Bar on the each column shows ± SEM on three replicates

Larue *et al.* (2012) reported that less than 0.005% of the applied MWCNT dose is taken up by plant roots and translocated to the leaves which this accumulation does not induce any modifications in photosynthetic activity nor cause oxidative stress in wheat plant leaves. Despite to this assumption, our results showed that MWCNTs are able to promote plant growth and chlorophyll content and decline MDA production in sorghum seedlings under control conditions. Similarly, Chai *et al.* (2013) reported that the reduction of shoot biomass, water content, and plant height recovered after addition of CNTs on *Spartina alterniflora* under Cd stress. Authors concluded that CNTs may alleviate the toxicity caused by Cd stress and improve plant growth under Cd^{2+} stress. These evidences support Khodakovskaya *et al.* (2011) statement on the carbon nanotubes effects on genes regulation and physiology of plants. However, the mechanism of how the nanotubes can trigger the expression of specific genes requires further experimentations.

Cadmium and Lead Accumulation

Contents of lead and cadmium were ignorable in seedlings did not received Pb/Cd in growth medium. Several-fold increase in Pb/Cd content was observed in samples from plants treated with heavy metals. Cd contents were nearly the same in control and MWCNTs-treated plants. Neither MWCNTs 10 nor 50 exposure affect cadmium content compared to MWCNT-free cadmium treated seedlings. Pb contents increased by MWCNTs treatments; the highest Pb accumulation was belonging to Pb/CNT 50 mg/l) in sorghum seedlings (Table 1).

Table 1 Cd (A) and Pb (B) accumulation (mg/g DW) by *Sorghum bicolor* seedlings exposed to MWCNTs

	Control	CNT 10 (mg/L)	CNT 50 (mg/L)
Pb 0 μM	0.0052 ± 0.0002	0.0043 ± 0.0031	0.0062 ± 0.0008
Pb 500 μM	0.1561 ± 0.0023	0.1920 ± 0.0047	0.1984 ± 0.0007
Cd 0 μM	0.0003 ± 0.0001	0.0006 ± 0.0003	0.0011 ± 0.0004
Cd 200 μM	0.0552 ± 0.0061	0.0526 ± 0.0043	0.0517 ± 0.0021

CNT 10 or 50: carbon nanotubes 10 or 50 mg/L

Heavy metals accumulation in Cd/Pb treated seedlings was significantly higher compared to control. Therefore, slight increasing in MDA production upon Pb treatment might be contributed to higher Pb accumulation in seedlings. Moreover, MWCNTs exposure caused a higher accumulation of Pb especially at 50 mg/L, increasing Chl content and higher seedlings growth. At MWCNTs exposure, the increased lead accumulation in seedlings may be due to alleviation in factors that brought about growth reduction, resulting in an increase in total metal accumulation. It was reported that CNTs not only adsorb and immobilize heavy metal on plants, but also it alleviates damages on growth parameters (Chai *et al.*, 2013). Chai *et al.* (2013) found that the reduction of plant height, shoot biomass, and water content,) on *S. alterniflora* under Cd stress (200 mg kg^{-1}) were recovered after addition of CNTs (same as our results on plant height), indicating that CNTs may alleviate the toxicity caused by heavy metal stress and improve plant growth under higher Cd stress (Chai *et al.*, 2013). Other explanations for these findings could be CNTs ability to increase heavy metal allocation to vacuoles. Researches show that MWCNTs target specific cellular substructures including nucleus, plastids and vacuoles (Serag *et al.*, 2010). CNTs ability to deliver different cargoes into different plant cell organelles has been proposed (Liu *et al.*, 2009). Besides, the ability of carbon nanotubes in activation of genes/proteins expression and hence growth promoting effects of CNT supposed to be associated to maintaining of growth parameters under stress condition (Villagarcia *et al.*, 2012). Under various environmental stresses, one of the most important physiological responses of plants is to undergo osmotic adjustment by accumulation of ions in the vacuole and synthesis of compatible solutes in the cytosol (Li *et al.*, 2010). MDA contents were increased in seedlings treated by Pb upon exposure to CNTs. From the results it is obvious that sorghum seedlings have a higher sensitivity to Cd than Pb. Likewise, Huang *et al.* (1974) reclaimed higher toxicity effects of cadmium on soybean plants rather to lead ions (Huang *et al.*, 1974). It was reported that the adsorption mechanism of heavy metals by CNTs may be related to electrostatic attraction, and reactions between heavy metal ions and the surface functional groups of CNTs (Rao *et al.*, 2007).

The cadmium accumulation was not increased in seedlings receiving CNTs, but CNTs

50 mg/l leads to higher MDA production and lower chlorophyll content. It seems that on Cd-treated seedlings the effect of CNTs on lipid peroxidation was not attributed to changing the accumulation of Cd. Same as our results, Chai *et al.* (2013) showed that CNTs induce reduction in Cd accumulation in shoots and roots of *S. alterniflora* under Cd stress. They stated that CNTs may absorb the added Cd in the vermiculate, thereby reducing the Cd available for uptake by *S. alterniflora* and relieve the toxicity caused by Cd stress (Chai *et al.*, 2013). It is probable that cumulative effects of higher concentrations of CNT (50 mg/l) together with cadmium ions caused a higher toxicity effects on sorghum seedlings which consequently induce oxidative stress and photosynthetic pigments disturbance.

CONCLUSION

Effects of MWCNTs on growth and physiological parameters in *Sorghum bicolor* seedlings were investigated. 500 µM $Pb(NO_3)_2$ and 200 µM $CdCl_2$ resulted in decrease in growth and pigment content, and an increase in MDA content. The results indicated that the exposition of MWCNTs significantly promote root and shoot growth of seedlings in control and Cd/Pb treated seedlings.

MWCNTs application exerted a significant beneficial effect on growth and chlorophyll content of Pb-exposed plants. Pb accumulation was also increased by MWCNTs. Obviously; both MWCNTs 10 and 50 mg/L displayed the ability to alleviate the phytotoxicity of Pb to growth of sorghum seedlings. However, the mechanism of how the nanotubes can trigger heavy metal tolerance mechanisms requires further experimentations. It seems that sorghum seedlings have more sensitivity to Cd toxicity than Pb. Our results suggested that CNTs application may have positive regulatory roles on sorghum seedlings under heavy metal stress. However, CNTs detrimental effects to the living organisms should be considered since little information is available about nano–particles eco–toxicological effects.

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