HEAVY METALS INDUCED LIPID PEROXIDATION IN SPINACH MEDIATED WITH MICROBES

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Abstract

Rapid growth of industrial sector and lack of proper disposal of industrial wastes heavily loaded our soil reservoirs with toxic metals which is a serious threat to all form of life. Among other mechanisms, lipid per-oxidation is a major threat to biological matrix. The aim of this research work was to evaluate the lipid peroxidation induced by heavy metals in spinach that is mediated by microbes (Bacillus spp. and Coryne bacterium spp.) isolated from contaminated soils irrigated with industrial effluents of Gadoon Industrial Estate, Swabi (GIES) and Hyatabad Industrial Estate, Peshawar (HEIP). The severity of the lipid peroxidation induced by heavy metals was determined by malondialdehyde (MDA) contents, glycine betaine (GB), proline (Pro), hydrogen per oxide (H2O2) contents, photosynthetic pigments (Chlorophyll “a”, Chlorophyll “b” and “Carotenoids), total soluble sugar (TSS), total soluble proteins (TP), and cell viability (EC) estimation. Heavy metals contaminated soil significantly affected the photosynthetic system of the plant by lowering the content of Chlorophyll “a”, Chlorophyll “b”, carotenoids, total soluble sugar and total soluble proteins, while electrolyte contents, glycine betaine, proline, hydrogen peroxide and malondialdehyde contents in terms of lipid peroxidation were increased. Whereas the seeds inoculated with microbes showed significant increase in photosynthetic pigments, total soluble sugar and proteins contents with low cell leakage, glycine betaine, proline, hydrogen peroxide and malondialdehyde contents showing decrease in oxidative stress produced by heavy metals. Present results revealed that microbes inoculated plants showed low degree of lipid peroxidation which also confirmed the key role of microbes in bioremediation. Interestingly, Coryne bacterium spp., shows improved resistance to heavy metals contamination than Bacillus spp.

Key words: Heavy metals, Lipid peroxidation, Soil micro biota, MDA, Industrial effluents, Cell membrane stability.

Introduction

During growth, plants are exposed to the various stress factors in nature (Jahangir et al., 2009). Among others, heavy metals are naturally present in soil, where rapid industrialization and agriculture activities boosts their concentration to toxic level (Bhatti et al., 2017), where it poses foremost threats to human health and ecosystem because of its high toxic nature, less solubility, mutagenic, carcinogenic, and non biodegradable. Furthermore, it plays key role in DNA damage, enzymes deformation and destruction of different membrane embedded structures of cells by producing toxic complexes which interfere with routine cellular function (Diels, 2002; Rajbanshi, 2008). The most drastic and commonly documented consequences of such heavy metal contamination are the creation of reactive oxygen species (ROS) that is formed as a byproduct of cellular respiration.

ROS level is increased during heavy metal stress which affects cellular comportments by damaging lipids, disintegration of lipo-membrane and inhibition of various enzyme (Khan et al., 2013a; Wu et al., 2012) that leads to the disruption of the natural balance between ROS production and cellular detoxifying system. Reactive oxygen species or oxidants are the peroxides, super oxides or singlet oxygen, which attack on carbon-carbon double bonds producing different cytotoxic compounds. Heavy metals cannot be degraded to harmless products but can only be transformed from one state to another and become less toxic (Lasat, 2002; Garbisu & Alkorta1997; Garbisu et al., 2003).

Lipids peroxidation is well known in plants, where these are exposed to different environmental abiotic stresses especially heavy metals stresses that disrupts important cellular organelles (Jahangir et al., 2008) and resulting in low biomass, low germination rate, stunted growth and reduced crop yield (Alloway, 1990). Soil micro biota on other hand grow rapidly and utilized different soil substances for their nutritional purposes and provide plants fixed nitrogen, inhibit the effect of various environmental biotic and a biotic stresses by acquisition of nutrient resources (Glick, 2012). Microbes adopt different ways to cope with heavy metals in soil as by immobilization of heavy metals, by direct uptake of heavy metals or by using energy for this purpose which is available in the form of ATPs (Tebo et al., 1997; Nies & Silver, 1995). Due to positive role of microbes in the soil, heavy metals are either biotransformed or immobilized and are not available for plants and so level of lipid peroxidation is reduced in this case. Plant growth promoting bacteria can support their host plant (Jahangir et al., 2008) by releasing chelating substances and change the chemistry and mobility of heavy metals through the process of reduction, accumulation, immobilization and acidification to overcome the heavy metal induced stress and so improves plant growth (Glick, 2010; Yousuf et al., 2010). Microorganisms resistant to higher metals concentration can be used for cycling of metals, bioremediations and reclamation of polluted sites (Jiang et al., 2008). Present study was proposed to investigate lipid peroxidation induced by heavy metals in spinach grown in contaminated soils and the mediated potential of microbes isolated from same soil.

Material and Methods

Soil, seed and bacterial strains collection: Heavy metals contaminated soil was obtained from industrial estate of Gadoon, Swabi and Hyatabad industrial estate, Peshawar, Khyber Pakhtunkhwa. The soil was analyzed for heavy metals contamination according to the approved methods of United States Environmental protection Agency (Anon., 3050b, 1996). Spinach (*Spinacia oleracea* L.) seeds were obtained from National Agricultural Research Center (NARC) Islamabad, Pakistan while heavy metals resistant bacterial strains were obtained from Plant Stress Molecular Biology Lab, Kohat University of Science and Technology, Kohat isolated from heavy metals contaminated soil.

Seeds Sterilization and microbial inoculation: Seeds were surface sterilized with 3.5% sodium hypochlorite solution and were inoculated with heavy metal resistant strains having cell suspension of 10^7–10^8 CFU/ml for 10 hours (Chanway & Nelson, 1990). Seeds were grown in pots having 1.5 kg control and heavy metal contaminated soil. The soil was air dried and passed through 2mm steel mesh. The treatment included a control soil (soil without heavy metals), heavy metals contaminated soil, and heavy metals contaminated soil with seeds inoculated with microbes. Pots were irrigated with tap water as required and harvested after 60 days. After harvesting, plants (cut 2 cm above the ground) were analyzed for lipid peroxidation by different methods.

Lipid peroxidation (TBARS) estimation: MDA contents were estimated by the method described by Velikova et al. (2000). Reaction mixture of 25 mg Trichloroacetic acid and 2.5 mg of Thiobarbituric acid was prepared in double de-ionized water. Similarly second reaction mixture was prepared by adding 1.5 ml of enzyme extract and 2.5 ml of first reaction mixture and incubated at 100°C for 10 min in hot water bath. The reaction was terminated by placing the reaction mixture at ultra low temperature for 10 min. To achieve proper homogenization, mixture was gently vortexed and then centrifuged at 1300 rpm for 15 min, to obtain clear crude enzyme extract. Absorbance was noted at 532 nm while non specific absorption at 600 nm to estimate the amount of MDA by an extinction coefficient of 155nM^-1 cm^-1.

Cell viability in term of electrical conductivity: Cell viability was determined as described by Jamil et al. (2011). Equal number of small strips of 1 cm were cut and placed in 20 ml double distilled water in closed tubes. The tubes along with plant strips were incubated for 24th at 10°C and then at 25°C to measure electrical conductivity (CV1). After that, samples were autoclaved for 40 min and electrical conductivity (CV2) was noted by using conductivity meter (BMS EC Meter EC-4001).

Determination of photosynthetic pigments: Photosynthetic pigments were determined by taking equal amounts of dried plant material and MgO (25 mg) along with 5 ml methanol in autoclaved closed caped tubes, to prevent evaporation of methanol. The mixture was completely homogenized by vortex and placed at room temperature for 15 min and then placed on shaker for overnight at room temperature. Samples were then centrifuged at 4000 rpm for 5 mins at 25°C. Supernatant was removed and absorbance were noted at three different wavelengths 666 nm, 653 nm, and 470 nm on UV-visible spectrophotometer using methanol as standard. Total pigments like carotenoids, chlorophyll “a” and chlorophyll “b” were determined by using the formulae proposed by Lichtentaler & Wellburn (1985).

\[
C_a = 15.65 A_{666} - 7.340 A_{653} \\
C_b = 27.05 A_{653} - 11.21 A_{666} \\
C_{ab} = 1000 A_{470} - 2.860 C_a - 129.2 C_b / 245
\]

Proline estimation: Proline contents of plant samples were estimated by using protocol of Bates et al. (1973) with slight modifications as described by Janil et al. (2012). Plant material (100 mg) was completely homogenized with the help of homogenizer with 5 ml of 3% sulfo-salicylic acid following centrifugation at 4000 rpm for 30 minutes at 25°C. Equal volume of supernatant (1000 ul) and acid ninhydrin solution (1.25 g ninhydrine in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) were thoroughly mixed, along with 1 ml glacial acetic acid and was placed in oven for an hour at 100°C. Reaction was stopped by placing the samples in ultra low temperature for 10 min. After these reaction mixtures were completely homogenized with 2 ml toluene and placed at 25°C until two layers were formed. Supernatant was separated from the mixture and absorbance was measured by spectrophotometer at 520 nm by using toluene as blank. Total Proline content was determined according to formula:

\[
(\mu g \text{ proline/ml} \times 2/115.5) / (0.1/5)
\]

Glycine betaine estimation: Methodology of Grieve & Grattan (1983) was used to calculate glycine betaine contents. Plant materials of 1000 µg were homogenized in 10 ml de ionized water, followed by complete vortex for 10 min and then filtration. Crude extract of 1000 µl was mixed with 1000 µl of 2M HCl. After that 500 µl mixture and 200 µl potassium tri-iodide solutions was mixed in glass tube, again vortex and cool down the reaction with occasional shaking for 2 hours. Mixture was supplemented with 2000 µl distilled water and 20 ml pre cooled 1-2 dichloromethane. Two clear layers were formed in the tube placed on ice bath. Optical density of the organic layer was measured at 365 nm. The concentrations of the Glycine betaine were calculated with the help of standard curve.

Hydrogen peroxide contents: Hydrogen per oxide contents were determined by using methodology of Nankano & Asada (1980). Reaction mixture of 4000 µl was prepared with 1000 µl Potassium phosphate buffer (PBS), and 2000 µl Potassium Iodide (KI) and 1000 µl of enzyme extract. Absorbance of the mixture was measured at 390 nm.

Determination of soluble proteins: Fresh spinach leaves were grind in liquid nitrogen and completely homogenized with the help of electrical homogenizer by adding 10 ml phosphate buffer having pH 7.8 and centrifuged at 14000 rpm at 4°C for 20 minutes. Supernatant was removed and stored at 4°C. Soluble
protein was measured using Bradford assay (Bradford, 1976). Reaction mixture of 2.02 ml was prepared having composition of 500 µl Bradford reagent and 2000 µl distilled water and 20 µl extracted protein. The mixture was shaken vigorously and kept at room temperature for 5 minutes. The absorbance was noted at 595 nm having distilled water as a blank.

**Determination of soluble sugar:** Soluble sugar was quantified by the method of Dey (1990) with slight modification. Fresh spinach leaves (50 mg) were grinded into fine powder in 3 ml pre warmed solution of 90% ethanol. The sample was incubated at 80°C for 1 hour and the top aqueous layer was transferred to another sterile tube. The debris were grinded again in 3 ml pre-warmed 90% ethanol solution and incubated again at 80°C for 1 hr and its supernatant was collected, again. Now both the supernatants were mixed and diluted to 15 ml with distilled water. Plant extract of 1 ml was taken and mixed with 1 ml solution of 5% phenol. The mixture was added slowly with 5 ml solution of concentrated H₂SO₄ and then diluted up to 10 ml with distilled water. The mixture was then incubated for 30 minutes and stirred vertically with glass rod. The absorbance was measured at 485 nm by using filtered distilled water as blank.

**Statistical analysis:** Analysis of variance (ANOVA) was performed by using statistics 9 software; verticals bar represent standard deviation (SD) while alphabets represents significant differences between treatments.

**Results**

**Thiobarbituric acid reactive substances – TBARS:** Malondialdehyde (MDA) contents were high in plants grown in contaminated soil of GIES and HIEP, by showing MDA contents as 16.72 and 13.95 µmol in contaminated soil of both industrial estates as compared with control i.e. 12.49 µmol (Fig. 1). On other hand, plants raised from microbes inoculated seeds showed low amount of MDA with both microbes “a” (Bacillus spp.) and “b” (Coryne bacterium spp.) as 12.19 and 11.52 µmol in GIES while 8.9 and 10.92 µmol in HIEP respectively (Fig. 1).

**Glycine betaine:** High level of Glycine betaine (GB) i.e. 3.18 and 2.62 mg/g was determined in plants grown in GIES and HIEP soils, while seeds inoculated with microbes showed low level of GB as compared to non inoculated seeds plants (Fig. 2A). Both microbes (a and b) alleviate the drastic effect of heavy metals by lowering the concentration of GB in plants as 1.98 and 1.48 mg/g in plants grown from seeds inoculated with microbe (a) and 1.62 and 0.96 mg/g in plants grown from seeds inoculated with microbe (b) in both GIES and HIEP soils as shown in Fig. 2A.

**Proline:** Increase level of proline was noted in non inoculated plants of GIES and HIEP as 0.76 and 0.96 µmol/g respectively, while plants raised from seed inoculated with microbe’s showed reduced amount of proline indicating positive role of microbes in alleviating heavy metals stress (Fig. 2B). Microbes inoculated plants showed low level of proline accumulation as 0.76 and 0.33 µmol/g in GIES soil with microbe a and b, while similar results was observed in HIEP soil as 0.44 and 0.6 µmol/g as shown in Fig. 2B.

**Hydrogen Peroxide contents:** High level of Hydrogen per oxide content i.e., 1.02 and 1.05 µmol was found in plants grown in GIES and HIEP soils, while seeds inoculated with microbes showed low level of Hydrogen peroxide as compared to non inoculated seeds plants (Fig. 3). Both microbes (a and b) alleviate the harsh effect of heavy metals by lowering the concentration of Hydrogen per oxide in plants as 0.83 and 0.76 µmol in plants grown from seeds inoculated with microbe a and 0.57 and 0.78 µmol in plants grown from seeds inoculated with microbe b in both GIES and HIEP soils (Fig. 3).

**Total soluble sugar:** Total soluble sugar content was decreased in plants grown in heavy metals contaminated soil of GIES and HIEP i.e., 20.48 and 21.56 mg/g as compared to control (25.26 mg/g) as shown in Fig. 4. Microbes inoculated plants showed high level of total soluble sugar as 23.36 and 24.7 mg/g inoculated with microbe (a and b) in GIES soil, while similar results were noted in HIEP soil as 24.6 mg/g and 23.8 mg/g (Fig. 4).

**Total soluble proteins:** A decrease was noted in TSP contents of the plants grown in heavy metals contaminated soil of GIES and HIEP i.e., 22.4 and 18.53 mg/g as compared to control i.e. 31.36 mg/g (Fig. 4). On other side, plants grown from seed inoculated with microbes lowers the drastic effect of heavy metals by increasing the level of TSP (27.2 and 22.2 mg/g) in GIES and HIEP soil (22.5 and 21.2 mg/g (Fig. 4).

**Cell viability:** Heavy metals concentration in contaminated soil caused increase in cell membrane injury. Seeds inoculated with microbes showed low level of conductivity as compared to non inoculated seeds (Fig. 5). High electrolyte leakage (86.93 and 80.7 µs/cm) was found in plants grown in GIES and HIEP soils respectively as compared to control (61.43 µs/cm) while microbes inoculated plants showed low level of electrolyte leakage i.e., 55.46 and 65.9 µs/cm in GIES soil inoculated with microbe (a) while electrolyte leakages were 58.1 and 69.9 µs in HIEP soil inoculated with microbe (b) as shown in Fig. 5.

**Photosynthetic pigments:** Photosynthetic pigments (chlorophyll “a” chlorophyll “b” and carotenes) were significantly affected by heavy metals present in contaminated soil of GIES and HIEP as shown in Table 1. Chlorophyll “a” contents in plants grown in control soil were 11.7 mg/g while these values were lower in plants grown in GIES and HIEP i.e. 8.1 and 6.1 mg/g. Similarly Chlorophyll “b” and Carotenoids values were 4.9 and 7.2 mg/g in control while in plants grown in GIES and HIEP soils, these values were 4.2 and 4.2 mg/g, and 3 and 4 mg/g respectively (Table 1). On other side significant increase was noted in plants grown from microbes inoculated seeds i.e. chlorophyll a, b and Carotenoids (11.8, 5.6 and 6.3mg/g) in control soil, while in contaminated soil of GIES these values were 9.8, 4, and 6mg/g with microbe (a) and 12.4, 5.1 and 6.3mg/g with microbe (b) respectively. Same pattern of results was noted in HIEP soil, where chlorophyll a, b and Carotenoids contents with microbe (a) were 8, 3.5 and 5.2 mg/g and 10.3, 4.6 and 5.3 mg/g with microbe (b) (Table 1).
Fig. 1. Effect of control and heavy metals contaminated soil on MDA content of spinach plant mediated with microbes. **Con**: Control; **GIES**: Gadoon Industrial estate; **HIEP**: Hyatabad Industrial estate; **Con a**: Control + *Bacillus* spp.; **Con b**: Control + *Coryne bacterium* spp.; **GIES a**: Gadoon Industrial estate + *Bacillus* spp.; **GIES b**: Gadoon Industrial estate + *Coryne bacterium* spp.; **HIEP a**: Hyatabad Industrial estate + *Bacillus* spp.; **HIEP b**: Hyatabad Industrial estate + *Coryne bacterium* spp.

Fig. 2. Effect of control and heavy metals on contaminated soil on Glycine betaine (GB) (A) and Proline contents (Pro) (B) of spinach plant mediated with microbes. **Con**: Control; **GIES**: Gadoon Industrial estate; **HIEP**: Hyatabad Industrial estate; **Con a**: Control + *Bacillus* spp.; **Con b**: Control + *Coryne bacterium* spp.; **GIES a**: Gadoon Industrial estate + *Bacillus* spp.; **GIES b**: Gadoon Industrial estate + *Coryne bacterium* spp.; **HIEP a**: Hyatabad Industrial estate + *Bacillus* spp.; **HIEP b**: Hyatabad Industrial estate + *Coryne bacterium* spp.

Fig. 3. Effect of control and heavy metals contaminated soil on Hydrogen peroxide (**H$_2$O$_2$**) of spinach plant mediated with microbes. **Con**: Control; **GIES**: Gadoon Industrial estate; **HIEP**: Hyatabad Industrial estate; **Con a**: Control + *Bacillus* spp.; **Con b**: Control + *Coryne bacterium* spp.; **GIES a**: Gadoon Industrial estate + *Bacillus* spp.; **GIES b**: Gadoon Industrial estate + *Coryne bacterium* spp.; **HIEP a**: Hyatabad Industrial estate + *Bacillus* spp.; **HIEP b**: Hyatabad Industrial estate + *Coryne bacterium* spp.

Fig. 4. Effect of control and heavy metals contaminated soil on Total soluble sugar (TSS) and Total Soluble proteins (TSP) contents of spinach plant mediated with microbes. **Con**: Control; **GIES**: Gadoon Industrial estate; **HIEP**: Hyatabad Industrial estate; **Con a**: Control + *Bacillus* spp.; **Con b**: Control + *Coryne bacterium* spp.; **GIES a**: Gadoon Industrial estate + *Bacillus* spp.; **GIES b**: Gadoon Industrial estate + *Coryne bacterium* spp.; **HIEP a**: Hyatabad Industrial estate + *Bacillus* spp.; **HIEP b**: Hyatabad Industrial estate + *Coryne bacterium* spp.
Leaf number and size: It was found that plant raised from seed inoculated with microbes grown in contaminated soil of both industrial estates was more in leaf number and size. Plant raised from seeds inoculated with microbe grown in control soil had 5 leaves by comparing with non inoculated control plant (4 leaves). Similarly microbes inoculated plants grown in GIES and HIEP contaminated soil had better growth and leaf number i.e. 6 and 5 leaves respectively, by comparing with non inoculated plants i.e., 5 leaves as shown in Fig. 6 A & B. Similarly plant raised from seeds inoculated with microbe was larger in size as compared to plant non inoculated with microbe (Fig. 6A). Negative correlation was observed between MDA and Chlorophyll “a” (-0.462), chlorophyll “b” (-0.367) and Carotenoids contents (-0.455), while regression values of chlorophyll “a” “b” and Carotenoids with MDA were 9.43 -0.29, 7.00 -0.19 and 15.98 -0.48 respectively as shown in Fig. 7.

Table 1. Effect of control and heavy metals contaminated soil on Chlorophyll “a”, Chlorophyll “b”, Total Chlorophyll (a+b) and Carotenoids contents of spinach plant mediated with microbes.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chlorophyll (a) (mg/g)</th>
<th>Chlorophyll (b) (mg/g)</th>
<th>Total Pigm (a+b) (mg/g)</th>
<th>Carotenoids (mg/g)</th>
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Discussion

Lipids peroxidation is the oxidative degradation of lipids caused by reactive oxygen species (ROS) due to oxidative stresses. In plants, reactive oxygen species (ROS) are routinely generated during respiration process in mitochondria or in endoplasmic reticulum and play important role in cell signaling (Sharma et al., 2012), but upon exposure to stress conditions the production of ROS become high enough to damage plants tissues, cell viability, root growth and chloroplast development due to peroxidation of lipids of various membrane embedded structures of the cell. Oxidative stresses are induced by various types of environmental factors like heavy metals, drought, and salt (Jahangir et al., 2009). Climatic changes and anthropogenic activites boosts the concentration of heavy metals which affect plant growth and development (Alloway, 1990).

Plant-microbes interaction on other hands shows positive effect on plant physiology and productivity by providing plants fixed nitrogen, reduced the negative effect of various environmental biotic and a biotic stresses (Glick et al., 2007), enhanced nodules formation in host plants by entophytic bacteria (Wang et al., 2007), minimizing the deleterious effect of plant pathogens and by facilitating plant to obtain acquisition of nutrient resources (Glick, 2012; Yasmin et al., 2013). Malondialdehyde (MDA) is one of the important indicators of lipid peroxidation, in our study we found high values of MDA content in the plants grown in soil of HIEP and GIES, while these values were reduced when plants grown from seeds primed with microbes indicating low level of lipid peroxidation. Microbes adopt different ways to cope with heavy metals in soil and immobilize the heavy metals entry into plants (Tebo et al., 1997).

Microbes uptake metals from contaminated medium by chemiosmotic gradient of lipomembrane or by using energy molecules in the form of ATPs, while some microbes selectively uptake metals from soil leaving soil free from metals (Nies & Silver, 1995). *Bacillus* spp are actively involved in the biotransformation process of metals, oxidation of manganese, arsenic, chromium, selenium are often used for energy production and electron acceptor for anaerobic respiration (Santini et al., 2000; Quitantana et al., 2001). It is clear from the literature that microbes immobilized and biotransformed the metals from very toxic form to less toxic form and reduced their bioavailability to the plant lowering the oxidative damage with low level of lipid peroxidation and MDA content (Barkay & Schaefer, 2001). Similarly (Islam et al., 2016) reported that seed inoculated with *P. vermicola* showed resistance by restricting excess to heavy metals uptake and decreased occurs in MDA and *H₂O₂* contents under copper stress.

Osmolytes like proline and glycine betaine have protective role in plants commonly used as indicators of environmental stresses (Girija et al., 2002; Pandit et al., 2011). According to our results the amount of proline and glycine betaine were increased in the contaminated soil while in microbes inoculated seed raised plant, these amount was reduced indicating that microbes increased plant resistance to heavy metals (Dimkpa et al., 2009). According to Barkay & Schaefer, (2001), microbes either bio-transformed or immobilized the metals by reducing their bioavailability to plant system minimizing the stress effect ultimately lowering the concentration of proline and glycine betaine. Cell membrane is the first line of defense against heavy metals in medium, and heavy metals affect cell membrane by increase leakage from the membrane (Hall, 2002).

Heavy metals induce different types of oxidative stresses and disrupt the function of mitochondrial respiration (Shanker et al., 2005). Soluble sugar such as glucose, sucrose and fructose are important metabolic sources and play key role in cell signaling during different types of a biotic stresses including heavy metals, by reducing the amount of soluble sugar which ultimately affect the seed germination and seedling growth (Rosa et al., 2004). Reduction in total soluble sugar content of plants grown in heavy metals contaminated soil may due to inhibition of photosynthetic activity leads to low production of glucose in grana of chloroplast, so low availability of glucose in cytosol for glycolysis, resulting decrease in sugar content in the cell. Secondly stimulated metabolic path ways for energy production also utilized the remaining glucose resulting lowering the total sugar content of the cell (John et al., 2008; Bhattacharyya & Choudhuri, 1997; Ericson & Alfinto, 1984; Costa & Spitz, 1997; Mohan & Hosetti, 1997; Davis et al., 1987). On other side microbes alleviated the drastic effect of heavy metals and reduced the toxicity of heavy metals by increasing level of total soluble proteins and sugar in the plant (Fig. 4). The increased level of soluble sugar and protein in the plants in contaminated soil may be due to less excess of the plant to heavy metals as microbes are proven to uptake and immobilized the metals present in the soil by

Fig. 7. Coefficient of linear correlation “R” and Regression line between MDA and Photosynthetic pigments i.e. chlorophyll “a”, “b” and Carotenoids.
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chemiosmotic gradient of lipomembrane, direct uptake of heavy metals, biotransformation of heavy metals from high toxic form to low toxic form and by utilizing the heavy metals for energy production (Barkay & Schaefer, 2001; Santini et al., 2000; Nies & Silver, 1995). Secondly heavy metals in a medium enhance the production of ethylene which reduce or inhibit plant growth (Jackson, 1991; Goldstain, 1986) on the other hand bacteria in soil protect plant from negative effect of heavy metals and shows positive effect on plants growth by reducing the level of ethylene by hydrolyzing with ACC deaminase activity (Glick et al., 1998).

A reduction in photosynthetic pigments was noted in plants grown in the contaminated soil of both industrial estates (Table 1). A reduction in the chlorophyll contents may be due to the replacement of Mg and Fe ions of chlorophyll by toxic heavy metals (Pourraut et al., 2011), inhibition of the respective enzymes system essential for plant photosynthesis like Rubisco, and carbonic anhydrase (Mobin & Khan, 2007), inhibition of the Calvin cycle (Ali et al., 2015), lowering CO₂ fixation, decrease accumulation of protein pigment complexes. Heavy metals toxicity also damage carotenoids which serve as antioxidants against ROS and minimize different photochemical damage (Sengar et al., 2008). On other side microbes tolerant to metals for energy production (Barkay & Schaefer, 2012). On other side microbes tolerant to metals for energy production (Barkay & Schaefer, 2012).

Different markers were used to analyze lipid peroxidation in spinach grown in contaminated soil. Present study confirmed that heavy metals induced lipid peroxidation by increasing proline, glycine betaine contents, hydrogen peroxide and electrical conductivity. Whereas decreasing total soluble sugar and total soluble proteins along with lowering photosynthetic ability of plants. Microbes have the potential to alleviate the drastic affect of heavy metals in a medium by lowering lipid per oxidation of important membrane bounded organelles.

Conclusion

Different markers were used to analyze lipid peroxidation in spinach grown in contaminated soil. Present study confirmed that heavy metals induced lipid peroxidation by increasing proline, glycine betaine contents, hydrogen peroxide and electrical conductivity. Whereas decreasing total soluble sugar and total soluble proteins along with lowering photosynthetic ability of plants. Microbes have the potential to alleviate the drastic affect of heavy metals in a medium by lowering lipid per oxidation of important membrane bounded organelles.

Acknowledgment

Financial support for this study was provided by HEC through research grant no. 4372, Pakistan.

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(Received for publication 17 November 2016)