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Biochemical and Physiological Responses of Rice (*Oryza sativa* L.) Grown on Different Sewage Sludge Amendments Rates

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Abstract Using sewage sludge, a biological residue from sewage treatment processes, in agriculture is an alternative disposal technique of waste. To study the biochemical and physiological responses of Rice (*Oryza sativa* L.) grown on different sewage sludge amendments (SSA) rates a field experiment was conducted by mixing sewage sludge at 0, 3, 4.5, 6, 9, 12 kg m^{-2} rate to the agricultural soil. Rate of photosynthesis and stomatal conductance increased in plants grown at different SSA rate. Chlorophyll and protein contents also increased due to different SSA rates. Lipid peroxidation, ascorbic acid, peroxidase activity and proline content increased, however, thiol and phenol content decreased in plants grown at different SSA rates. The study concludes that for rice plant sewage sludge amendment in soil may be a good option as plant has adequate heavy metal tolerance mechanism showed by increased rate of photosynthesis and chlorophyll content and various antioxidant levels.

Keywords Sewage sludge amendment · Heavy metals · Physiological responses · *Oryza sativa*

Management of sewage sludge is problematic and if not dealt properly may lead to environmental pollution. Due to high economic and environmental costs of incineration and land filling operation, land application of sewage sludge may be a more preferred option (Singh and Agrawal 2008). Majority of studies indicated that crop production have been benefitted from land application of sewage sludge (Gaskin et al. 2003; Singh and Agrawal 2008, 2010a, b), but the presence of toxic metals such as Cd, Ni, Pb and Zn may accumulate in plant tissues and can cause food chain contamination (Chaney 1990; Singh and Agrawal 2008, 2010a, b).

Heavy metal stress stimulates the generation of free radicals and reactive oxygen species in plants (Halliwell and Gutteridge 1993). Presence of free radicals can disrupt the normal metabolism through oxidative damage to plant cellular components as these species bear strong oxidizing property and can attack all types of biomolecules. Under normal conditions, formation and removal of reactive oxygen species (ROS) are in balanced state, but in stressed conditions ROS are produced due to various environmental factors, this balance is disturbed resulting in enhanced production of ROS. A balance between ROS and their destruction is necessary to maintain metabolic functions and efficiency both under normal and stressed conditions. To mitigate and repair the damage initiated by ROS, plants have a defense system that helps in eliminating ROS before any damage to sensitive parts of the cells may occur (Moller 2001).

Singh and Sinha (2005) observed an increase in carotenoid and total chlorophyll contents in leaves of *B. juncea* grown in tannery sludge amendment @ 10%, 25%, 35%, 50%, 75% and 100% at 30 and 60 DAS, however, these pigments decreased at and beyond 35% tannery sludge amendments at 90 DAS. Increments in protein content of plants under heavy metals stress have been reported (Lou et al. 2004). Protein content in leaves of *B. juncea* increased at 10%–100% tannery sludge amendments at 30 and 60 DAS, however, at 100% amendment it decreased at 90 DAS (Singh and Sinha 2005). Excess of heavy metals can cause accumulation of proline in plants due to water deficit induced by heavy metals. Lipid peroxidation and

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proline content increased significantly in *B. juncea* due to tannery sludge amendments (Singh and Sinha 2005). Maximum increase (132%) in lipid peroxidation was observed at 100% tannery sludge amendment, whereas proline content increased maximally (103%) at 35% tannery sludge amendment at 90 DAS. Foliar thiol and ascorbic acid contents of *B. juncea* also increased at most of the tannery sludge amendment ratios.

In view of the above, field experiment was carried out on rice plant grown at different sewage sludge amendment (SSA) rates to assess the biochemical and physiological responses due to accumulation of metals in the plant.

Materials and Methods

The experiment was conducted at the agricultural farm of Banaras Hindu University, Varanasi, India between July and November 2005. Sewage sludge was collected from 80 MLD Dinapur sewage treatment plant (DSTP). Field was prepared by using standard agronomic practices. Eighteen plots of 1.5 × 1.5 m size having 0.25 m margin between the plots were prepared, and air dried and well grounded sewage sludge was mixed in soil at 3, 4.5, 6, 9 and 12 kg m^{-2} sewage sludge amendments (SSA) rates, respectively. There were three replicate plots of each treatment and plots were left for 15 days for stabilization. Unamended plots were used as control. Treatments were designated as T_0 for unamended control, T_1 for 3 kg m^{-2}, T_2 for 4.5 kg m^{-2}, T_3 for 6 kg m^{-2}, T_4 for 9 kg m^{-2} and T_5 for 12 kg m^{-2} sewage sludge amendments (SSA), respectively for convenience. Seeds were grown in seed beds without any SSA. Each plot was flooded with water to generate water logged condition. Fifteen days after nursery preparation, three seedlings were transplanted together at 15 cm distance from each other in plots already prepared after amending sewage sludge at different rates. Time to time irrigation was done to keep water logged condition. Depending upon the weed intensity, hand weedings were done. Rice (*Oryza sativa* L.) cv Pusa sugandha 3, a highly recommended cultivar for northern zone of India was used as test plant. Thinning was done after germination of seeds to maintain a distance of 15 cm between the plants.

Soil samples collected in triplicate from each treatment were air dried, crushed, passed through a sieve of 2 mm mesh size and then stored separately for further physico-chemical analyses. For analysis of heavy metals in soil samples, 1 g air-dried sample was digested in 20 ml of tri acid mixture (HNO_3:H_2SO_4:$HClO_4$:5:1:1) for 8 h at 80 °C following the method described by Allen et al. (1986). After complete digestion solution was filtered and the filtrate was analyzed separately for heavy metals using Atomic Absorption Spectrophotometer (Model 2380, Perkins Elmer, Inc., Norwalk, CT, USA).

The pH of the soil at different treatments was measured in the suspension of 1:5 (w/v) with the help of pH meter (Model EA940, Orion, USA) standardized with pH 4, 7 and 9.2 reference buffers. Conductivity was measured by conductivity meter (Model 303, Systronics, India). Organic carbon and total nitrogen contents of the soil samples were determined by Walkley and Black's rapid titration method (Allison 1973) and Gerhardt automatic analyzer (Model KB 8S, Germany), respectively. The available P in soil and sewage sludge samples was quantified by $NaHCO_3$ extraction method given by Olsen and Sommers (1982). The rates of photosynthesis (Ps) and stomatal conductance (Cs) were measured in fully expanded leaves using portable photosynthesis system (Lcpro, ADC BioScientific Ltd., Hoddesdon, Herts) at ambient climatic conditions between 9 and 11 am at photo synthetically active radiation ranging from 1,000 to 1,200 µmol m^{-2} s^{-1} on three randomly selected plants of each treatment at 75 days after sowing (DAS). The chlorophyll fluorescence measurements were also performed on the same leaf during 10–11 am using portable plant efficiency analyzer (MK2 9414, Hansatech Instruments Ltd., UK). Leaf clips for dark adaptation were placed on the adaxial side of the leaves 30 min before the measurement at excitation irradiance intensity of 200 µmol m^{-2} s^{-1}. Initial fluorescence (Fo), maximal fluorescence (Fm) variable fluorescence (Fv) and Fv/Fm ratios were measured.

Fully expanded fresh leaves were sampled manually at 50 and 80 DAS and kept in deep freezer for further estimation of photosynthetic pigments, lipid peroxidation and different metabolites. Chlorophyll and carotenoid contents expressed as mg g^{-1} dry leaf was estimated according to the method of Machlachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Protein content in the fresh leaves was analyzed by the method of Lowry et al. (1951). Foliar ascorbic acid and proline contents were measured by the method of Keller and Schwager (1977) and Bates et al. (1973), respectively. Peroxidase activity was measured using the method of Britton and Mehley (1955). Total phenol and thiol contents were measured following the methods of Fahey et al. (1978) and Bray and Thorpe (1954), respectively. The MDA (Malondialdehyde) level representing the index of lipid peroxidation was measured by the method given by Heath and Packer (1968).

The data were subjected to one-way analysis of variance (ANOVA) using SPSS version 10 software. Duncan's multiple range test was performed to test the significance of difference between the treatments. Precision and accuracy of heavy metal analyses were also assessed through repeated analysis of the samples against National Institute of Standard and Technology, Standard Reference Material

(SRM 1570) for all the heavy metals. The results were found to be within ±2% of the certified value.

Results and Discussion

The sewage sludge of DSTP used for amending agricultural farm soil was neutral in pH and had high electrical conductance (2.28 mS cm^{-1}), organic carbon (5.52%), total N (1.73%), available P (716.7 mg kg^{-1}) and Total Fe (6,059 mg kg^{-1}) contents (Table 1). The concentrations of Ni, Zn, Mn, Pb, Cr, Cd and Cu were 47.17, 785.3, 186.2, 60, 35.5, 154.5 and 317.7 mg kg^{-1}, respectively in sewage sludge of Dinapur STP (Table 1). Physico-chemical as well as biological properties of sewage sludge vary with wastewater composition as well as treatment processes (Melo et al. 2002; Sharma et al. 2006). Sewage sludge of STPs, Okhla, Delhi showed variations in pH from slightly acidic to alkaline (pH 6.8–9.6; Shrivastava and Banerjee 2004). Electrical conductivity of sewage sludge from DSTP, was several times higher than that of the value reported from Delhi (0.56 mS cm^{-1}; Shrivastava and Banerjee 2004).

It was found that in present study due to SSA treatments soil pH decreased, but EC increased significantly (Table 1). This trend may be ascribed to lower pH and higher EC of sewage sludge of Dinapur as compared to the agricultural farm soil. Reduction in soil pH may be attributed to release of humic acid as a result of biodegradation of amended sewage sludge which is rich in organic carbon. Parkpain et al. (2000) however, reported increase in soil pH upon sewage sludge amendment.

Organic C, total N, available P and total Fe contents of soil increased due to SSA amendment significantly (Table 1). Increase in soil nutrients due to sewage sludge application has been reported by several workers (Parkpain et al. 2000; Martinez et al. 2003; García-Orenes et al. 2005; Singh and Agrawal 2007, 2008, 2009). Land application of sewage sludge has been shown to provide potential benefits for agricultural soils in form of N (Binder et al. 2002) and P (Hogan et al. 2001). Sewage sludge amendment in soil led to higher concentrations of total heavy metals (Table 1). Total heavy metals concentrations were highest at higher SSA rate for all the heavy metals under study. Concentration of Zn was highest in sewage sludge amended soil followed by Mn, Cu, Pb, Ni, Cd and Cr (Table 2). Use of sewage sludge as soil amendment has been shown to present potential environmental risks of heavy metals in soil (Singh and Agrawal 2007; Wong et al. 2001). Significant positive correlations between plant heavy metal uptake and total heavy metal content in soil have been reported (Singh and Agrawal 2007, 2009, 2010a, b).

Sewage sludge amendments increased the contents of chl a, b and total significantly at both the ages of observations as compared to the plants grown in unamended soil (Fig. 1). Total chl contents in rice plants were 1.60, 1.85, 1.91, 2.04, 2.41 and 2.43 mg g^{-1} in T_0, T_1, T_2, T_3, T_4 and T_5 plants, respectively at 80 DAS. Increment in total chl content was maximum (88%) in plants grown at 12 kg m^{-2} SSA at 50 DAS. Maximum increments of 101% and 99% in chl a and b contents, respectively were observed in T_5 (12 kg m^{-2}) plants at 50 and 80 DAS, respectively. Carotenoid content also increased significantly at both the ages of observations at all the SSA rates (Fig. 1). Maximum increment (77%) in carotenoid content was observed in T_5 plants at 50 DAS as compared to those grown in unamended control soil.

Table 1 Physico-chemical properties of sewage sludge, soil (T_0) and different SSA rates at 0 DAS of rice plants (Mean ± 1SE)

Parameters	Sewage sludge	T_0	T_1	T_2	T_3	T_4	T_5
pH (1:5)	7.0 ± 0.01	8.17 ± 0.02^{a}	8.14 ± 0.05^{ab}	8.11 ± 0.02^{abc}	8.01 ± 0.05^{c}	8.00 ± 0.03^{c}	7.93 ± 0.04^{bc}
E. C. (mS cm^{-1})	2.28 ± 0.0	0.24 ± 0.01^{d}	0.26 ± 0.01 ^{cd}	0.27 ± 0.01^{c}	0.29 ± 0.01^{bc}	0.31 ± 0.01^{b}	0.37 ± 0.01^{a}
Organic C (%)	5.52 ± 0.12	0.78 ± 0.02^{e}	1.26 ± 0.02^{d}	1.39 ± 0.03^{c}	1.44 ± 0.02^{c}	1.54 ± 0.03^{b}	1.77 ± 0.02^{a}
Total N (%)	1.73 ± 0.02	0.15 ± 0.00^{f}	0.16 ± 0.00^{e}	0.16 ± 0.00^{d}	0.18 ± 0.00^{c}	0.19 ± 0.00^{b}	0.21 ± 0.00^{a}
Avail. P (mg kg^{-1})	716.7 ± 60.1	54.10 ± 2.76^{f}	80.70 ± 1.59^{e}	91.77 ± 1.68^{d}	108.81 ± 1.82^{c}	123.70 ± 1.10^{b}	132.12 ± 2.22^{a}
Total Fe (mg kg^{-1})	6,059 ± 104.61	517.6 ± 16.6^{e}	732.3 ± 21.1^{d}	864.3 ± 12.2^{c}	951.7 ± 24.6^{c}	1,179.7 ± 55.9^{b}	1,384.7 ± 63.6^{a}
Ni (mg kg^{-1})	47.17 ± 0.32	12.5 ± 0.59^{d}	16.0 ± 0.68^{c}	18.4 ± 0.54^{c}	18.2 ± 0.31^{c}	21.2 ± 1.23^{b}	24.2 ± 1.2^{a}
Mn (mg kg^{-1})	186.2 ± 1.33	120.8 ± 2.2^{c}	130.6 ± 5.9^{c}	136.6 ± 4.5^{bc}	147.7 ± 4.8^{b}	148.2 ± 4.8^{b}	175.2 ± 7.3^{a}
Zn (mg kg^{-1})	785.3 ± 16.69	63.1 ± 3.0^{e}	109.6 ± 3.3^{d}	147.4 ± 4.4^{c}	162.9 ± 5.2c	184.7 ± 6.5^{b}	221.8 ± 8.6^{a}
Pb (mg kg^{-1})	60.0 ± 5.77	11.3 ± 0.5^{e}	15.5 ± 0.8^{d}	16.5 ± 0.8 ^{cd}	18.5 ± 0.8^{c}	25.3 ± 0.9^{b}	29.0 ± 1.2^{a}
Cr (mg kg^{-1})	35.5 ± 0.76	5.5 ± 0.6^{d}	6.8 ± 0.6 ^{cd}	8.6 ± 0.7^{c}	11.2 ± 0.6^{b}	12.7 ± 1.2^{b}	15.4 ± 0.8^{a}
Cd (mg kg^{-1})	154.5 ± 2.52	2.03 ± 0.2^{e}	8.5 ± 0.6^{d}	10.7 ± 0.7^{c}	12.7 ± 0.5^{c}	16.9 ± 0.8^{b}	21.3 ± 0.9^{a}
Cu (mg kg^{-1})	317.7 ± 1.92	15.1 ± 0.5^{f}	19.6 ± 1.0^{e}	25.7 ± 0.7^{d}	31.6 ± 1.7^{c}	42.3 ± 1.8^{b}	48.6 ± 1.4^{a}

T_0, Unamended soil; T_1, 3 kg m^{-2}; T_2, 4.5 kg m^{-2}, T_3, 6 kg m^{-2}; T_4, 9 kg m^{-2}; T_5, 12 kg m^{-2}

Different letters in each group show significant difference at $p < 0.05$

Table 2 Variations in selected physiological characteristics of rice plants grown at different SSA rates (Mean ± 1SE)

Parameters	T_0	T_1	T_2	T_3	T_4	T_5
Photosynthesis rate (μmol m^{-2} s^{-1})	8.76 ± 0.38^{c}	10.02 ± 0.54^{c}	13.01 ± 1.39^{b}	13.13 ± 0.95^{b}	16.83 ± 0.63^{a}	15.62 ± 1.19^{ab}
Transpiration (mmol m^{-2} s^{-1})	4.27 ± 0.85^{a}	3.04 ± 0.60^{b}	2.93 ± 0.45^{b}	2.75 ± 0.55^{b}	2.79 ± 0.25^{b}	3.06 ± 0.12^{b}
Stomatal conductance (cm s^{-1})	2.60 ± 0.31^{bc}	2.64 ± 0.26^{bc}	3.05 ± 0.48^{ab}	2.77 ± 0.15^{b}	2.81 ± 0.48^{b}	3.56 ± 0.28^{a}
Initial fluorescence (Fo) (millivolt)	551.7 ± 13.8^{c}	627.67 ± 4.3^{b}	662.0 ± 4.9^{ab}	687.7 ± 19.8^{a}	691.0 ± 8.7^{a}	693.3 ± 16.2^{a}
Maximal fluorescence (Fm) (millivolt)	$2,891.0 \pm 89.3^{ab}$	$2,990.3 \pm 91.0^{a}$	$3,065.7 \pm 70.4^{ab}$	$2,865.7 \pm 67.4^{ab}$	$2,867.7 \pm 119.0^{ab}$	$2,633.3 \pm 74.9^{b}$
Variable fluorescence (Fv) (millivolt)	$2,306.0 \pm 46.2^{a}$	$2,362.7 \pm 87.1^{a}$	$2,403.7 \pm 66.0^{a}$	$2,178.0 \pm 59.3^{a}$	$2,176.7 \pm 113.8^{a}$	$1,940.0 \pm 75.6^{a}$
Fv/Fm ratio	0.807 ± 0.00^{a}	0.790 ± 0.01^{b}	0.784 ± 0.00^{bc}	0.760 ± 0.01^{bc}	0.758 ± 0.01^{bc}	0.736 ± 0.01^{c}

T_0, Unamended soil; T_1, 3 kg m^{-2}; T_2, 4.5 kg m^{-2}, T_3, 6 kg m^{-2}; T_4, 9 kg m^{-2}; T_5, 12 kg m^{-2}

Different letters in each group show significant difference at $p < 0.05$

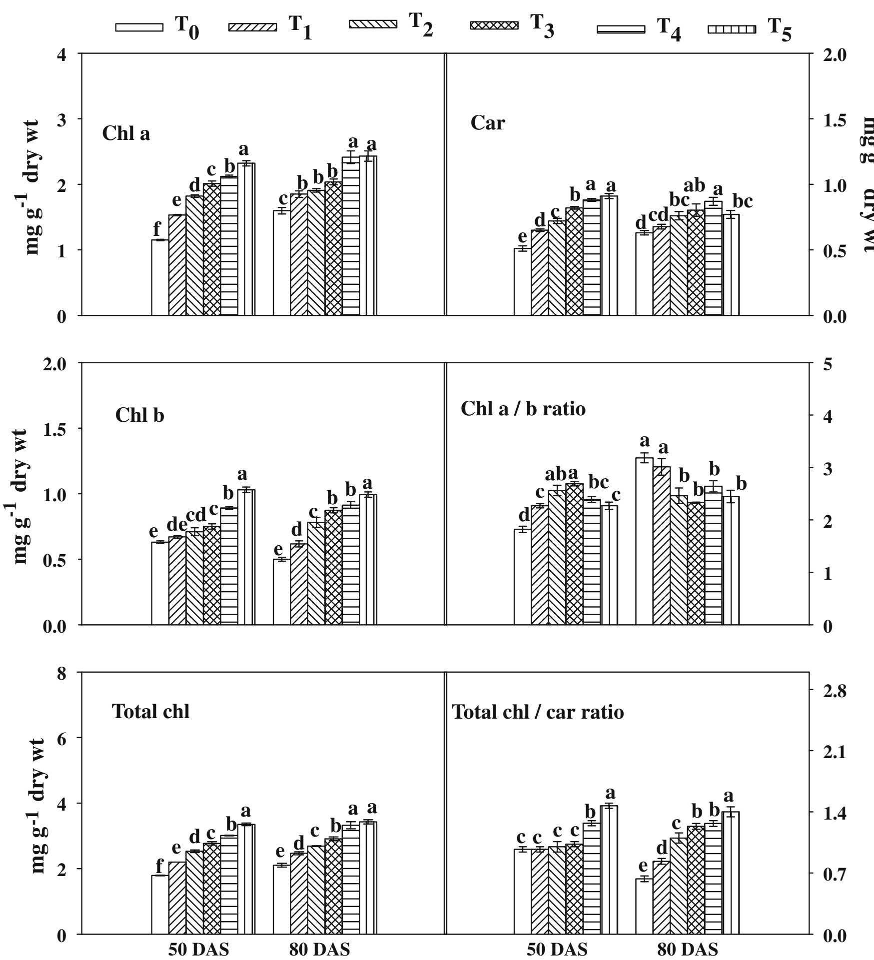

Fig. 1 Chl a, chl b, total chlorophyll and carotenoid contents, Chl a/b and total chl/carotenoid ratios in rice plant grown at different SSA rates at different ages (Mean ± 1SE). *Bars* with *different letters* in each group show significant difference at $p < 0.05$

Chl a/b and total chl/carotenoid ratios were affected differently at various SSA rates. Chl a/b ratio increased significantly at 50 DAS, but decreased significantly at 80 DAS at all SSA rates (Fig. 1). Increment in chl a/b ratio was maximum (48%) in T_3 (6 kg m^{-2}) plants at 50 DAS as compared to T_0 plants. Total chl/carotenoid ratio increased significantly at 80 DAS at all SSA rates, but at 50 DAS, significant increments were observed at T_4 and T_5 treatments as compared to T_0.

El-Sabour et al. (1997) reported increase in total chlorophyll content under organic waste compost application to *Helianthus annus*. Singh and Agrawal (2007) also reported decrease in total chlorophyll in Palak (*Beta vulgaris* var. Allgreen H1) grown in 20% and 40% sewage sludge amended soil. Carotenoids are non-enzymatic antioxidants, which protect the chlorophyll molecules against oxidative stresses (Halliwell 1987). Increase in carotenoid content may thus be attributed to the plant defense strategy to overcome the heavy metal stress.

Stomatal conductance and photosynthetic rate (Ps) increased (Table 2) significantly in plants grown in different SSA as compared to those grown in unamended soil. Reduction in photosynthetic rate has been reported under high heavy metal concentrations (Mysliwa-Kurdziel and Strzalka 2002; Krupa and Baszynski 1995). High nutrient availability to plant due to SSA can be attributed to increase in plant photosynthesis. The increase in photosynthesis rate can also be directly correlated to increases in total chlorophyll content of respective plants grown under various SSA rates. However, increase of stomatal conductance may be due to high nutrient availability through SS amendment which nullified the heavy metal toxicity.

Chlorophyll fluorescence measurement technique reflects the changes in Chlorophyll a fluorescence due to altered PS II activity. Initial fluorescence (Fo) is known to be very sensitive to any stress that interferes with the structural integrity of PS II, while variable fluorescence (Fv) is sensitive to the changes in rates of electron transfer and membrane ultrastructure (Mohammadian et al. 2003). Significant increase in Fo observed during the present study (Table 2) indicates photoinhibition. Fv/Fm ratio varying from 0.78 to 0.85 are associated with healthy and non-stressed plants (Demming and Bjarkman 1987). In the present study, the values of Fv/Fm were 0.80, 0.79, 0.78, 0.76 and 0.74, respectively for the plants grown in control, 3, 4.5, 6, 9 and 12 kg m^{-2} SSA rates (Table 2). Indicating a stress on photosynthetic system of plants under sewage sludge amendment as compared to unamended control.

Protein content increased significantly in plants grown at different rates of SSA as compared to those grown in unamended soil, increments being higher at 12 kg m^{-2} SSA at 50 DAS and at 9 kg m^{-2} SSA at 80 DAS (Fig. 2). Increase in protein content may be due to higher organic matter content of sewage sludge amended soil, which led to slow release of N in the soil. In the present study, N content of sewage sludge amended soil was 7%, 7%, 20%, 27% and 40% higher at 3, 4.5, 6, 9 and 12 kg m^{-2} SSA, respectively as compared to unamended soil. Wollgieh and Newmann (1995) reported an induction of heat shock protein in tomato cultivars under metal stress condition. Foliar thiol content decreased significantly at all SSA rates at both the ages of observations (Fig. 2). Decrease in thiol content was also reported in sunflower treated with Cu and Cd (Gallego et al. 1996), in bladder campion (*Silene cucubalus*) grown in presence of Cu (De Vos et al. 1992) and in palak (*B. vulgaris*; Singh and Agrawal 2007) and lady's finger (*Abelmoschus esculentus*; Singh and Agrawal 2009) grown at 20% and 40% SSA ratios. Decrease in thiol content may be correlated with increase in protein content, as thiols are utilized in the formation of protein.

Lipid peroxidation measured as MDA concentration increased significantly at different SSA rates in rice plants as compared to the control (Fig. 2). Membrane injury as a result of oxidative stress caused by reactive oxygen species (ROS) generated by toxic heavy metal has been related to an increase in peroxidation in plants (De Vos et al. 1989). The magnitude of lipid peroxidation was higher at higher SSA rates at both the ages of observation suggesting higher oxidative stress at higher amendment rates. Similar result has been reported by Singh and Agrawal (2007) in palak (*B. vulgaris*) and Singh and Agrawal (2009) in lady's finger (*A. esculentus*) grown at 20% and 40% SSA ratios.

Significant increase in foliar proline content at increasing SSA rates at both the ages of observation during present study suggests that elevated levels of heavy metals in plants under sewage sludge amendment have affected the permeability of membranes, causing water stress like condition leading to proline accumulation (Pesci and Reggiani 1992). Phenol, a secondary metabolite decreased significantly in plants grown at different SSA rates as compared to the control at both the ages of observation (Fig. 2). A decrease in phenol content has been reported in tea (*Camellia sinensis*) leaves under heavy metal stress (Basak et al. 2001). Singh and Agrawal (2007) also reported reduction in phenol content of palak (*B.vulgaris*) at 20% and 40% SSA.

Ascorbic acid, a powerful antioxidant scavenging free oxy-radicals (Smith et al. 1989), content increased significantly in plants under different SSA rates as compared to those grown in unamended soil at both the ages of observations (Fig. 2). However, ascorbic acid content did not varied significantly between different SSA rates. Thus, increase in ascorbic acid content is an indicative of defensive response against the oxidative stress caused by elevated levels of heavy metals under SSA rates. Significant increase in peroxidase activity of plants under different SSA rates also indicates heavy metal induced production of ROS. The increment in peroxidase activity was more at 40% SSA suggesting higher oxidative stress than that observed at 20% SSA. Chaoui and Ferjani (2004) reported increases in cell wall peroxidases of pea (*Pisum sativam* cv. Douce province) seedlings under Cd treatment.

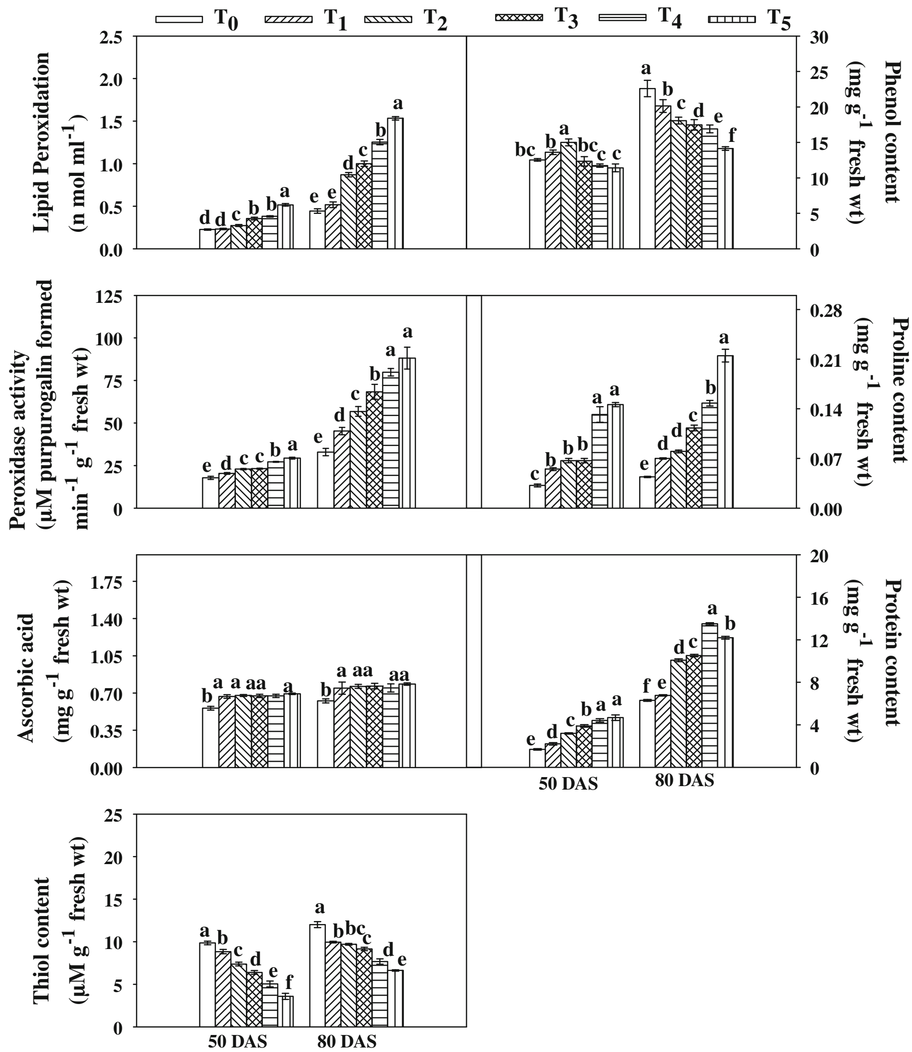

Fig. 2 Lipid peroxidation, ascorbic acid, phenol, protein, thiol and proline contents and peroxidase activity of rice plants grown at different SSA rates (Mean ± 1SE). *Bars* with *different letters* in each group show significance difference at $p < 0.05$

Similar result has been reported by Singh and Agrawal (2007) in palak (*B. vulgaris*) and Singh and Agrawal (2009) in lady's finger (*A. esculentus*) grown at 20% and 40% SSA ratios.

The present study clearly showed that organic carbon, total N, available P and exchangeable nutrients contents in soil increased due to sewage sludge amendment. Concentrations of heavy metals such as Cu, Cd, Cr, Zn, Pb, Ni and Mn also increased due to SSA, which led to accumulation of these metals in various plants parts. The higher accumulation of heavy metals in plants led to reduced transpiration rate, disturbed photochemical light quenching, increased lipid peroxidation and proline and protein contents, however, increase in photosynthetic rate and chlorophyll pigments have been reported due to different SSA.

The results of the present study suggests that rice may be a good option to be grown on sewage sludge amended soil as the plants showed tolerance under elevated heavy metal concentrations by increased rate of photosynthesis and chlorophyll content and various antioxidant levels.

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