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Research article

Physiological response of metal tolerance and detoxification in castor (*Ricinus communis* L.) under fly ash-amended soil

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ABSTRACT

Castor (*Ricinus communis* L.) is a metal tolerant plants and its ability to survive in highly polluted sites as well as its capacity for metal accumulation. There are very few reports on their physiological mechanism of metal tolerance and detoxification under fly ash. Therefore, an *in-situ* experiment was designed to study its biomass accumulation, photosynthetic response and antioxidative metabolism under different levels of fly ash amendments. Significant *($P < 0.05$)* increase in plant biomass and metal tolerance index was observed in *R. communis* under 50 % fly ash in soil amendments in comparison to the control plants. In addition, photosynthetic activity was not significantly altered under fly ash amended soil in comparison to the garden soil, but these responses/activities were remarkable lowered under bare fly ash. The induction of antioxidant enzymes was also observed in different tissue over control under fly ash treatments. The bioconcentration factor (BCF) of Al, Fe, Zn, Mn, Cu and Cr in *R. communis* were recorded greater than one under fly ash (50%) with soil amendments. Therefore, it concluded that it can be used for phytoremediation of fly ash and fly ash (50%) with soil amendments enhanced phytoremediation ability.

1. Introduction

Fly ash is a waste material of coal-based industries and its storage and disposal have become a major concern worldwide (Pandey, 2013). The managing fly ash deposits becomes imperious in ecological stand points as fly ash contains toxic metals (Haynes, 2009; Kisku et al., 2018). To overawed this problem, phytorestoration is the need of the hour for managing the fly ash dump site (Gajić et al., 2013). However, establishing vegetation cover in fly ash dump site is difficult because of higher concentration of metals and devoid of essential nutrients with severely alkaline properties that negatively affect the plant growth in fly ash (Verma et al., 2014). In this regard, understanding the physiological response of plants to unfavourable properties of fly ash provides the selection of the plants for phytorestoration (Gajić et al., 2013). Recent studies suggested on the use of high value industrial crops for better management and restoration of fly ash deposit sites (Pandey, 2013; Verma et al., 2014; Panda et al., 2018a).

The castor is a promising species used for phytoremediation with its ability to survive in highly polluted sites (Adhikari and Kumar, 2012). It is a commercial non-edible oil crop and It has many industrial applications such as its oil is used for paints, cosmetics, lubricants and biofuel, etc (Huang et al., 2011; Adhikari and Kumar, 2012). Recently, researchers have identified that the *R. communis* L. has the ability to accumulate metal and suitable for phytoremediation in fly ash deposits (Pandey, 2013; Pandey et al., 2014). However, its growth and physiological response of *R. communis* L. under different levels of fly ash is still lacking. Phytoremedial potential of plants can be measured through its elevated antioxidative potential with increased capacity to scavenge reactive oxygen species (ROS) (Nadgórska-Socha et al., 2013). Therefore, the plant antioxidant defence system may be important to reveal its tolerance mechanisms to different metals present in contaminated soil (Bisoi et al., 2017). However, relatively few reports have been published on the oxidative metabolism in higher plants under fly ash including Castor (Bisoi et al., 2017; Nadgórska-Socha et al., 2013). Thus, the study aims to: a) assess the growth and photosynthesis in *R. communis* under fly ash amendments; b) evaluating the metal accumulation potential of *R. communis* under different levels of fly ash; c) assessing the adaptive physiological response of *R. communis* under different levels of fly ash and d) find out suitable levels of fly ash in soil amendments for better phytoremediation.

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2. Material and methods

2.1. Characterisation of fly ash and garden soil

The fly ash of NALCO (National Aluminium Corporation Limited), Koraput, India was used for the current experiment. The soil used for the study was taken from the experimental garden of Central University of Odisha. A total of three different treatments were prepared by mixing of fly ash and garden soil, which were denoted as T_1: 100% garden soil; T_2:100% fly ash; T_3: 50 % fly ash +50% garden soil. After that these mixtures were kept separately in plastic pots (45 × 30 cm diameter and height). The pH of the substrates was measured in a pH meter (D1-707, Digison model) and electrical conductivity (EC) was estimated in a conductive meter (D1-909, Digison model) as per the method of Panda et al. (2018a). Total organic carbon was estimated according to Bisoi et al. (2017). The samples were also analysed for the available Nitrogen (N), Phosphorus (P) and Potassium (K) in the laboratory following APHA (2017).

2.2. Plant growth condition and plant growth

Five healthy seeds of *R. communis* L. were sown on plastic pots in three replications for each treatment condition. Two seedlings of the studied plant were selected and kept per pot after thinning. Plants were grown in experimental garden under natural condition and irrigated with running water. The plants were maintained for 120 days and used for measurement of different parameters. The growth of the plant was recorded in each replication from different fly ash soil amendments. The plant samples were dried for the determination of total plant biomass at 80 °C, until a stable reading was obtained. The metal tolerance index (MTI) of *R. communis* L. was calculated on the basis of biomass accumulation under different treatments based on the formula of Qihang et al. (2011).

2.3. Analysis of metal concentration

The analysis of metals concentrations of Al, Fe, Zn, Mn, Cu and Cr were carried out in the laboratory by taking the oven dried (80 °C) samples of substrates and different plant tissue like root and shoot. The metal contents were determined by digesting 1 g each of oven dried sample with a triacid consisting of nitric acid: sulphuric acid: perchloric acid with a ratio of 6:1:2 at 100 °C. Different metal concentration were measured in an Atomic Absorption Spectrophotometer (Analyst AA-200, Parkin Elmer) following the method of APHA (2017).

Bioconcentration factor (BCF) was determined for shoot and root of *R. communis* according to the equation of Pandey (2012): BCF = (metal concentration in plant tissue/metal concentration in substrate).

Translocation factor (TF) was calculated by the ratio of particular metal concentration in the shoot to that of root tissue (Pandey, 2012).

2.4. Measurement of photosynthetic parameters

The measurement of different photosynthetic parameters such as rate of photosynthesis (P_N) and stomatal conductance (gs) of *R. communis* were carried out by a portable photosynthetic system (CI-304, CID, USA) as described by Panda et al. (2018a).

Photosynthetic pigments (chlorophyll and carotenoid) were estimated spectrophotometrically by taking fresh leaf tissue in ice cold 80% acetone. After measurement of absorbance chlorophyll and carotenoid content were calculated following to the equations of Arnon (1949) and Lichtenthaler and Welburn (1983), respectively.

Chlorophyll fluorescence measurements were carried out by JUNIOR-PAM (WALZ, Germany) fluorescence meter on the same leaves, which were previously used for the measurement of the photosynthetic gas exchange parameters. Minimum fluorescence (Fo), maximum fluorescence (Fm) and photochemical efficiency of photosystem (PS) II (Fv/Fm) were measured after keeping the samples in dark for 20 min. The non-photosynthetic quenching (NPQ) and photochemical quenching (qP) were measured in light adapted samples (Maxwell and Johnson, 2000).

2.5. Assay of antioxidative enzymes, lipid peroxidation and protein content

Plants of *R. communis* L. were harvested after 120 days of growth and were then analyzed for different antioxidant enzyme activities. The activity of superoxide dismutase was measured by photoreduction of nitro blue tetrazolium (NBT) following Choudhury and Choudhury (1985). The activity of ascorbate peroxidase was determined by ascorbate oxidation method following Nakano and Asada (1981). The guaiacol peroxidase activity was assessed as per Rao et al. (1995). Catalase activity was performed by following the method of Cakmak and Marschner (1992). The crude extract was used for the estimation of protein content according to Lowry et al. (1951). Malondialdehyde (MDA) content was determined as the product of lipid peroxidation according to Panda (2007).

2.6. Statistical analysis

Raw data on various parameters were subjected to analysis of variance test by the software CROPSTAT (IRRI, Philippines). Mean values were compared by *Fisher's* least significance difference test.

3. Results and discussion

3.1. Characterisation of fly ash and garden soil

General physico-chemical parameters of fly ash, garden soil and fly ash amended soils were presented in Panda et al. (2020). The garden soil showed 6.64 ± 0.11 pH, 0.25 ± 0.02 mg kg^{-1} nitrogen and 0.16 ± 0.01 mg kg^{-1} organic carbon (Table 1). The fly ash showed higher pH, EC and P with low contents of N, K and OC over garden soil. In addition, the concentration of different metals viz. Al, Fe, Cr, Mn and Cu in fly ash were more than the garden soil except Zn. The levels of Zn and Cu in garden soil were in normal range and Mn level was very low (Kabat Pendias, 2011). The results were consistent with the reported value in different fly ash deposits by Bisoi et al. (2017), Gajić et al. (2018) and Panda et al. (2018a). Fly ash (50%) in soil amendments led to improve the pH, EC and P compared to garden soil treatment, whereas metal concentrations were significantly decreased compared to bare fly ash treatment (Table 1). The increase of pH in fly ash amended soil may be due to the alkaline nature

Table 1. Physicochemical parameters of garden soil (GS) and fly ash (FA) 50% and 100%. Data are the mean of three replications ± standard deviation. LSD: least significance difference (Panda et al., 2020).

Treatments	pH	EC (μS cm^{-1})	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	OC (%)	Al (μg g^{-1})	Fe (μg g^{-1})	Cr (μg g^{-1})	Zn (μg g^{-1})	Mn (μg g^{-1})	Cu (μg g^{-1})
GS	6.64 ± 0.11	64.5 ± 8.0	0.25 ± 0.02	0.08 ± 0.01	0.71 ± 0.07	0.16 ± 0.01	148 ± 16	89.0 ± 6	10.0 ± 3.0	65.6 ± 0.6	64.0 ± 6.6	12.2 ± 2.3
FA -50%	7.21 ± 0.04	95.1 ± 6.5	0.16 ± 0.06	0.14 ± 0.03	0.49 ± 0.04	0.14 ± 0.02	500 ± 26	520 ± 20	30.1 ± 2.5	56.2 ± 2.0	75.6 ± 2.3	21.6 ± 2.5
FA-100%	8.50 ± 0.10	135 ± 10.0	0.11 ± 0.02	0.32 ± 0.05	0.41 ± 0.05	0.09 ± 0.02	1655 ± 21	1378 ± 47	52.0 ± 1.6	51.0 ± 0.8	119 ± 2.3	32.2 ± 1.3
LSD ($P < 0.05$)	0.09	18.3	0.05	0.05	0.15	0.02	43.23	48.13	8.32	4.31	14.5	4.23

of fly ash addition in garden soil. Similarly, Panda et al. (2018b) reported that the fly ash significantly increases the EC and P of the soil amendments which is helpful for plant growth. Thus, fly ash amendment in soil suggestively improves soil physical parameter and lower heavy metal content that may be safe for soil amendment.

3.2. Growth parameters

The growth of *R. communis* under bare fly ash treatment was significantly inhibited in comparison to other treatments (Figure 1). Soil amendments with 50 % fly ash showed higher plant growth than other treatments. The percentage of plant biomass was increased by 37% in fly ash amended soil over garden soil whereas, 80% of decrease of biomass was noticed in bare fly ash. The impaired growth of plants might be due to increasing level of toxic metal concentrations and less availability of essential mineral elements and nutrients in the fly ash (Verma et al., 2014). Hence, it indicates that accumulation of nutrients on fly ash substrate is less which could be the cause in decreased growth of *R. communis*. In the present study, improvement of growth of *R. communis* in fly ash in soil amendments was observed due to the presence of some physical and chemical properties in fly ash which are favourable for plant development.

The higher MTI in plants are more tolerant to the metals and such plants can grow easily in the metal polluted area (Pandey, 2013). The MTI of *R. communis* under different treatments showed a significant increase in MTI under fly ash amended soil than that of garden soil and 100% fly ash (Figure 1). Based on the results, *R. communis* is recognized as a metal tolerant plant and can grow better in fly ash amended soil. There are various studies also support our findings that *R. communis* is emerging as a primary choice for phytomanagement due to its metal tolerant ability (Adhikari and Kumar, 2012; Zacchini et al., 2009).

3.3. Leaf photosynthetic parameters

Photosynthesis efficiency of *R. communis* under different fly ash treatments was shown in Table 2. Photosynthetic rate (P_N) was significantly decreased under bare fly ash substrate in comparision to garden soil however, it was not significantly altered under fly ash-amended soil (Table 2). Similarly, the stomatal conductance and chlorophyll content were also reduced in bare fly ash substrate in comparison to the garden soil. The reduction of P_N was 44%, gs was 41% and Chl was 13% in bare fly ash in comparison to the garden soil. The reduction of chlorophyll pigments under fly ash may be due to higher concentration of metal inhibit chlorophyll synthesis and may be due to oxidative degradation of chlorophyll by ROS (Pandey, 2013). The improvement of carotenoid content in *R. communis* under fly ash treatments may be due to photoprotection reaction induced under metal stress as reported in other crop plants by Gajić et al. (2013).

The value of photochemical parameters of PSII were not significantly different under fly ash-amended soil in comparison to garden soil (Table 2). The value of Fm, Fv/Fm and qP in *R. communis* was significantly inhibited in 100% fly ash substrate in comparison to garden soil (Table 2). In contrast, Fo and NPQ was significantly increased under fly ash treatments. Based on the findings, inhibition of photosynthesis in *R. communis* under high fly ash condition may be due to inhibition of photochemical activity, inhibition of stomatal conductance along with the pigment damage as it was reported earlier in other crops (Guidi et al., 2011; Panda et al., 2018b).

3.4. Metal accumulation in R. communis

The behaviour of metal accumulation in shoot and root tissue of *R. communis* in different treatments are shown in Figure 2. The concentration of Mn, Zn, Cu, Cr, Al and Fe in *R. communis* was significantly higher in 50 % fly ash in soil amendments. The concentration of different metal also remarkable more in roots tissue than the shoot under different treatments except Fe. Consistent with our study, Pandey (2013) also studied the *in-situ* metal availability in *R. communis* under fly ash pond and reported larger amount of metals in root. It has been established that phytoremediation and revegetation potentiality of any crop estimated by using the values of translocation factor (TF) and bioconcentration factor (BCF). BCF of studied metals Mn, Zn, Cu, Cr, Al and Fe in tissue of *R. communis* was found to be greater than 1 (Table 3). It indicates *R. communis* have remediation potential of studied metals, however metal remediation capacity was higher in fly ash amended soil compared to other treatment. Plants grown under fly ash amended soil shows >1 TF value for Fe and Zn only. It suggests that except Fe and Cu, other metals like Mn, Cu, Cr and Al were unable to move towards aerial parts by the plant, but accumulated in the root tissues of *R. communis*.

3.5. Oxidative stress and antioxidative response

Induction of MDA level was observed in different tissue of *R. communis* L. under fly ash treatments in comparison to garden soil

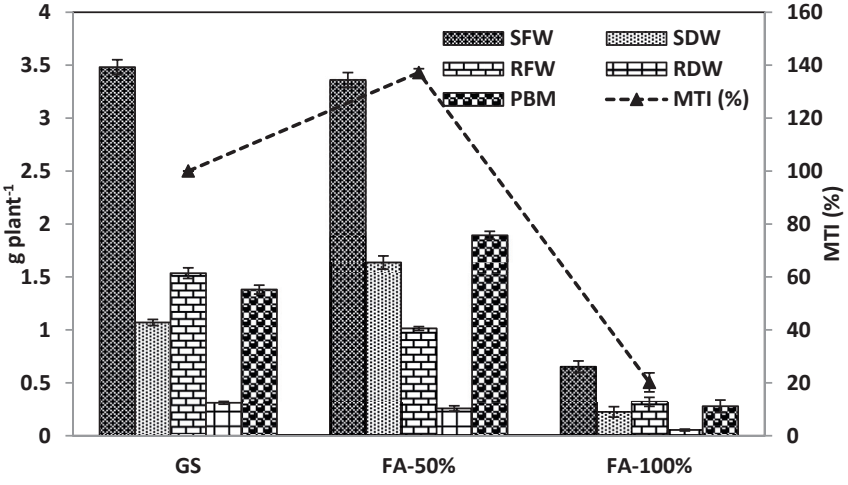

Figure 1. Plant growth and metal tolerance index (MTI) of *R. communis* L. at garden soil (GS) and fly ash (FA) 50% and 100%. Data are the mean of three replications with vertical bar represents standard deviation. SFW: shoot fresh weight; SDW: shoot dry weight; RFW: root fresh weight; RDW: root dry weight; PBM: plant biomass.

Table 2. Photosynthetic parameters of *R. communis* L. at garden soil (GS) and fly ash (FA) 50% and 100%. Data are the mean of three replications ±standard deviation. Mean followed by a common letter in the same column are not significantly different at the 5 % level by *Fisher's* least significance difference (LSD) test.

Treatment	P_N (μmol CO_2 m^{-2} s^{-1})	gs (mmol H_2O m^{-2} s^{-1})	Chlorophyll (mg g^{-1})	Carotenoid (mg g^{-1})	Fo (rel.)	Fm (rel.)	Fv/Fm (ratio)	qP (rel.)	NPQ (rel.)
GS	18.51 ± 1.20^a	77.50 ± 5.23^a	1.63 ± 0.08^a	0.142 ± 0.006^a	295 ± 13^a	1360 ± 25^a	0.789 ± 0.005^a	0.981 ± 0.019^a	0.021 ± 0.003^a
FA-50%	16.32 ± 1.58^a	69.91 ± 4.32^a	1.60 ± 0.05^a	0.203 ± 0.008^b	346 ± 15^b	1325 ± 32^a	0.745 ± 0.008^a	0.962 ± 0.018^a	0.043 ± 0.004^b
FA-100%	10.52 ± 1.31^b	46.23 ± 3.41^b	1.43 ± 0.09^b	0.242 ± 0.020^c	454 ± 18^c	1224 ± 27^b	0.639 ± 0.006^b	0.871 ± 0.012^b	0.054 ± 0.007^c
LSD ($P < 0.05$)	2.22	9.2	0.09	0.040	19.2	35.7	0.046	0.023	0.012

P_N: CO_2 photosynthetic rate; gs: stomatal conductance; Fo: minimum chlorophyll fluorescence yield obtained with dark-adapted leaf; Fm: maximum Chl fluorescence yield obtained with dark-adapted leaf; Fv/Fm: maximal photochemical efficiency of PSII; qP: photochemical quenching; NPQ: non-photochemical quenching.

Table 3. Bioconcentration factor (BCF) and translocation factor (TF) of different metals of *R. communis* L. at garden soil (GS) and fly ash (FA) 50% and 100%. Data are the mean of three replications ±standard deviation. Means followed by a common letter in the same column are not significantly different at the 5 % level by *Fisher's* least significance difference (LSD) test. S: shoot tissue; R: root tissue.

Substrate	Mn			Cu			Zn		
	BCF-R	BCF-S	TF	BCF-R	BCF-S	TF	BCF-R	BCF-S	TF
GS	0.97 ± 0.09^a	0.86 ± 0.07^a	0.88 ± 0.01^a	1.18 ± 0.21^b	0.85 ± 0.14^b	0.72 ± 0.01^c	0.90 ± 0.01^c	0.54 ± 0.01^a	0.59 ± 0.02^a
FA-50%	1.02 ± 0.01^a	0.47 ± 0.01^b	0.46 ± 0.01^c	1.46 ± 0.14^a	1.57 ± 0.13^a	1.07 ± 0.01^a	1.06 ± 0.04^a	0.53 ± 0.05^a	0.43 ± 0.03^b
FA-100%	0.63 ± 0.01^b	0.32 ± 0.01^c	0.53 ± 0.01^b	0.79 ± 0.02^c	0.64 ± 0.02^c	0.89 ± 0.02^b	0.96 ± 0.02^b	0.45 ± 0.03^b	0.47 ± 0.04^b
LSD ($P < 0.05$)	0.04	0.03	0.02	0.10	0.07	0.04	0.02	0.02	0.01
Substrate	Fe			Cr			Al		
	BCF-R	BCF-S	TF	BCF-R	BCF-S	TF	BCF-R	BCF-S	TF
GS	0.96 ± 0.02^b	1.06 ± 0.03^b	1.10 ± 0.02^b	0.34 ± 0.07^c	0.20 ± 0.04^c	0.61 ± 0.14^c	0.43 ± 0.09^a	0.25 ± 0.03^a	0.56 ± 0.03^a
FA-50%	1.14 ± 0.04^a	1.16 ± 0.07^a	1.02 ± 0.02^a	1.14 ± 0.06^a	0.92 ± 0.05^a	0.81 ± 0.08^b	0.16 ± 0.02^b	0.10 ± 0.02^b	0.64 ± 0.04^a
FA-100%	0.81 ± 0.07^c	0.83 ± 0.05^c	1.02 ± 0.03^a	0.70 ± 0.02^b	0.64 ± 0.08^b	0.91 ± 0.04^a	0.07 ± 0.02^c	0.05 ± 0.01^b	0.65 ± 0.06^a
LSD ($P < 0.05$)	0.03	0.02	0.02	0.04	0.02	0.06	0.05	0.05	0.51

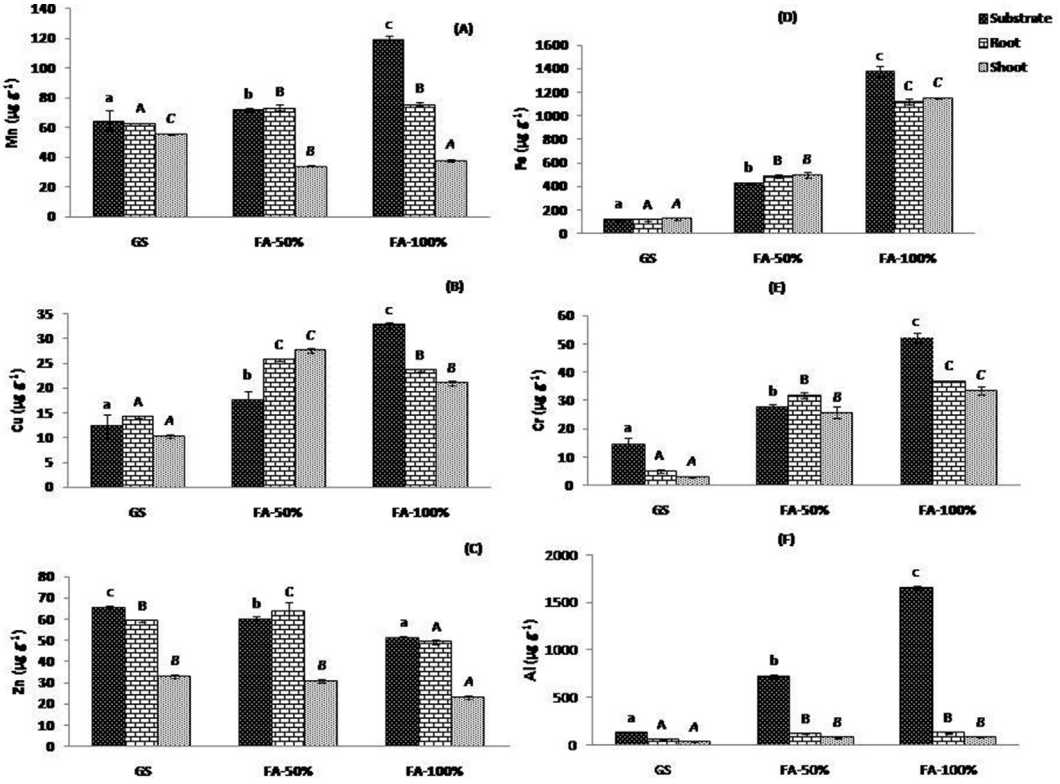

Figure 2. The concentrations of selected metals (μg g^{-1}dwt) such as Mn, Cu, Zn, Fe, Cr and Al in different substrates along with root and shoot tissue of *R. communis* L. Data are the mean of three replications with vertical bar represents standard deviation. Same alphabets shown in the figure among the treatments are not significant different at $P < 0.05$. *GS*: garden soil; FA: fly ash.

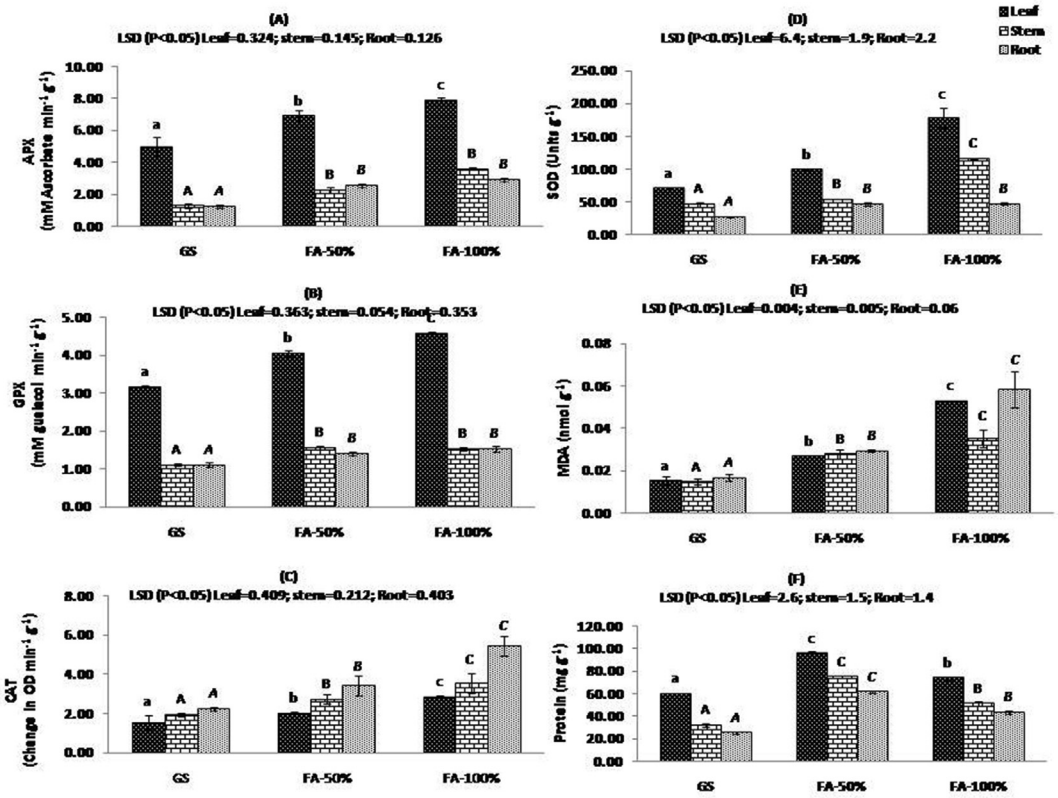

Figure 3. The activities of antioxidative enzymes, lipid peroxidation and protein content in different plant part (leaf, stem and root) of *R. communis* L. at garden soil (GS) and fly ash (FA) 50% and 100% treatments. Data are the mean of three replications with vertical bar represents standard deviation. Same alphabets shown in the figure among the treatments are not significant different at $P < 0.05$. APX: ascorbate peroxidase; GPX: guaiacol peroxidase; CAT: catalse; SOD: superoxide dismutase; MDA: malondialdehyde.

(Figure 3). Malondialdehyde is a product of lipid peroxidation and is used for quantification of membrane damage under oxidative streaa (Panda, 2007). Higher concentration of metals in fly ash may damage cell membrane in *R. communies*, inhibit metabolic process ultimately leading to growth inhibition.

The changes of activities of antioxidant enzymes in *R. communies* L. in different tissues was illustrated in Figure 3 A-D. Over control, there was increased level of APX, CAT, GPX and SOD enzyme activities were observed under fly ash substrates. Leaf tissues showed higher activities of APX, GPX and SOD followed by tissues of stem and root however, activity of CAT was greater in root part. The induction of these enzyme activity might be assumed as responses of oxidative stress caused by metals of fly ash and it showed the metal tolerance response. Protein content of different tissues of *R. communis* L. plant grown in 50% and 100% fly ash was more than that of plant grown on garden soil (Figure 3 F). The increment of protein content under fly ash might be generation of stress responsive proteins under fly ash.

4. Conclusion

In conclusion, fly ash amendments in garden soil significantly enhanced the soil physico-chemical properties and also improved the growth and biomass of *R. communis*. The inhibition photosynthetic activity in *R. communis* under high level of fly ash may be due to decrease of photochemical activity of PSII and loss of chlorophyll pigments. Induction of antioxidant enzymes in *R. communis* under fly ash showed the metal tolerance response of plants and can able to survive better under fly ash amendments. Based on the higher BCA value, *R. communis* can able to accumulate more amount of Mn, Cu, Zn, Fe and Cr metals in different tissue and can be recommended for suitable candidates for phytoremediation of fly ash.

Declarations

Author contribution statement

Debabrata Panda: Conceived and designed the experiments; Wrote the paper.

Lopamudra Mandal: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Jijnasa Barik, Bandana Padhan, Sidhant S. Bisoi: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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