Growth, biochemical, and antioxidant response of beetroot (*Beta vulgaris* L.) grown in fly ash-amended soil

Adnan Shakeel¹ · Abrar Ahmad Khan¹ · Khalid Rehman Hakeem²

Received: 23 March 2020 / Accepted: 8 July 2020 / Published online: 15 July 2020
© Springer Nature Switzerland AG 2020

Abstract

A pot experiment was conducted under net house conditions to study the ameliorative role of different levels of fly ash as a soil amendment, on the growth markers, biochemical attributes, and the antioxidant enzyme activity of beetroot. Seeds of beetroot were sown in earthen pots containing growth substrate, consisting of field soil amended with different levels of fly ash (0%, 5%, 10%, 15%, 20%, and 25% weight/weight). Beetroot grown in 15% fly ash-amended soil had significantly ($p \leq 0.05$) higher plant growth and pigment content as compared to the control. With the improvement in nitrate reductase activity, the contents of total protein and carbohydrate also increased significantly at 15% fly ash. The proline content and the total antioxidant activity of beetroot increased significantly with increasing fly ash levels. The elevated levels of proline and antioxidant activity might be responsible for scavenging the oxidative stress caused by the higher concentration of fly ash in the soil. The principal component analysis presented significant correlations among the various considered parameters. Two principal components explained a total of 97.12% overall data variability in plants treated with different levels of fly ash. Therefore, this work suggests that the application of fly ash as a soil amendment is suitable to improve the growth and yield of beetroot.

Keywords Antioxidant activity · EDX · Fly ash · Nutrients · Scanning electron microscopy

1 Introduction

The utilization of coal burning in power generation is a key factor for the development of countries across the globe. Due to the urge of rapid development, this coal burning is accelerating that generates various pollutants and increases the number of fly ash (FA) dumpsites. In India, FA generation during the period 2017–2018 was 196.44 million tons from 624.88 million tons of coal [7]. Despite its use in cement, sanitary, and brick industries, large quantities are thrown away as waste into dumpsites [40]. Therefore, eco-friendly approaches that would increase the utilization of FA while keeping in limits its harmful effects are required. FA can be utilized for economic profit in an

environmentally friendly manner rather than dumping it as a waste [20].

FA has potential benefits for utilization in agricultural soils due to the presence of some essential as well as beneficial nutrients, viz. Ca, Mg, K, S, P, Si, Al, Fe, Cu, etc. [32]. Most of these elements fall in the criteria of essentiality of nutrients whose deficiency might affect the growth and yield of crops. FA is a dual-edged waste which, if used at low levels, can be useful for crop productivity. The amendment of agricultural soils with FA improves soil pH, permeability, electrical conductivity, porosity, and water-holding capacity of the soil [9, 29, 32]. Because of its soil ameliorating properties, FA has [2, 32] been successfully tested for the yield improvement in various crops including Indian

✉ Adnan Shakeel, adnanshakeel1717@gmail.com; ✉ Khalid Rehman Hakeem, kur.hakeem@gmail.com; khakim@kau.edu.sa | ¹Section of Plant Pathology and Environmental Botany, Faculty of Life Sciences, Aligarh Muslim University, Aligarh 202002, India. ²Department of Biological Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia.

mustard, radish [33], safed Musli [12], rice [5], wheat [23], turnip, onion, garlic, potato, and carrot [38].

Beetroot (*Beta vulgaris* L.) belongs to the family Chenopodiaceae. It is commonly used as a root vegetable. It ranks among the ten most potent vegetables concerning its antioxidant property [15]. Being a rich source of minerals, carotenoids, betalains, flavonoids, nitrates, and vitamins makes it an efficient source of nutrition for humans [27]. Carotenoids, vitamins, and polyphenols of beetroot have been reported with antioxidant, anti-inflammatory, anti-carcinogenic, and hepato-protective activities and have been found to reduce diabetes, cardiovascular diseases, and lowering of hypersensitivity [36]. Taking into account, the economic importance of beetroot and the soil ameliorating properties of FA, it was, therefore, planned to assess the growth, biochemical, and antioxidant response of beetroot to different FA levels amended to the soil.

2 Materials and methods

2.1 Plant and growth conditions

A pot experiment was conducted in a complete randomized block design under natural conditions at the Department of Botany, Aligarh Muslim University, India. An average day/night temperature of 24 °C/18 °C (± 3 °C) with relative humidity of $70 \pm 5\%$ was observed during the experiment. Before sowing, the seeds of beetroot were surface-sterilized with 0.1% HgCl_2 solution for 2 min. Soil from the agricultural field was collected, mixed with FA in different concentrations, and autoclaved at 20 lb pressure for 20 min. The treatments were formulated as w/w% ratio of field soil and FA: control = 100:0; F5% = 95:5; F10% = 90:10; F15% = 85:15; F20% = 80:20, and F25% = 75:25, respectively.

After properly mixing the soil (sandy loam) with FA, earthen pots of 10 cm height were filled with 3 kg of each type of mixture, and the number of replicates for each treatment was maintained three ($n = 3$). In total, 18 pots (6 treatments $\times$ 3 replicates) were prepared for the experiment. Six seeds were sown in each type of soil–FA mixture. One week after the germination of seeds, thinning of seedlings was done to maintain one healthy plant in each pot. Pots were irrigated regularly with the required amount of water.

2.2 Fly ash and soil analysis

For the experimental work, FA was collected from the ash pond of the Harduaganj Thermal Power Plant, which is located 15 km away from Aligarh city. The physicochemical characteristics of soil and FA were analyzed before the

mixing and sowing of the crop. For elemental analysis, an energy-dispersive X-ray analyzer (EDX) was employed. The ultrastructure of soil and FA was observed through scanning electron microscopy (SEM) of make JEOL, JSM-6510 LV, Japan (Fig. 1). The pH of soil and FA was determined by a digital pH meter.

2.3 Determination of growth biomarkers

At maturity, the plants were uprooted at 90 DAS (days after sowing) from the pots and washed with tap water to remove the soil. Measurement of shoot and root length was taken with the help of a meter scale. Fresh weight was recorded with the help of a digital weighing balance. For dry weight, plants were dried in the oven at 80 °C for 48 h. The number of leaves was manually counted, and the leaf area was measured by the graph paper method.

2.4 Determination of leaf characteristics

Stomatal area and the number of stomata were analyzed with the help of a scanning electron microscope (SEM) of make JEOL, JSM-6510 LV, Japan. For SEM studies, leaves were coated with gold.

2.5 Determination of proline content

Proline content was determined by the protocol of Bates et al. [3].

2.6 Determination of photosynthetic pigments

Chlorophyll and carotenoid contents were estimated according to the method of Maclachlan and Zalick [17]. The absorbance was read at 663 and 645 nm against 80% acetone blank on a spectrophotometer.

2.7 Determination of nitrate reductase and protein content

Nitrate reductase (NR) activity in leaves was measured by preparing an enzyme extract using the method of Jaworski [14]. Protein content in the edible part was measured by using the method of Bradford [6] with bovine serum albumin as standard.

2.8 Assay of antioxidant enzyme activity

Total antioxidant enzyme activity from the edible part of beetroot was determined by the method suggested by Shimada et al. [34]. An aliquot of 100 μL samples was added to 3 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) working solution. Absorbance was recorded at 517 nm

Fig. 1 Energy-dispersive x-ray (EDX) profiling of soil (a) and fly ash (b)

through a spectrophotometer. Lower absorbance indicated higher antioxidant activity of the sample. Antioxidant activity was expressed as percent scavenging of DPPH radical, and the following formula was used:

$$\text{IC 50\%} = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100.$$

IC 50 is the inhibition concentration of the sample at which the DPPH radicals were scavenged by 50%. The smaller the IC 50 value, the higher the antioxidant activity and vice versa.

2.9 Statistical analysis

All data are presented as mean $\pm$ standard error ($n = 3$). Analysis of variance (ANOVA) and DMRT tests were used to determine significant differences at $p \leq 0.05$ by using SPSS version 17.0 software. Different letters indicate significant differences between treatments at $p \leq 0.05$, ($n = 3$). The principal component analysis was performed through Origin Pro (2020) software.

3 Results and discussion

3.1 Physicochemical characterization of soil and fly ash

Favorable soil conditions and its richness in essential plant nutrients support healthy plant growth with improved yield. To measure the nutrient status of soil, various techniques are employed. Energy-dispersive X-ray spectroscopy (EDX) is a useful technique for studying the elemental composition of soil [19]. In this study, EDX technique was employed, and it showed that FA contains some essential as well as beneficial plant nutrients (Fig. 1). The nature of FA was alkaline with a pH 8.4 as compared to the 7.6 pH of the field soil. The SEM analysis showed that FA has small spherical particles in comparison with soil (Fig. 2). The EDX profiling showed that the FA contains some essential and beneficial plant nutrients, viz. Ca, Mg, K, Al, Fe, Cu, and Si (Fig. 1).

Fig. 2 Scanning electron micrograph of soil (a) and fly ash (b)

3.2 Effect of fly ash on growth markers, pigment content, and yield of beetroot

Application of FA as a soil amendment caused a significant improvement in all the growth markers, pigment content as well as the yield attributes of beetroot. A remarkable enhancement was observed at 15% FA in terms of shoot length, shoot fresh and dry weight, number of leaves, and leaf area, respectively (Table 1). Similarly, the yield of beetroot in terms of root fresh and dry weight, and root circumference was enhanced at 15% FA (Table 1). The photosynthetic pigment contents including chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents increased maximally at 15% FA-amended soil (Table 2). The scanning electron microscopy revealed that the FA rate of 15% significantly increased the stomatal area of beetroot

leaves as compared to the control (Fig. 3). The observations showed that with a gradual increase in FA rates from 0 to 15%, all the growth markers, yield, and pigment content parameters were significantly improved over control. The possible reason for this improvement is the enhancement of the nutrient status of soil (Ca, Mg, K, P, and S) and improvement in soil characteristics, including soil pH (Fig. 1), [32]. The improvement in the soil pH is an important factor that facilitates the availability of essential plant nutrients [24]. Calcium enrichment makes a significant contribution to the observed improvement because it acts as a secondary messenger and facilitates cell wall formation [18]. The present study also reported the improvement in photosynthetic pigment content with the FA amendment up to 15%. This may be attributed to the enrichment of Mg, which is the central component of chlorophyll [21]. Likewise, K is also

Table 1 Effect of different fly ash levels amended to the soil on the growth and yield attributes of beetroot

Attributes	Treatments					
	Control	F5%	F10%	F15%	F20%	F25%
Shoot length (cm)	20.36 ± 1.57 ^c	21.87 ± 1.09 ^{bc}	23.43 ± 1.03 ^b	26.89 ± 1.07 ^a	24.33 ± 1.06 ^b	21.14 ± 1.10 ^{bc}
Shoot fresh weight (g)	25.5 ± 0.94 ^c	27.2 ± 1.10 ^{bc}	29.23 ± 0.97 ^b	32.81 ± 1.09 ^a	30.33 ± 1.11 ^b	27.26 ± 0.93 ^{bc}
Shoot dry weight (g)	7.03 ± 0.64 ^c	8.59 ± 0.40 ^{bc}	9.69 ± 0.33 ^b	11.67 ± 0.31 ^a	9.07 ± 0.60 ^b	8.11 ± 0.63 ^{bc}
Root length (cm)	10.99 ± 0.95 ^c	11.18 ± 1.05 ^{bc}	13.55 ± 1.33 ^b	15.76 ± 1.80 ^a	13.44 ± 1.34 ^b	11.01 ± 0.94 ^{bc}
Root fresh weight (g)	77.31 ± 3.94 ^c	80.98 ± 2.97 ^{bc}	84.13 ± 5.71 ^b	88.12 ± 8.68 ^a	85.14 ± 3.72 ^b	79.21 ± 1.00 ± ^{bc}
Root dry weight (g)	16.7 ± 0.94 ^c	18.54 ± 1.02 ^{bc}	21.09 ± 1.10 ^b	24.66 ± 0.97 ^a	21.6 ± 0.95 ^b	19.12 ± 0.99 ^{bc}
Root circumference (cm)	16.6 ± 0.95 ^c	17.8 ± 0.91 ^{bc}	19.3 ± 0.94 ^b	23.7 ± 0.97 ^a	21.9 ± 1.05 ^b	19.11 ± 0.96 ^b
Leaves (plant ⁻¹)	9.48 ± 0.35 ^c	11.53 ± 0.76 ^{bc}	13.69 ± 0.73 ^b	15.22 ± 0.42 ^a	13.37 ± 0.44 ^b	10.71 ± 0.32 ^{bc}
Leaf area (cm ² leaf ⁻¹)	10.6 ± 1.56 ^c	11.4 ± 1.47 ^{bc}	13.21 ± 0.97 ^b	16.64 ± 1.14 ^a	13.32 ± 0.81 ^b	11.67 ± 1.39 ^{bc}
Stomatal area (µm ²)	25.62 ± 0.98 ^d	26.0 ± 1.04 ^d	29.88 ± 1.06 ^{bc}	33.27 ± 0.90 ^a	30.94 ± 1.00 ^{bc}	27.49 ± 0.93 ^{cd}

Data represent mean of three independent replicates ± standard error (SE). Different letters indicate significant differences between treatments at $p < 0.05$, ($n = 3$)

Table 2 Effect of different fly ash levels amended to the soil on the photosynthetic pigments of beetroot

Attributes	Treatments					
	Control	F5%	F10%	F15%	F20%	F25%
Chlorophyll <i>a</i> (mg g ⁻¹ FW)	0.48 ± 0.005 ^c	0.51 ± 0.007 ^c	0.54 ± 0.005 ^b	0.57 ± 0.004 ^a	0.55 ± 0.06 ^b	0.51 ± 0.003 ^c
Chlorophyll <i>b</i> (mg g ⁻¹ FW)	0.25 ± 0.008 ^c	0.27 ± 0.005 ^c	0.30 ± 0.004 ^b	0.32 ± 0.007 ^a	0.30 ± 0.006 ^b	0.27 ± 0.006 ^c
Total chlorophyll (mg g ⁻¹ FW)	0.73 ± 0.06 ^c	0.78 ± 0.005 ^{bc}	0.84 ± 0.009 ^b	0.91 ± 0.007 ^a	0.85 ± 0.008 ^b	0.79 ± 0.003 ^{bc}
Carotenoids (mg g ⁻¹ FW)	0.21 ± 0.009 ^c	0.23 ± 0.007 ^{bc}	0.27 ± 0.008 ^b	0.32 ± 0.009 ^a	0.30 ± 0.006 ^b	0.28 ± 0.004 ^{bc}

Data represent mean of three independent replicates ± standard error (SE). Different letters indicate significant differences between treatments at $p < 0.05$, ($n = 3$)

Fig. 3 Scanning electron micrograph showing the effect of different fly ash levels amended to the soil on the stomatal apertures of beetroot at control (a), its enlarged view (c), and beetroot grown in 15% fly ash-amended soil (b) and its enlarged view (d)

an important metal ion with a dominant function of turgor pressure generation for the opening of stomata. Therefore, the observed improvement in the stomatal area (Table 1, Fig. 3) is due to the K enrichment of the soil. Although phosphorous is present in small amounts in FA, it could help in better cell division for improved leaf area. The addition of S also makes a considerable contribution to plant growth and yield improvement as it is required in Fe–S cluster formation of photosynthetic apparatus, sulfur-containing proteins such as methionine, electron transport, and secondary metabolism [13]. Besides, FA also contains some essential micronutrients such as Mn, Cu, and Fe (Fig. 1) that also help in the improvement in plant performance. Mn has a vital role to play during photolysis of water, while Cu is a component

of plastocyanin. Fe acts as a co-factor during photosynthetic electron transport [31].

However, when FA level was increased beyond 15%, all the growth, yield, and the pigment parameters of beetroot were reduced significantly in comparison with the 15% FA. The possible reason for the reduction in photosynthetic pigment is the breakdown of chlorophyll pigment at higher FA levels. This happens possibly due to the substitution of Mg by metal ions such as Ni, Cd, Zn, Cr, Pd, and Cu [16]. At higher levels, they can also interfere and inhibit the normal functioning of enzymes involved in the Calvin cycle of photosynthesis and hence reduce the overall growth performance [4, 39]. These results are in conformity with Ashfaq and Inam [2], Gond et al. [10], and Shakeel et al. [32]. FA contains some hazardous metals such as Cd,

Pb, Ti, and Cr which were accumulated by plants in a dose-dependent manner. At lower levels, these metals are accumulated by plants at very low concentrations and below the permissible limit which is safe for human consumption [8, 22, 42]. However, the use of FA in agroecosystems at higher levels may pose a threat to the soil ecosystem due to the presence of toxic heavy metals. These heavy metals may be uptaken and accumulated by the plants, and through food chain, humans may be affected [30, 35]. The response of plants to the accumulation of these metals varies in a species-specific manner. Wheat (*Triticum aestivum*) grown in 60% FA-amended soil accumulated all the heavy metals below the permissible limit, while as in mung bean (*Vigna radiata*) and urad bean (*Vigna mungo*) only 20% FA was found safe and all the heavy metals accumulated above the permissible limit beyond 20% FA [22]. For pigeon pea (*Cajanus cajan*), 25% FA was found safe for its cultivation which not only increased its yield but also

ensured the accumulation of heavy metals below toxic levels [26]. All these studies suggest that the application of FA at lower levels acts as a soil conditioner that enhances the growth and yield of crops.

3.3 Effect of fly ash on biochemical parameters of beetroot

Application of FA as a soil amendment caused a significant change in the biochemical attributes of beetroot. Results from Fig. 4 depict that the activity of NR was enhanced significantly by 38.65% as compared to the control at 15% FA level. Likewise, the protein content and the total carbohydrate content of the edible part of beetroot were increased significantly by 28.01% and 27.89% as compared to control at 15% FA level, respectively (Fig. 4). Nitrate reductase is the primary enzyme required during nitrogen metabolism. Because FA is deficient in nitrogen, a significant decrease in

Fig. 4 Effect of different fly ash levels amended to the soil on the nitrate reductase activity of leaves, total protein, and total carbohydrate content in the edible part of beetroot. Data represent mean

of three independent replicates $\pm$ standard error (SE). Different letters indicate significant differences between treatments at $p < 0.05$, ($n = 3$)

NR activity was observed at higher FA levels, which agrees with the observations of Qurratul et al. [28]. Gupta et al. [11] suggested that this reduction in the NR activity might be due to the binding of metal ions to –SH groups of the enzymes. The present study also reported the increased levels of total carbohydrates in the edible portion of beetroot at up to 15% FA, which reduced latter at higher FA levels. These changes correlate with the improved as well as reduced levels of photosynthetic pigments at lower and higher FA rates. Similar results were also reported for protein content in the edible portion of beetroot. Ashfaq and Inam [2] credited K for the improvement in protein content at lower FA rates as it plays an important role in the activation of enzymes required for protein synthesis. The activity of NR is directly proportional to the protein synthesis, which is clear from the above observations. With improved NR activity at up to 15% FA level, the total protein content also increased in the same fashion and reduced when NR activity falls at higher FA levels. Amm et al. [1] suggested that at higher FA levels, the protein content decreases because of the disruption of protein folding and proper protein activity by heavy metals.

3.4 Oxidative stress and its alleviation by antioxidant enzymes

Application of different levels of FA as a soil amendment caused a significant and continuous increase in oxidative stress as compared to control. In response to this oxidative stress, the total antioxidant activity, along with proline, showed a significant increment at all the FA rates

as compared to control (Fig. 5). Therefore, in the present study, the proline and the antioxidant activity increased significantly by 25% and, 25.62% at 15% FA level in response to scavenge the oxidative stress that is generated by different levels of FA amended to the soil (Fig. 5). These results show adherence to the observations of Ashfaq and Inam [2], Pandey et al. [25], and Wang et al. [41].

Principal component analysis (PCA) plotting the effect of different levels of FA amended to the soil on the growth, yield, pigment content, and biochemical attributes of beetroot is illustrated in Fig. 6. The PC 1 shows 80.30%, and PC 2 shows 16.82% of the original information. Significant positive correlations were found between plant growth parameters, viz. shoot length, shoot fresh, and dry weight, yield parameters, viz. root length, root fresh, and dry weight, and photosynthetic pigment parameters, viz. chlorophyll *a*, chlorophyll *b*, and carotenoid content (Fig. 6). The proline and the total antioxidant activity of beetroot showed a strong positive correlation with each other, but their correlation with other studies parameters was found negative (Fig. 6). Our results are in agreement with the criteria developed by Sneath and Sokal [37], who believed that data should represent at least 70% of the total data variability.

4 Conclusion

In an attempt to investigate the soil ameliorative and growth-promoting properties of FA, a pot experiment was conducted in which FA was mixed with field soil in

Fig. 5 Effect of different fly ash levels amended to the soil on the proline content of leaves and total antioxidant activity in the edible part of beetroot. Data represent mean of three independent repli-

cates $\pm$ standard error (SE). Different letters indicate significant differences between treatments at $p < 0.05$, ($n = 3$)

Fig. 6 The biplots of principal component analysis (PCA), showing the effect of different fly ash levels amended to the soil on the growth, yield, pigment content, and biochemical attributes of beetroot. *SL* shoot length, *SF* shoot fresh weight, *SD* shoot dry weight, *N* leaves no. of leaves, *L* area leaf area, *Stm* stomatal area, *Circum* root circumference, *Root L* root L, *Root F* root fresh weight, *Root D* root dry weight, *Chla* chlorophyll *a*, *Chlb* chlorophyll *b*, *T chl* total chlorophyll, *Crts* carotenoid content, *Nitrate R* nitrate reductase activity, *Protein* total protein content, *TC* total carbohydrate content, *Proline* proline content, *Antioxidant* total antioxidant activity

different concentrations (0%, 5%, 10%, 15%, 20%, and 25% w/w). In a dose-dependent manner, FA affected the growth, yield, pigment content, and biochemical attributes of beetroot. Among the different concentrations of FA amended to the soil, 15% FA proved to be most effective in enhancing all the growth, yield, pigment, biochemical attributes, and antioxidant system of beetroot. A remarkable improvement was observed at 15% FA in the plant growth of beetroot (shoot length, shoot fresh and dry weight, number of leaves, leaf area, and stomatal aperture), yield (root length, root fresh and dry weight, root circumference), pigment content (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids), biochemical markers (nitrate reductase activity, total carbohydrate, and total protein content), and antioxidant system (proline and total antioxidant activity). Therefore, this work recommends that the application FA at low levels as a soil amendment is a cost-effective approach to improve the growth and yield of beetroot.

Acknowledgements The authors are thankful to USIF, Aligarh Muslim University, for providing access to scanning electron microscope (SEM) and energy-dispersive X-ray (EDX) facilities.

Compliance with ethical standards

Conflict of interest The authors declare that no conflict of interest exists.

References

1. Amm I, Sommer T, Wolf DH (2014) Protein quality control and elimination of protein waste: the role of the ubiquitin-proteasome system. *Biochem Biophys Acta* 1843:182–196
2. Ashfaq F, Inam A (2019) Interactive effect of potassium and fly ash: a soil conditioner on metal accumulation, physiological and biochemical traits of mustard (*Brassica juncea* L.). *Environ Sci Pollut Res* 26(8):7847–7862
3. Bates LS, Walden RT, Teare ID (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39:205–207
4. Bertrand M, Poirier I (2005) Photosynthetic organisms and excess of metals. *Photosynthetica* 43(3):345–353
5. Bisoi SS, Mishra SS, Barik J, Panda D (2017) Effects of different treatments of fly ash and mining soil on growth and antioxidant protection of Indian wild rice. *Int J Phytoremediat* 19(5):446–452
6. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72(1–2):248–254
7. CEA (2017–2018) Report on fly ash generation at coal/lignite based thermal power stations and its utilization in the country for the year 2017–2018. Central Electricity Authority, New Delhi, India
8. Dahiya HS, Budania YK (2018) Prospects of fly ash application in agriculture: a global review. *Int J Curr Microbiol Appl* 7:397–409
9. Debnath S, Mittal A (2019) Effect of fly ash on permeability of soil. In: *Recycled waste materials*, pp 97–10
10. Gond DP, Singh S, Pal A, Tewary BK (2013) Growth, yield and metal residues in *Solanum melongena* grown in fly ash amended soil. *J Environ Biol* 34:539–544
11. Gupta AK, Sinha S (2006) Role of *Brassica juncea* L. Czern. (var. vaibhav) in the phytoextraction of Ni from soil amended with fly-ash: selection of extractant for metal bioavailability. *J Hazard Mater* 136:371–378
12. Hadke PB, Wankhade SG, Ingle SN (2019) Consequence of fym and fly ash application on yield, nutrient uptake, and quality of safed musli grown on inceptisols. *Int J Curr Microbiol Appl Sci* 8(5):263–273
13. Hell R, Hillebrand H (2001) Plant concepts for mineral acquisition and allocation. *Curr Opin Biotechnol* 12:161–168
14. Jaworski EG (1971) Nitrate reductase assay in intact plant tissues. *Biochem Biophys Res Commun* 43:1274–1279
15. Kale RG, Sawate AR, Kshirsagar RB, Patil BM, Mane RP (2018) Studies on evaluation of physical and chemical composition of beetroot (*Beta vulgaris* L.). *IJCS* 6(2):2977–2979
16. Kupper H, Kupper F, Spiller M (1998) In situ detection of heavy metal substituted chlorophylls in water plants. *Photosynth Res* 58(2):123–133
17. MacLachlan S, Zalik S (1963) Plastid structure, chlorophyll concentration, and free amino acid composition of chlorophyll mutant barley. *Can J Bot* 41(7):1053–1062
18. Marschner P (2012) Mineral nutrition of higher plants, 3rd edn. Academic Press, London
19. McCully ME, Canny MJ, Huang CX, Miller C, Brink F (2010) Cryo-scanning electron microscopy (CSEM) in the advancement of functional plant biology: energy dispersive X-ray microanalysis (CEDX) applications 1. *Funct Plant Biol* 37:1011–1040
20. Mohan SKR, Jayabalan KP, Rajaraman A (2012) Properties of fly ash-based coconut fibre composite. *Am J Eng Appl Sci* 5:29–34
21. Moorby J, Besford RT (1983) Mineral nutrition and growth. In: Lauchli A, Bielecki RL (eds) *Encyclopedia of plant physiology*, vol 15B. Springer, Berlin, pp 81–527
22. Mahale NK, Patil SD, Sarode DB, Attarde SB (2012) Effect of fly ash as an admixture in agriculture and the study of heavy metal accumulation in wheat (*Triticum aestivum*), mung bean (*Vigna*

- radiata*), and urad beans (*Vigna mungo*). Pol J Environ Stud 21(6):1713
23. Ochecova P, Tlustos P, Szakova J (2014) Wheat and soil response to wood fly ash application in contaminated soils. Agron J 206(3):995–1002
 24. Pandey VC, Singh N (2010) Impact of fly ash incorporation in soil ecosystems. Agric Ecosyst Environ 136:16–27
 25. Pandey VC, Singh JS, Kumar A, Tewari DD (2010) Accumulation of heavy metals by chickpea grown in fly ash treated soil: effects on antioxidants. Clean: Soil, Air, Water 38:1116–1123
 26. Pandey VC, Abhilash PC, Upadhyay RN, Tewari DD (2009) Application of fly ash on the growth performance and translocation of toxic heavy metals within *Cajanus cajan* L: implication for safe utilization of fly ash for agricultural production. J Hazard Mater 166(1):255–259
 27. Panghal A, Virkar K, Kumar V, Dhull SB, Gat Y, Chhikara N (2017) Development of probiotic beetroot drink. Curr Res Nutr Food Sci 5(3):257–262
 28. Qurratul JS, Khan R, Mahmooduzzafar STO (2013) Soil amendments of fly ash: effects on function and biochemical activity of *Carthamus tinctorius* L. Isr J Plant Sci 61(1–4):12–24
 29. Raj S, Mohan S (2014) Approach for improved plant growth using fly ash amended soil. Int J Emerg Technol Adv Res 4(6):709–715
 30. Rao CS, Lakshmi CS, Tripathi V, Dubey RK, Rani YS, Gangaiah B (2020) Fly ash and its utilization in Indian agriculture: constraints and opportunities. In: Ghosh SK, Kumar V (eds) Circular economy and fly ash management. Springer, Singapore, pp 27–46
 31. Scholnick S, Keren N (2006) Metal homeostasis in cyanobacteria and chloroplasts. Balancing benefits and risks to the photosynthetic apparatus. Plant Physiol 141:805–810
 32. Shakeel A, Khan AA, Ahmad G (2019) The potential of thermal power plant fly ash to promote the growth of Indian mustard (*Brassica juncea*) in agricultural soils. SN Appl Sci 1(4):375
 33. Sharma B, Singh RP (2019) Physiological, biochemical, growth, and yield responses of radish (*Raphanus sativus* L.) plants grown on different sewage sludge–fly ash mixture (SLASH) ratios. In: Ghosh S (ed) Waste valorisation and recycling. Springer, Singapore pp 539–552
 34. Shimada K, Fujikawa K, Yahara K, Nakamura T (1992) Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 40:945–948
 35. Singh PK, Tripathi P, Dwivedi S, Awasthi S, Shri M, Chakrabarty D, Tripathi RD (2016) Fly-ash augmented soil enhances heavy metal accumulation and phytotoxicity in rice (*Oryza sativa* L.); a concern for fly-ash amendments in agriculture sector. Plant Growth Regul 78(1):21–30
 36. Slavov A, Karagyzov V, Denev P, Kratchanova M, Kratchanov C (2013) Antioxidant activity of red beet juices obtained after microwave and thermal pretreatments. Czech J Food Sci 31(2):139–147
 37. Sneath PHA, Sokal RR (1973) Numerical taxonomy: the principles and practice of numerical classification. Freeman, San Francisco, 573 pp
 38. Tripathi RC, Jha SK, Ram LC (2010) Impact of fly ash application on trace metal contents in some root crops. Energy Source Part A 32(6):576–589
 39. Vazquez MD, Poschenrieder CH, Barcelo J (1987) Chromium VI induced structural and ultrastructural changes in bush bean plants (*Phaseolus vulgaris* L.). Ann Bot 59(4):427–438
 40. Verma SK, Singh K, Gupta AK, Pandey VC, Trivedi P, Verma RJ, Patra DD (2014) Aromatic grasses for phytomanagement of coal fly ash hazards. Ecol Eng 73:425–428
 41. Wang YL, Wei MY, Chen SJ (2008) Effects of Cr6+ on the growth and physiological and biochemical characteristics of *Mentha crispata*. J Anhui Agric Sci 36:7100–7102
 42. Yu CL, Deng Q, Jian S, Li J, Dzantor EK, Hui D (2019) Effects of fly ash application on plant biomass and element accumulations: a meta-analysis. Environ Pollut 250:137–142

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.