Evaluation of Coal Fly Ash for Modulating the Plant Growth, Yield, and Antioxidant Properties of *Daucus carota* (L.): A Sustainable Approach to Coal Waste Recycling

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Abstract: In search of a safe, cost-effective, and sustainable method for the disposal and management of coal fly ash (CFA), seeds of carrot were sown in earthen pots containing growth substrate consisting of field soil amended with different concentrations of weathered CFA at w/w % ratios. Results suggested that CFA added many essential plant nutrients to the growth substrate and improved some important soil characteristics such as pH, electric conductivity, porosity, and water holding capacity. The growth substrate containing 15% of CFA proved most suitable for growing carrots. Plants grown in 15% CFA amended soil had significantly (*p* ≤ 0.05) enhanced plant growth, yield, photosynthetic pigments, nitrate reductase activity, protein, and carbohydrate contents as compared to the control. The activity of antioxidant enzymes such as SOD and CAT was significantly upregulated in 15% CFA amended soil as compared to the control. The biomineralization of various elements in the edible part of the carrot was well under the limits and no toxic metal was detected in the edible part of the carrot. The present study, therefore, attempts to delineate the application of weathered CFA as a soil amendment in agroecosystems to improve the productivity of lands through a cost-effective and an ecofriendly manner.

Keywords: antioxidants; coal fly ash; carrot; growth; heavy metals; nutrients; soil

1. Introduction

The problem of solid wastes is growing all over the world, and therefore, ecofriendly approaches are being explored to mitigate their harmful effects on the environment [1]. Charred biowaste and biofuels are being considered as potential substitutes for pollution-causing solid biofuels such as coal [2,3]. Coal is an essential non-renewable resource that serves as a raw material for the generation of electricity in thermal power plants. Its combustion produces large quantities of coal fly ash (CFA) which, if not managed properly, creates potential threats to the environment. CFA is an amorphous mixture of various elements. These elements include both essential plant nutrients as well as some non-essential elements [4,5]. The annual generation of CFA in India is approximately 131.9 million tons, of which 73.13 million tons are used annually [6]. The available data show that about half of the CFA is wasted. Therefore, there is a need for sustainable approaches to boost its proper usage in various fields. Instead of discarding it as a waste, methods are now being built for its use in agriculture in an environmentally friendly way [7]. In India, 1.93% of CFA is used in the agricultural industry [6]. It has been categorized as a Green List waste by the Organization for Economic Cooperation and Development (OECD) and is not considered as a waste under the Basel Convention of 1992. Due to the presence
of some essential macro- and micro-nutrients (Ca, Mg, K, S, P, Si, Al, Fe, Cu), it has a good potential for use in agricultural soils [8]. It has been assessed for improving the important physicochemical characteristics of soil including pH, porosity, electrical conductivity, bulk density, and water holding capacity [5,9]. Some reports also suggest its role in improving the biological properties of soil [10]. To date, CFA has been evaluated for various crops such as pumpkin [5], chickpea [11], beetroot [4], Indian mustard [8,12], radish [13], white musli [14], rice [15], wheat [16], turnip, onion, garlic, and potato [17]. Ahmad et al. [5] reported that pumpkin exhibited a significant enhancement in growth and yield at 30% CFA level. A significant enhancement has been reported in the growth, yield, antioxidant activity and phytohormone level of chickpeas at 40% CFA level [11]. Studies on beetroot demonstrate that 15% CFA level significantly increased all the growth, yield, biochemical, and antioxidant parameters of beetroot [4]. In Indian mustard, 30–40% CFA level has been found optimum for the improvement in growth, yield, antioxidant activity [8,12]. In rice, 50% CFA level has been reported to improve its photosynthetic pigments, protein content, and antioxidant activity [15]. All these studies demonstrate a species-specific response of plants towards CFA. Moreover, at higher concentrations, CFA has been found to be toxic to plants; therefore, lower levels of CFA at which plant growth is significantly improved need to be explored.

Vegetables comprise a key element of world agriculture due to their high nutritional value, widespread cultivation, and economic importance. India holds the second rank in vegetable production globally, with an annual production of 184.39 million tons from a cultivated area of 10.25 million hectares [18]. Carrot (Daucus carota L.), a member of Apiaceae family, is an important nutritious root vegetable. Its roots are used mainly as crunchy salad or in juice preparation. It is also used in sweets and carrot pudding, cooked with mixed vegetables, and preserved by pickling and canning [19]. With an annual production of 1147.08 thousand metric tons, India ranks among the top 15 countries in carrot production [20]. Phytochemicals that contribute to the nutritional value of carrots comprise mostly four types, viz.: carotenoids, phenolic compounds, polyacetylenes, and ascorbic acid [21]. In recent years, the consumption of carrot and its derivatives has increased progressively due to its recognition as an important source of natural antioxidants. The anticancer activity of β-carotene, which is the precursor of vitamin A, has raised its demand in pharmaceutical applications [22].

The challenge of CFA management due to its bulk generation is a global problem that needs to be addressed by the scientific community. Many developing nations like India still have a bulk amount of CFA that is not utilized but treated as a waste. Therefore, there is an urgent need to explore more possible ways towards the utilization of CFA to mitigate its potential as a waste and exploit its use towards the achievement of sustainable goals. A major constraint to the application of CFA in agroecosystems is the presence of heavy metals in it. In Indian CFA, however, these heavy metals are present below the permissible limits [7] and therefore its application at low concentrations is an ecofriendly approach to crop improvement. Another concern related to its agronomic use is the boron toxicity because CFA is a good source of boron. Therefore, aged or weathered CFA should be used because due to leaching, the amount toxic heavy metals and boron is reduced and thus CFA becomes safe for agronomic purposes [7]. Considering all these issues, this study was carried out to determine the optimum level of CFA suitable for growing carrot.

2. Materials and Methods
2.1. Experimental Site and Plant Growth Conditions

A pot experiment was conducted in a complete randomized block design under natural conditions with an average temperature of 28 °C at the Department of Botany, Aligarh Muslim University, India. The CFA used during the study was collected from the ash pond of Harduaganj Thermal Power Plant, which is situated about 15 Kms away from the Lab of Environmental Botany, Aligarh Muslim University (Aligarh, India). After the collection of soil from an agricultural field, it was autoclaved at 15 lb pressure for 20 min.
and then mixed with CFA at w/w % ratios. After sterilization, the mixture was filled in pots of 10-inch height. The number of replicates for each treatment was maintained at three, i.e., for each object three pots were used. In total, 18 pots were used—6 treatments with 3 replicates for each treatment (6 × 3 = 18). The control pots were filled with 3 kg of soil and the treatments were amended with different CFA levels on w/w basis. The treatments were formulated as: C = 100:0; T1 = 95:5; T2 = 90:10; T3 = 85:15; T4 = 80:20; T5 = 75:25 (w/w ratios of field soil: CFA), respectively. Six seeds were sown in each type of soil—CFA mixture. One week after the germination of the seeds, thinning of seedlings was carried out to maintain one healthy plant in each pot. The pots were irrigated on alternate days with the required amount of water. The plants were harvested at the phenological growth phase (BBCH) of principal growth stage 4 coded as 49, at which the expansion of the root was complete and typical conical form, and a desired size was attained. Data were collected from all the plants/pots maintained during the experiment. Analysis was also performed for all the replicates of different treatments.

2.2. Soil and CFA Analysis

For the experimental work, weathered CFA, which was aged and leached, was collected from the ash pond of Harduaganj Thermal Power Plant. Physicochemical characteristics were determined for soil, CFA, and the growth substrate containing 15% CFA. The ultrastructure of CFA was observed through scanning electron microscopy (SEM) of make JEOL (Tokyo, Japan), JSM-6510 LV, Japan, by following our previous study [4]. pH was measured by the method of Jackson [23]. A digital pH meter was used and calibrated through standard buffers of pH 4.0, 7.0, and 9.2. The texture of soil and CFA was determined by the traditional feel method, which involves rubbing of moistened soil/CFA between the thumb and fingers. EC was determined by the method of Rayment and Higginson [24]. After weighing a 10-gram air dried soil/fly ash, 1:5 soil/CFA: water suspension was prepared in a bottle to which 50 mL of deionized water was added. The suspension was mechanically stirred at 15 rpm for 1 hour. Using 0.01M potassium chloride solution as a reference, EC was read through a conductivity meter. For determining the water holding capacity, the method of Priha and Smolander was followed [25]. N (nitrogen) content was determined by the standard Kjeldahl method [26]. P (phosphorous) content was determined by the protocol of Dickman and Bray [27]. K (potassium) content was determined by using flame photometry. Calcium, sodium, chlorides, and sulphates were determined by the method of Chopra and Kanwar [28]. Carbonate and bicarbonate contents were measured by the protocol of Richards [29].

2.3. Determination of Growth Biomarkers

At maturity, i.e., 60 days after sowing, the plants were uprooted from the pots and washed with tap water to remove the soil. Measurements of shoot and root length were taken with the help of a meter scale. Fresh weight was recorded with the help of a digital weighing balance. For dry weight, shredded plants were dried in the oven at 80 °C for 48 h. The leaves were counted manually.

2.4. Determination of Leaf Characteristics

The number of stomata and the ultra structural dimensions of stomata were determined with the help of scanning electron microscopy (SEM) of make JEOL, JSM-6510 LV, Japan. For SEM studies, leaf sample was prepared as outlined in our previous study [4].

2.5. Determination of Biochemical Parameters

2.5.1. Determination of Photosynthetic Parameters

The content of chlorophyll and carotenoids was measured according to the Maclachalan and Zalik protocol [30]. At 663 and 645 nm, the absorbance was read against a blank 80% acetone on a spectrophotometer.
2.5.2. Determination of Nitrate Reductase Activity, Protein, and Carbohydrate Content

In leaves, nitrate reductase (NR) activity was calculated by preparing an enzyme extract using the Jaworski process [31]. By using the Bradford method [32] with bovine serum as normal, protein content was calculated. The carbohydrate content was measured by using the Hedge and Hofreiter assay [33].

2.6. Quantification and Histochemical Localization of Superoxide Anion (O$_2^-$)

The estimation of the O$_2^-$ content was conducted as per a standardized protocol defined by Wu et al. [34]. The superoxide content was calculated by a standard curve of sodium nitrite, and the content was demonstrated as $\mu$ mole g$^{-1}$ FW. The localization of superoxide anions was conducted as per a standardized protocol defined by Kaur et al. [35]. Images were captured with the help of a stereomicroscope.

2.7. Antioxidant Enzyme Assay

The extraction of 0.1 g root sample was carried out in a 2 mL of 50 mM sodium phosphate buffer (pH 7.0) containing EDTANa$_2$ (1 mM) and polyvinylpyrrolidone (0.5%). It was followed by the centrifugation of sample at 13,000 $\times$ g for half an hour and the extract was used for enzymatic analysis. The SOD (superoxide dismutase) activity was measured spectrophotometrically by following a standardized protocol of Dhindsa et al. [36] based on the ability of SOD to inhibit the photochemical reduction of nitro blue tetrazolium. The CAT (catalase) activity based on the consumption of H$_2$O$_2$ was measured spectrophotometrically at 240 nm by adopting the standardized protocol of Beers and Sizer [37].

2.8. Assessment of Biomineralization in the Edible Part of Carrot

The edible part of the carrot (root) was oven dried for the analysis of various elements and heavy metals. EDX technique combined with mapping and SEM was used for the assessment of biomineralization and localization of various elements accumulated in the edible part of the carrot under different CFA treatments by following the EDX protocol of Ensikatand and Weigend [38]. Further, ICP-MS was also used to assess the concentration of accumulated minerals in the edible roots of carrot. The various isotopes used in ICP-MS were $^{39}$K, $^{24}$Mg, $^{23}$Na, $^{44}$Ca, $^{57}$Fe, $^{55}$Mn, $^{64}$Zn, $^{28}$Si, $^{63}$Cu, $^{52}$Cr, $^{208}$Pb, and $^{114}$Cd. The data acquisition parameters of the ICP-MS instrument were as follows: sweeps/reading = 20, readings/replicate = 1, replicates = 3, dwell time per AMU (ms) = 50, integration time = 1000 (ms) nebulizer gas flow = 1.03 L/min, plasma gas flow = 15 L/min, lens voltage = 7.50 V, plasma power = 1275 W.

2.9. Statistical Analysis

The mean ± standard deviation ($n=3$ replicate pots) of all data is provided. Variance analysis (ANOVA) with a DMRT test, using SPSS version 17.0 software, was used to evaluate significant differences at $p<0.05$. Origin pro (2021) software was used for principal component analysis.

3. Results

3.1. Effects of CFA Amendment on the Physicochemical Characteristics of the Soil

The scanning electron microscopy (SEM) revealed that the particles in CFA were spherical while the shape of soil particles was non uniform (Figure 1). Table 1 shows the improvement in soil characteristic after its amendment with 15% CFA. The addition of 15% CFA to the soil improves its EC by 66.91%, porosity by 19.44%, and water holding capacity by 15.38%, respectively. The content of P and K was also improved with 15% CFA amendment to the soil, but the N content was decreased because CFA lacks the N element. The content of Ca, Na, carbonate, bicarbonate, sulphate, and chloride was also improved with the addition of 15% CFA to the soil.
Figure 1. Ultrastructure of the soil (a) and CFA (b) through scanning electron microscopy.

Table 1. Physicochemical characteristics of the soil, coal fly ash (CFA), and 15% CFA amended soil.

| Characteristics          | Soil               | CFA               | Soil + 15% CFA     |
|--------------------------|--------------------|-------------------|-------------------|
| Color                    | Brownish           | Greyish white     | Off white         |
| Texture                  | Sandy loam         | Silty             | Mixed             |
| Shape                    | Flaky              | Spherical         | Mixed             |
| pH                       | 6.97 ± 0.84        | 8.63 ± 0.63       | 7.71 ± 0.56       |
| EC (µmhos cm⁻¹)          | 269.44 ± 15.22     | 899.2 ± 30.2      | 449.33 ± 17.2     |
| Porosity (%)             | 36.87 ± 2.33       | 59.22 ± 3.11      | 43.13 ± 2.23      |
| Water holding capacity (%) | 39.67 ± 2.12  | 55.21 ± 2.04      | 45.21 ± 3.41      |
| N (%)                    | 2.22 ± 0.32        | —                 | 1.77 ± 0.23       |
| P (g kg⁻¹ soil)          | 2.53 ± 0.27        | 0.023 ± 0.002     | 2.21 ± 0.76       |
| K (mg L⁻¹)               | 21.10 ± 1.23       | 14.1 ± 0.93       | 26.11 ± 1.74      |
| Calcium (mg L⁻¹)         | 16.32 ± 1.5        | 20.7 ± 1.46       | 22.2 ± 2.6        |
| Sodium (mg L⁻¹)          | 11.6 ± 0.43        | 14.0 ± 0.54       | 13.3 ± 1.6        |
| Carbonate (mg L⁻¹)       | 75.6 ± 3.11        | 67.5 ± 2.87       | 89.3 ± 1.87       |
| Bicarbonate (mg L⁻¹)     | 16.5 ± 0.96        | 14.5 ± 0.33       | 19.5 ± 0.53       |
| Sulphate (mg L⁻¹)        | 14.5 ± 0.57        | 22.4 ± 1.23       | 20.55 ± 2.22      |
| Chloride (mg L⁻¹)        | 23.4 ± 1.22        | 16.5 ± 1.16       | 31.27 ± 2.12      |

Data represent mean of three independent replicates ± standard error (SE). The date represents the total element content.

3.2. Effects of CFA Amended Soil on the Growth and Yield of Carrot

The results presented in Tables 2 and 3 demonstrate the impact of different CFA levels amended to the soil on the growth markers and yield attributes of carrot. Among the different CFA levels, 15% level was found most optimal at which significant ($p \leq 0.05$) increments in growth and yield of carrot were observed. At 15% CFA level, shoot length increased by 16.96%, shoot fresh weight by 20.01%, shoot dry weight by 25.65%, and number of leaves per plant by 30.3% as compared to the control (Table 2). The yield attributes such as root length increased by 40.03%, root fresh weight by 23.67%, root dry weight by 38.18%, and root circumference by 36.16% as compared to the control (Table 3). The scanning electron microscopy revealed that the plants grown in 15% CFA have a significantly higher number of stomata with improved stomatal apertures on their leaves (Figure 2).
Figure 2. Scanning electron micrograph showing the effect of different CFA levels amended to the soil on the stomatal aperture and stomata density in carrot leaves. Data represent mean of three independent replicates ± standard error (SE). Different letters placed over the bars indicate significant differences between treatments while similar letters indicate non-significant differences between treatments at $p < 0.05$, ($n = 3$) according to one-way ANOVA and DMRT (Duncan multiple range test).
The growth and yield markers of carrot observed in this work showed a dose-dependent response to CFA. A gradual increase in CFA concentration in amended soils elicited a dual response from the test plants. Application of CFA up to 15% (T3) caused improvements in all the biochemical attributes of the carrot. However, further increment in CFA concentration (T4, T5) did not favor the plants and thus these parameters started to decline (Tables 2 and 3). A progressive increase in growth and yield attributes was observed from T1 to T3. However, these attributes have a progressive decrease in their values with further CFA amendments, i.e., from T4 to T5 treatments. One-way ANOVA showed that T3 (15% CFA) is the best CFA concentration among different treatments at which significant ($p \leq 0.05$) enhancement in growth and yield attributes of carrot was observed.

### 3.3. Effects of CFA Amended Soil on the Leaf Photosynthetic Pigments and Biochemical Markers in the Roots of Carrot

The photosynthetic pigments including chlorophyll ‘a’, chlorophyll ‘b’, and carotenoid contents increased significantly ($p \leq 0.05$) at 15% CFA amended soil as compared to the control (Figure 3). Chlorophyll ‘a’ improved by 38.11%, chlorophyll ‘b’ by 29.20%, and carotenoid content by 61.53% as compared to the control. The activity of nitrate reductase increased by 61.2% with a subsequent increase in protein content by 41.8% and carbohydrate content by 37.46%, respectively, at 15% CFA level as compared to the control (Figure 3). One-way ANOVA showed that T3 (15% CFA) is the optimum concentration among different treatments at which significant ($p \leq 0.05$) improvement in all the biochemical attributes of carrot was observed.
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3.4. Effects of CFA Amended Soil on the Superoxide Anion (O\(_2\)•\(^-\)) in the Leaves of Carrot

The results presented in Figure 4 demonstrate the direct effect of different CFA levels amended to the soil on the production of O\(_2\)•\(^-\) anions in the leaves of carrot. The concentration of O\(_2\)•\(^-\) radicals increased correspondingly with the CFA levels. At 15% CFA level, the O\(_2\)•\(^-\) concentration was raised by 20.01% as compared to the control. However, the highest O\(_2\)•\(^-\) content was found at 25% CFA level (Figure 4C), while the least content of O\(_2\)•\(^-\) was found in the control plants (Figure 4A). The order of O\(_2\)•\(^-\) anion generation was T5 > T4 > T3 > T2 > T1 > C.
3.4. Effects of CFA Amended soil on the Superoxide Anion (O$_2$•$^-$) in the Leaves of Carrot

The results presented in Figure 4 demonstrate the direct effect of different CFA levels amended to the soil on the production of O$_2$•$^-$ anions in the leaves of carrot. The concentration of O$_2$•$^-$ radicals increased correspondingly with the CFA levels. At 15% CFA level, the O$_2$•$^-$ concentration was raised by 20.01% as compared to the control. However, the highest O$_2$•$^-$ content was found at 25% CFA level (Figure 4C), while the least content of O$_2$•$^-$ was found in the control plants (Figure 4A). The order of O$_2$•$^-$ anion generation was T5 > T4 > T3 > T2 > T1 > C.

Figure 4. Effects of different CFA levels amended to the soil on the generation of superoxide anion radical (O$_2$•$^-$) and its localization in the leaves of carrot at Control (A), 15% CFA (B), and 25% CFA (C). Data represent mean of three independent replicates ± standard error (SE). Different letters placed over the bars indicate significant differences between treatments while as similar letters indicate non-significant differences between treatments at $p < 0.05$, ($n = 3$) according to one-way ANOVA and DMRT (Duncan multiple range test).

3.5. Effect of CFA Amended Soil on the Antioxidant Enzyme Activity in the Edible Part of Carrot

The amendment of soil with different CFA levels demonstrated a significant impact on the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in the edible part of the carrot as compared to the control (Figure 5). A significant improvement in the activity of SOD (57.14 %) and CAT (69.23 %) was observed at 15% CFA level as compared to the control. However, the highest activity of both the enzymes was observed at 25% CFA level and least activity was observed in the control plants, respectively (Figure 5). The order of antioxidant activity in terms of SOD and CAT was T5 > T4 > T3 > T2 > T1 > C.
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Figure 5. Effects of different CFA levels amended to the soil on the antioxidant enzyme activity in the edible part, i.e., root of carrot. Data represent mean of three independent replicates ± standard error (SE). Different letters placed over the bars indicate significant differences between treatments while similar letters indicate non-significant differences between treatments at $p < 0.05$, $(n = 3)$ according to one-way ANOVA and DMRT (Duncan multiple range test).

3.6. Effects of CFA Amended Soil on Biomineralization in the Edible Part of Carrot

The amendment of soil with CFA added various essential nutrients as well as some non-essential elements to the soil. Metal accumulation in the edible part of the carrot was a principal concern during the study and analyzed through ICP-MS. The results from ICP-MS demonstrate that with increasing CFA concentrations, the content of various essential and non-essential elements increased correspondingly in the edible carrot roots (Table 4). By increasing the concentration of CFA from 5% to 25%, the concentration of elements except N increased consequently in a dose-dependent fashion (Table 4; Figure 6). At 15% CFA level, various elements were localized through EDX mapping in the edible part of the carrot, viz.: C, N, O, Mg, K, Ca, Na, Fe, Mn, Si, and Zn (Figure 6). It is important to note that none of the potential toxic heavy metals like Cd, Pb, Cr, etc. were accumulated by the carrot at 15% CFA level. Therefore, this level was recommended as the best treatment during this experimental study. However, before its recommendation to farmers, field experiments to confirm these reports from pot experiments are necessary. We do not know how CFA will be effective in various weather conditions in an open space (field).
Table 4. Effect of different CFA levels amended to the soil on the biomineralization of carrot analyzed through ICP-MS.

| Treatments | C       | N       | O       | K       | Mg      | Na      | Ca      | Fe      | Mn      | Zn      | Si      | Cu      | Cr      | Pb      | Cd      |
|------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| C          | 44.16 ± 0.63 | 10.06 ± 0.28 | 44.15 ± 0.55 | 0.69 ± 0.02 | 0.65 ± 0.08 | ND      | ND      | ND      | ND      | ND      | ND      | ND      | ND      | ND      |
| T1         | 44.13 ± 0.58 | 8.40 ± 0.32 | 44.45 ± 0.45 | 0.71 ± 0.05 | 0.66 ± 0.26 | 0.96 ± 0.01 | 0.05 ± 0.07 | 0.15 ± 0.04 | 0.27 ± 0.045 | 0.17 ± 0.021 | 0.12 ± 0.011 | ND      | ND      | ND      |
| T2         | 42.63 ± 0.75 | 7.10 ± 0.45 | 46.80 ± 0.65 | 0.73 ± 0.06 | 0.91 ± 0.17 | 0.96 ± 0.04 | 0.04 ± 0.004 | 0.58 ± 0.033 | 0.31 ± 0.013 | 0.19 ± 0.015 | 0.14 ± 0.006 | ND      | ND      | ND      | ND      |
| T3         | 39.67 ± 0.37 | 6.52 ± 0.37 | 49.73 ± 0.45 | 0.77 ± 0.021 | 1.03 ± 0.29 | 0.46 ± 0.07 | 0.06 ± 0.002 | 0.65 ± 0.041 | 0.37 ± 0.021 | 0.22 ± 0.022 | 0.16 ± 0.005 | ND      | ND      | ND      | ND      |
| T4         | 38.21 ± 0.51 | 5.16 ± 0.18 | 52.12 ± 0.29 | 0.79 ± 0.032 | 1.07 ± 0.21 | 0.66 ± 0.05 | 0.08 ± 0.006 | 0.69 ± 0.033 | 0.39 ± 0.002 | 0.24 ± 0.012 | 0.19 ± 0.005 | 0.21 ± 0.02 | 0.23 ± 0.02 | ND      | ND      |
| T5         | 35.15 ± 0.74 | 5.02 ± 0.43 | 55.06 ± 0.86 | 0.81 ± 0.096 | 1.09 ± 0.43 | 0.46 ± 0.004 | 0.10 ± 0.002 | 0.72 ± 0.002 | 0.41 ± 0.004 | 0.26 ± 0.002 | 0.22 ± 0.007 | 0.29 ± 0.002 | 0.27 ± 0.011 | 0.17 ± 0.002 | 0.13 ± 0.002 |

Units = g kg\(^{-1}\) DW for macroelements and µg kg\(^{-1}\) DW for microelements; DW = dry weight; ND = not detected. C, N, and O were determined through EDX.
Figure 6. Biomineralization and localization of elements through the transverse section of the carrot root grown in soil (control) and 15% coal fly ash (15% CFA) analyzed through EDX coupled with SEM.

3.7. Principal Component Analysis

To simplify and summarize the complex data of this work, principal component analysis (PCA) or factor analysis was performed through Origin pro-2021. It was used to determine the relationship between growth, yield, biochemical characteristics, and antioxidant attributes of carrot under the impact of different CFA levels (Figure 7). The PC1 shows 81.15%, and PC2 reveals 17.08% of the total data variability. The loading plot shows two groups of parameters. One group included all the growth, yield, and biochemical parameters, which exhibit a positive correlation with each other. The other group included superoxide anion (O2•−), SOD, and CAT, which exhibit a positive correlation with each other. Our results agree with the criteria developed by Sneath and Sokal [39] which suggests that data should represent at least 70% of the total data variability.
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Figure 7. Principal component analysis (PCA): loading plot of principal component 1 and principal component 2 obtained from growth, yield, biochemical, and antioxidant attributes of carrot. (SL = length; RL = root length; FWS = fresh weight of shoot; FWR = fresh weight of root; DWS = dry weight of shoot; DWR = dry weight of root; Chl a = chlorophyll a; Chl b = chlorophyll b; NRA = nitrate reductase activity; TP = total protein content; TC = total carbohydrate content; $O_2^-$ = Superoxide anion; SOD = superoxide dismutase; CAT = catalase).

4. Discussion

Plants thrive better under favorable soil conditions and thus produce the desired yield. Various factors are included under favorable soil conditions such as pH, soil porosity, EC, water holding capacity, and the nutrient status of the soil. The physicochemical properties of CFA alone were numerically higher than compared to the soil. However, the amendment of 15% CFA to the soil improved all these characteristics except N content. The increase in EC is possibly due to the richness of inorganic elements and heavy metals present in CFA [40]. CFA enhances the potential of soil to retain water through the changes in soil structure and texture [41]. The water holding capacity of the soil is determined by its surface area and porosity. The improvement in pH of the soil is attributed to the sulfur content and CaO content of the CFA [42]. The use of CFA in improving the soil characteristics such as pH, electric conductivity, soil texture, porosity, bulk density, and water holding capacity has also been reported by various studies [43–45]. Although CFA contains low K and Cl content as compared to the soil, the additive effect of CFA and the change in pH level may possibly have improved their content in the substrate containing 15% CFA.

Soil amendment with CFA is a fast-emerging and promising area of research in agricultural soil since it is a source of important plant nutrients [46]. Our results show that 15% CFA amendment to the soil significantly improved all the growth markers, yield attributes, photosynthetic pigments, biochemical parameters, and antioxidant enzyme activity of carrot as compared to the control (Figure 8). The possible reason for this improvement is the modification of soil characteristics including soil structure, pH, water holding capacity, and EC. It also enriches the nutrient status of the soil with essential plant nutrients such as Ca, Mg, K, P, and S. Soil pH is an important factor that influences the physical, chemical, and biological properties of soil and is therefore regarded as the “master soil variable” [47]. It also plays a vital role in the availability and uptake of nutrients by plants and thus affects the growth and yield of crops [48]. Ca is an essential secondary
macronutrient that serves a dual role in plants, i.e., not only being an essential factor for the stability of the cell wall and membrane but also serving in many developmental and physiological processes \[49,50\]. Therefore, Ca enrichment of the soil through 15% CFA amendment has possibly contributed to the improved plant growth and yield of carrot. Our results further represented that the photosynthetic pigment content of carrot was significantly improved with the 15% CFA amendment to the soil (Figure 3). This is possibly due to the enrichment of soil with Mg, which is the central atom of chlorophyll molecule amid four N atoms \[51\]. It also acts as an activator and stabilizes the nucleic acids for several enzymes required in plant growth processes \[52,53\]. The improvement in the plant growth and stomatal aperture of carrot can also be attributed to the enrichment of soil with K ion. It is the most abundant cationic essential element, which is important for ensuring optimal plant growth \[54\]. K is an activator of dozens of important enzymes that are involved in important biochemical processes such as protein synthesis, sugar transport, N and C metabolism, and photosynthesis in plants. K is also very important for cell growth, which is an important process for the function and development of plants \[55\]. In terms of the growth-promoting mechanism of K, it is generally agreed that K stimulates and controls ATPase in the plasma membrane to generate acid stimulation, which then triggers cell wall loosening and hydrolase activation \[56\], thus promoting cell growth. K has strong mobility in plants and plays an important role in regulating cell osmotic pressure and balancing the cations and anions in the cytoplasm \[57\]. Therefore, the observed improvement in the plant growth, yield, and stomatal attributes are possibly due to the amendment of soil with CFA, which adds K ion to the soil. Moreover, CFA also contains some essential micronutrients such as Fe, Mn, and Cu that assist in improving plant performance, as well. Fe serves as a co-factor during photosynthesis and is involved in various physiological and biochemical pathways in plants. It acts as a component of many vital enzymes, such as electron transport chain cytochromes, and is therefore needed for a broad range of biological functions \[58,59\]. Mn participates in many important processes in plants such as photosynthesis, respiration, translation, and activation of hormones \[60\]. It is actively involved in the photosystem II (PSII) water splitting reaction. Cu is also involved in some important biochemical pathways in plants because it is an integral component of certain electron transfer proteins, e.g., plastocyanin, cytochrome c-oxidase, and laccase \[61\]. The texture of the growth substrate containing 15% CFA could not be determined in this study, which is therefore a limitation of this study. Our results show adherence to the observations of Sarangi and Mishra \[62\], who observed better growth and yield of groundnut, ladyfinger, and radish at 15% CFA. Additionally, our previous study reported that 15% CFA improves growth, yield, and antioxidant enzyme activity of a root vegetable, beetroot \[4\].

The principal enzyme vital for the metabolism of nitrogen in plants is the nitrate reductase. Since CFA has insufficient nitrogen content, substantial inhibition of NR activity has been observed at higher levels of CFA, which agrees with previously reported studies \[4,63\]. The binding of metal ions to –SH groups of the nitrate reductase may be another cause for this reduction \[64\]. The current study also recorded a rise in total carbohydrate levels at 15% CFA in the edible portion of carrot, which was reduced at higher CFA levels (Figure 3). This enhancement is related to the changes in the photosynthetic pigments and rate of photosynthesis at different levels of CFA (Figure 3) \[65\]. Likewise, the protein content in the edible portion of the carrot was significantly increased at the 15% CFA level (Figure 3). This increment is due to the K enrichment of the soil, which plays an important role in the activation of enzymes required for protein synthesis \[12\].
Despite the presence of essential plant nutrients, CFA contains several heavy metals that could create stress in plants [66]. This stress may trigger the generation of reactive oxygen species (ROS) such as superoxide anion (O$_2^{•-}$), which may cause damage to plants through cell disintegration and lipid peroxidation [67]. In this study, we observed that the content of O$_2^{•-}$ increased correspondingly with the levels of CFA (Figure 4). This is possibly due to the enrichment of heavy metals in the soil that triggers ROS. However, plants maintain the stress homeostasis by employing the antioxidant machinery to combat the adverse effects of ROS [68]. SOD and CAT are very important enzymatic antioxidants that regulate the homeostasis of harmful radicals in plants [69]. As a first line of defense, SOD helps to scavenge O$_2^{•-}$ radicals by converting it to O$_2$ and H$_2$O$_2$. This H$_2$O$_2$ is converted into H$_2$O by the activity of CAT [70]. Therefore, in this study, the activity of SOD and CAT enhanced correspondingly with increasing CFA levels to scavenge the radicals generated through heavy metals stress (Figure 5). A similar trend in the activity of antioxidants was observed in our previous study [4].

The evaluation of the biomineralization and metal accumulation by the carrot roots was of primary concern while attempting the utilization of CFA in agricultural ecosystems. The application of SEM coupled with EDX and ICP-MS was followed in this study for the assessment of biomineralization and elemental composition because these techniques have been found very useful to study the locations of biominerals in plant tissues with their respective compositions [71,72]. In this study, the biomineralization of different metallic elements was found to be directly proportional to the level of CFA amendment, and the highest level was observed when 25% CFA was amended to the soil (Table 4). However, at 15% CFA level, none of the toxic elements were in the edible part of the carrot (Figure 6). Though 15% CFA was found safe and beneficial to the carrot, it should not be used repeatedly in agricultural soils since it contains toxic heavy metals, which may accumulate in the food chain due to continuous CFA application. Keeping in mind the presence of heavy metals in CFA, weathered CFA was used, which is comparatively...
safe and ecofriendly for agronomic uses as compared to unweathered CFA [7,39]. The current study did not consider the deficiency of N element in CFA, which is the primary macronutrient essential for plants. Therefore, revised approaches through modified CFA to address this limitation are required. Sewage sludge, which is also used a soil amendment like CFA, has a limitation in that it does not allow the recycling of P element present in it. To address that problem, modified sewage sludge has been used to recycle the P element present in it [73,74]. Similar approaches through modified CFA can be used to address N availability in CFA amended soils. Considering the financial benefits of this work, it provides a cost-effective approach towards the management of solid wastes in an ecofriendly manner.

5. Conclusions

The results from this work clearly demonstrate that the augmentation of field soil with an optimum level of weathered CFA (15%) improved the physicochemical characteristics and nutrient status of the soil, which ultimately resulted in improved growth and yield of carrot. A dose-dependent response of carrot to CFA amended soil was observed and, more importantly, no toxic heavy metal was accumulated by carrot at 15% CFA level. This study recommends the use of weathered CFA in agroecosystems as it becomes safe and free of toxic elements present in unweathered CFA. However, further studies are required to investigate and identify the key elements present in CFA and the molecular mechanisms through which these elements are linked to different plant metabolic pathways. Furthermore, the hypothesis of the present study needs to be confirmed further at molecular level before its recommendation to farmers.

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