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GROWTH CHARACTERISTICS OF BEAN (*PHASEOLUS VULGARIS L.)* PLANT EXPOSED TO BLACK CARBON UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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ABSTRACT

Urbanization has created stressed conditions for the vegetation by inducing air pollution through automobile use. However, the role of fine particulate matter inclusive of black carbon interaction in the growth of a plant is less understood. Hence the effect of atmospheric and terrestrial black carbon particulate interaction with a plant is attempted. Simulation experiments were carried out to understand the plant-soil and air-soil interaction towards the deposition of particulate aerosols. Plant soil interaction is studied through carbon black employment in potted plants. While air-plant interaction is studied through wind-tunnel experiments and open road-side potted plants. Leaf traits such as leaf surface area, length, and width are studied through imaging techniques. Further, morphological and chemical aspects of deposited particulates are studied by employing SEM-EDX analysis. Dominance in plant growth is observed in a natural environment with a shoot reaching 38.6cm at the end of the experiment. Reduced growth is observed in stressed outdoor followed by indoor conditions. Analysis by leaf traits showed that leaf surface area, length, and width were lower in plants growing under stressed conditions than the non-stressed conditions. The morphological and source apportionment of plant deposited particulate indicates that the source was mainly from biomass burning, vehicular, and road dust which is a characteristic of our study region. Further, an additional approach of implementing carbon black for plants under stressed condition is attempted which tend to show better results in plant growth even under stressed conditions. Future works to consider other gaseous pollutants, plant scale effects, and efficient usage of carbon black can help unveil a wide-ranging of possibility towards the creation of sustainable and polluted adapted plant species.

KEY WORDS : Wind tunnel, Black carbon, Leaf traits, Imaging techniques, Source apportionment and morphology.

INTRODUCTION

Urbanization drive across the tropics is on the expansion phase as humans started to inhabit in large numbers in tune with the population explosion. Landscape dynamics is experienced in natural and agricultural regions as a result of urbanization. This alteration is envisioned to affect the plant community in those regions concerning water availability, urban heat island, and pollution (Miles *et al.*, 2019). Urban studies concerning the effect of particulate matter on plants have been gaining significance in recent years as urban ambient pollution serves as a major contributor of Particulate Matter of aerodynamic size 2.5μm ($PM_{2.5}$) across the globe which is depicted in Figure 1. India is found to contribute majorly to the emissions of particulate matter inclusive of Black Carbon (BC) which is the pressing issue in terms of health and the environment of the current Anthropocene period (Boys *et al.*, 2014; Huang *et al.*, 2014; Maher *et al.*, 2013). BC can inhibit the germination and growth of plants due to the presence of free radicals (Liao *et al.*, 2014) and/or phytotoxic compounds and heavy metals (Rogovska *et al.,* 2012). BC is the result of incomplete combustion of fossil fuels, biofuel, biomass, natural gas, and vegetation (Klimont *et al.,* 2017). BC can be understood as a continuum from partly charred plant material through char and charcoal to graphite and soot particles re-condensed from the gas phase, with no general agreement on clear-cut boundaries (Crutzen *et al.,* 2016). It is reported that black carbon fractions below 0.45 µm have a higher content of oxygen and polar functional groups, but lower aromaticity and less condensed aromatic clusters compared with bulk particulates (Qu *et al.,* 2016). These results indicate that black carbon is active and inclined to transport both laterally and vertically in terrestrial and aquatic ecosystems (Major *et al.,* 2010). Also, it tends to bind with soil organic matter and minerals to form micro aggregates via organo-mineral interactions (Han Weng *et al.,* 2017). Previous works of literature have well demonstrated that black carbon plays an important role in the immobilization of pollutants, plant growth, and crop yield (Khan *et al.,* 2013; Sun and Lu, 2014). Hence, it is foreseeable that black carbon (including its micro aggregates) will more easily enter into the rhizosphere and contact with the plant roots relative to bulk black carbon and can regulate growth. However, this paradox can be better clarified and understood by examining the interaction between black carbon and plant roots in the rhizosphere, which is one of the most critical zones for plants to communicate with the environment.

Understanding soil dynamics is essential in terms of plant growth which is best known with particle size the fractionalisation of soil as fine particulates account for stable organic carbon content in the soil (Samson *et al.,* 2020). Carbon black is found to be a better alternative for implementing the fine particulate fraction into the soil to study its role in the growth of a plant as BC is the main component in biochar or carbon black and is found to be a promising soil amendment to improve the soil conditions (Schmidt *et al.,* 1999; Jiang *et al.,* 2019). The improvements in soil nutrients pave the way for good quality soil which is facilitated by ingredients in biochar (Dai *et al.,* 2017; Ding *et al.,* 2016). However, a different type of biochar produces unpredictable results on its application towards different soils making it difficult to understand its role in plant growth (Zhu *et al.,* 2017). Fine particulate matter (especially BC) in the atmosphere may interact with the plant in terms of deposition which may further inhibit the growth potential of a plant in addition to the above-mentioned role of fine particles in the soil.

Plant particulate interaction is majorly studied in terms of deposition of particulates in leaves of trees. In tropics, an average of 7.5-32.1 µg per cm^2 of particulate matter can be with-hold by woody plant species (Popek *et al.,* 2013), in shrubs, it ranged from 2-16 mg per cm^2 (Leonard *et al.,* 2016) and conifer species were found to capture more particulate than broad-leaved trees (Hwang *et al.,* 2011). In addition to the above studies, modelling has been used to simulate the experimental conditions, and remote sensing studies are also employed to cover extensive spatial distributions which have been gaining popularity in recent days. However, there were still large uncertainties exists about the lack of larger spatial coverage of studies dealing with particulate interaction with plants, discrepancies in modelled data, shorter timescale studies (usually weeks), and non-uniform method adaptation (Prajapati, 2012; Ram *et al.,* 2015). Therefore, a real-time study is in need to address and understand the role of globally significant disruptive pollutants such as black carbon (BC) in above and below ground soil. This will be better explained in an agricultural region as particulate aerosols contamination status on plant-soil interaction is of immense importance in terms of plant growth. Madurai region is identified to be ideal for this experiment as the fine particulate load is high in this region which is equivalent to Delhi (a megacity) (Bhaskar *et al.,* 2018) and then soils in the urban and sub-urban areas of the city are being extensively used for agricultural purposes especially for vegetation production. Thus, the study aims to address the following objectives 1) what is the effect of deposition of black carbon in plant growth characteristics? 2) Does environmental condition play a role in the growth of a plant? 3) Does particulate deposition affectthe leaf traits of plants? 4) What is the effect of the implementation of biochar on soil characteristics? 5) What are the characteristics of particulates deposited in plants?

MATERIALS AND METHODOLOGY

Experimental setup

Outdoor

The plants were grown in pots with the characteristics (Length × Breadth× Height) of 7.5cm ×7cm × 12cm in an open area that was exposed to the prevailing environmental conditions and was watered based on moisture stress. Moisture stress is identified using soil moisture probes which were placed in the soil column at three different levels: near the bottom, at the middle, and the top. These soil moisture probes were used to determine how much water each treatment receive, to maintain some drought pressure. Treatments were only watered when the soil moisture dropped below 40 centibars. The soil used to grow the plants were taken from a treeless grassland in Madurai Kamaraj University, Madurai, India. The Soil is oven-dried at 60°C to remove any moisture before dry sieving through 2 mm mesh sieve. Dry materials of black carbon and soil were then oven-dried at 100°C for 24 hours to create homogenous mixtures with an initial water content of zero as the employed temperature (100°C) ensures water loss but minimizes chemical impacts as it is significantly lower than pyrolysis temperature (Barnes *et al.,* 2014). The concentration of the dried Carbon black was applied to the soils to represent typical deposition rates in soils. Annual varieties of bean (*Phaseolus vulgaris L.*) plants are chosen for this study. Seeds were germinated in Petri dishes and seedlings were transplanted after approximately three days. Plant seeds were sown in pots which are then measured for a daily increase in leader shoot length (n=24) were performed using a tape measure for all plants used in the study. The experimental design includes two replicates for each treatment.

Indoor

A wind tunnel is set up to study the impact of particulate matter on the growth of selected plant species. A glass chamber with the dimensions of 120cm×75cm×40cm is used to enclose the vegetation and the particulate source (Figure 1.1). The setup is placed near a window for allowing sufficient light to reach the plant chamber. The pollutant source is generated using incense sticks (Cycle pure incense sticks; widely used in India) which is used to generate a continuous supply of particulate aerosols through an axial fan with a suction flow generation ability of up to 18m s^{-1}. Omnidirectional flow is ensured in the deposition chamber by proper positioning of inlet and outlet tubes. Incense sticks were made out of bamboo sticks, charcoal powder, wood powder, and perfumes which closely resemble burning wood (Chang *et al.,* 2007). The emission from incense sticks includes particulates of hydrophilic and hydrophobic nature though its ratio varies with brand and additives (Li and Hopke, 1993). Particles generated through incense sticks may have varying size distribution ranging from 0.1 to 10μm which is similar to traffic emissions and remained consistent under fluctuating humid conditions (Chiam *et al.,* 2019). The distance between the particulate source and vegetation is maintained to be 30cm-50cm to avoid point source influence favouring high concentration at one point. Another axial fan is placed at the other end of the chamber to ensure the turbulence of the air inside the chamber for better mixing. A wind anemometer (Lutron-42, Taiwan) is placed at the centre to measure the wind speed inside the chamber. Wind speeds were regulated to mimic the environmental conditions i.e., 3 m/s, and the pressure drop inside the chamber is negligible.

Estimation of PM deposited on plants

We obtained the total leaf surface area of plants, leaf length, and leaf width using the image software Image J (Rasband, W.S., Image J, U. S. National Institute of Health, Bethesda, Maryland, USA, HTTPS:// imagej.nih.gov/ij/,1997-2012) following (Osunkoya *et al.,* 2010). We hypothesized that the loading of particulates on leaves from different parts of the plants were the same. The particulates with leaves were placed in a glass container of 250ml and agitated for 60 seconds in double-distilled water to wash off particles from surfaces. This mechanism is similar to the number of particulates removed by rainfall (Dzierzanowski *et al.*, 2011). The resultant water is then filtered using Type 42 (retention 2.5μm) filter paper placed in a glass filter funnel. Filters were then dried and subjected for further analysis.

SEM and EDX analysis

Filtered samples were cut in the centre of the filter paper and then attached to the stub with double-sided adhesive tape. The samples were gold coated to enhance the electrical conductivity before analyzing those using Scanning Electron Microscope (SEM, VEGA3TESCAN, Bruker, USA). The images were taken at different magnifications. Besides, the particulate composition was investigated by Energy-Dispersive X-ray (EDX) analysis.

Statistical analysis

One-way analysis of variance (ANOVA) was performed for each time interval to determine significant differences between the treatments.

Significant effects of various treatments were detected using the t-test.

RESULTS AND DISCUSSION

Soil Characterisation

pH

Soil chemical analysis showed that the addition of carbon black induced an increment in pH levels at the end of the experiment. In detail, an average inclination of pH of Outdoor Planted Soil with Black carbon (OPSB) changed from 6.31 to 7.73, and in Outdoor Planted Soil with More Black carbon (OPSMB) variation is found to be 8.01. Similarly, in roadside treatment (traffic) of plants pH changed from 6.3 to 6.9 and indoor an average pH of 7.42 is observed at the final measurement. Our results claimed that increment in pH is dominant in OPSB and OPSMB setup than the control setup which is similar to the results achieved in different carbon black application rates in clayey soil (Mavi *et al.*, 2018). However, the overall pH of the soil increased with the increase in application rates of BC or carbon black. One of the causes of the increase of pH in treatments may be due to growth stimulation by plants. Such increment in pH was observed in several studies (Gaskin *et al.*, 2010; Yuan and Xu, 2011; Alburquerque *et al.*, 2014) which may be triggered for numerous reasons and it is found to vary across different soil types. Generally, pH tends to be dependent on several factors such as the nature of biochar used, chemical characteristics of biochar, pyrolytic temperature, soil environmental conditions and other factors (Shah *et al.*, 2017; Rajpal and Nagabovanali, 2020). The influence of pH in altering the growth of the plant can be understood with the help of regression analysis. A significant positive contribution is observed in outdoor grown plants with the regression coefficient of R^2=0.753 while the lower significant contribution is observed in roadside plants (traffic) growth with the regression coefficient of R^2=0.68.

Electrical Conductivity (EC)

Different treatments pose different responses of conductivity concerning BC application in soil. Soil electrical conductivity (EC) was increased with the application of different carbon black rates on soil placed in pot experiments. The highest increase in soil EC was observed in OPSMB and OPSB which is presented in Table 1. Similarly, the highest reduction was noticed in an indoor setup. On the whole, an increment in conductivity of soil is observed among plants grown under different environmental setup. Increment of conductivity of soils may be due to BC dynamics which is reported in several studies (Gundale and DeLuca, 2007; Chintala *et al.*, 2014; Berek *et al.*, 2018). Proliferation in conductivity of soils may also be attributed due to the solubilization of ions present in the carbon black. However, the effect may diverge based on salt contents in biochar material (Spokas, 2010). The contribution of electrical conductivity towards plant growth in the different setup were Control (0.94), OPSB (0.98), OPSMB (0.98), TPSB (0.99) and IPSB (0.96). Our results are in line with a study by Stadler *et al.* (2015) which showed a higher relationship between growth and conductivity and is found to promote plant height between 0.10 to 0.20 mm. Higher electrical conductivity in soil denotes the number of nutrients which is available in the root zone favouring plant growth (Ding *et al.*, 2018).

Soil Organic Carbon (SOC)

An upsurge of Soil Organic Carbon content (SOC) in the soil relative to carbon black application is

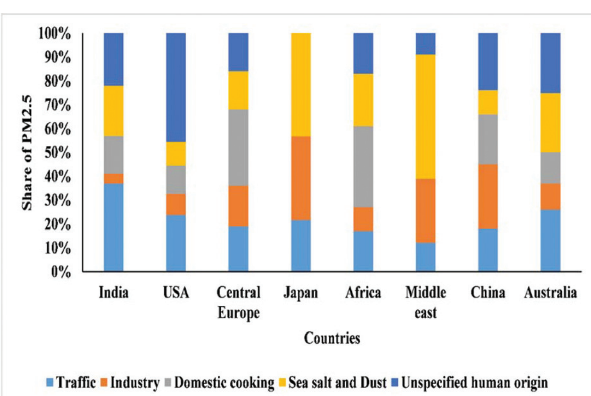

Fig. 1. Indoor Experimental setup of the study.

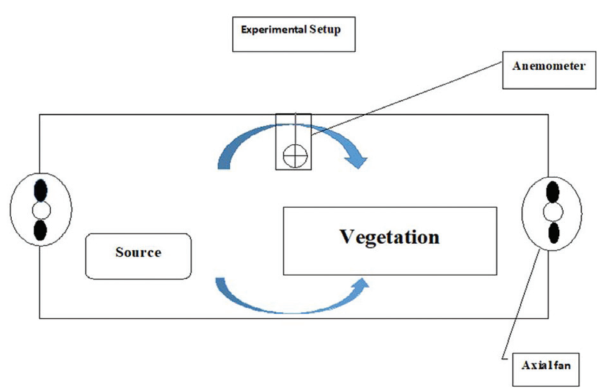

Fig. 1.1. Indoor Experimental setup of the study.

noticed in the study. SOC increase in percent from initial soil organic carbon is found to be in the order of Indoor (128%), roadside (136%), OPSB (226% and OPSMB (237%) respectively. This is substantiated by multiple regression analysis with OPSB (R^2=0.98), roadside (R^2=0.93), Indoor (R^2=0.93), Control (R^2=0.94). The above characteristics are a result of factors such as higher pyrolytic temperature during carbon preparation and also due to the higher stability of amended carbon black which favoured soil with stable SOC of least mineralisation capacity. Several studies about carbon amendment also reported similar results (Nguyen *et al.,* 2008; Liang *et al.,* 2008). Generally, soil organic matter will be high in carbon black soil which may significantly promote the soil carbon content in poor carbon soil (Zhang *et al.,* 2019).

Cation Exchange Capacity (CEC)

The CEC value was found to be slightly increased in different carbon black treatments of soils placed outdoor than the traffic (roadside) and indoor treatments respectively. A study done in clay-loam soil has shown that CEC increases concerning the application rates of black carbon which is ideal with our results as˙CEC value is found to be higher in OPSMB 8.68 > OPSB 8.42 > Control 8.2 (Sultan *et al.,* 2020) as carbon black has more available cation exchange sites on its surface which are because of activation of the surface area. This increased surface area plays a vital role in retaining nutrient contents leading to augmentation of sodium and potassium ions (Table 1) in carbon black amended soils (Hollister *et al.,* 2013). A study by Lehmann *et al.* (2003) also iterated that the increase of pH augments the free bases such as potassium and magnesium in the soil. The soil characteristics such as pH, TOC, and CEC of non-carbon black setup remained almost unchanged as a lesser number of particulate black carbon, or fine aerosol is found to deposit in the soil (Xu *et al.,* 2017). The role of the exchangeable ions in the growth of the plants was in the following order: roadside > outdoor > indoor > control. Roadside soils are exposed to vehicular metal and ionic depositions which will be leached into the soils over the period. Such accumulated ions are available in the root zone to be exchanged with a plant (Alsbou and Al-Kashman, 2018). Hence, it is inferred that the extent of available cations is high in roadside setup than in another experimental setup. Characteristics of the soil during pre and post-experiment stages were displayed in Table 1.

Growth characteristics

Effect of carbonaceous aerosol on a plant, growth parameters were recorded between treatments with significant differences observed among them. Growth characteristics (in cm) of a plant under different environmental condition is presented in Figure 2. In detail, the outdoor growth of beans is found to be 38.2 cm in the non-BC soil setup. While the plant in the OPSB setup has shown considerably better performance in the growth of beans (38.6cm) than the outdoor setup. While the bean plant placed in the pots has not grown in OPSMB treatment of plants. Initially, bean plant requires less moisture and water content to begin the phase of growth which is affected by the addition of a higher amount of carbon black to the soils (Hadid, 2016) as the

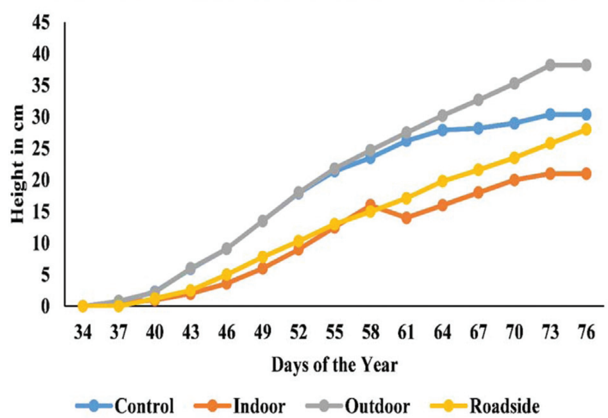

Fig. 2. Growth characteristics (in cm) of a plant under different environmental condition

Table 1. Characteristics of the soil during pre and post experiment stages.

Parameters	Initial	Soil	Outdoor	Outdoor (more BC)	Road side	Indoor
pH	6.31	7±0.08	7.73±0.09	8.01	7.1±0.08	7.42±0.05
Electrical Conductivity (µs)	25	26.6±0.9	36.1±0.8	39.5±0.7	32.7±1.6	25.7±0.2
Total Organic Carbon (%)	0.75	1.8±0.08	2.4±0.04	2.5±0.2	1.77±0.03	1.71±0.01
Na (mg/l)	1.1	1.2±0.2	2.3±0.4	2.81±0.15	1.5±0.2	1.2±0.12
K(mg/l)	0.97	1.02±0.1	3.2±0.2	3.5±0.03	2±0.4	1.2±0.16
CEC (C mol/kg)	8.2	8.28±0.1	8.42±0.12	8.68±0.02	8.4±0.1	8.22±0.09

addition of carbon black results in waterlogging of soils. BC has known to possess a higher water retention capacity than soil. Similarly, a roadside exposed traffic setup of non-carbon black soil has shown unique growth results in beans (23.5cm) during the experimental period. Interestingly in indoor, beans have the lowest maturity at the end of the experimental period with a height of 21cm. The reduction in the growth of plants in the traffic (roadside) and the indoor plant is due to the point source deposition of particulate on the plant stomata which hinders the photosynthetic process leading to reduced growth (Popek *et al.,* 2018). A study demonstrated that carbon-dominated leaves also tend to show a similar effect by decreasing the stomatal and epidermal cell size (Kulshreshtha *et al.* 1980). Similar reduced plant growth is observed in several urban particulate deposition studies (Leonard *et al.,* 2016; Xu *et al.,* 2020). There is a significant difference in growth is observed between the different treatments which is substantiated by the results obtained from ANOVA ($F_{5, 88}$ =2.149, p=0.06). Overall, the addition of carbon black has increased the ability of the bean plant to grow under different environmental conditions. Besides this, the simultaneous or individual factors can also act together to produce the increased growth which may be because of increment in pH, slow and steady release of nutrients, appropriate moisture level, less plant stress, improved microbial activities, and favourable environmental conditions. This is becauseof a study conducted on beans showing significant plant growth by using different concentrations of carbon black (Silva *et al.,* 2017).

Leaf Traits

Leaf traits (Area, width, and length) of the plants grown under different environmental conditions were represented in Figures 3-5. The total leaf surface area of plants in the different environmental setup was found to be 18.4cm^2, 17.3 $cm^{2,}$ and 10.6 cm^2 for Outdoor, Traffic, and Indoor respectively. Leaf lengths are found to larger by 45% in OPSB followed by Traffic Planted Soil with Black carbon (TPSB) with 13% and 10% in Traffic Planted Soil (TPS) than in the control soil. While the smaller length of leaves is observed in Indoor Planted Soil with Black carbon (IPSB) by 3% and Indoor Planted Soil (IPS) by 4% respectively. A similar variation is observed in the leaf width during the experimental period. An experimental study on the effect of deposition of fine particulates in annual plant species also reported a reduction in growth parameters of leaves which is similar to our results (Rai *et al.,* 2010). Overall leaf surface area, length, and width remain almost unchanged within the treatments, i.e. TPS vs TPSB and IPS vs IPSB. However, a change in characteristics is observed between the treatments with the plants grown in

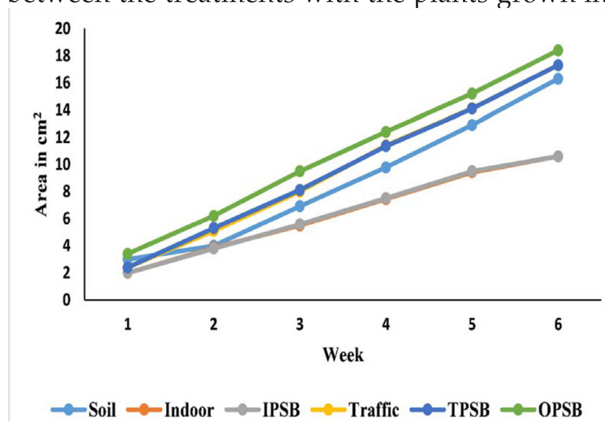

Fig. 3. Leaf area (in cm^2) of a plant under different environmental condition

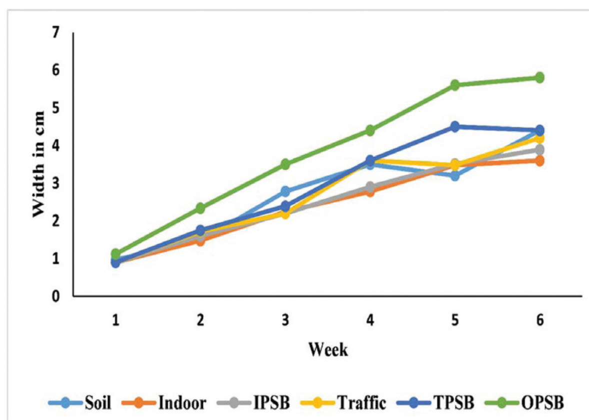

Fig. 4. Leaf width (in cm) of a plant under different environmental condition

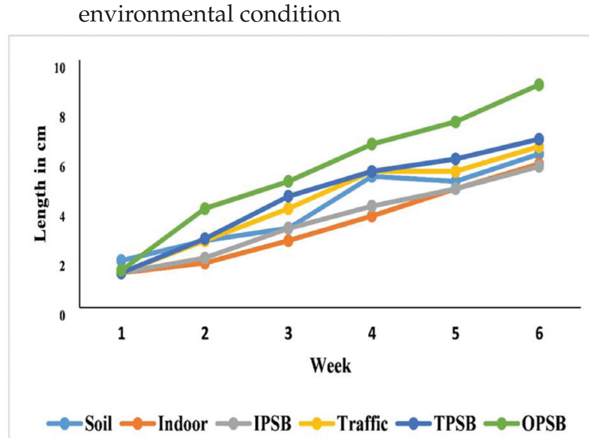

Fig. 5. Leaf length (in cm) of a plant under different environmental condition

OPSB treatment followed by TPSB>TPS>IPSB>IPS respectively. A study on the Lettuce (*Lactuca Sativa L.*) plant showed a similar increase of leaf traits concerning the addition of carbon black to soil (Trupiano *et al.*, 2017). Leaf traits were inter compared among treatments using the ANOVA technique to ensure the significance of the impact of black carbon on the growth of plants under different environments. The results show that there is a significant difference in traits of plants is observed between the different treatments which were presented in Table 2. An approach to identify the ability of the plants to grow under the stressed condition under biochar amendment is attempted. Plants under traffic conditions are subjected to PSB treatment which poses an average increment in the growth rate of 5.2% for beans than the control treatment. In the case of indoor has contributed an average increment of 3.8% for beans than the control treatment. Deposition of BC is found to cause various effects on plants such as stomatal plugging, leaf shading, and leaf temperature increment (Yamaguchi and Izuta, 2017). Besides the induced stress, the addition of carbon black in soil favoured the amelioration of growth in plants under stressed conditions. Our results suggest that the black carbon may be amended as a potential nutrient supplement with special reference to the plant under a stressed condition. However, an implementation should be carried out once extensive studies concerning biochar application rates and its environmental consequence are completed.

Table 2. ANOVA of leaf traits different experimental setup

Setup/traits	df	F value	Significance
Leaf width	5	410.309	0.001
Leaf Area	4	353.07	0.001
Leaf Length	5	2290.2	0.001

Morphological and elemental characteristics

Analysis of morphological characters will help in the determination of the particulate sources of the plants. The particulate sample is collected from plants placed in the outdoor setup which includes natural and traffic (roadside) placed pots. The samples were collected only at the end of the experiment to verify the source of particles and this

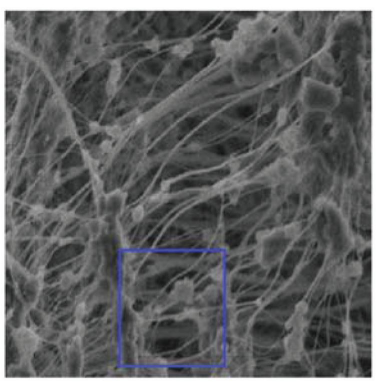

Fig. 6 (a)

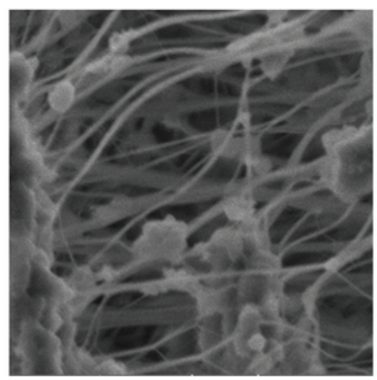

Fig. 6 (b)

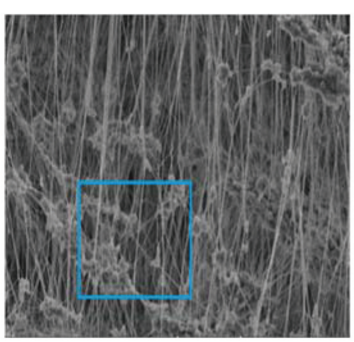

Fig. 6 (b)

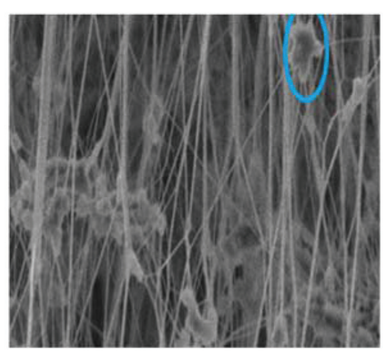

Fig. 6 (c)

Fig. 6(a) Represents the microscopy image of outdoor pots (b) Represents microscopic magnified part of outdoor pots. (c) Represents microscopic image of traffic exposed pots (d) represents microscopic magnified part of traffic exposed pots, individual particle (circled) sample collection is not intended for quantification of particles deposited as samples were random. The particulates screened in the microscope are represented in Fig 6 (a-d). From the figure, the observed particles with irregular shapes and angular edges came from natural sources, such as soil dust or transport sources which lead to particle abrasion. Spherical particles may come from biomass burning which can be identified through smooth surfaces and regular shapes. The particle here with a size of more than 10 µm are aggregates of fine and ultra-fine particles that are the result of vehicular emission and biomass burning. The EDX results indicated that the main elemental composition of particulates placed in an outdoor setup was C> F>O>Si>Al> Mg. The particles mainly composed of Carbon, Silicon, and Magnesium have come from natural sources (Song *et al.*, 2015). A similar result is obtained during a source apportionment study conducted in the study region with a major contribution from dust and vehicular emissions (Bhaskar *et al.*, 2018).

CONCLUSION

The study examined the characteristic effect of fine particles on plant growth and its characteristics under natural, stressed-outdoor, and indoor conditions. The study has been carried out using a commonly available annual variety of vegetables that can be found in both agricultural and garden environments. In natural conditions application of carbon black similar to atmospheric deposition rate is employed. While in stressed outdoor and indoor conditions plants were allowed to exposure to direct particulate sources. Dominance in plant growth is observed in the natural environment with shoot length reaching 38.6cm at the end of the experiment. Reduced growth is observed in traffic followed by indoor conditions. Analysis of leaf traits showed that leaf surface area, length and width were lower in plants growing under stressed conditions than in non-stressed conditions. The morphological and source apportionment of plant deposited species indicates that the source was mainly from biomass burning, vehicular and road dust which is a characteristic of our study region. Further, an additional approach of implementing carbon black for plants under stressed conditions is performed which tends to show better results in plant growth even under stressed conditions. This approach may help us in the reduction of the impact made by varying particulate sources. Our findings show that plants tend to adapt to changing environmental factors that involve both short-term physiological responses and long-term physiological, structural, and morphological modifications.

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