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Characterization and phytotoxicity studies of suspended particulate matter (SPM) in Chennai urban area

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Abstract

Airborne suspended particulate matter (SPM) collected from urban areas of Chennai, Tamil Nadu, India were characterized by high resolution scanning electron microscopy (HRSEM) along with Energy Dispersive X ray (EDX) microanalysis system. The imaging technique showed the presence of complex branched or layered structure with their size ranging from 26 to 34 nm. The EDX analysis revealed the presence of several elements such as, C, O, Na, Mg, Al, Si, Cl, K, Ca, Ti, Fe, Zn and Pb. The aim of this study was to find the effects of various concentrations of diesel and petrol exhaust particles (DEPs and PEPs) on *Bacopa monnieri*, a traditional medicinal plant. The results of the present study showed that there were significant changes ($p<0.05$) in plant morphology (% shoot induction and number of shootlets /explants) and its biochemical properties (% inhibition of DPPH radical , total chlorophyll, chlorophyll a, chlorophyll b and total protein) with different concentrations of DEP in a dose dependent manner as compared to control. Significant decrease in % inhibition of DPPH radical was evident in the PEP treated group when compared to the control. No significant changes were observed in other parameters for PEP treated group. *Bacopa monnieri* was found to be more sensitive to DEPs as compared to PEPs. The results in the present study obtained helped to understand the interaction of vehicle exhaust nanoparticles with the ecosystem.

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Introduction

Air pollution is one of the major prevalent problems faced by Asian countries (Mohanraj *et al.,* 2011). The major component of air pollution is suspended particulate matter (SPM) - $PM_{2.5}$ and PM_{10} (aerodynamic diameter less than 2.5µm and 10µm respectively). It is a combination of inorganic and organic substances present in air as both solids and liquids (Tasic *et al,* 2006). Although many advances have been made with emission control technologies, motor vehicles contribute to the major source of airborne SPM. According to a study report by the Chennai Metropolitan Authority (CMDA), levels of particulate matter in the city range from 274 to 1470mg m^{-3}, which is quite higher than the WHO's prescribed limit of 200mg m^{-3} (WHO, 2002). Diesel exhaust particles (DEPs) and petrol exhaust particles (PEPs) are the major source of nano and micro sized particles in urban areas (Buzea et al, 2007) contributing to SPM.

Studies have revealed that due to small dimensions of DEPs, they make way into lungs and cause hypertension (Brook *et al.,* 2002), genotoxicity (Dybdahl *et al,* 2004), lung inflammation (Doornaert et al, 2003), infertility (Kim, 2004) and oxidative stress. Previous literatures have stressed on testing nanoparticles *in vitro* (Durga *et al.,* 2012; Arul Prakash *et al.,* 2011). Most of the research done so far has emphasized the effects of DEPs on animal models and cell lines, hence, demonstrating its toxicity to animals and humans. As an effect of public activity, environmental nanoparticles are introduced into air, soil and land. With reference to ecotoxicity, there has been considerable focus on marine species rather than terrestrial groups, and very few studies have been performed on terrestrial plants (Jiang *et al.,* 2009). Higher plants strongly interact with their terrestrial and atmospheric environments and are affected as a result of exposure to engineered and environmental nanoparticles (Monica and Cremonini, 2009).

Bacopa monnieri is a traditional medicinal plant widely distributed in Chennai, Tamil Nadu. The plant has extensive medical uses in ayurvedic treatment of asthma and epilepsy (Rajani *et al.,* 2004). It is rich in potential alkaloids such as brahmine and herpestine. It is also used in Ayurveda for the treatment of indigestion, anemia, ulcers, ascities, enlarged spleen, inflammations, leprosy and tumours. The DEPs and PEPs have the capacity to agglomerate (Park *et al.,* 2004) and hence settle down on soil. Evidences also suggest that inorganic fractions of these particles are water soluble and hence can contaminate nearby water bodies and adversely affect plant growth. Thus in this study, the effect of different concentrations [250 (low concentration), 500,750 (Medium concentration) and 1000 (high concentration) mg l^{-1} denoted as C1, C2, C3 and C4] of vehicle exhaust DEP and PEP on plant morphology was studied.

Materials and Methods

Analysis of collected SPM : Chennai in Tamil Nadu was selected as study area. The study sites chosen were T. Nagar and Adyar.The SPM (PM_{10} and $PM_{2.5}$) were collected on glass fibre filters by a high volume sampler (Envirotech APM 460N4, New Delhi, India), kept at a height of 2m from ground level. The collected samples were sealed and refrigerated at 4°C till further analysis. The collected SPM was analyzed with HRSEM/EDX (FEI Quanta FEG 200) according to EPA guidelines (US-EPA, 2002). Around 300 particles per SPM sample were analyzed for their size, morphology and chemical composition.

Collection and characterization of vehicle exhaust particles: Light duty multi-cylinder diesel and petrol engines (ALLMECH Pvt Ltd) operating on standard diesel and petrol fuel at a speed of 1500rpm was used to collect DEP and PEP, as previously described by Sagai *et al.* (1993). The average diameter of collected particles was less than 2.5μm in size. The morphological analysis was done using High Resolution Transmission Electron Microscope (HR-TEM) (JEOL 3010). The elemental analysis for two samples, DEPs and PEPs, was performed using HRSEM/EDX (FEI Quanta FEG 200).

In vitro studies

Shoot proliferation : Nodal and inter-nodal explants were collected from healthy plants of *Bacopa monnieri* (Purchased from National Institute of Siddha, Chennai). The explants were sterilized according to Pati *et al.* (2006). Murashige and Skoog medium (MS medium, Sigma Aldrich) supplemented with 0.8% w/v agar and 30g l^{-1} sucrose was used, with a combination of 6-benzylaminopurine (BAP; 2.0 mg l^{-1}), a- naphthalene acetic acid (NAA; 0.05 mg l^{-1}) (Himedia, India) for explant propagation. To this different concentrations of DEPs (Treatment I) and PEPs (Treatment II) were added. DEPs or PEPs were not added to control tubes. The medium pH was maintained at 5.8. 30ml of the medium was dispensed into conical flasks (Erlenmeyer Borosil, India), covered with aluminium foil and autoclaved at 121°C for 20 min. All the flasks containing medium were stored in culture room. Culture flasks were maintained at 25°±2°C in a culture room provided with 16/8 hr light/dark period [light intensity maintained by fluorescent tubes (Philips, India)]. Each experiment was conducted in 3 replicates and repeated 3 times.

At the end of the experiment, percentage of shoot induction and the number of shootlets per explant was studied. From shoot, the plant extract was prepared. Estimation of chlorophyll, protein and % DPPH inhibition was performed.

Preparation of plant extract : The extracts were prepared following the procedures of Pizzale *et al.* (2002). Extraction of about 10 g of proliferated shoot material of *B.monnieri* (control, different concentrations of treatment I and treatment II) with 150 ml of methanol (Merck, extra pure) was done with an Ultra Turax mixer at 13,000 rpm for a minute. The extract was filtered using Whatmann filter paper. The filtered extract was then kept under vacuum at 40°C.

Anti-oxidant assay (DPPH) : Methanolic extract of plant was used to determine the anti-oxidant activity. DPPH is a purple colour, free radical with an absorbance at 517 nm. Upon scavenging of free radicals, DPPH changes to yellow colour. This assay depicts free radical scavenging activity of the plant. The anti-oxidant activity of methanolic extract was measured according to the procedure described by Cotelle *et al.* (1996). 100 μl of each extract (control, different concentrations of treatment I and treatment II) of *B.monnieri* was taken in microtiter plate. To this, 100 μl 0.1% methanolic DPPH was added in dark for 30 min. The samples were observed for colour change. Percent inhibition of DPPH free radical by the extracts was calculated.

Protein estimation : Protein concentration in the methanolic extracts of *B.monnieri* (control, different concentrations of treatment I and treatment II) was estimated (Lowry *et al.,* 1951). About 0.5 ml of alkaline copper solution was added to 1 ml of plant extract and was kept at room temperature for 10 min. To this 0.5 ml of 1N folin-phenol reagent was added and allowed to stand at room temperature for 30 min. Absorbance was recorded at 660 nm using a UV visible spectrophotometer (Chemito, India).

Estimation of chlorophyll pigment : 1gram of shoot sample was taken and dissolved in 50 ml of 96% methanol, followed by homogenization at 1000 rpm for 1 min. This was followed by filtration and centrifugation for 10 min at 2500 rpm. The absorbance of separated supernatant was read at 400–700 nm by a UV–Vis spectrophotometer. It was observed that chlorophyll a and b gave maximum absorbence at 662 nm and 646 nm respectively, and quantification of total chlorophyll pigment was

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performed according to the procedure described by Lichtentaler and Wellburn (1985).

Statistical analysis: All experiments were performed in triplicates. The results were expressed as mean± SD. Data were analyzed by standard statistical analysis one-way ANOVA (Sokal and Rohlf, 1969) with Duncan's test for multiple comparisons to determine significance between different groups. The results were considered statistically significant if 'p' value was .05 or less.

Results and Discussion

The quality of air in many Indian cities is worsening due to rise in the total number of vehicles (major contributors of SPM) (Bhatia, 2001). SPM- PM_{10} and $PM_{2.5}$ collected at two different sampling points in Chennai urban region were analyzed with HRSEM-EDAX. SEM images showed the presence of complex structures of irregular shape along with fibrous structures. The fibrous structure formation might be due to high temperature and incomplete combustion of fuels. The images also showed the presence of nanometer size particles of respirable range (Fig.1). Nearly 300 particles were analyzed. The relative size distribution of the collected particles from both the sampling sites showed a huge peak around 0.0-0.5µm range (Fig.2). Size is the main contributing factor for the toxicity of nanoparticles. Small size particles have greater surface area to volume ratio and hence have increased biological and chemical activity (Liu *et al.,* 2009). These particles have capacity to enter small airways and get deposited in alveoli and cause pulmonary and extra-pulmonary effects. They cause diseases that mainly involve three tissues; respiratory passage, respiratory membrane and blood vessels (Mohanraj and Azeez, 2004).

The chemical composition of collected SPM showed the presence of elements such as C, O, Na, Mg, Al, Si, Cl, K, Ca, Ti, Fe, Zn and Pb. Carbon was found to be major element. The presence of carbon was mainly due to SPM from vehicle traffic (Diesel and Petrol vehicles) and heating systems. Elemental carbon and organic carbon are the dominant carbon compounds in SPM. They effect health and are carcinogenic and mutagenic. OC fraction contains hydrocarbons and poly aromatic hydrocarbons (PAHs) that cause infertility, hormonal imbalance

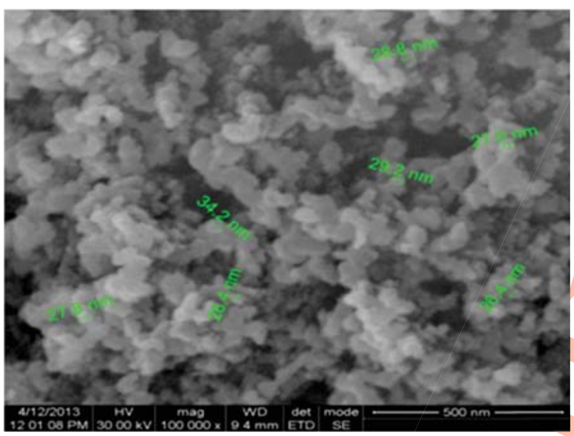

Fig.1 : HRSEM images of nano-size respirable SPM

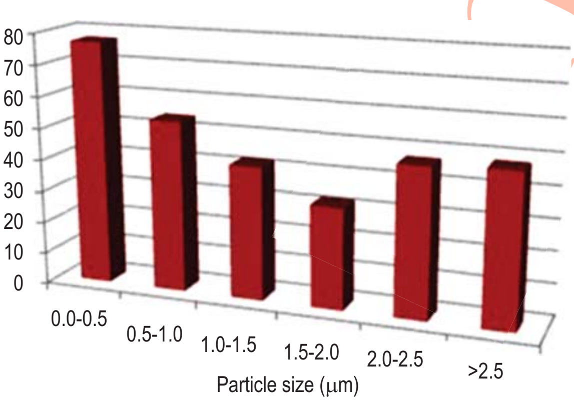

Fig. 2 : Relative size distribution of SPM sample

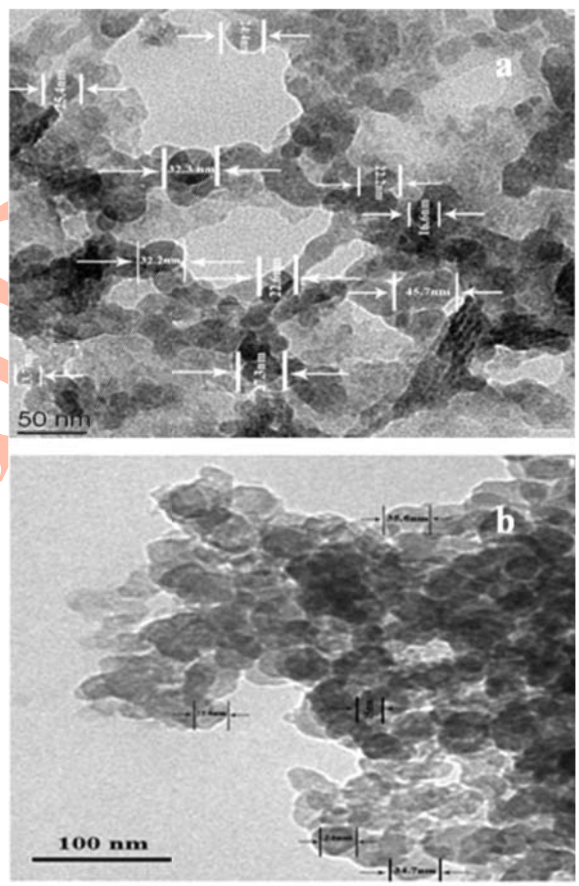

Fig. 3 : (a) HRTEM micrograph Of PEP and (b) DEP

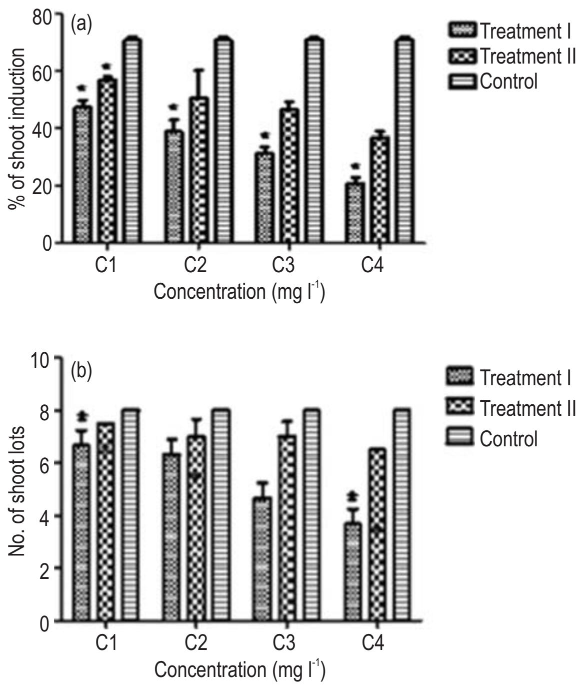

Fig. 4 : Effect of DEPs and PEPs on (a) percentage shoot induction and (b) No. of shootlets per explant of *Bacopa monnieri*. Treatment I and Treatment II refers to different concentrations (C) of DEPs and PEPs. C1, C2, C3 and C4 denotes 250 (low concentration), 500, 750 (medium concentration) and 1000 (high concentration) mg l^{-1} of DEPs/PEPs; (*p<0.05).

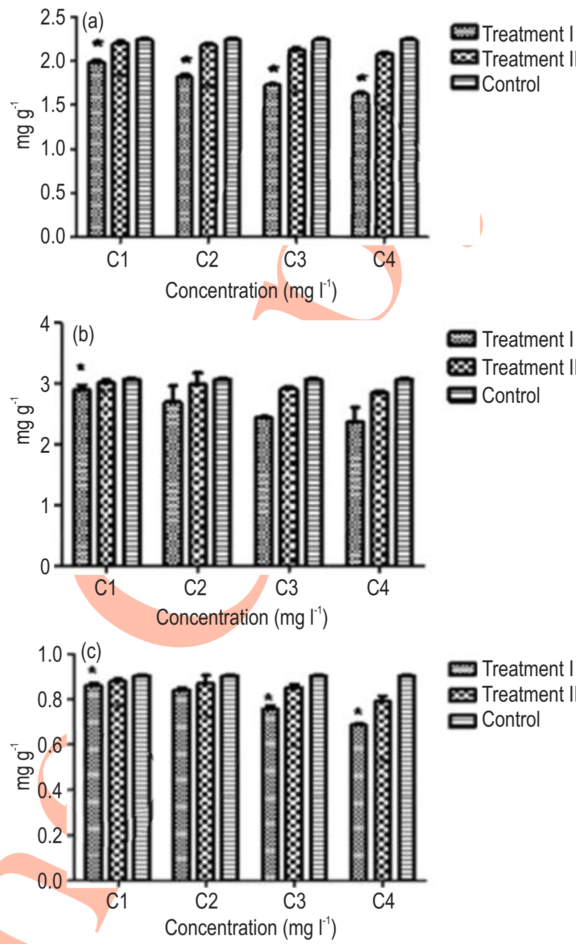

Fig. 5: Effect of DEPs and PEPs on (a) total chlorophyll, (b) chlorophyll a, (c) chlorophyll b content of *Bacopa monnieri.* Treatment I and Treatment II refers to different concentrations (C) of DEPs and PEPs. C1, C2, C3 and C4 denotes 250 (low concentration), 500, 750 (medium concentration) and 1000 (high concentration) mg l^{-1} of DEPs/PEPs; (*p<0.05)

and impairment of immune function (Mumtaz and George, 1995). Silica oxides and alumino-silicates (Si with K, Al, Ca and Fe) comprise of silica particles. They mainly originate from coal combustion processes and are manly less than 1µm in size. The oxides of Ti, Al, Pb and Zn are dominant in the sub-micometer region. Flyash is the major source of these particles (Tasic et al., 2006).

Vehicle exhaust particles (DEPs and PEPs) are the key contributors to air pollution in India and are the main contributors of SPM. Hence collected DEPs and PEPs were used in this study. The composition of diesel and petrol exhaust particles (DEPs and PEPs) varies by engine condition, type of engine, its operation, engine performance and the fuel used (McDonald *et al.*, 2011). Thus, as stated by Helland *et al.* (2007), toxicity of each kind of particle has to be investigated separately. Results indicated that PEPs contained slightly larger size particles. Elemental analysis for DEPs and PEPs were performed using HRSEM/EDX technique. Carbon was found to be the major element. In comparison to DEPs; PEPs contained more carbon. Apart from carbon, PEPs depicted elements like calcium, sulphur, iron, aluminium, lead and silica in trace amounts as compared to DEPs, which contained only oxygen, carbon and sulphur.

The overall results of the present study suggest that treatment of *Bacopa monnieri* explants with different concentrations of DEPs (treatment I) and PEPs (treatment II) induced significant phytotoxicity in the treated groups. This was evident from decrease in % shoot induction (Fig.4a); decrease in number of shootlets/ explants (Fig.4b); decrease in % inhibition of DPPH radical and decrease in the total chlorophyll and protein content of DEP treated groups as compared to control groups. PEP treated group was found interfere significantly with % inhibition of DPPH radical (p< 0.05) only as compared to control. No significant changes were found in other parameters of the PEP treated groups.

Literature report suggests that air pollution from motor vehicles significantly affect nearby ecosystems (Bignal *et al.,*

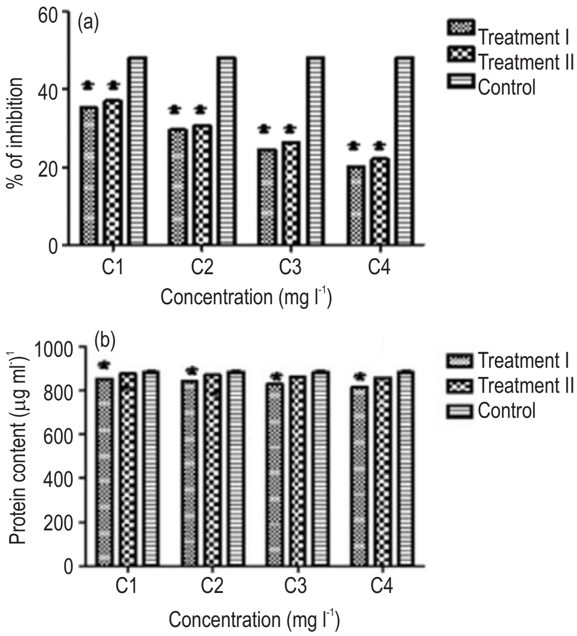

Fig. 6 : Effect of DEPs and PEPs on (a) percentage inhibition of DPPH and (b) total protein content of *Bacopa monnieri*. Treatment I and Treatment II refers to different concentrations (C) of DEPs and PEPs. C1, C2, C3 and C4 denotes 250 (low concentration), 500, 750 (medium concentration) and 1000 (high concentration) mg l^{-1} of DEPs/PEPs; (*p<0.05)

2008). Biochemical parameters such as total protein, chlorophyll a, b, total chlorophyll, sugars, lipids and starch gradually reduced in polluted plants when compared with control (Uma and Rao, 1996). The present results could possibly be due to interaction of vehicle exhaust nanoparticles in the MS medium with plant growth regulators (PGRs) and hormones necessary for shoot proliferation. Extracts of *Bacopa monnieri* act as natural anti-oxidants. They are significant in preventing various disorders caused by the production of free radicals. Studies revealed that *Bacopa monniera* showed anti-oxidant effects by various mechanisms like disruption of oxidative chain reactions, chelating metal ions, scavenging free radicals (Russo *et al.,* 2003) and by improving the activities of anti-oxidant enzymes (Bhattacharya *et al.,* 1999). Swathi *et al.* (2013) showed that extracts of *Bacopa monniera* exhibited hepatoprotective and antioxidant effects against carbon tetrachloride induced liver damage in animals. Sharma *et al.* (2012) investigated the modulatory role of *B. monniera* against radiation induced oxidative stress and hence proved its anti-oxidant activity *in vitro*. Studies by Jyoti and Sharma, 2006 showed the ameliorating activity *B. monniera* against aluminium induced oxidative stress in rat brain thus, revealing its anti-oxidant potential *in vivo.* The results of this study suggest that the plant was under oxidative stress; there was a significant percent inhibition of DPPH radical in treatment I and treatment II (Fig.6a) as compared to control ($p< 0.05$) indicating that the vehicle exhaust nanoparticles have interfered with free radical scavenging activity/ antioxidant potential of the medicinal plant.

The metabolic pathways in plants like glycolysis, Krebs cycle and photosynthesis are regulated by a series of enzymes (proteins). The process of photosynthesis constantly starts when light energy is captured by photosynthetic reaction proteins containing chlorophylls. Proteins like RuBP carboxylase present in leaf play a vital role as both storage proteins and as efficient proteins, for catalysing chemical reactions (Onyango, 1996). Studies revealed that enhanced protein denaturation (Tripathi and Gautam, 2007), breakdown of existing proteins to amino acids and decrease in denovo synthesis are the main causes of reduction in the total protein content (Singh and Jothi, 1999). Raajasubramanian *et al.* (2011) showed that cement dust pollution decreased the total protein content of groundnut plant by nearly 50% when compared with control. In the present study, combined stress could have negatively depleted cellular actions leading to noticeable decrease in the total protein content of treatment I group ($p<0.05$) as compared to control group. No significant changes were found in Treatment II group (Fig.6b).

The photosynthetic capacity of a plant depends on photosynthetic pigment composition (chlorophyll) (Netondo *et al.,* 2004). A similar decrease was seen in ground nut, gram and maize crop (Pandey *et al.,* 1999; Raajasubramanian *et al.,* 2011). Literatures showed that high concentration of air pollutants such as sulphur dioxide (SO_2) degrade chlorophyll to inactive Mg^{++} and phaeophytin (Constantinidoa and Kozlowski, 1979). A considerable reduction in the total chlorophyll content of leaves suggest that chloroplast are the major site of interruption by pollutants (Randall *et al*., 1977). These air pollutants enter plant tissues through stomata and hence, cause reduction in chlorophyll pigment and partial chloroplast denaturation in the polluted leaves (Raajasubramanian *et al.,* 2011). In the present study, a significant decrease ($p < 0.05$) in dose dependent manner in the total chlorophyll, chlorophyll a and b content of treatment I was evident when compared with control (Fig.5a, b, c). Significant changes were not observed in treatment II. The results of treatment I can be contributed to the reason that the chloroplasts are the primary site of attack by this air pollutant (DEP).

Hence, relative toxicity was found to be DEPs > PEPs. It is concluded that *Bacopa monnieri* is more sensitive to DEPs as compared to PEPs.

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