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Atmospheric particulate matter (PM) effect on the growth of *Solanum lycopersicum* cv. Roma plants

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HIGHLIGHTS

- Particulate matter (PM10) is detrimental for *S. lycopersicum* cv. Roma plant growth.
- Root exposure to PM10 is accompanied by Reactive Oxygen Species production.
- Chlorophyll content is diminished in PM10 exposed plant compared to control.
- Carotenoid content is increased in PM10 exposed plant compared to control.

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ABSTRACT

This study shows the direct effect of atmospheric particulate matter on plant growth. Tomato (*Solanum lycopersicum* L.) plants were grown for 18 d directly on PM10 collected on quartz fiber filters. Organic and elemental carbon and polycyclic aromatic hydrocarbons (PAHs) contents were analyzed on all the tested filters. The toxicity indicators (i.e., seed germination, root elongation, shoot and/or fresh root weight, chlorophyll and carotenoids content) were quantified to study the negative and/or positive effects in the plants via root uptake.

Substantial differences were found in the growth of the root apparatus with respect to that of the control plants. A 17–58% decrease of primary root elongation, a large amount of secondary roots and a decrease in shoot (32%) and root (53–70%) weights were found. Quantitative analysis of the reactive oxygen species (ROS) indicated that an oxidative burst in response to abiotic stress occurred in roots directly grown on PM10, and this detrimental effect was also confirmed by the findings on the chlorophyll content and chlorophyll-to-carotenoid ratio.

1. Introduction

Atmospheric particulate matter (PM) has received wide attention due to its adverse impacts on human health and the environment (Polichetti et al., 2009; Perrone et al., 2010).

PM is a complex mixture of suspended solid and liquid particles with different physical and chemical properties, originating from natural and anthropogenic sources. According to the aerodynamic diameter, particles can be classified as PM10 (<10 μm diameter) and PM2.5 (<2.5 μm). The chemical composition of PM10 varies greatly and strongly depends on combustion sources and atmospheric conditions (Lighty et al., 2000; Solomon and Sioutas, 2008; Amodio et al., 2012). PM10 consists of major components representing the main part of the total mass of particles, and trace components usually represent less than 1% of total particle mass (Amodio et al., 2010). Organic matter, sulphate, nitrate, ammonium and elemental carbon (EC) are the main PM contributors. Organic carbon (OC) and elemental carbon (EC) originate from combustion processes; primary OC arises from combustion, geological and natural sources, while secondary OC is formed when the atmospheric oxidation products of Volatile Organic Compounds (VOCs) undergo gas–particle transfer. EC is essentially a primary pollutant emitted during the incomplete combustion of fossil and biomass carbonaceous fuels (Seinfeld and Pandis, 2006; Sánchez de la Campa et al., 2009; Snyder et al., 2010). Among the PM trace components, Polycyclic Aromatic Hydrocarbons (PAHs) constitute

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a major class of environmental pollutants (Amodio et al., 2009, 2010). These compounds are generated by the combustion of organic matter in mobile sources, such as motor vehicles, as well as in stationary sources, such as power plants and residential heating. Many PAHs, particularly the larger five- and six-ring compounds that can be metabolized to diol epoxides, are mutagens and carcinogens (de Kok et al., 2006; Binkova et al., 2007).

A number of epidemiological and toxicological studies link PM10 mass to adverse effects on human health, including respiratory and cardiac diseases (Polichetti et al., 2009). In addition, increased emissions of air pollutants induce damages to crops, ornamental plants, and trees (Larcher, 2003). PM can affect green plants either via deposition on the biomass above ground, i.e., leaf surface penetration, or indirectly via soil–root interaction (Žalud et al., 2012).

At the cellular level, many data indicate that PM has several mechanisms of adverse cellular effect, such as cytotoxicity through oxidative stress mechanisms, reactive oxygen species (ROS) production, oxidative damage to DNA and harm to photosynthetic machinery (Risom et al., 2005). To avoid potential damage, plant cells contain several enzymatic and non-enzymatic antioxidant scavenging systems that address ROS detoxification. Under unstressed conditions, the formation and scavenging of ROS are in balance. However, several forms of biotic and abiotic stress increase the generation of ROS resulting in cellular damage, manifested in the inactivation of enzymes or cell death, if the amount of ROS generated exceeds the capacity of the scavenging systems (Melillo et al., 2006). The capacity of the plant cell antioxidative and photoprotective defense against harmful ROS is determined by the pool size of the antioxidants and protective pigments (e.g., carotenoids) (Sharma et al., 2012). A change in antioxidant content may reflect the impact of environmental stresses on plant metabolism (Polle and Rennenberg, 1994). ROS overload can cause the degradation of photosynthetic pigments and damage to photosynthetic machinery, which in turn decrease photosynthesis (Hasan et al., 2011; Masood et al., 2012; Ahammed et al., 2013).

However, the effects of pollutants on plant biochemistry and physiology may depend on each individual pollutant or their combination. The effects also vary according to the exposure regime, in terms of both temporal variation in pollutant concentration and cumulative dose (Weber et al., 1993)

Few data are available on PM components migration via soil–vegetable transport and their effect on plant growth (Žalud et al., 2012; Tremlová et al., 2013).

In our research, a model experiment was conducted to demonstrate and evaluate the environmental impact of the chemical components in PM10 on plants via root uptake. Among vegetables, tomato (*Solanum lycopersicum* L.) was chosen because it is one of the world's most important crops (FAO, 1995), has been reported to accumulate high amounts of PAHs in the fruit (Rojo Camargo and Toledo, 2003) and is the main crop in the neighborhood of the PM10 collection site (Canosa di Puglia, Apulia, Southern Italy). Plants were directly grown on PM10 collected on quartz fiber filters for 18 d with the purpose of simulating the putative environmental conditions in which pollutants are recovered in the soil, e.g., those conditions following deposition or rain precipitation, and come in contact with roots. The goal was to evaluate the effect of PM10 collected in an urban background site on tomato seedling growth, biomass, ROS accumulation in roots, and its stress on the photosynthetic pigment content.

2. Materials and methods

2.1. Sampling method

A monitoring campaign of PM10 was conducted at an urban background site of Canosa (Apulia, Southern Italy) on four different days (indicated by 1, 2, 3 and 4). PM10 samples were collected for 24 h on quartz fiber filters (QM-A Whatman 20 × 25 cm) using a high-volume sampler operating at a flow rate of approximately 1.7 m^3 min^{-1} (Graseby-Andersen, Smyrna, GA, USA). The filters were conditioned and weighed before and after sampling using a system supplied with temperature and humidity control (20 °C and 50% RH) (Activa Climatic, Aquaria, Milano, Italy). The sensitivity of the analytical balance (Gibertini mod. E154, Milano, Italy) was 0.1 mg. The filters were then cut off into two parts, one of which was used for the growth of tomato and one for the chemical characterization of the PM10. The concentrations of PM10 and components detected during the different days are listed in Table 1.

2.2. Chemical characterization

A portion of each sample filter (a circle of 47 mm in diameter) was used for PAH determination.

The filter was extracted by means of a microwave-assisted solvent extraction (Milestone s.r.l. model Ethos D, Sorisole (BG), Italy). The extracted samples were then analyzed using a gas chromatograph (Agilent 6890 PLUS, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a programmable temperature vaporization injection system (PTV) and interfaced to a quadrupole mass spectrometer, operating in electron impact ionization (Agilent MS-5973 N). The quantitative determination of several PAHs (see the list in supplementary material) was performed using Perylene-D12 as the internal standard (IS). The analytical performances of the whole procedure (extraction recovery, extraction linearity, analytical repeatability, LOD) were verified in a previous work (Bruno et al., 2007). Rectangular punches (normally 1.50 cm^2) of each filter were analyzed for organic and elemental carbon (OC and EC) by a thermal optical method (Sunset Laboratory Inc., Tigard, OR, USA). To remove possible carbon contamination, quartz fiber filters were pre-cleaned in a muffle furnace, according to the NIOSH method 5040. In this method, speciation of organic, carbonate and elemental carbon is accomplished through temperature and atmosphere control. Passing a He/Ne laser light through the filter allows for continuous monitoring of the filter transmittance, and an optical feature corrects for pyrolytically generated OC. The evolved carbon was quantified by a Flame Ionization Detector (FID). Instrument calibration was achieved through injection of a known volume of methane into the sample oven (Birch and Cary, 1996).

2.3. Plant material

Seeds of tomato (*S. lycopersicum* L. cv. Roma) were surface sterilized for 3 min in 95% ethanol, placed in 1% sodium hypochlorite for 10 min (3 under shaking and 7 still) and washed thoroughly in sterilized distilled water. For the germination test, ten seeds were sown, in duplicate, in Petri dishes on 47 mm circular quartz filters obtained from the sampling campaign or on blank filters (control). Filters were moistened with sterile distilled water and seeds were allowed to germinate for 6 d in a growth chamber at 26 °C in the dark. For long-term cultivation, surface sterilized seeds were sown on control filters in Petri dishes and allowed to germinate as reported above. Successfully germinated seeds, each with a primary root 1 cm long, were transferred to 8 × 10 cm sampled filters in growth pouches filled with 6–7 ml of Hoagland's solution. Each pouch held 5 seedlings. Root systems were shielded against light during growth by wrapping each pouch in an aluminum cover. Due to the transparency of the plastic pouch, root development could be followed daily. The pouches were placed vertically inside a transparent plastic box inside the growth chamber at 26 °C, with an 18/6 h light/dark photoperiod and a photosynthetic

Table 1
Daily concentrations of PM10, OC, EC and PAHs collected on sampled filters. ∑ BaPeq was calculated for each filter by the sum of the BaP equivalent concentration obtained multiplying the concentration of each PAH for the corresponding TEF value (see Table S1 for more details).

	PM10 (μg^{-1} m^3)	OC (μg^{-1} m^3)	EC (μg^{-1} m^3)	∑PAHs (ng^{-1} m^3)	∑BaPeq (ng^{-1} m^3)
Filter 1	22.40	5.98	1.21	2.54	0.56
Filter 2	19.70	6.17	0.93	5.14	1.28
Filter 3	24.30	6.46	0.69	2.15	0.44
Filter 4	38.90	6.88	1.93	4.70	1.07
Mean	26.33	6.37	1.19	3.63	0.84

photon flux density (PPFD) of approximately 450 μmol m^{-2} s^{-1}. To compensate for liquid evaporation, Hoagland's solution (3 ml) was added to pouches every 2 d.

2.4. Measurements of plant growth

The germination percentage was determined (3 and 6 d after sowing) by comparing the number of seeds that developed a primary root to the total number of seeds planted in each dish. Primary root elongation was determined by measuring the straightened primary root from the bottom of the stem to the end of the primary root at 7 d from the transfer to the growth pouch. After 18 d, the plants were harvested by gently removing them from the filter surface. Roots were properly washed and dried on filter paper in order to eliminate excess water. The biomass of each plant part, the shoot (stem and leaves emerging from the pouches) and root, was determined by direct measurement of its fresh weight. For the biochemical studies, samples of roots were immediately used for ROS determination, whereas the shoots were frozen in liquid nitrogen and stored at −80 °C.

2.5. Determination of ROS release

Roots from plants grown for 18 d in the growth pouches were excised and pre-incubated for 30 min in a K-phosphate buffer 20 mM, pH 6. Each root sample was incubated in a working solution (Melillo et al., 2006), containing 50 μM 2′,7′-dichlorofluorescein-diacetate (DCFH-DA) (Sigma, St Louis, MO, USA) in a K-phosphate buffer 20 mM pH 6 with 0.2 g ml^{-1} of porcine liver esterase (Sigma) for 30 min at 25 °C on a shaker. Fluorescence (excitation 488 nm, emission 525 nm) caused by the oxidation of DCFH to DCF was measured by a fluorometer (GloMax®-Multi Jr, Promega, Madison, WI, USA).

2.6. Pigment extraction and determination

A pool of tomato leaves (0.1 g) from the plants grown on each filter was pestled in a mortar with the appropriate amount of liquid nitrogen. Grounded leaves were added to 1 ml of 80% acetone in distilled water (v/v) containing a small amount of sodium ascorbate to avoid any pigment oxidation. The pigment extract was hence separated by 5 min at 3000 × *g* centrifugation. Chlorophylls *a* (Chl*a*) and *b* (Chl*b*) and total carotenoid content were spectrophotometrically determined (Cary 5000-Agilent) by the Porra method (Porra et al., 1989; Wellburn, 1994).

2.7. Statistical data analysis

Student's *t*-test (STATISTICA 8 StatSoft, Inc.) is one of the most commonly used techniques for testing a hypothesis on the basis of a difference between sample means. In this study, morphometric and ROS data were subjected to one-tailed *t*-test (with 95% and 99% confidence) in order to verify that data collected on sampled filters were significantly lower or higher than data collected under the control conditions.

3. Results and discussion

3.1. Chemical characterization

Organic carbon, PM10, EC, and PAHs concentrations of each filter are summarized in Table 1 and found to be in line with those collected in other Apulia background sites (Amodio et al., 2011). PM10 levels were found below the Directive 2008/50/EC limit value for the daily average PM10 concentration (50 μg m^3). The EU Council Directive 2004/107/CE sets a threshold value (1 ng^{-1} m^3) for the concentration of benzo(a)pyrene (BaP) in ambient air to avoid, prevent or reduce harmful effects of PAHs on human health and the environment as a whole. BaP, used as a marker for the PAHs carcinogenic risk in ambient air, was measured below the EC target value (1 ng^{-1} m^3) on the sampled filters; the average concentration was 0.49 ng^{-1} m^3. The assessment of PAHs carcinogenic potency (i.e., total BaP equivalent concentration (BaPeq)) is obtained by the sum of the BaPeq concentration for each PAH. The BaPeq concentration is determined by its toxic equivalent factor (TEF), representing its relative carcinogenic potency, using BaP as a reference compound to adjust its original concentration (Nisbet and LaGoy, 1992). ∑BaPeq was calculated for each filter (listed in Table 1); its average value was 0.85 ng^{-1} m^3, suggesting a moderate impact of PAHs exposure on human health.

3.2. Effect of PM on plant development

Germination was not impacted by atmospheric PM in any of the utilized filters, although a delay was observed. On the third day after sowing, only tomato seeds placed on the control filters germinated (40%). However, most of the germination occurred within 6 d for seeds placed on control or PM10 absorbed filters. The final germination was 90% on the control filters and 85% on the sample filters.

Germinated seeds were transferred to modified growth pouches comprising the PM10 absorbed filter enclosed in a thin transparent plastic envelope that was open at the top. The upper part of the filter was folded to support the germinating seeds and allow roots to grow through holes in the fold and along the filter surface. Root growth can hence be easily monitored (Fig. 1).

Biomass accumulation reflecting the life-sustaining activities of the plant is an optimum index for evaluating various stresses on a plant. The pouch experiment revealed the PM10 toxic effect on the tomato plants. Previous experiments showed that the porous structure of tomato roots allows the uptake of chemicals which reach the shoot portion of the plant (Maliszewska-Kordybach and Smreczak, 2000; Ahammed et al., 2012). The tomato response to PM10 contamination was assessed on the basis of decreased growth of both the root and shoot portions of the plants. Primary root elongation was shortened by the treatment during the whole experimental period. A significant negative influence on root elongation was observed for all filters. The primary root elongation in the treatments was found as 64.7% in filter 1, 84.0% in filter 2, 54.6% in filter 3, and 42.0% in filter 4, compared with the elongation on control filter. Furthermore, early seedling growth parameters,

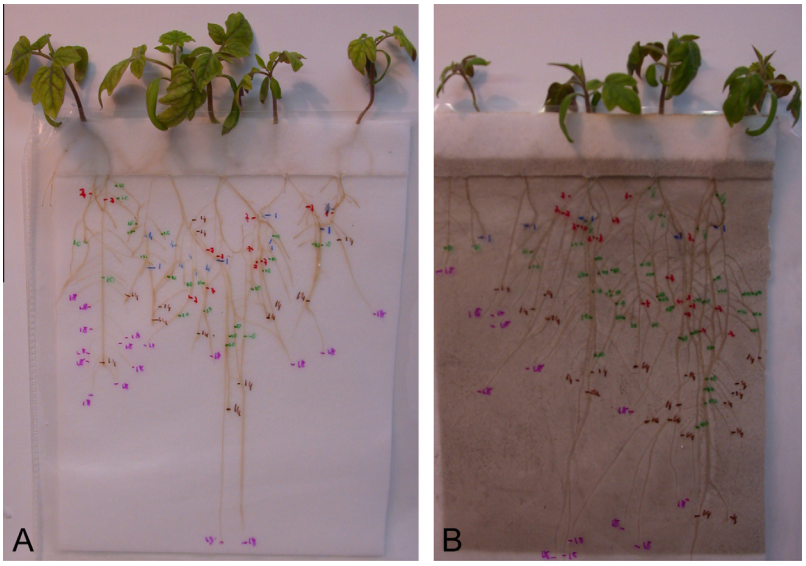

Fig. 1. Growth pouches holding 5 tomato (*S. lycopersicum* L. cv. Roma) seedlings, photographed 18 d after seedling transfer from germination plates. A, Control filter and B, filter absorbed with PM10. The position of primary root apices was marked at 1 and 7 d (marks 1 and 7 and corresponding colours) and the presence of lateral roots was marked at 10, 14 and 18 d (marks 10, 14 and 18 respectively and corresponding colours) after seedling transfer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
The effect of PM10 on plant growth parameters.

	Root elongation (cm)	Root weight (g)	Shoot weight (g)
Control	4.8 ± 0.5	0.23 ± 0.02	0.31 ± 0.05
Filter 1	3.1 ± 0.4*	0.11 ± 0.02**	0.22 ± 0.04*
Filter 2	4.0 ± 0.7	0.08 ± 0.04**	0.21 ± 0.10
Filter 3	2.6 ± 0.5**	0.09 ± 0.04**	0.21 ± 0.08
Filter 4	2.0 ± 0.6**	0.07 ± 0.03**	0.20 ± 0.06*

Data represent mean values ± standard error ($n = 5$). Asterisks indicate statistically significative differences with the control (Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$).

i.e., fresh shoot and root weights, were remarkably decreased in all tested filters. Compared with the control plants, seedlings grown on PM10 exhibited a root weight decline of 52.2% on filter 1, 65.2% on filter 2, 61% on filter 3 and 70% on filter 4. Similarly, the shoot growth was reduced by the treatment showing a lower weight relative to the control of 29.1% for filter 1, 32.3% for filters 2 and 3, and 35.5% for filter 4. Root elongation, root weight, and shoot weight values and *t*-test output are summarized in Table 2.

Moreover, plants grown on filters absorbed with PM10 showed the development of a highly branched root system to the detriment of the primary root, characterized by the stimulated formation and emergence of thinner and longer lateral roots (Fig. 1B). These results are in agreement with several authors who observed similar responses to PAHs (Alkio et al., 2005; Baldyga et al., 2005; Kummerova et al., 2013). Indeed, a generic exposure induces a specific 'stress-induced morphogenic response' (SIMR) phenotype, characterized by an inhibition of root elongation, and enhanced lateral roots formation (Potters et al., 2007).

3.3. Effect of PM on ROS concentration

Plants continuously produce ROS as the result of various metabolic pathways, but excess ROS accumulation leads to oxidative stress and cell death (Apel and Hirt, 2004). Given that both ROS and SIMRs are common components of many distinct stresses, it is attractive to hypothesize that ROS are intermediates between the stress and the development of the SIMR phenotype. Indeed, ROS are related to signal transduction responses and play an important role in signaling, hence affecting root growth and development (Jovanovic et al., 2007).

To assess if the direct exposition of roots to PM10 induced an oxidative stress, the relative ROS concentration was evaluated by measuring the fluorescence arising from the oxidation of DCFH-DA in both control and treated roots. The fluorescence observed in PM10 exposed plants was significantly increased compared with the control plants (Table 3). The ROS generation in roots grown on PM10 was 32% in filter 1, 71% in filter 2, 89% in filter 3 and 93% in filter 4 higher in comparison with the control. Fig. 2 shows that ROS values obtained for roots grown on sampled filters are rather variable but still significantly higher than the control values.

The obtained evidence suggests that the atmospheric particulate matter collected on all the examined filters was able to trigger ROS production in the tomato roots. Therefore, it can be hypothesized that, in presence of PM10, as in response to other stresses (Niu et al., 2013), changes in the ROS concentration and distribution in specific root cells occurred, thus influencing the root growth response.

3.4. Effect of particulate matter on pigments

Photosynthetic pigment levels can be directly related to stress physiology, as concentrations of carotenoids increase and chlorophylls generally decrease under stress conditions, such as drought, heat stress, disease or pollutant exposure (Penuelas and Filella, 1998; Pavlik et al., 2012). A change in the chlorophyll/carotenoid ratio can, therefore, be a good indicator of the stress levels in plants (Netto et al., 2005). Consequently, the physiological stress level can be assessed by following the dynamics of photosynthetic pigment concentrations (Yang et al., 2010).

A significant influence on the photosynthetic pigment (chlorophyll *a*, *b* and carotenoids) content was observed for all filters (Table 3). Compared with the control plants, a decrease in total chlorophyll content was observed for filters 1, 2 and 3. An increase was observed only for filter 4, most likely due to the higher level of EC in this case (see Table 1). A significant increase in carotenoid content was observed for all filters: 1.98, 1.25, 1.61 and 1.39 times higher than that in the control plants for filters 1, 2, 3 and 4, respectively.

Table 3
Content of reactive oxygen species in tomato roots and photosynthetic pigments in tomato leaves grown on different filters.

	ROS content (FSU μg^{-1} FW)	Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	Chl *a* + Chl *b* (mg g^{-1} FW)	Car (mg g^{-1} FW)	Chl *a*/Chl *b*	Chl (*a* + *b*)/Car
Control	16 ± 1	0.40 ± 0.01	0.11 ± 0.01	0.52 ± 0.02	0.10 ± 0.01	3.6 ± 0.3	5.1 ± 0.4
Filter 1	21 ± 2*	0.33 ± 0.01	0.19 ± 0.01	0.50 ± 0.02	0.20 ± 0.01	1.7 ± 0.1	2.5 ± 0.2
Filter 2	27 ± 5*	0.33 ± 0.04	0.13 ± 0.01	0.46 ± 0.04	0.13 ± 0.01	2.4 ± 0.4	3.7 ± 0.4
Filter 3	30 ± 3**	0.19 ± 0.04	0.12 ± 0.03	0.31 ± 0.07	0.16 ± 0.01	1.6 ± 0.7	1.9 ± 0.5
Filter 4	30 ± 5**	0.47 ± 0.04	0.18 ± 0.01	0.65 ± 0.05	0.15 ± 0.01	3.2 ± 0.4	4.3 ± 0.5

For photosynthetic pigment contents values are means of three determinations ± standard error.
For ROS content data represent mean values ± standard error ($n = 5$). Asterisks indicate statistically significative differences with the control (Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$).

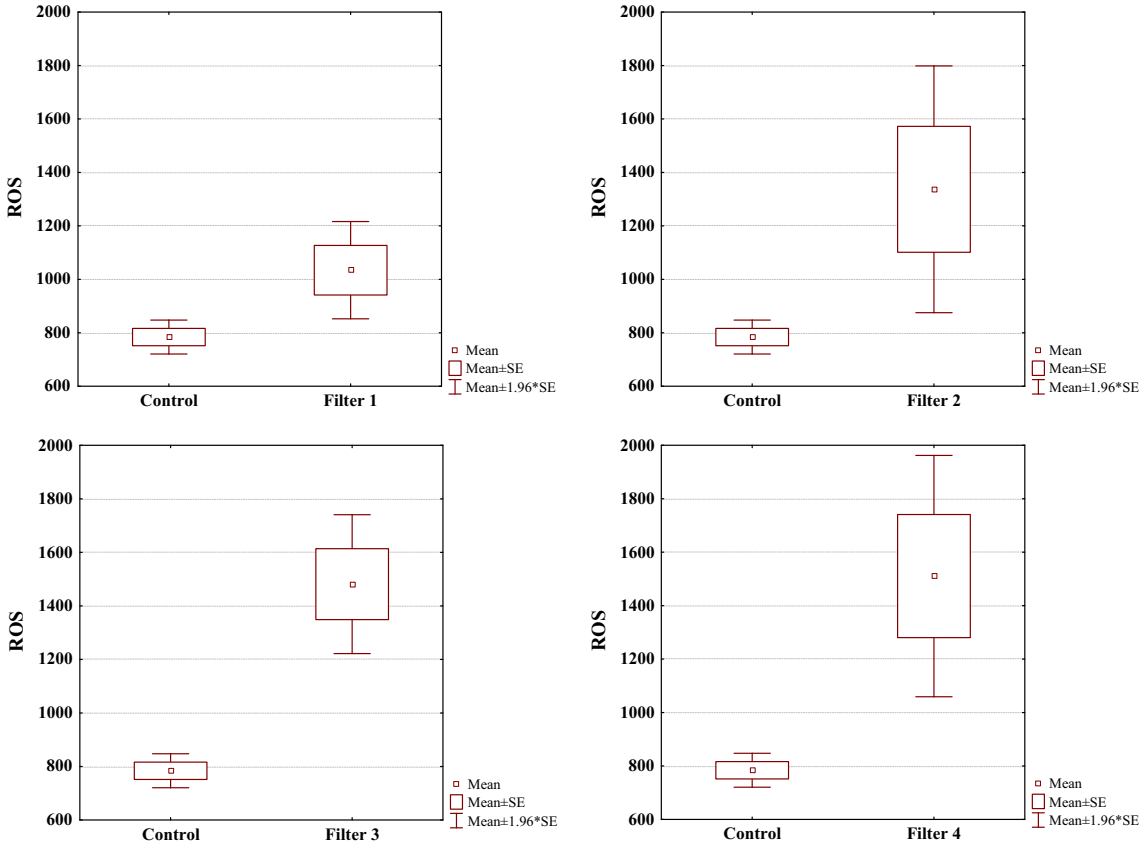

Fig. 2. *t*-Test graph of ROS values measured for seedlings grown in contact with Filters 1, 2, 3, 4 and control. Mean, mean + SE (standard error), and the upper and lower 95% confidence limits (calculated as mean + 1.96 SE, where 1.96 is the 0.975 quantile of the normal distribution) are visualized.

Pigment parameters were used to calculate two stress indicators: Chl *a*/Chl *b* and Chl(*a* + *b*)/carotenoid content. A decrease in these two ratios was observed for all filters, confirming the detrimental effect of PM10 exposure on photosynthetic activity.

An inhibition of photosynthetic processes is often a key mechanism of toxic action on many harmful substances, including PAHs (Kummerova et al., 2006; Pavlik et al., 2012). The decline of photosynthetic activity may be a protective response to limit ROS byproduct in chloroplasts, as also reported for PAH exposure in *Arabidopsis thaliana* (Liu et al., 2009) and in pea plants (Kummerova et al., 2006).

The decrease of chlorophyll content in treated plants can be caused by the direct influence of PM on their biosynthesis or by indirect PM-induced destruction. High ROS levels may have also directly or indirectly contributed to the decline in the observed chlorophyll levels (Table 3).

ROS scavenging or detoxification in plants is achieved by an efficient antioxidative enzymatic system and non-enzymatic antioxidants, including carotenoids (Sharma et al., 2012). As antioxidants, carotenoids scavenge $^{1}O_2$ to inhibit oxidative damage and quench triplet sensitizer ($^{3}Chl^{*}$) and excited chlorophyll (Chl^{*}) molecules to prevent the formation of $^{1}O_2$, hence protecting the photosynthetic apparatus. Carotenoids also serve as precursors to signaling molecules that influence plant development and biotic/abiotic stress responses (Sharma et al., 2012). The increase in carotenoid content could be related to the protective response against oxidative stress induced by PM exposure.

4. Conclusions

An experimental set-up based on transparent pouches to follow the growth of the shoot and the root portions of tomato seedlings on quartz filters was designed in the present paper. According to experiments performed using this set-up, several results indicate that PM10, collected on quartz filter, has a robust effect on the growth of tomato plants. Overall, a clear stress-induced

morphogenic response appears to be associated with the intimate contact between the growing plants and the PM. This interaction also induces changes in the photosynthetic apparatus, particularly in the case of the photoprotective carotenoids.

By specifically focusing on high-PM10 conditions for the quartz filters, this PM characterization campaign obtained consistent plant responses that otherwise would most likely not be visible in the presence of lower PM concentrations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.05.054.

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