Ecotoxicology and Environmental Safety 79 (2012) 101–107

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Trace elements present in airborne particulate matter—Stressors of plant metabolism

Milan Pavlík [a], Daniela Pavlíková [b,*], Veronika Zemanová [b], František Hnilička [c], Veronika Urbanová [b], Jiřina Száková [b]

[a] Isotope Laboratory, Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Vídeňská 1083, 14220 Prague, Czech Republic
[b] Department of Agro-Environmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 16521 Prague, Czech Republic
[c] Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 16521 Prague, Czech Republic

ARTICLE INFO

Article history:
Received 25 August 2011
Received in revised form
12 December 2011
Accepted 13 December 2011
Available online 31 December 2011

Keywords:
Airborne particulate matter
Amino acids
Gas-exchange parameters
Lactuca serriola L.
Toxic elements

ABSTRACT

Changes of amino acid concentrations (glutamic acid, glutamine, asparagine, aspartate, proline, tryptophan, alanine, glycine, valine and serine), gas-exchange parameters (net photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration) and nitrate levels in *Lactuca serriola* L. under airborne particulate matter (PM) contamination reported here reveal their role in plant chronic stress adaptation. Results of the pot experiment confirmed the toxic effect of trace elements present in PM for lettuce. PM applied to soil or on the lettuce leaves were associated with the strong inhibition of above-ground biomass and with the enhancement of plant trace element contents. The significant changes of amino acid levels and leaf gas-exchange parameters of the plants showed strong linear dependences on PM contamination (R^2=0.60–0.99). PM application on leaves intensified toxic effect of trace elements (As, Pb, Cr and Cd) originating from PM by shading of the leaf surface. The plant accumulation of nitrate nitrogen after PM contamination confirmed to block nitrate assimilation.

1. Introduction

Air pollution is one of the most important environmental problems in densely populated and industrialized areas. Airborne particulate matter (PM) in urban air has been at the center of recent concerns, mainly due to its adverse health effects on the urban population. Among the characteristics of particulate matter that relate to its toxicity could be mentioned the presence of trace metals (Rodriguez et al., 2010). Motor vehicles have been demonstrated to be a major contributor of particle-bound trace metals (Valavanidis et al., 2006) in urban areas. As plants are immobile and more sensitive in terms of physiological reaction to the common air pollutants than humans and animals, they better reflect local conditions. High accumulation due to airborne particulate matter was found for Pb, Cr and Cd, especially in leafy vegetables (Voutsa et al., 1996). The major contribution of most trace elements to vegetable leaves was from atmosphere. According to De Temmerman and Hoenig (2004) the leaves accumulate the deposited airborne trace elements; however, they are also influenced by soilborne metals. Mechanisms of air pollution toxicity are very complex and depend on various physiological and biochemical properties of plants. The PM deposited on the leaf surface can affect the plant's metabolism by blocking light, obstructing stomatal apertures, increasing leaf temperature and altering pigment and mineral contents of the leaf (Kuki et al., 2008). Surface dust deposits may alter the optical properties of leaves, particularly the surface reflectance in the visible and short wave infrared radiation range (Hope et al., 1991). In response to these adverse effects various biochemical changes also occur such as decreased chlorophyll content and increased ascorbic acid content of leaves. The significant negative correlation between dust load and leaf pigment content in vegetation near the highway was confirmed by Prusty et al. (2003). According to Poma et al. (2002) in plant tissues an enhancement of the specific activity of the stress-related enzyme peroxidase was monitored and were confirmed genotoxic activities associated with the coarse (PM 10 smaller than 10 μm) and the fine fraction (PM 2.5 smaller than 2.5 μm) of airborne particulates. Field transect studies have shown significant negative correlations between air pollutant concentrations and net photosynthesis, biomass accumulation and yield of crop plants (Agrawal, 2005).

Plant stress metabolism has been usually studied in experiments focused on short-term effects of stress agens, i.e. in the form of an acute stress. This form of stress, however, does not

* Corresponding author. Fax: +420 234381801.
E-mail address: pavlikova@af.czu.cz (D. Pavlíková).

0147-6513/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2011.12.009

reflect correctly the environmental conditions (di Toppi and Gabbrielli, 1999). Plants usually grow and develop in conditions of long-term chronic stresses—they take up small quantities of several toxicants after a long time period. For this reason, the PM study has been focused on complex changes of plant metabolism (plant growth, photosynthesis, N metabolism, etc.) under chronic stress. Wuytack et al. (2011) recommended the use of plant characteristics, such as growth, total biomass production and specific leaf area, as diagnostic monitoring tools. Amino acid metabolism may play an important role for plant growth and development as well as in plant stress resistance, by osmotic adjustment and the accumulation of compatible osmolytes, detoxification of active oxygen species and risk elements and intracellular pH regulation (Singh, 1999).

We suppose that the impact of trace elements originated from airborne particulate matter on photosynthesis and nitrogen utilization by plants can result in changes of amino acids concentrations of leafy vegetable. The present study was conducted to examine the relationship between amino acids concentrations and leaf gas-exchange parameters as a result of lettuce contamination by trace elements contained in airborne particulate matter.

2. Materials and methods

2.1. Plant material and cultivation conditions

Adaptation of lettuce (*Lactuca serriola* L. var. capitata cv. Detenicka atrakce) plants to trace elements present in air pollution was investigated in pot experiment repeated for two years. For this experiment, lettuce plants (seeds obtained from SEMO Ltd. Smržice, Czech Republic) were planted into plastic pots containing the soil as specified below. The plants (one plant per pot) were cultivated from April to June under natural light and temperature conditions at the experimental hall of the Czech University of Life Sciences Prague, Czech Republic. The water regime was controlled and the soil moisture was kept at 60% maximum water-holding capacity.

Airborne particulate matter using for experiment was sampled in air condition units in large buildings near highway (Prague, Czech Republic) with heavy traffic (87 thousand of motor vehicles per day). We got the dust mostly from vehicular traffic with a dominance of fine particles. The fraction <0.065 mm was used for this experiment.

For cultivation of lettuce plants, 5 kg of chernozem ($pH_{KCl}=7.2$, $C_{ox}=1.83\%$, CEC$=258$ mval kg^{-1}) was thoroughly mixed with 0.5 g N, 0.16 g P and 0.4 g K applied in the form of ammonium nitrate and potassium hydrogen phosphate for all treatments. Lettuce was exposed to a PM applied to soil (the soil was spiked prior to the experiment with 30 g PM per pot) or to the application of suspended PM on the leaves during the vegetation (Table 1). Lettuce leaves were sprayed by suspended PM eight times (3.75 g PM $\times$ 100 mL^{-1} per pot for every spraying) during 4 weeks. The soil contents of toxic elements were analyzed as total contents and the portions of available elements are very low in this soil therefore the effect of these contents on plants are not high. Each treatment was performed in five replications. Lettuce plants were planted up to the stage of full leaves development (75 days after planting). The lettuce leaves were washed up after harvest. The plants from each pot were analyzed individually.

2.2. Analyses

2.2.1. Analyses of trace elements

Plant samples were decomposed using the dry ashing procedure as follows: an aliquot ($\sim$1 g) of the dried and powdered biomass was weighed into a borosilicate glass test-tube and decomposed in a mixture of oxidizing gases ($O_2+O_3+NO_x$) at 400 °C for 10 h in a Dry Mode Mineralizer Apion (Tessek, Czech Republic). The ash was dissolved in 20 mL of 1.5% HNO_3 (v/v) (electronic grade purity, Analytika Ltd., Czech Republic) and kept in glass tubes until the analysis. Aliquots of the certified reference material RM NCS DC 73350 poplar leaves (purchased from Analytika, CZ) were mineralized under the same conditions for quality assurance.

Table 1
Experimental design.

Treatment	Fertilization	Particulate matter <0.065 mm (30 g per pot)
1	NPK	0—control
2	NPK	Soil
3	NPK	Plant leaves

Table 2
Total concentrations of selected trace elements in tested soil and in airborne particulate matter determined at the beginning of experiment. The values represent the means of data obtained in the experiment ($n=2$, i.e. two experimental years).

	As	Cd	Cr	Pb
	mg kg^{-1}			
Chernozem	39.4 ± 0.01	0.69 ± 0.07	65.5 ± 2.76	30.4 ± 2.2
Airborne particulate matter	24.5 ± 0.85	2.06 ± 0.27	125.5 ± 3.27	131.5 ± 8.9

Total element concentrations in PM and in soil were determined in digests obtained by two-step decomposition as follows: 0.5 g of the sample was decomposed by dry ashing in a mixture of oxidizing gases ($O_2+O_3+NO_x$) in an Apion Dry Mode Mineralizer (Tessek, CZ) at 400 °C for 10 h; the ash was then dissolved in a mixture of HNO_3+HF (2:1 v/v), evaporated to dryness at 160 °C and dissolved in diluted Aqua Regia (Pavlíková et al., 2008).

The concentrations of trace elements were determined by ICP-OES with axial plasma configuration (Varian VistaPro, Varian, Australia). The toxic effects of As, Cd, Cr and Pb for plant was evaluated for their highest concentration in PM (Table 2).

2.2.2. Analysis of free amino acids

The amino acids from methanol$+H_2O$ extracts were determined using EZ-faast amino acid analysis procedure (Phenomenex, USA). Samples were analyzed for amino acid contents by GC–MS using the Hewlett Packard 6890N/5975 MSD (Agilent Technologies, USA). Samples were separated on a ZB-AAA 10 m $\times$ 0.25 mm amino acid analysis GC column under these conditions: the carrier gas (He) flow was kept constant at 1.1 ml min^{-1}. The oven temperature program was the following: initial temperature 110 °C, a 30 °C min^{-1} ramp to 320 °C. The temperature of the injection port was 280 °C. A 1.5–2 µl sample was injected in split mode (1:15, v/v). MS conditions were as follows: MS source 240 °C, MS quad 180 °C, auxiliary 310 °C, electron energy was 70 eV, scan m/z range 45–450 and sampling rate was 3.5 scan s^{-1} (Pavlík et al., 2010a).

The complex of free amino acids was determined (alanine, glycine, valine, leucine, isoleucine, threonine, serine, tryptophan, proline, asparagine, aspartate, methionine, glutamic acid, glutamine and lysine). The concentrations of the free glutamine, lysine and methionine were below detection limit of GC.

2.2.3. Analysis of nitrate nitrogen in plant biomass

Dried above ground biomass was extracted by hot distilled water (1:10, w/v). Contents of N–NO_3^- were determined using segmental flow-analysis using a colorimetric method on a SKALAR^{plus} SYSTEM (Skalar, Netherlands).

2.2.4. Determination of gas-exchange parameters

The net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were measured in the leaves *in situ* using the portable gas-exchange system LCpro+(ADC BioScientific Ltd., Hoddesdon, Great Britain) from 10:00 to 11:30 of Central European summer time the both experimental years. The irradiance was 802–821 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR), the temperature in the measurement chamber was 25–26.5 °C, the CO_2 concentration was 550 ± 50 cm^3 m^{-3}, the air flow rate was 205 ± 30 µmol s^{-1} and the duration of the measurement of each sample was 15 min after the establishment of steady-state conditions inside the measurement chamber (Holá et al., 2010). From these data, the water use efficiency was estimated (WUE$=P_N/E$).

For calculation of linear correlation (R^2) Statistica for Windows version 7.0 CZ was used (StatSoft, Inc., Tulsa, OK).

3. Results

Results of the pot experiment revealed the toxic effect of PM for lettuce plants. Plant response to the PM contamination was assessed on the basis of a decreased lettuce leaves dry matter and increased concentrations of trace elements in the above-ground biomass (Table 3). The strong inhibition of above-ground biomass was observed on the treatment 3 (PM application on lettuce leaves). Compared to the untreated control, the biomass yield of