

Accumulation of Airborne Toxic Elements and Photosynthetic Performance of *Lolium multiflorum* L. Leaves

Authors:

Klaudia Borowiak, Anna Budka, Marta Lisiak-Zielińska, Anetta Hanke, Janina Zbierska, Danuta Baranek, Dariusz Kayzer, Renata Gaj, Anna Szymczak-Graczyk, Jolanta Kanclerz

Date Submitted: 2021-02-03

Keywords: intercellular CO₂, transpiration rate, air pollution, trace elements, net photosynthesis rate, stomatal conductance

Abstract:

In this study, we aimed to investigate the accumulation of airborne trace elements in *Lolium multiflorum* leaves concerning photosynthetic activity parameters. Five sites for four 28-day series of plant exposition were selected. The concentration of trace elements in leaves was measured after each series, while photosynthetic activity parameters were measured three times during each series. Net photosynthesis rate (PN) and stomatal conductance (gs) were mostly negatively associated with all analyzed trace elements, unlike to CO₂ concentrations (Ci). Arsenic was found with opposite trend in two exposure series. The high accumulation of Cd and Pb in plants recorded at two sites was mostly related to lowest PN and gs. Similar tendency for PN was found at sites and series with the highest Cr and Ni content in plants. *L. multiflorum* revealed a medium-level accumulation of trace elements and a low tolerance of the photosynthetic process to the presence of trace elements in ambient air.

Record Type: Published Article

Submitted To: LAPSE (Living Archive for Process Systems Engineering)

Citation (overall record, always the latest version):

LAPSE:2021.0003

Citation (this specific file, latest version):

LAPSE:2021.0003-1

Citation (this specific file, this version):




LAPSE:2021.0003-1v1

DOI of Published Version: <https://doi.org/10.3390/pr8091013>

License: Creative Commons Attribution 4.0 International (CC BY 4.0)

Article

Accumulation of Airborne Toxic Elements and Photosynthetic Performance of *Lolium multiflorum* L. Leaves

Klaudia Borowiak ^{1,*}, Anna Budka ², Marta Lisiak-Zielińska ¹, Anetta Hanć ³,
Janina Zbierska ¹, Danuta Barańkiewicz ³, Dariusz Kayzer ², Renata Gaj ⁴,
Anna Szymczak-Graczyk ⁵ and Jolanta Kanclerz ⁶

¹ Department of Ecology and Environmental Protection, Poznan University of Life Sciences, 60-637 Poznan, Poland; marta.lisiak@up.poznan.pl (M.L.-Z.); janina.zbierska@up.poznan.pl (J.Z.)

² Department of Mathematical and Statistical Methods, Poznan University of Life Sciences, 60-637 Poznan, Poland; anna.budka@up.poznan.pl (A.B.); dariusz.kayzer@up.poznan.pl (D.K.)

³ Department of Trace Analysis, Faculty of Chemistry, Adam Mickiewicz University, 61-614 Poznan, Poland; anettak@amu.edu.pl (A.H.); danutaba@amu.edu.pl (D.B.)

⁴ Department of Agricultural Chemistry and Environmental Biogeochemistry, Poznan University of Life Sciences, 60-637 Poznan, Poland; grenata@up.poznan.pl

⁵ Institute of Construction and Geoengineering, Poznan University of Life Sciences, 60-637 Poznan, Poland; anna.szymczak-graczyk@up.poznan.pl

⁶ Institute of Land Improvement, Environmental Development and Geodesy, Poznan University of Life Sciences, 60-637 Poznan, Poland; jolanta.kanclerz@up.poznan.pl

* Correspondence: klaudia.borowiak@up.poznan.pl

Received: 16 July 2020; Accepted: 17 August 2020; Published: 19 August 2020



Abstract: In this study, we aimed to investigate the accumulation of airborne trace elements in *Lolium multiflorum* leaves concerning photosynthetic activity parameters. Five sites for four 28-day series of plant exposition were selected. The concentration of trace elements in leaves was measured after each series, while photosynthetic activity parameters were measured three times during each series. Net photosynthesis rate (P_N) and stomatal conductance (g_s) were mostly negatively associated with all analyzed trace elements, unlike to CO_2 concentrations (C_i). Arsenic was found with opposite trend in two exposure series. The high accumulation of Cd and Pb in plants recorded at two sites was mostly related to lowest P_N and g_s . Similar tendency for P_N was found at sites and series with the highest Cr and Ni content in plants. *L. multiflorum* revealed a medium-level accumulation of trace elements and a low tolerance of the photosynthetic process to the presence of trace elements in ambient air.

Keywords: air pollution; trace elements; net photosynthesis rate; stomatal conductance; intercellular CO_2 ; transpiration rate

1. Introduction

Increasing heavy metal concentrations in soil and ambient air, caused by a higher intensity of human activities, can limit the growth and yield of plants [1,2]. Trace elements in the ambient air can cause a physiological imbalance in plants, influencing the biogeochemical balance and stability of habitats [3]. Physiological and biochemical processes aiming to maintain or regain cell homeostasis, disturbed due to negative stress factors, are very important for the further determination of potential resistance to these stress factors, such as trace elements in ambient air. The defensive mechanisms of plants for specific elements employ one of two strategies: avoidance or tolerance of stress. The first

strategy involves various processes limiting the absorption of toxic ions into the cells. The second one allows the plant to absorb the element, but its negative effects are minimized by various processes inside the cell [4].

There are heavy metals (HM), such as cobalt, copper, iron, manganese, molybdenum, nickel, and zinc are necessary elements for plant life functions; however, an increase in their concentration can cause HM stress and impact various processes in plant metabolism [4]. Due to their high reactivity, they can have a direct influence on growth, senescence, and various processes that produce energy in plants. There is many several approaches conducted by plants to survive the toxicity of HM, such as the protein pump in the cytoplasmic membrane, capture of trace elements in the cell, and creation of connections with ligands, e.g., phytochelatins or free amino acids. Hyperaccumulators can collect up to a few percent of HM in dry matter of aboveground plant organs [5]. Mechanisms responsible for this ability were examined by many investigators, but there are still a lot of uncertainties and discussions [6].

The photosynthetic process is very sensitive to the presence of trace elements in the environment. Disturbances of photosynthesis are the first symptom of their toxic effect [7]. The influence on photosynthesis includes injuries of the leaf structure, the ultrastructure of chloroplasts and chloroplast membranes, disturbances in chlorophyll synthesis, especially in seedlings and new leaf growth [8], inhibition of PSI and PSII activity, and disturbances in electron transport [7,9]. Heavy metals can affect on leaf and chloroplast structure and might be revealed by inhibition of leaf growth as well as disorganization of chloroplast ultrastructure. Lead and cadmium can cause the disappearance of chloroplast thylakoids and increase the number of lipid droplets (plastoglobules) [10].

Interactions of HM with enzymatic proteins and other biomolecules are the main cause of negative effects on certain photosynthetic reactions. Trace elements can cause cell oxidative stress and the creation of reactive oxygen species (ROS). This can result in many negative effects on lipids, pigments, membranes, and enzyme activity. To decrease this process, the plant defense system includes some enzymatic and non-enzymatic mechanisms to achieve the redox balance [11]. The enzymatic system includes enzymes like peroxidases (POX), superoxide dismutase (SOD), and catalases (CAT), and non-enzymatic scavengers like glutathione (GSH) or ascorbate (AsA) [12]. The effect of the antioxidative mechanism relates mainly to the balance of redox and avoiding cell and tissue damage, especially chloroplasts and mitochondria [13].

The possibility of the accumulation of HM in plants is widely used for the determination of their concentrations in different environments, but the use of plants with a standardized response is critical in the production of reproducible results [14]. Lichens and mosses are the most popular air pollution bioindicators of trace elements [15–20], but their use in highly polluted areas might be limited due to their low tolerance levels; higher plants can be used, as they are more tolerant. Several plants are used as bioindicators of air pollution, such as tobacco (*Nicotiana tabacum* L. [21], white campion (*Melandrium album*), and black locust (*Robinia pseudoacacia*) [22], or other trees and shrubs [23]. There is also great interest in standardized Italian ryegrass (*Lolium multiflorum* L.) as an easy-to-apply and low-cost HM bioindicator [24]. This plant belongs to the *Poaceae* family and is widely cultivated as a pasture plant and material for bio-ethanol production [25]. Standardized Italian ryegrass cultivated in controlled conditions can be used for the monitoring of trace elements (in anthropogenic areas, with high replicability and comparable results [26]). Other factors do not influence the final result of heavy metal accumulation, because all exposed plants are cultivated in the same conditions (water and soil properties). The most important factors affecting plant HM accumulation levels are their concentrations in the air and meteorological conditions [27].

Due to its high biomonitoring potential and tolerance, we can also evaluate the potential of plant species tolerant to HM in ambient air, as well as investigating the mechanisms of response (such as photosynthetic activity) to stress factors. In this study, our aims were to: (i) examine whether there were any significant differences between photosynthetic activity parameters and the accumulation of trace elements of plants exposed in sites varying in environmental characteristics;

(ii) evaluate whether there were any significant differences between gas exchange parameters during the growing season; (iii) determine the types of relations between HM concentrations in leaves and photosynthetic activity parameters of *L. multiflorum* and evaluate the potential of photosynthetic activity as a biomarker of airborne trace elements stress.

2. Materials and Methods

2.1. Experimental Design

The experiment was carried out during the growing season in 2011. The investigation schedule was provided according to the standardized method of the German Engineering Association [28]. 5 L pots with a standard mixture of peat and sand were used with the same amount of seeds sown in it. The deionized water was used to plants irrigation. A solution of laboratory chemicals (analytical grade) was used so that no heavy metals in readily available form were introduced into the substrate during cultivation in the greenhouse. It contained 5.8 g KH_2PO_4 , 8.5 g KNO_3 , and 5.3 g NH_4NO_3 per liter of deionized water. The last fertilizer application was at least one day before transport to the exposure site. Plants were cut to 4 cm before exposure to sites varying in environmental conditions. The control site (site 1) and five exposure sites were selected for these investigations, located in Poznan city and surrounding areas. Two sites were located in an urban area (sites 2 and 3), one site in a suburban area (site 5), one site in an agricultural area (site 6), and one site located in the Agro-ecological Landscape Park (site 4) (Figure 1). Each of four exposure series lasted 28 ± 1 days in 2011 growing season (16.05–12.06; 13.06–10.07; 11.07–07.08; 08.08–04.09). A similar set of five plants as at exposure site was cultivated in greenhouse conditions as a control. Deionized water was supplied through glass fiber wicks located in pots and water tanks placed underneath the pots. The construction made it possible to ensure plants were 130 cm above the ground at every site, to obtain comparable results of the plant response to air pollution by trace elements.

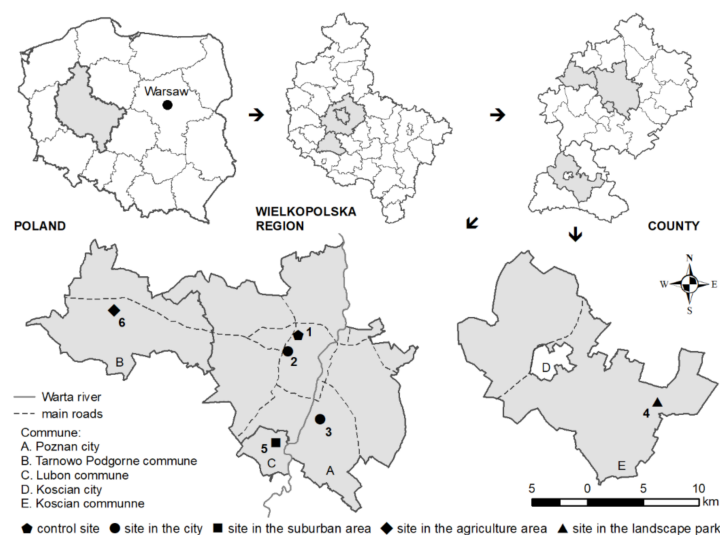


Figure 1. Location of exposure sites. Source: own study based on data from National Geodetic and Cartographic Resource.

2.2. Trace Elements Analysis

Lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), and arsenic (As) concentrations in leaves were measured after every exposure series. Five samples (replicates) were taken from each site. Leaves were dried and 0.5 g of dry weight was placed in 9 mL of ultrapure HNO_3 . Samples were mineralized in MARS5 microwave equipment. The whole process was divided into three phases: reaching certain parameters, maintaining the parameters (pressure 300 PSI, temperature 175 °C) for

15 min, and cooling for the next 15 min. The digested samples were quantitatively transferred into 10 mL volumetric flasks, and the final volume adjusted to the mark with deionized water. Procedural blanks and reference materials were carried out in the same way as the samples in each digestion run. The digestion process for each sample was replicated three times.

Elements were determined in each prepared sample using an inductively coupled plasma mass spectrometer (ICP-MS) equipped with a dynamic reaction cell (Elan DRCII, Perkin Elmer, Toronto, Ontario, Canada). An ICP-MS spectrometer equipped with a Meinhard concentric nebulizer, cyclonic spray chamber, Pt cones, and quadrupole mass analyzer was used for this study. Typical instrument operating conditions for the ICP-MS spectrometer were: RF power 1150 W, plasma Ar flow rate 15 L/min, nebulizer Ar flow rate 0.98 L min⁻¹, and auxiliary Ar flow rate 1.2 L min⁻¹. Whilst tuning the ICP-MS, compromise conditions for maximum signal intensity of the analyte (²⁴Mg⁺, ¹¹⁵In⁺, ²³⁸U⁺), minimum ratio of oxide (¹⁴⁰Ce¹⁶O⁺/¹⁴⁰Ce < 3%), and doubly charged ions (¹²⁸Ba²⁺/¹²⁸Ba⁺ < 3%) were found. The proper conditions of ICP-MS operation were checked by using a solution containing Mg, In, and U at a concentration of 1 µg L⁻¹ and Ba concentration of 10 µg L⁻¹ (Smart Tune Solution—Elan DRC II/plus, Atomic Spectroscopy Standard, PerkinElmer Pure). Calibration curves were established using aqueous standards of relevant elements. The standard solutions were prepared using 10 mg L⁻¹ Multi-element ICP-MS Calibration Std 3 (Atomic Spectroscopy Standard, PerkinElmer, Canada). The isotopes of Sc⁴⁵ and Rh¹⁰³ were prepared from appropriate solutions with a concentration of 1000 mg L⁻¹ (Merck, Darmstadt, Germany). All standards were prepared daily after subsequent appropriate dilution with high purity deionized water (Millipore, Burlington, MA, USA). The correction with internal standards, scandium and rhodium at the concentration of 1 µg L⁻¹, allowed us to correct for matrix-induced variation and instrumental drift.

To verify the analytical performance of the applied method, we validated the selected parameters. Calibration curves were determined by the interpolation method. For all analytes, the calibration curves demonstrated very good linearity, and the correlation coefficients were 0.999 for all elements. The limit of detection (LOD) was considered three times the standard deviation (SD) for the ten independent blank samples. The estimated detection parameters were as follows: As—0.005 µg/g; Cd—0.003 µg g⁻¹; Cr—0.01 µg g⁻¹; Ni—0.01 µg g⁻¹; Pb—0.009 µg g⁻¹. Precision values, expressed as relative standard deviations, were estimated as As—2.3%; Cd—1.6%; Cr—2.3%; Ni—2.5%; Pb—2.1%. Establishing traceability of measurement was performed by applying: Water reference material NIST 1643e (NIST, Gaithersburg, MD, USA) and trace elements in spinach leaves NIST SRM 1570a (NIST, Gaithersburg, MD, USA). In the course of the study, the control materials were run every 10 samples to ensure analytical accuracy. The calculated recoveries for NIST SRM 1570a were As—95%; Cd—98%; Cr—93%; Ni—96%; Pb—98%; NIST 1643e recovery values ranged from 95% to 101%.

2.3. Gas Exchange Measurements

Before (Day 0), in the middle (Day 14), and at the end (Day 28) of each exposure series, net photosynthetic activity (P_N), stomatal conductance (g_s), and intercellular CO₂ concentration (C_i) were measured. Leaves without mechanical injury and fully developed leaves were chosen. Gas exchange investigations were conducted with the aid of the portable photosynthesis system Ci 340 aa (CID BIOSCIENCE Inc., Camas, WA, USA). The constant conditions in the leaf chamber were maintained to ensure similar conditions of measurements at every site: CO₂ inflow concentration (390 µmol (CO₂)mol⁻¹), photosynthetic photon flux density (PPFD) 1000 µmol (photon) m⁻²s⁻¹, a chamber temperature of 23 °C, and relative humidity of 50% ± 3%. Gas exchange measurements were performed between 10:00 and 15:00 h. Five plants (replicates) were analyzed at every site.

2.4. Statistical Analysis

Results were analyzed with a factorial ANOVA with “exposure site” and “day of measurement” as fixed factors. Before ANOVA, the normality and homogeneity of data were checked. Shapiro–Wilk’s test and Bartlett’s test were conducted, respectively. The data normality and Box–Cox’s transformation

were performed in case of a lack of data normality and homogeneity. The differences between measured parameters were analysed with the aid of Tukey's test, while principal component analysis (PCA) was performed to determine the structure and rules between variates. In this analysis, the orthogonal transformation of observed variates to a new set of non-correlated variates (components) was performed. Pearson's correlation coefficients were presented in the form of a matrix of correlations. The data were analyzed with the statistical software STATISTICA 13.1 R platform version 3.6.1.

3. Results

3.1. Trace Elements Accumulation and Meteorological Conditions

The results varied between sites and series. The possibility of trace elements accumulating changes during the growing season, which might be related to changing meteorological conditions. Hence, the variation between sites might be more important for further recommendations for certain areas of environmental management and we were more focused on site variability. One-way ANOVA within a certain series revealed a highly significant effect of site on each element accumulation in plants (Table 1). Trace element concentrations in *L. multiflorum* leaves varied between exposure series. Chromium and nickel accumulation levels were mostly the highest in plants located in urban areas (sites 2 and 3); however, higher levels were also noted at the agricultural site (site 6) (Table 2). The highest levels of these two elements were observed after the second and third exposure series, which might be related to the highest precipitation levels (Table 3). The Agro-ecological Landscape Park (site 4) was found with the highest arsenic accumulation in plants in all series (excluding series 1). Although arsenic-based pesticides are no longer used, elevated arsenic levels may be connected with a windblown dispersion of fine particulate material containing arsenic. Cadmium and lead accumulation levels were the highest at urban sites. The highest cadmium levels were observed after the third series, the highest lead levels after the second series (Table 2).

Table 1. One-way ANOVA results (*F* statistics) of element accumulation in leaves at six sites (including control). The site is an influencing factor. *** statistically significant at level $\alpha \leq 0.001$.

Parameter	1st Series	2nd Series	3rd Series	4th Series
Cr	75.80 ***	123.90 ***	60.49 ***	98.96 ***
Ni	15.87 ***	15.23 ***	31.26 ***	117.97 ***
As	6.80 ***	428.58 ***	68.45 ***	12.62 ***
Cd	16.68 ***	22.74 ***	14.70 ***	348.14 ***
Pb	15.16 ***	325.14 ***	76.00 ***	35.88 ***

Table 2. Trace elements concentrations (means \pm SD, $n = 5$) in *L. multiflorum* leaves measured after four exposure at exposure and control sites.

Series	Site	Cr ($\mu\text{g g}^{-1}$)	Ni ($\mu\text{g g}^{-1}$)	As ($\mu\text{g g}^{-1}$)	Cd ($\mu\text{g g}^{-1}$)	Pb ($\mu\text{g g}^{-1}$)
	1 (control)	0.233 \pm 0.025	0.690 \pm 0.035	0.018 \pm 0.006	0.093 \pm 0.001	0.000 \pm 0.000
1–4	2	1.581 \pm 0.072	1.932 \pm 0.067	0.295 \pm 0.051	0.156 \pm 0.021	0.342 \pm 0.029
	3	1.570 \pm 0.083	1.881 \pm 0.074	0.519 \pm 0.011	0.224 \pm 0.040	0.304 \pm 0.014
1	4	1.723 \pm 0.067	2.035 \pm 0.071	0.425 \pm 0.011	0.149 \pm 0.016	0.388 \pm 0.028
	5	1.879 \pm 0.350	1.742 \pm 0.246	0.268 \pm 0.013	0.162 \pm 0.016	0.396 \pm 0.013
	6	1.520 \pm 0.086	1.796 \pm 0.321	0.376 \pm 0.007	0.248 \pm 0.026	0.481 \pm 0.017
2	2	2.876 \pm 0.262	1.493 \pm 0.235	0.069 \pm 0.006	0.190 \pm 0.004	0.883 \pm 0.066
	3	2.114 \pm 0.101	1.468 \pm 0.081	0.031 \pm 0.007	0.312 \pm 0.012	1.086 \pm 0.176
	4	2.204 \pm 0.219	1.437 \pm 0.101	0.533 \pm 0.006	0.128 \pm 0.009	0.275 \pm 0.020
	5	2.049 \pm 0.068	1.254 \pm 0.040	0.088 \pm 0.006	0.111 \pm 0.011	0.322 \pm 0.020
	6	2.508 \pm 0.227	1.594 \pm 0.206	0.049 \pm 0.001	0.119 \pm 0.017	0.164 \pm 0.022

Table 2. Cont.

Series	Site	Cr ($\mu\text{g g}^{-1}$)	Ni ($\mu\text{g g}^{-1}$)	As ($\mu\text{g g}^{-1}$)	Cd ($\mu\text{g g}^{-1}$)	Pb ($\mu\text{g g}^{-1}$)
3	2	2.872 \pm 0.218	1.923 \pm 0.083	0.044 \pm 0.004	0.285 \pm 0.021	0.234 \pm 0.027
	3	3.384 \pm 0.159	2.255 \pm 0.100	0.036 \pm 0.005	0.347 \pm 0.006	0.210 \pm 0.031
	4	4.469 \pm 0.858	2.222 \pm 0.603	0.225 \pm 0.016	0.322 \pm 0.016	0.167 \pm 0.020
	5	1.829 \pm 0.133	1.205 \pm 0.050	0.041 \pm 0.004	0.122 \pm 0.006	0.038 \pm 0.018
	6	3.168 \pm 0.382	1.955 \pm 0.116	0.046 \pm 0.007	0.225 \pm 0.023	0.106 \pm 0.002
4	2	2.512 \pm 0.117	1.762 \pm 0.057	0.053 \pm 0.009	0.381 \pm 0.044	0.632 \pm 0.082
	3	2.368 \pm 0.159	1.587 \pm 0.063	0.061 \pm 0.003	0.413 \pm 0.086	0.181 \pm 0.022
	4	1.921 \pm 0.223	1.397 \pm 0.105	0.100 \pm 0.011	0.173 \pm 0.010	0.028 \pm 0.002
	5	1.768 \pm 0.145	1.416 \pm 0.152	0.041 \pm 0.013	0.215 \pm 0.024	0.069 \pm 0.009
	6	2.373 \pm 0.102	1.718 \pm 0.061	0.066 \pm 0.011	0.195 \pm 0.014	0.673 \pm 0.064

Table 3. Mean values of meteorological parameters (temperature, air humidity, and ultraviolet B (UVB) radiation) and the sum of precipitation for series.

Series	Temperature ($^{\circ}\text{C}$)	Air Humidity (%)	UVB Radiation ($\text{W}\cdot\text{m}^{-2}$)	PrecipitationSum (mm)
16.05–12.06	17.67	64.97	246.19	10
13.06–10.07	18.20	76.34	103.89	86
11.07–07.08	17.00	81.00	171.72	155
08.08–04.09	16.00	78.25	181.36	43
Means	17.00	76.62	173.92	294 (sum)

3.2. Gas Exchange Parameters

Gas exchange parameters varied between sites and series; however, the differences in plant response might also be seen within each series due to the growth of plants and changes in physiological plant response at each site. Two-way analysis of variance revealed a highly significant effect of site and term of measurement interaction on the net photosynthesis rate in all exposure series, while g_s and C_i results were statistically dependent on site only during the second and third series (Table 4).

Table 4. Two-way ANOVA results of photosynthetic activity parameters measured before, in the middle, and after exposure in four series and six sites (including control). *F* statistics of the interaction of site and term of measurement within the series. *** statistically significant at level $\alpha \leq 0.001$, ** statistically significant at level $\alpha \leq 0.01$, ns—not significant.

Parameter	1st Series	2nd Series	3rd Series	4th Series
P_N	8.331 ***	7.393 ***	8.208 ***	16.633 ***
g_s	1.225 ns	66.845 ***	6.083 ***	1.387 ns
C_i	1.542 ns	3.327 **	10.649 ***	12.788 ns

The net photosynthesis rate varied between series and sites. The lowest level was mostly noted at urban sites in all exposure series. The mid-series measurements during the first series revealed a decrease at both urban sites and an increase at the end; however, these values did not reach the level before exposure. The highest level of P_N was observed after exposure for the first series (Figure 2), which might be connected to the relatively low levels of trace elements in comparison to the other series (Table 2).

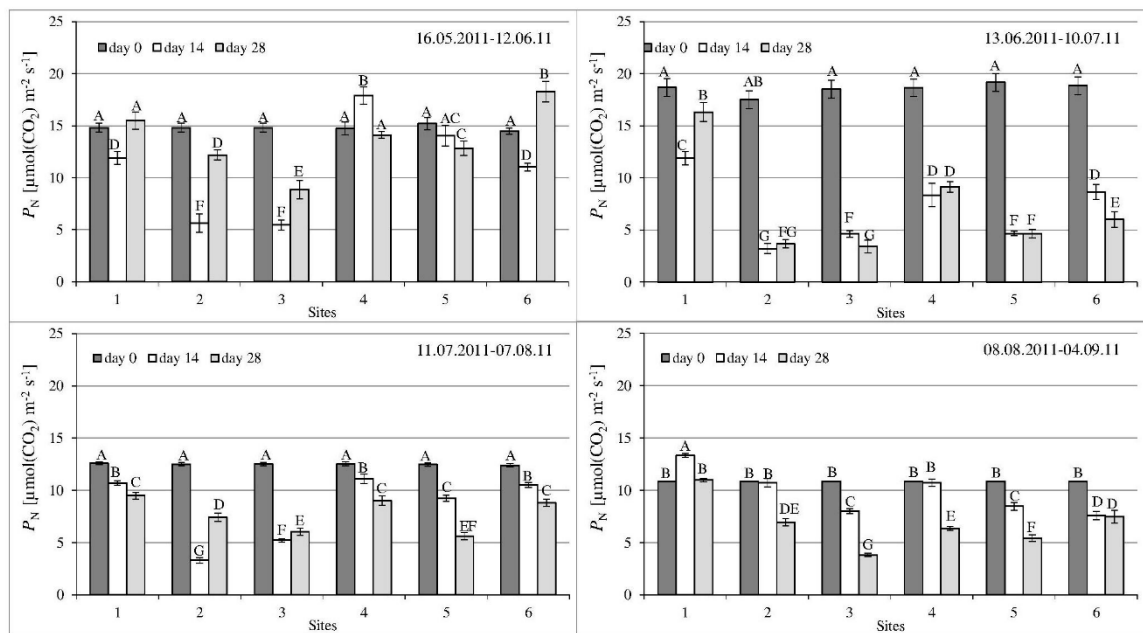


Figure 2. Net photosynthesis rate (P_N) of *L. multiflorum* measured at control and exposure sites during four series. Vertical bars represent standard errors; different letters denote significance difference at level $\alpha \leq 0.05$ between all treatments within each series.

Overall, the lowest levels of P_N were noted after exposure in the second and fourth series. Moreover, a decrease of P_N at the end of exposure was observed in most exposed plants of the fourth series (excluding site 6), whereas the response pattern varied in other series. Usually, the highest level of P_N was recorded at sites 4 and 6 (Figure 2).

Stomatal conductance was related to P_N response, and usually, a decrease at the end of exposure was noted; however, the lowest levels were noted in the last two series and there were no differences between plants from almost all sites at the end of exposure in the fourth series (Figure 3). Inter-cellular CO_2 concentrations also varied between sites and series, and the highest levels were noted in the second series, where the lowest levels of P_N were also recorded (Figures 2 and 4). An increase of C_i at the end of the exposure series was noted—this was especially valid for the last series. Additionally, during the fourth series, a higher increase of C_i was found in plants located at urban areas (sites 2 and 3); however, during the second series, a relatively high level of C_i was noted in plants exposed at site 4, where a high level of P_N was also observed. In some exposure sites, a decrease of P_N and g_s after 14 days of exposure was noted and then an increase, but not higher than the level at day 0. In some cases, this was related to the opposite level of C_i (Figures 2 and 4).

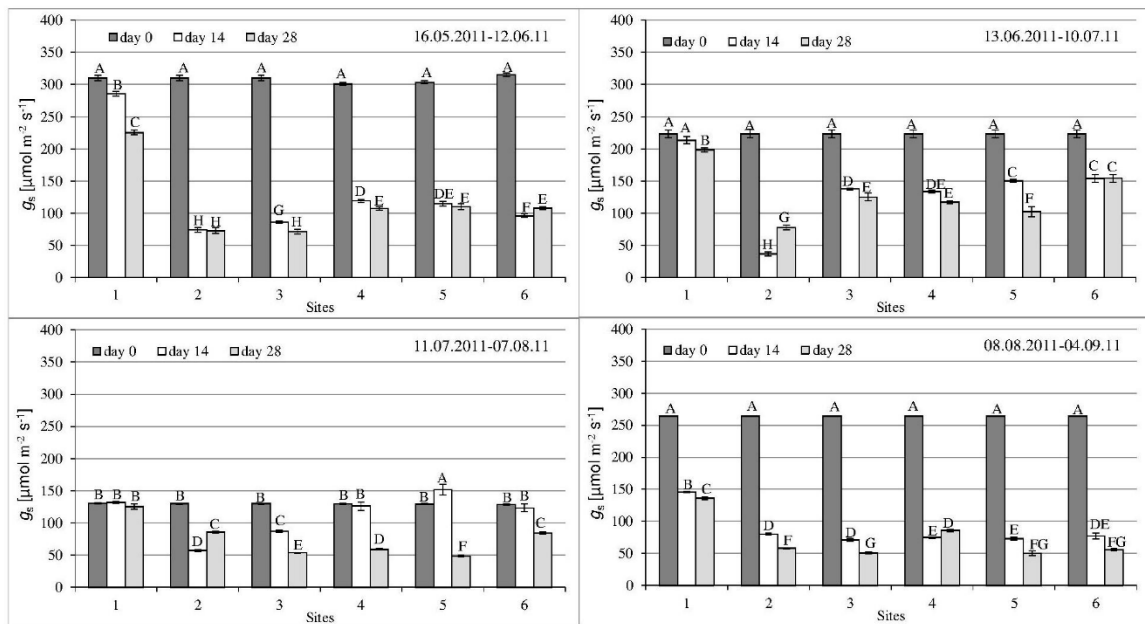


Figure 3. Stomatal conductance (g_s) of *L. multiflorum* measured at control and exposure sites during four series. Vertical bars represent standard errors; different letters denote significance difference at level $\alpha \leq 0.05$ between all treatments within each series.

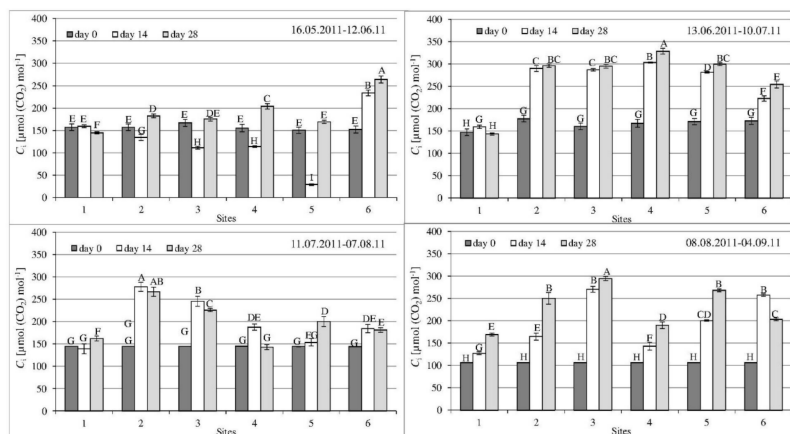


Figure 4. Intercellular CO_2 concentration (C_i) of *L. multiflorum* measured at control and exposure sites during four series. Vertical bars represent standard errors; different letters denote significance difference at level $\alpha \leq 0.05$ between all treatments within each series.

3.3. Relations between Trace Element Accumulation and Photosynthetic Activity

PCA revealed that after the first exposure series P_N and g_s had negative relations to all measured trace elements, whereas C_i revealed a positive or no relation to trace element concentrations. A positive location of trace elements of control site to P_N and g_s was noted opposite to the exposure sites. In the rest of the series, C_i revealed a positive relation to trace elements (excluding As). A similar location of the control site concerning P_N and g_s was found in the rest of the series. Arsenic was found in a position indicating no relation to photosynthetic parameters and trace elements (Figure 5).

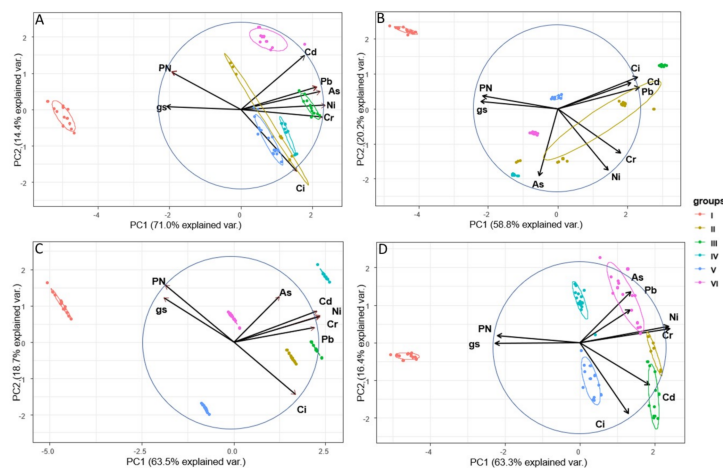


Figure 5. Principal component analysis of photosynthesis activity parameters and trace elements concentrations (arrows) and samples from each exposure sites (points) in four exposure series (A–D, respectively). Ellipses represent the confidence intervals of points.

The accumulation and photosynthesis activity level in ambient air conditions might be the synergistic result of many factors, including other air pollutants (such as oxidative and particulate matter), meteorological conditions, and trace elements accumulation. To analyze these possibilities, a matrix of correlation was performed. All data from the experiment and the sites were taken into consideration. A positive relation was found between P_N and such parameters as, SO_2 , O_3 , and UVB radiation, whereas g_s revealed a positive relation to air temperature. A negative relation was found between P_N and Cr, Cd, Pb, NO_x , and air humidity. A strong negative relation was also found between g_s and Cd (Figure 6).

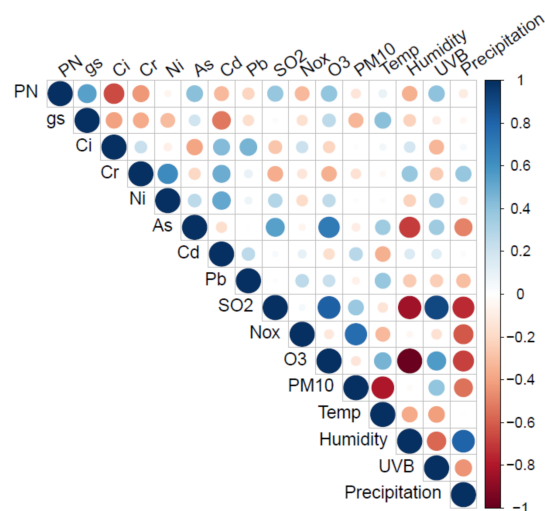


Figure 6. Matrix of correlations between photosynthesis activity parameters, trace elements accumulations, meteorological parameters, and other air pollutants.

4. Discussion

Air pollution by trace elements can cause several negative effects on the environment, including plants. The main sources of trace elements in the Wielkopolska region are transport, households, and agriculture. Air pollution caused by these sources is hard to eliminate due to population growth, vehicular traffic, and food production. The accumulation of elements in plant tissue might be related to meteorological conditions, such as precipitation. The highest precipitation was noted in the third series (Table 3), together with the elevated accumulation of some elements, such as chromium, nickel,

and cadmium. The lower levels of arsenic and lead may be associated with rainfall, which may interfere with metals' bioavailability characteristics [29].

Plant growth is influenced by many synergistic and antagonistic factors that determine the final result. We have correlated trace elements accumulation and photosynthesis activity parameters to meteorological parameters and other air pollutants. Some oxidative air pollutants also influenced plant photosynthesis and the potential to accumulate trace elements. A positive relation was found between P_N , tropospheric ozone and solar radiation. A positive relation between ozone and UVB is related to the conditions of ozone creation, where solar radiation is a key factor [30]. Tropospheric ozone is a well-known phytotoxic air pollutant and harms many crop plants [31]; however, we observed that trace elements were more negatively-related to photosynthesis plant activity than this air pollution. NO_X air pollution related negatively to P_N , while some authors previously found a positive role of NO_X and SO_2 on plant response at certain levels in control conditions [32]. We can conclude that some negative effects of elements on plants, such as Cd, Cr, and oxidative air pollutants, such as NO_X , might be important. So, it is important to take into consideration all possible influencing factors during ambient air condition experiments.

Air pollution can cause a decrease in plant productivity due to its direct effect on plants and an indirect effect on the environment, such as soil and water [33]. Most exposed plants usually reveal some physiological changes before visible symptoms occur [34]. The negative role of airborne trace elements in particulate matter on photosynthetic activity, as well as on nitrate assimilation, was already proven [35]; however, there is a lack of studies in ambient air conditions confirming these findings.

Negative effect of trace elements on photosynthetic activity can be observed. On the other hand, in the case of cadmium hyperaccumulators and tolerant species, an elevated photosynthetic activity can also be found as an adjustment to stress due to tolerance of their carbon assimilation enzyme (Rubisco) [36] and carbonic anhydrase [37]. Shanying et al. [38] indicated a protective role of organic-Cd complex formation and changing the interactions of Cd with other elements, as a tool to protect the photosynthetic process against excessive Cd levels in hyperaccumulator species; however, many authors have pointed out the non-essential function of cadmium in plants and the negative effect of its excess on photosynthesis and biochemical processes [39,40].

Although toxic levels of Cd in plant tissues, which are considered to be 3–30 mg kg⁻¹ [41,42], were not recorded in our experiment, decreases of P_N and g_s were found as well as a negative relation to Cd in all exposure series. This might indicate a low level of tolerance of *L. multiflorum* to Cd in the atmosphere, as well as the potential of P_N and g_s to be early-stage indicators of Cd stress without visual symptoms. Decreasing stomatal conductance was also found as an effect of Cd influence on plants [43,44].

There have been relatively few studies on the effect of arsenic on photosynthesis; however, a chemical relation between arsenate and phosphate was reported, which enables the replacement of the latter in ATP (adenosine triphosphate), which in turn may cause an imbalance in growth and plant metabolism [45]. Rahman et al. [46] found a negative effect of arsenic on chlorophyll content, which in turn can disrupt photosynthesis.

Nickel was found to be an essential element for plants in amounts between 0.01 and 5 µg g⁻³ DW [47]. Nevertheless, our results show that some effects on photosynthetic activity can already be observed within this range. A negative relation of Ni accumulation and P_N , as well as g_s , was found. Similarly, as for Cd, it may indicate low tolerance of photosynthesis and further potential as a biomarker of trace element stress without visible symptoms. A nickel-caused decrease of P_N and g_s was already found in wheat, which was related to stressed carbon metabolism and similarly, as in the case of Cd, with a decrease of Rubisco [48]. The relation between Ni leaf concentration and P_N decrease was also found in poplar [49]. The authors related the reduction of the photosynthetic process to mesophyll conductance and metabolism impairment. Some hyperaccumulators of Ni, such as *Arundo donax* L. [50], also showed no response or even an increase of photosynthetic activity, which may indicate that *L. multiflorum* can be tolerant or even a sensitive indicator without visible symptoms.

Similarly to Cd and Ni, chromium affects photosynthesis through a disturbance in Rubisco [51,52], CO₂ fixation, and electron transport [9,53]. Decreases of P_N , g_s , and transpiration rate were observed in chromium-treated *Lolium perenne* plants [50], pea [52], and *Datura innoxia* [54].

Lead was also found to be a disruptor of the photosynthetic process due to inadequate carbon dioxide concentration because of stomatal closure, obstruction of the electron transport system, and distorted chloroplast ultrastructure [55]. We have noted a decrease of P_N and g_s , which caused a reduction of CO₂ availability for photosynthesis, similar to reports by previous authors.

5. Conclusions

Net photosynthesis rate (P_N) and stomatal conductance (g_s) were mostly negatively associated with all analyzed trace elements, unlike to CO₂ concentrations (C_i). Arsenic was found with opposite trend in two exposure series. The high accumulation of Cd and Pb in plants recorded at two sites was mostly related to lowest P_N and g_s . Similar tendency for P_N was found at sites and series with the highest Cr and Ni content in plants. Based on our results, we can conclude that *L. multiflorum* is a plant showing low tolerance of the photosynthetic process to the presence of trace elements in ambient air and, in turn, its photosynthetic parameters are potential indicators of stress caused by trace elements in the air without visible symptoms. The plant response was also connected with the other factors occurring in the ambient air conditions, such as other pollutants and meteorological parameters.

Author Contributions: K.B., J.Z., and D.B. conceived the idea and planned the experiments. K.B., A.B., M.L.-Z., A.S.-G., J.K. and R.G. carried out the experiments. A.B., D.K. and A.S.-G. planned and carried out the statistical analyses. A.H., M.L.-Z. and R.G. contributed to sample preparation. K.B. and A.B. contributed to the interpretation of the results. K.B. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the NATIONAL SCIENCE CENTRE, grant number N 0540/B/P01/2011/40.

Acknowledgments: We would like to thank the Adam Mickiewicz University Botanical Garden and Research Station in Turew of the Institute for Agricultural and Forest Environment of the Polish Academy of Sciences for their provision of space for exposure sites. I would also like to thank the Adam Mickiewicz University Botanical Garden and Research Station in Turew of the Institute for Agricultural and Forest Environment of the Polish Academy of Sciences for their provision of space for exposure sites.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Bergqvist, C.; Herbert, R.; Persson, I.; Greger, M. Plants influence on arsenic availability and speciation in the rhizosphere, roots and shoots of three different vegetables. *Environ. Pollut.* **2014**, *184*, 540–546. [[CrossRef](#)] [[PubMed](#)]
- Tiwari, K.K.; Singh, N.K.; Rai, U.N. Chromium phytotoxicity in radish (*Raphanus sativus*): Effects on metabolism and nutrient uptake. *Bull. Environ. Contam. Toxicol.* **2013**, *91*, 339–344. [[CrossRef](#)] [[PubMed](#)]
- Ahsan, N.; Renaut, J.; Komatsu, S. Recent developments in the application of proteomics to the analysis of plant responses to heavy metals. *Proteomics* **2009**, *9*, 2602–2621. [[CrossRef](#)] [[PubMed](#)]
- Emamverdian, A.; Ding, Y.; Mokhberdoran, F.; Xie, Y. Heavy metal stress and some mechanisms of plant defense response. *Sci. World J.* **2015**. [[CrossRef](#)] [[PubMed](#)]
- Rascio, N.; Navari-Izzo, F. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Sci.* **2011**, *180*, 169–181. [[CrossRef](#)] [[PubMed](#)]
- Lone, M.; He, Z.; Stoffella, P.; Yang, X. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. *J. Zhejiang Univ. Sci. B* **2008**, *9*, 210–220. [[CrossRef](#)]
- Ali, H.; Khan, E.; Sajad, M.A. Phytoremediation of heavy metals—Concepts and applications. *Chemosphere* **2013**, *91*, 869–881. [[CrossRef](#)]
- Myśliwa-Kurczel, B.; Strzałka, K. Influence of metals on biosynthesis of photosynthetic pigments. In *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*; Prasad, M.N.V., Strzałka, K., Eds.; Springer: Dordrecht, The Netherlands, 2002; pp. 201–227. [[CrossRef](#)]

9. Singh, M.; Kumar, J.; Singh, S.; Singh, V.P.; Prasad, S.M.; Singh, M.P.V.V.B. Adaptation strategies of plants against heavy metal toxicity: A short review. *Biochem. Pharmacol.* **2015**, *4*, 161.
10. Clijsters, H.; Van Assche, F. Inhibition of photosynthesis by heavy metals. *Photosynth. Res.* **1985**, *7*, 31–40. [[CrossRef](#)]
11. Lin, Y.L.; Chao, Y.Y.; Huang, W.D.; Kao, C.H. Effect of nitrogen deficiency on antioxidant status and Cd toxicity in rice seedlings. *Plant Growth Regul.* **2011**, *64*, 263–273. [[CrossRef](#)]
12. Li, F.; Qi, J.; Zhang, G.; Lin, L.; Fang, P.; Ta, A.; Xu, J. Effect of cadmium stress on the growth, antioxidative enzymes and lipid peroxidation in two Kenaf (*Hibiscus cannabinus* L.) plant seedlings. *J. Integr. Agric.* **2013**, *12*, 610–620. [[CrossRef](#)]
13. Dagupta, N.; Datt, P.N.; Das, S. Photosynthesis and antioxidative enzyme activities in five Indian mangroves with respect to their adaptability. *Acta Physiol. Plant* **2010**, *33*, 803–810. [[CrossRef](#)]
14. Vaverkova, M.; Adamcova, D. Heavy metals uptake by select plant species in the landfill area of Stepanovice, Czech Republic. *Pol. J. Environ. Stud.* **2014**, *23*, 2265–2269. [[CrossRef](#)]
15. Bačeva, K.; Stafilov, T.; Šajn, R.; Tănăselia, C. Moss biomonitoring of air pollution with heavy metals in the vicinity of a ferronickel smelter plant. *J. Environ. Sci. Health A* **2012**, *47*, 645–656. [[CrossRef](#)]
16. Giordano, S.; Adamo, P.; Spagnuolo, V.; Tretiach, M.; Bargagli, R. Accumulation of airborne trace elements in mosses, lichens and synthetic materials exposed a turban monitoring stations: Towards a harmonisation of the moss-bag technique. *Chemosphere* **2013**, *90*, 292–299. [[CrossRef](#)]
17. Pajak, M.; Jasik, M. Heavy metal (Zn, Pb, Cd) concentration in soil and moss (*Pleurozium schreberii*) in the Brynica district, southern Poland. *Iforest* **2011**, *4*, 176–180. [[CrossRef](#)]
18. Gerdol, R.; Marchesini, R.; Iacumin, P.; Brancaloni, L. Monitoring temporal trends of air pollution in an urban area using mosses and lichens as biomonitors. *Chemosphere* **2014**, *108*, 388–395. [[CrossRef](#)]
19. Demková, L.; Bobul'ská, L.; Árvay, J.; Jezný, T.; Ducsay, L. Biomonitoring of heavy metals contamination by mosses and lichens around Slovinky tailing pond (Slovakia). *J. Environ. Sci. Health A* **2016**, *52*, 30–36. [[CrossRef](#)]
20. Will-Wolf, S.; Jovan, S.; Amacher, M.C. Lichen elemental content bioindicators for air quality in upper Midwest, USA: A model for large-scale monitoring. *Ecol. Indic* **2017**, *78*, 253–263. [[CrossRef](#)]
21. Budka, A.; Kayzer, D.; Borowiak, K.; Zbierska, J.; Wolna-Maruwka, A.; Schroeter-Zakrzewska, A.; Chlebowska, A. Visible tobacco leaf injury indices as indicators of cumulative tropospheric ozone effect. *Arch. Environ. Prot.* **2014**, *40*, 53–65. [[CrossRef](#)]
22. Nadgórska-Socha, A.; Kandziora-Ciupa, M.; Ciepał, R.; Barczyk, G. *Robinia pseudoacacia* and *Melandrium album* in trace elements biomonitoring and air pollution tolerance index study. *Int. J. Environ. Sci. Technol.* **2016**, *13*, 1741–1752. [[CrossRef](#)]
23. Liang, J.; Fang, H.L.; Zhang, T.L.; Wang, X.X.; Liu, Y.D. Heavy metal in leaves of twelve plant species from seven different areas in Shanghai, China. *Urban For. Urban Green.* **2017**, *27*, 390–398. [[CrossRef](#)]
24. Illi, J.C.; Vancetta, T.; Alves, D.D.; Osório, D.M.M.; Bianchin, L.; De Quevedo, D.M.; Juchem, F. Integrated assessment of air pollution by metals and source apportionment using ryegrass (*Lolium multiflorum* Lam.) in southern Brazil. *Environ. Sci. Pollut. Res.* **2017**, *24*, 2790–2803. [[CrossRef](#)]
25. Fang, Z.; Lou, L.; Tai, Z.; Wang, Y.; Yang, L.; Hu, Z.; Cai, Q. Comparative study of Cd uptake and tolerance of two Italian ryegrass (*Lolium multiflorum*) cultivars. *PeerJ* **2017**, *5*, e3621. [[CrossRef](#)]
26. Borowiak, K.; Zbierska, J.; Barańkiewicz, D.; Hanć, A.; Budka, A.; Kayzer, D.; Kawala, A. Biomonitoring of Air Pollution by Trace Elements Using Italian Ryegrass (*Lolium multiflorum* L. 'Lema'). *Pol. J. Environ. Stud.* **2014**, *23*, 681–688.
27. VDI 3957 Part 2. German proposal to grass culture. In *Biological Measuring Techniques for the Determination and Evaluation of Effects of Air Pollutants on Plants (Bioindication)*. Method of Standardised Grass Exposure; Beuth Verlag: Berlin, Germany, 2003.
28. Klumpp, A.; Ansel, W.; Klumpp, G.; Breuer, J.; Vergne, P.; José Sanz, M.; Rasmussen, S.; Ro-Poulsen, H.; Ribas Artola, A.; Peñuelas, J.; et al. Airborne trace element pollution in 11 European cities assessed by exposure of standardised ryegrass cultures. *Atmos. Environ.* **2009**, *43*, 329–339. [[CrossRef](#)]
29. Alves, D.D.; Osório, D.M.M.; Rodrigues, M.A.S.; Illi, J.C.; Bianchin, L.; Benvenuti, T. Concentrations of PM_{2.5-10} and PM_{2.5} and metallic elements around the Schmidt Stream area, in the Sinos River Basin, southern Brazil. *Braz. J. Biol.* **2015**, *75*, 43–52. [[CrossRef](#)]

30. Jasaitis, D.; Vasiliauskienė, V.; Chadyšienė, R.; Pečiulienė, M. Surface ozone concentration and its relationship with UV radiation, meteorological parameters and radon on the eastern coast of the Baltic Sea. *Atmosphere* **2016**, *7*, 2. [[CrossRef](#)]
31. Singh, A.A.; Agrawal, S.B. Tropospheric ozone pollution in India: Effects on crop yield and product quality. *Environ. Sci. Pollut. Res.* **2017**, *24*, 4367–4382. [[CrossRef](#)]
32. Muneer, S.; Kim, T.H.; Choi, B.C.; Lee, B.S.; Lee, J.H. Effect of CO, NO_x and SO₂ on ROS production, photosynthesis and ascorbate–glutathione pathway to induce *Fragaria×annasa* as a hyperaccumulator. *Redox Biol.* **2014**, *2*, 91–98. [[CrossRef](#)]
33. Seyyednejad, S.M.; Niknejad, M.; Koochak, H. A review of some different effects of air pollution on plants. *Res. J. Environ. Sci.* **2011**, *5*, 302–309. [[CrossRef](#)]
34. Liu, Y.J.; Ding, H. Variation in air pollution tolerance index of plants near a steel factory: Implications for landscape-plant species selection for industrial areas. *WSEAS Trans. Environ. Dev.* **2008**, *4*, 24–32.
35. Pavlík, M.; Pavlíková, D.; Zemanová, V.; Hnilička, F.; Urbanová, V.; Száková, J. Trace elements present in airborne particulate matter—stressors of plant metabolism. *Ecotoxicol. Environ. Saf.* **2012**, *79*, 101–107. [[CrossRef](#)]
36. Pérez-Romero, J.A.; Redondo-Gómez, S.; Mateos-Naranjo, E. Growth and photosynthetic limitation analysis of the Cd-accumulator *Salicornia ramosissima* under excessive cadmium concentrations and optimum salinity conditions. *Plant Physiol. Biochem.* **2016**, *109*, 103–113. [[CrossRef](#)]
37. Pereira, M.P.; de Almeida Rodriguez, L.C.; Corrêa, F.F.; de Castro, E.M.; Ribeiro, V.E.; Pereira, F.J. Cadmium tolerance in *Schinus molle* trees is modulated by enhanced leaf anatomy and photosynthesis. *Trees* **2015**, *30*, 807–814. [[CrossRef](#)]
38. Shanying, H.E.; Yang, X.; He, Z.; Baligar, V.C. Morphological and physiological responses of plants to cadmium toxicity: A review. *Pedosphere* **2017**, *27*, 421–438. [[CrossRef](#)]
39. Tóth, T.; Zsiros, O.; Kis, M.; Garab, G.; Kovács, L. Cadmium exerts its toxic effects on photosynthesis via a cascade mechanism in the cyanobacterium, *Synechocystis* PCC 6803. *Plant Cell Environ.* **2016**, *35*, 2075–2086. [[CrossRef](#)]
40. Tran, T.A.; Popova, L.P. Functions and toxicity of cadmium in plants: Recent advances and future prospects. *Turk. J. Bot.* **2013**, *37*, 1–13. [[CrossRef](#)]
41. Solís-Domínguez, F.A.; González-Chávez, M.Z.; Carrillo-González, R.; Rodríguez-Vázquez, R. Accumulation and localization of cadmium in *Echinochloa polystachya* grown within a hydroponic system. *J. Hazard. Mater.* **2007**, *141*, 630–636. [[CrossRef](#)]
42. Chen, L.; Long, X.H.; Zhang, Z.H.; Zheng, X.T.; Rengel, Z.; Liu, Z.P. Cadmium accumulation and translocation in two Jerusalem artichoke (*Helianthus tuberosus* L.) cultivars. *Pedosphere* **2001**, *21*, 573–580. [[CrossRef](#)]
43. Perfus-Barbeoch, L.; Leonhardt, N.; Vavasseur, A.; Forestiers, C. Heavy metal toxicity: Cadmium permeates through calcium channels and disturbs the plant water status. *Plant J.* **2002**, *32*, 539–548. [[CrossRef](#)]
44. Shi, G.R.; Cai, Q.S. Photosynthetic and anatomic responses of peanut leaves to cadmium stress. *Photosynthetica* **2008**, *46*, 627–630. [[CrossRef](#)]
45. Punshon, T.; Jackson, B.P.; Meharg, A.A.; Warczack, T.; Scheckel, K.; Guerinot, M.L. Understanding arsenic dynamics in agronomic systems to predict and prevent uptake by crop plants. *Sci. Total Environ.* **2017**, *581*, 209–220. [[CrossRef](#)]
46. Rahman, M.A.; Hasegawa, H.; Rahman, M.M.; Islam, M.N.; Miah, M.A.M.; Tasmen, A. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. *Chemosphere* **2007**, *67*, 1072–1079. [[CrossRef](#)]
47. Seregin, I.V.; Kozhevnikova, A.D. Physiological role of nickel and its toxic effects on higher plants. *Russ. J. Plant Physiol.* **2006**, *53*, 257–277. [[CrossRef](#)]
48. Gajewska, E.; Niewiadomska, E.; Tokarz, K.; Słaba, M.; Skłodowska, M. Nickel-induced changes in carbon metabolism in wheat shoots. *J. Plant Physiol.* **2013**, *170*, 369–377. [[CrossRef](#)]
49. Velikova, V.; Tsonev, T.; Loreto, F.; Centritto, M. Changes in photosynthesis, mesophyll conductance to CO₂, and isoprenoid emissions in *Poplar nigra* plants exposed to excess nickel. *Environ. Pollut.* **2011**, *159*, 1058–1066. [[CrossRef](#)]
50. Papazoglou, E.G.; Karantounias, G.A.; Vemmos, S.N.; Bouranis, D.L. Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. *Environ. Int.* **2005**, *31*, 243–249. [[CrossRef](#)]

51. Vernay, P.; Gauthier-Moussard, C.; Hitmi, A. Interaction of bioaccumulation of heavy metal chromium with water relation, mineral nutrition and photosynthesis in developed leaves of *Lolium perenne* L. *Chemosphere* **2007**, *68*, 1563–1575. [[CrossRef](#)]
52. Rodriguez, E.; Santos, C.; Azevedo, R.; Moutinho-Pereira, J.; Correia, C. Chromium (VI) induces toxicity at different photosynthetic levels in pea. *Plant Physiol. Biochem.* **2012**, *53*, 94–100. [[CrossRef](#)]
53. Dhir, B.; Sharmila, P.; Saradhi, P.P. Photosynthetic performance of *Salvinia natans* exposed to chromium and zinc rich wastewater. *Braz. J. Plant Physiol.* **2008**, *20*, 61–70. [[CrossRef](#)]
54. Vernay, P.; Gauthier-Moussard, C.; Jean, L.; Bordas, F.; Faure, O.; Ledoigt, G.; Hitmi, A. Effect of chromium species on phytochemical and physiological parameters in *Datura innoxia*. *Chemosphere* **2008**, *72*, 763–771. [[CrossRef](#)]
55. Leal-Alvarado, D.A.; Espadas-Gil, F.; Sáenz-Carbonell, L.; Talavera-May, C.; Santamaria, J.M. Lead accumulation reduces photosynthesis in the lead hyper-accumulator *Salvinia minima* Baker by affecting the cell membrane and inducing stomatal closure. *Aquat. Toxicol.* **2016**, *171*, 37–47. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).