

Impact of expressway on physiology of plants and accumulation of risk elements in forest ecosystems

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Citation: Kuklová M., Hniličková H., Hnilička F., Pivková I., Kukla J. (2019): Impact of expressway on physiology of plants and accumulation of risk elements in forest ecosystems. *Plant Soil Environ.*, 65: 46–53.

Abstract: This study analyses the effects of expressways on physiology and risk elements content in plants (*Quercus cerris* L., *Prunus spinosa* L., *Melica uniflora* Retz.) and soils. Study forest stands are located at a distance of 30 m to 8100 m from the expressway 'R1 Nitra – Tekovské Nemce' (southwest Slovakia). The effect of distance from the road on the content of cadmium (Cd) in soils indicated an increase of the element in mineral layers in the 30 m variant; excessive Cd values were recorded in O-horizons and in the background zone. Also copper (Cu) showed a marked enrichment of surface horizons caused by car traffic. The results of redundancy discriminant analysis showed that the most important environmental variables related to physiological parameters of plants were the content of Cd and Cu in plants ($P < 0.05$) and the content of Cu in soils ($P < 0.05$). However, *M. uniflora* species responded most sensitively to a decrease of average chlorophyll content as a consequence of excessive accumulation of Cd in leaf tissues. The obtained results indicate risk of exposure to risk elements in soils and plants of forest stands under conditions of polluted air, but also under specific environmental conditions.

Keywords: road traffic; accumulation of toxic elements; air pollution; soil contamination; photosynthesis

Road traffic is one of the most significant anthropogenic factors that negatively affect the environment. Particular attention is paid to the problem of contamination of adjacent soils (Werkenthin et al. 2014) and vegetation (Galal and Shehata 2015). Car exhausts, oil residues, tire particles together with natural biogenic material can be absorbed to the surface of dust and can represent a possible vector of contamination of most of the territory (Omar et al. 2007).

In recent years, many studies focused on the concentration, distribution and source identification of heavy metals (HM) in roadside dust (Phi et al. 2018). With the additional influence of wind and airflow, very fine particulate matter can be transported and deposited up to a distance of 250 m (Zechmeister et

al. 2005). Many authors stated that the concentrations of HM with distance to the road decrease (e.g. Hjortenkrans et al. 2008).

In general, the most recognised and examined HM in roadside environments are Cd, Cr, Cu, Pb, Ni, and Zn (Kayhanian et al. 2012). The continuous enrichment of pollutants in roadside soils exposes vegetation, animals, and soil micro-flora to long lasting stress (Kocher et al. 2005). Various physiological and biochemical processes in plants can be affected by HM. In plants, metals exert their toxic action mostly by damaging chloroplasts and disturbing photosynthesis. Inhibition of photosynthesis is the consequence of interference of metal ions with photosynthetic enzymes and chloroplast

Supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and by the Slovak Academy of Sciences, Projects No. 2/0005/17 and 2/0120/17; and by the European Regional Development Fund, Project No. CZ.02.1.01/0.0/0.0/16019/0000845.

<https://doi.org/10.17221/585/2018-PSE>

membranes (Aggarwal et al. 2012). At a cellular level, an accumulation of HM can cause damage leading to the formation of reactive oxygen radicals (Rai et al. 2016). The soil copper (Cu) contamination resulted in the decline of photosynthetic pigments (Lin and Jin 2018). Results of John et al. (2008) indicated that higher concentrations of Cd and Pb showed a decrease in growth, pigment, proline, protein and sugar content in biomass yield. Plants growing in metal-polluted sites can exhibit altered metabolism, growth reduction, lower biomass production and metal accumulation (Roje et al. 2018).

The study transect (Southwest of Slovakia) is an anthropic-biotic complex, which consists of a set of natural and man-in part, or completely altered dynamic systems, as well as the newly created artificial elements. The region has an economy based predominantly on industrial and commercial activities. An increase of sulphur dioxide (SO₂), nitrogen oxides (NO_x), ozone (O₃) and particulate matter pollution was reported in this region. Contaminants represent mainly lead, copper, cadmium and arsenic (Report 2013). The expressway is an important tool of transportation in this region since November 2011. Due to the fact that soils and plants could be a main source for dust and a reservoir for the deposition of dust, it is important to assess the effects of traffic on the distribution and levels of heavy metals in roadside soils and plants. Our hypothesis was that the physiological characteristics and content of toxic elements in plants species are more optimal in the background zone than near the highway, because of the higher level of traffic. Therefore, for a better understanding of the spatial distribution of the measured physiological parameters and toxic element concentrations (Cd, Cu) in plants (*Quercus cerris* L., *Prunus spinosa* L., *Melica uniflora* Retz.) and soils, this study has focused on forest ecosystems located near highway and in the background zone.

MATERIAL AND METHODS

Study plots. The expressway ‘R1 Nitra – Tekovské Nemce’ (at a distance of 41.5 km) is situated in Southwest Slovakia and passes mostly through agricultural landscape in which forest geobiocoenoses are preserved in the form of larger or smaller enclaves. The research plots are situated in the segment of geobiocoene type of *Querci-Fageta typica* (G1, G3, G4) and *Fagi-Querceta typica* (G1, G5). Plot G1 represents a stand at the age of 70–90 years, plots G2–G4

of 60–80 years and plot G5 of 60–80 years. Study plots G1 to G4 are located at the distance of 30 m (edge of the forest stand) and 50 m (closed forest stand) from the expressway; the control plot G5 at a distance of approximately 8000 m (edge of the forest stand) and 8100 m (closed forest stand) from R1.

Soil analyses. Soil samples were taken from segments of geobiocoenoses located on plots G1 (Dystric Luvisol), G2 (Haplic Luvisol), G3 (Dystric Cambisol), G4 (Protostagnic Cambisol) and G5 (Dystric Cambisol) in July 2016. Surface humus (O-horizon) and mineral soil samples (from layers 0–5, 10–20 and 20–30 cm) were taken by random sampling at each of five plots at a distance of 30 m (plots G1–G4) and 8000 m (G5) from the expressway in triplicate. Values of soil reaction were determined potentiometrically by a digital pH meter Inolab pH 720 (Weilheim, Germany). Nitrogen total (N_t) and carbon total (C_t) content was determined by the NCS analyzer FLASH 1112 (Hanau, Germany). Total contents of Cd and Cu in soil samples were determined in the *aqua regia* extract (HNO₃ + HCl 1:3) by the methods AAS-F (Cd) and AAS (Cu) using an instrument Thermo iCE 3000 (Cambridge, UK) and a GBC SensAA analyser (Braeside, Australia).

Plant analyses. The samples of *Quercus cerris* L. leaves (taken from the bottom third of the tree crown), *Prunus spinosa* L. leaves and *Melica uniflora* Retz. stalks were obtained by stratified random sampling of each studied plot. Samples for chemical analyses of plants (100 to 200 leaves) were taken at a distance of 30 m (edge of the forest stand) and 50 m (closed forest stand) from the expressway (plots G1–G4); from control plot at a distance of 8000 m (edge of the forest stand) and 8100 m (closed forest stand). Total content of Cd and Cu in plant samples was determined in extract of concentrated HNO₃ by the method of AAS-GTA.

Physiological parameters of plants at a distance of 30 m from the road (G1–G4) were compared to the control variant (G5; 8000 m from the road). Samples of plants were not cleaned from airborne dust. The rate of photosynthesis (P_n) was measured on the upper surface of leaves (the middle part of the leaf blade) *in situ* using the portable gas exchange system LCpro+ (ADC BioScientific Ltd., Hoddesdon, UK). The maximum quantum efficiency of photosystem II (F_v/F_m) was measured *in situ* with the portable fluorometer OSI 1 FL (ADC BioScientific Ltd., Hoddesdon, UK). The methodology of measured physiological parameters is given in the paper by Kuklová et al. (2015). The chlorophyll (*Chl*) content was measured with

the CCM-300 Chlorophyll Content Meter (Opti-Sciences, Inc., Hudson, USA).

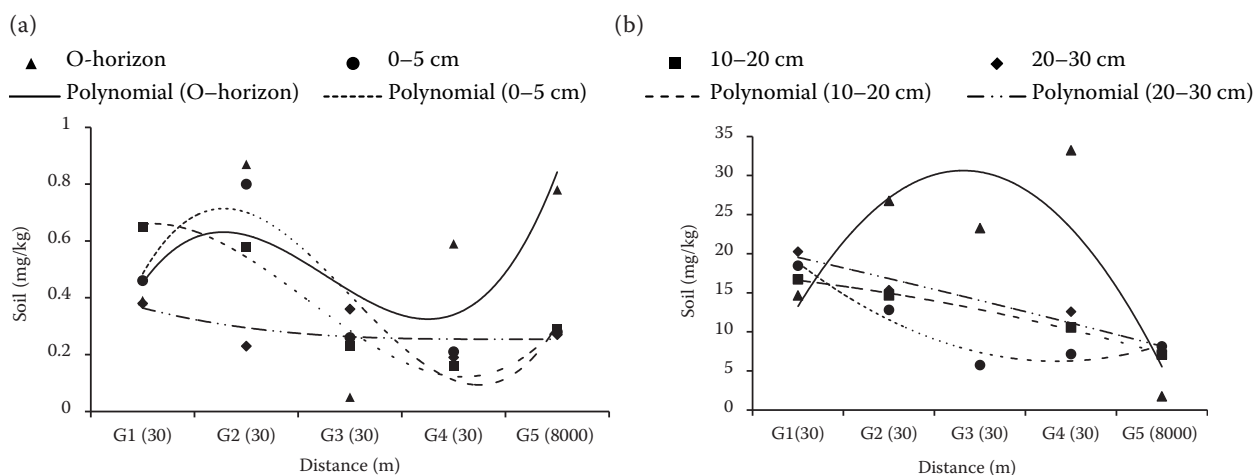
Data analysis. Data were analysed with the use of a program Statistica 9 (Tulsa, USA). The variability of measured characteristics between the studied plots was tested by one-way ANOVA model and Fisher-*LSD* (least significant difference) test. The impact of distance on the content of risk elements in soil layers was analysed with the regression method, using polynomial functions. A redundancy discriminant analysis (RDA), in the R Project for Statistical Computing was used to evaluate the relationships between average toxic concentration values of soils and plants (independent variables) and physiological parameters of plants (dependent variables).

RESULTS AND DISCUSSION

Soil analyses. Among significant variables controlling the distribution and enrichment of toxic elements in soils are pH, grain size and the amount of organic matter in the soil (Amadi and Nwankwoala 2013). N_t and C_t contents were determined in 0–5 cm soil layers. Within the top layers there are concentrated maximum amounts of pollutants (Kuklová et al. 2016) and the most significant impact of plant litter on chemical properties of soils (Hagen-Thorn et al. 2004). From the total amount of carbon (C) content found in

top soil layers, the following descending order can be set (%): 4.43 ± 0.21 (G1) > 3.69 ± 0.18 (G2) > 3.48 ± 0.17 (G4) > 2.59 ± 0.12 (G5) > 2.11 ± 0.11 (G3). According to the classification of Šály and Ciesarik (1991) they are therefore slightly (G3, G5) to moderate (G1, G2, G4) humic soils. The values of C/N ratio on study plots varied from 11.2 to 14.6, indicating a rather rapid process of forest litter decomposition. However, the influence of plants on the chemical properties of soil is most significantly reflected in the pH value (Augusto et al. 2002). The active soil reaction in the upper (0–5 cm) mineral soil layers situated along the road varied from 4.4 (G3) to 5.6 (G4), in Dystric Cambisol on the control plot (G5) pH value of 4.70 was detected, thus pointing at acidic to strongly acidic soils in the segments of studied geobiocoenoses.

Variability of Cd in soil profiles revealed that the coefficients of regression equations indicated a higher mutual similarity of these functions only with respect to the 0–5 cm and 10–20 cm soil layers and the same response to distance from the roadway (Figure 1a). The courses indicate an increase of the Cd content in these layers on plots near the road (G1, G2). Excessive values (0.59 ± 0.20 – 0.87 ± 0.29 mg/kg) were recorded at O-horizons on plots near the expressway (G4, G2) but also in the background zone. According to Wiczorek et al. (2005) the concentrations of Cd in



Soil layer (cm)	Cadmium		Copper	
	regression equation	R^2	regression equation	R^2
O-horizon	$y = 0.0792x^3 - 0.6568x^2 + 1.584x - 0.554$	0.368	$y = -5.2614x^2 + 29.635x - 11.096$	0.718
0–5	$y = 0.0833x^3 - 0.7536x^2 + 1.8931x - 0.738$	0.815	$y = 1.5536x^2 - 11.956x + 29.242$	0.952
10–20	$y = 0.04x^3 - 0.3114x^2 + 0.5386x + 0.392$	0.969	$y = -0.2317x^2 - 0.949x + 17.769$	0.996
20–30	$y = -0.0025x^3 + 0.0339x^2 - 0.1536x + 0.486$	0.322	$y = -0.0417x^2 - 2.592x + 22.163$	0.938

Figure 1. Effect of distance from the road (30 m and 8000 m) on (a) the cadmium and (b) the copper content in soil layers ($n = 3$, $P \leq 0.05$). R^2 – determination coefficient

<https://doi.org/10.17221/585/2018-PSE>

soil samples in Poland (road S 51) were not correlated to the distance from road, either; however, unlike our results they were generally low (mean level was 0.015 mg/kg). The calculated worldwide mean is 0.53 mg/kg Cd in surface soils, and apparently all higher values reflect the anthropogenic impact on the Cd status in top soils (Kabata-Pendias 2011).

In the studied soil profiles, the Cu content mostly declined to the lower layers, yet, on plot G1 and G5 it slightly increased (Figure 1b). According to Oliver (1997), Cu shows relatively little variations in soil profiles and this fact can be related to the presence of Fe and Al oxides and also to the value of pH. Regression equations describing the effect of distance from roadway on the Cu content indicated a similar response in soil layers of 10–20 cm and 20–30 cm and showed almost linear courses with minimum values in the background zone (Figure 1b). On the contrary, the most of Cu (> 20 mg/kg) in O-horizons on plots near expressway illustrates a typical response of chemical impurities in surface horizons caused by car traffic. However, Kabala and Singh (2001) state that soil properties contribute more to the relative distribution of metal fractions in the studied profiles to the source of pollution than distance and direction do.

Plant analysis. Average Cd concentrations in plants were mostly higher within the edges of forest stands and reached several times higher values of the closed forest stands. Particularly, the highest average Cd content was observed in *M. uniflora* on control plot (Figure 2). This fact could be caused by leaf surface of

grass with varying degree and coverage with bristles, which can retain dust (Jankowski et al. 2015). The results correspond with conclusions of Kachova and Atanassova (2014) that density of tree stands established right to the roads plays a role not only of a mechanical barrier for the contaminants suspended from automobile traffic but also as HM accumulators. Based on the findings, the average content of Cd in the studied plants of the edges of forest stands and closed forest stands along road was mostly above common values of Cd in plants (0.1–0.2 mg/kg) and it indicated pollution (Figure 2). Also at distances of 8000 and 8100 m *P. spinosa* (0.49–0.67 mg/kg) and *M. uniflora* (1.01 mg/kg) showed values above limit. Cd is a mobile metal and is easily received by roots of plants (Zacchini et al. 2011), causing plants chlorosis and inhibition of growth. This element significantly influences stomatal behaviour, photosynthesis, respiration and water and nutrient uptake (Cocoza et al. 2014). Depending on the species, accumulation of Cd was significantly diversified for *Q. cerris* and *M. uniflora* (Figure 2). At the distance of 30 m *Q. cerris* accumulated approximately 4 to 17 times more cadmium than at 50 to 8100 m from the road. At distances of 30 m and 8000 m *Melica uniflora* received approximately 11 to 20 times more Cd than in the closed forest stand (8100 m from the road). According to Jankowski et al. (2015) somewhat lower Cd contents in plants were recorded at *Arrhenatherum elatius* (L.) by J. Presl et C. Presl (0.251 mg/kg) and in other grasses (0.324–0.345 mg/kg) in a part of the E30 route compared to the forest

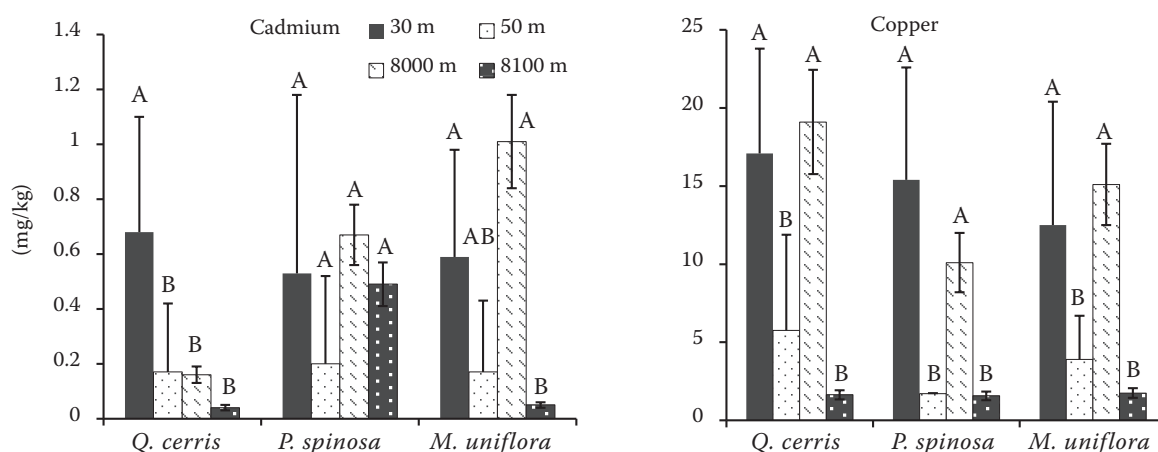


Figure 2. Effect of distance from the road on cadmium and copper content (arithmetic mean \pm standard deviation) in plant species (one-way ANOVA, *Quercus cerris* – Cd: $F_{(3,10)} = 4.155$, $P = 0.037$; *Q. cerris* – Cu: $F_{(3,10)} = 8.885$, $P = 0.0036$; *Prunus spinosa* – Cd: $F_{(3,10)} = 0.871$, $P = 0.488$; *P. spinosa* – Cu: $F_{(3,10)} = 10.394$, $P = 0.002$; *Melica uniflora* – Cd: $F_{(3,10)} = 8.396$, $P = 0.004$; *M. uniflora* – Cu: $F_{(3,10)} = 6.140$, $P = 0.012$)

stands of the R1. In another study, Cd content found in plants was in the range of 0.3–2.9 mg/kg in Hungary (Nasr radi et al. 2004).

Like cadmium, average Cu concentrations in plants were mostly higher within the edges of forest stands and reached several times higher values compared to closed forest stands (Figure 2). At distances of 30 m and 8000 m *Q. cerris* accumulated approximately 3 to 11 times more copper than in the closed forest stands (50 m and 8100 m from the road). *P. spinosa* showed high differences in accumulation of Cu between 30 m and 50 m distance from the road (species received 9 times more of copper in the edge of the forest than in the closed forest stand). From the edges of forest stands (30 m and 8000 m) *Melica uniflora* received approximately 3 to 8.5 times more Cu than of 50 m and 8100 m from the road. In general, uptake and allocation of Cu in plant organs, such as photosynthetic tissues, is high and active (Jasion et al. 2013). Based on the findings, Cu content in the forest edges of 30 m and 8000 m distances was above common values in plants (6–12 mg/kg) and it indicated pollution. In the study of Malinowska et al. (2015), the highest concentration of Cu, regardless of the distance from the road in Poland, was found in *Taraxacum* sp. (14.37 mg/kg), the lowest for *Rumex acetosa* L. (7.55 mg/kg) and *Vicia cracca* L. (8.17 mg/kg).

In the soil of geobiocoenosis G1 (near road) C content was 1.72–2.10 times, Cu 2.27–3.22 times and Cd 1.64–1.77 times higher in comparison with geobiocoenoses G3 and G5 in which the C content in soils was the lowest. According to various authors, excessive values of Cd in contaminated areas remain in the top 30 mm of soil. However, the higher content of element in plants is reflected in the highest transfer coefficient (TC). Better ability to cumulate Cd was observed in plant species from geobiocoenoses G3 (TC *Q. cerris* 1.16; *P. spinosa* 2.40; *M. uniflora* 0.18) and G5 (0.39; 1.65; 2.49) compared to G1 (0.79; 0.17; 2.04), respectively. Also the values of Cu TC of plants from G3 (1.08; 1.06; 96) and G5 (3.13; 1.64; 2.47) were higher, compared to G1 (*Q. cerris* 1.49; *P. spinosa* 0.38; *M. uniflora* 0.58). The explanation consists in different C content in soils and higher Cu fixation by humus, although the TC values can also be influenced by the depth into which the root system penetrates, mainly in trees (fall of element concentration with soil depth).

Heavy metal contamination alters many physiological and metabolic processes in plants. The presented results reflect the combined effect of HM accumulation. The *Chl* content fluctuated in the

range of 143 to 423.3 mg/m² (Figure 3). Significantly lower values were determined in species growing along the road (*Q. cerris* on G1, *P. spinosa* with *M. uniflora* on G3). On other plots, significant differences in the chlorophyll content compared to the control plot were not determined for the studied species. Schützendübel and Polle (2002) state that the decrease in *Chl* content under stress (pollution or temperature stress) may be attributed to either its degradation or to reduced biosynthesis. Heavy metals reduce accumulation of photosynthetic pigments by substituting Mg²⁺ in chlorophyll (Rai et al. 2016). The soil Cu contamination resulted in a decline of photosynthetic pigments (Lin and Jin 2018) and a decrease of P_n in plants. Also Aggarwal et al. (2012) state that heavy metals (such as Hg, Cu, Cr, Cd, and

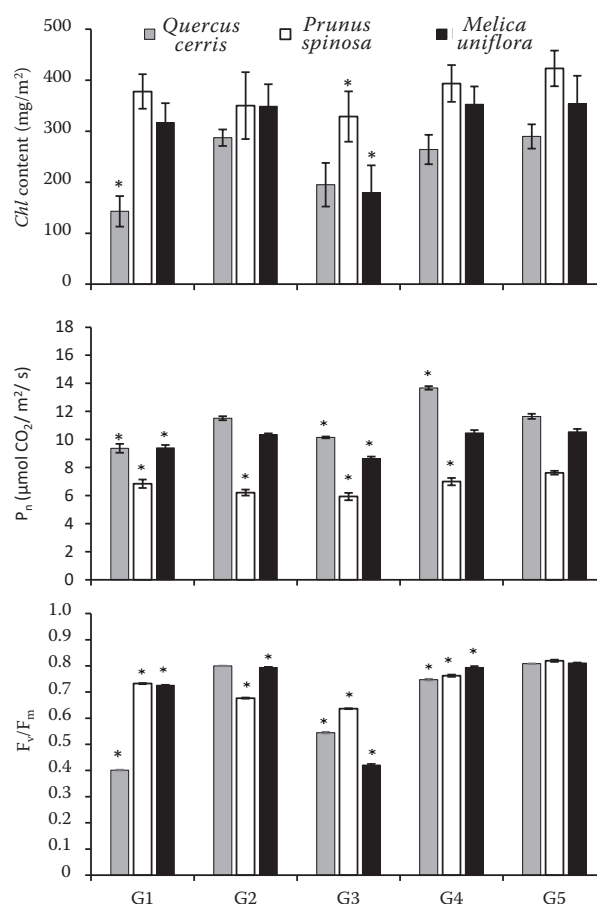


Figure 3. Rate of photosynthesis (P_n), chlorophyll (*Chl*) content and maximum quantum efficiency of photosystem II (F_v/F_m) (arithmetic mean ± standard deviation) at a distance of approximately 30 m from the road (G1–G4) compared to the control variant (G5; 8000 m from the road). *indicates significant differences (P < 0.05) compared to the control variant (G5)

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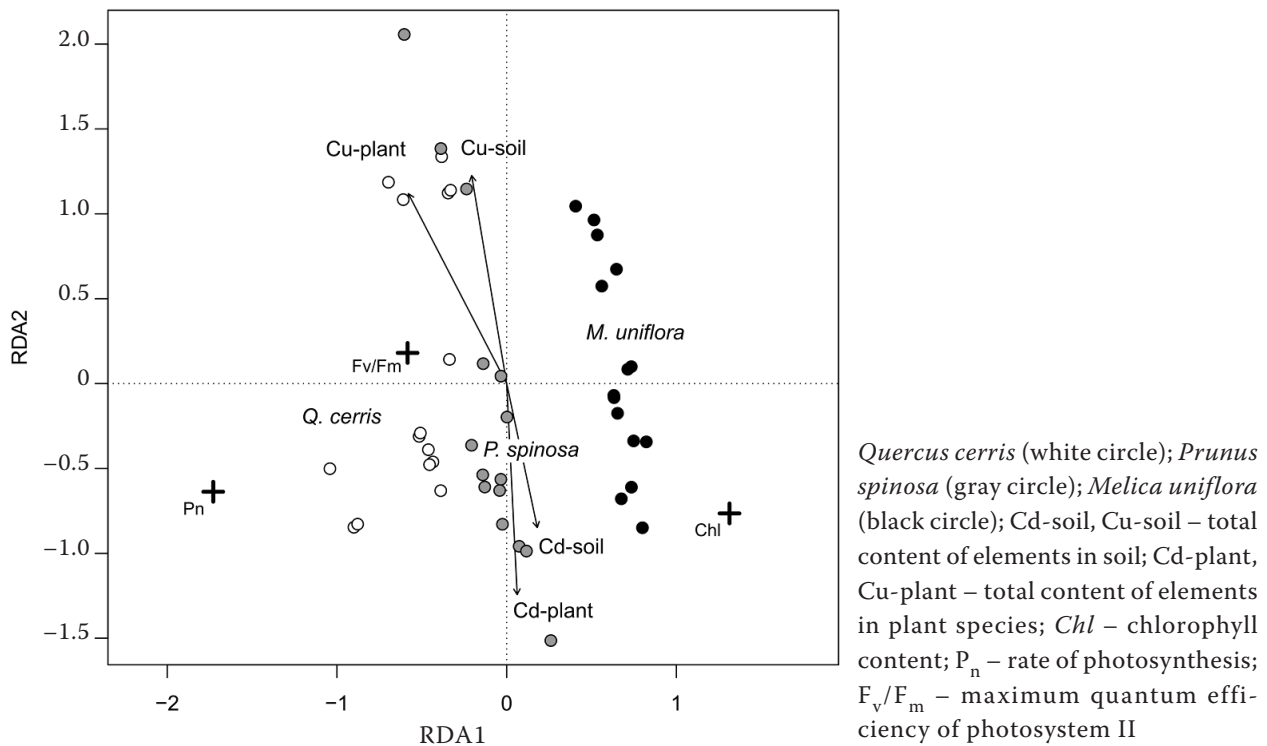
Zn) have been found to decrease the *Chl* content in various plants in most cases.

The P_n was measured and the obtained values are in the range 5.9 to 13.7 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (Figure 3). On plots G1 and G3 (distance of 30 m) for all monitored species, the P_n was significantly lower than that from the control plot. Lower P_n was also measured on G2 and G4 plots in *M. uniflora*. In contrast, *Q. cerris* on G4 had higher P_n values than on the control plot. The photosynthesis is indirectly reduced by heavy metal accumulation in leaves, which influences the functioning of the stomata and hence rate of photosynthesis and transpiration (Aggarwal et al. 2012). The photosynthesis can also be influenced by airborne dust, Bao et al. (2015) state that road dust decreases P_n and total chlorophyll. Lower values of net photosynthetic rates of ten woody species in

response to air pollution in Southern China were presented by Zhang et al. (2013).

Pollutants have a direct effect on plants by affecting PSII and PSI (Rai et al. 2016). Significantly lower values of F_v/F_m in comparison with the control plot were measured at all monitored sites except for *Q. cerris* on G2 (Figure 3). The decline in the value of F_v/F_m reflects the gradual inactivation of PSII, dissipation of a large amount of excitation energy in the form of heat (Dąbrowski et al. 2015), or a blockage of electron transport (Tuba et al. 2010). Kuklová et al. (2015) state that F_v/F_m was also lower (< 0.6) for plants growing on air pollutants-loaded plot in Žiar nad Hronom (Slovakia).

Results of the RDA analysis (Figure 4) showed that variability of *Chl* content, P_n and F_v/F_m values is species-specific ($P < 0.001$), significantly depend-



Variable	<i>df</i>	Variance	<i>F</i>	<i>P</i> -value	Signif. code
Plant species	2	1.2927	18.6692	0.001***	$P < 0.001$
Cd	1	0.0439	1.2670	0.268	ns
Cu	1	0.1228	3.5456	0.017*	$P < 0.05$
Cd	1	0.1161	3.3529	0.028*	$P < 0.05$
Cu	1	0.1089	3.1463	0.033*	$P < 0.05$

Figure 4. Ordination diagram showing the results of ANOVA by the redundancy discriminant analysis (RDA) analysis among average risk concentration values of soils and plants in relation to physiological parameters of plants. ns – not significant

ent on the content of Cd and Cu in study plants ($P < 0.05$) and significantly dependent on the content of Cu in soils ($P < 0.05$). However, *M. uniflora* species responded most sensitively to a decrease of average *Chl* content in plant as a consequence of higher accumulation of Cd in leaf tissues. RDA analysis revealed that explanatory variables mostly play an important role in dispersion of the physiological parameters of plants along the first and second canonical axis. The proportion of the total variance, which is explained by all environmental factors, is 52.7% (0.527). The first axis explained 44%, the second axis 8.7% of the variability of all analysed data.

The results showed that average Cd and Cu concentrations in plants within the forest edges of 30 m and 8000 m distances were mostly above common values in plants and it indicated pollution. The content of Cd in soils revealed an increase of an element mostly in top layers of 30 m from the road. Also Cu points marked enrichment of surface horizons caused by car traffic. The results of RDA showed that the most important environmental variables related to physiological parameters of plants were the content of Cd and Cu in plants and content of Cu in soils. However, *M. uniflora* species responded the most sensitively to a decrease of average *Chl* content in plant as a consequence of excessive accumulation of Cd in leaf tissues. The continuity of such research in terms of the studied and other elements concentrations under conditions of polluted air leads to predicting quality, growth and reproduction of plant species under a range of environmental conditions.

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Received on September 7, 2018

Accepted on December 3, 2018

Published online on January 17, 2018