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## **SPECIFIC LEAF AREA AND RELATIVE WATER CONTENT OF ITALIAN RYEGRASS LEAVES EXPOSED TO HEAVY METALS IN AMBIENT AIR CONDITIONS\***

SPECYFICZNA POWIERZCHNIA LIŚCI I WZGLĘDNA ZAWARTOŚĆ WODY  
W LIŚCIACH ŻYCICY WIELOKWIATOWEJ  
EKSPONOWANEJ NA METALE CIĘŻKIE W WARUNKACH ZEWNĘTRZNYCH

**Summary.** Heavy metals in the air can induce negative effects on vegetation, such as lower growth and development. This in turn can cause a decrease of crop yield and negatively influence protective functions of plants against air pollutant distribution. Biomonitoring of trace elements using higher plants is widely tested. Italian ryegrass is one of the commonly used heavy metal bioindicators, which can be used for evaluation of other effects on plants. Specific leaf area (SLA) and relative water content (RWC) of this species were analysed in the present study. Plants were exposed at five sites differing in environmental conditions. The experiment revealed an increase of leaf thickness due to increased heavy metal concentrations in ambient air, while higher trace element concentrations caused a decrease of relative water content of exposed plants. Overall, based on our results, we can conclude that trace element concentrations in ambient air were high enough to affect *Lolium multiflorum* L. water relations, but too low to influence leaf blade thickness.

**Key words:** relative water content, specific leaf area, lead, arsenic, cadmium, nickel, Italian ryegrass

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## Introduction

The air pollution by heavy metals can affect the physiological status of plants and animals. Particulate matter is the main donor of heavy metals (FARMER 2004). Plants absorb trace elements mainly from the soil, but particulate matter is also an important source (STARCK 2002, SIWEK 2008).

After absorption trace elements can interact in the plant cell in three ways, i.e. either combine with biomolecules or disturb the protein structure or their enzymatic activity stability. This is especially valid for  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  ions. Moreover, trace elements can cause displacement of important metals, which are cofactors in enzymatic proteins. This is the reason for inhibition of the activity of many compounds. The last effect can induce oxidative stress due to reactive oxygen species (ROS) creation and then inhibition of antioxidative enzyme activity. Oxidative stress is a great danger for cell balance, because ROS can affect many biomolecules, such as lipids, proteins and nucleic acids, and further disturb metabolic processes – photosynthesis and respiration (KRZESŁOWSKA 2004).

Moreover, heavy metals can disturb water balance in plants, and as a result lower relative water content can be observed. Heavy metals can cause a decrease of the transpiration process, cell osmotic pressure and xylem water potential (PARYS et AL. 1998). These disturbances are usually connected with negative effects on photosynthesis, biological membranes and enzymatic protein activity (POSCHENRIEDER and BARCELÓ 2004). At a cellular level trace elements can cause cell membrane injuries and blocking of water canals. These processes can cause a water deficit, and in turn a decrease of cell turgor. Due to cell turgor decline, higher abscisic acid (ABA) synthesis can be observed, which is responsible for closing stomata. This mechanism is crucial for decreasing the transpiration process (KRZESŁOWSKA 2004, POSCHENRIEDER and BARCELÓ 2004).

Heavy metals influence absorption, transport and excretion of water in the whole plant. They can cause limitation of root growth and in turn decrease the water uptake from the ground. Thus, the transport of water via stomata can be significantly lower (POSCHENRIEDER and BARCELÓ 2004). The final trace element effect on plants is decrease of their growth. It can be caused indirectly due to imbalance of biomolecule functioning (enzymes, proteins, nucleic acids), changes in metabolic processes (photosynthesis and respiration), changes in cell structure or defence cell response to heavy metals. The direct cause of the plant's decreased growth can be the limited number of created cells, due to imbalance of mitosis and lower frequency of mitotic division of meristematic cells (KRZESŁOWSKA 2004). Moreover, trace elements can disturb the cell elongation process, which is also connected with lower RWC (POSCHENRIEDER and BARCELÓ 2004). The cell elongation process is dependent on cell wall flexibility, cell turgor and proper cytoskeleton structure. Heavy metals disturb cell wall flexibility, as well as decreasing cell turgor (KRZESŁOWSKA 2004).

The water loss and lower plant growth can be an effect of heavy metal occurrence in ambient air. These can have an effect on agriculture and horticulture, as well as on environmental management at a local and regional level, due to plant sensitivity to trace elements. Moreover, some plant species can be used for measurement of heavy metal concentrations in ambient air, as well as for research of plant response to these xenobiotics. Higher plants are especially useful for air pollution biomonitoring, due to lower

growth of mosses and lichens in highly polluted areas (TRACZEWSKA 2011). Ryegrass can accumulate high levels of heavy metals with no visible symptoms of their occurrence. On the other hand, this fodder crop can be a good indicator of potential biomagnification of pollutants in the food chain, and in turn the potential effect on human health and animals. Hence, the bioindication method using Italian ryegrass (*Lolium multiflorum* L.) was determined in the early 1960s in Germany and with modification is still widely used (KLUMPP et AL. 2009). The aim of the present study was to examine the effect of selected trace elements on plant water status and leaf thickness using a well-known bioindicator, for further determination of the potential effect of these elements on other plant species growing in ambient air conditions.

## Materials and methods

### Experimental design

The experiment was carried out during the growing season in 2012. The investigation schedule was provided according to the standardized method of the German Engineering Association (KLUMPP et AL. 2009). Similar amounts of seeds were sown into 5-l pots filled with a standard mixture of peat and sand. Plants were watered with deionized water to avoid the additional application of heavy metals. Moreover, plants were fertilized (according to their needs) during growth in the greenhouse. The last fertilization was at least one day before transport to the exposure site. Whenever plants reached 8-10 cm height, and also one day before exposure, they were cut to 4 cm. After six weeks of cultivation in greenhouse conditions pots with plants were transported to exposure sites. Five sites were selected for these investigations, located in Poznań city and surrounding areas. Sites varied in the air quality characteristics – there were two city sites (site no. 1 and 2), one site in a suburban area (no. 4), one site in an agricultural area (no. 5) and one site located in an agro-ecological landscape park (no. 3) (Fig. 1). Plants were exposed for  $28 \pm 1$  days. Five exposure series were carried out in the year 2012: 14.05-10.06, 11.06-8.07, 09.07-5.08, 6.08-02.09 and 3.09-1.10. Five plants were exposed at every site. A similar set of plants was cultivated in greenhouse conditions as a control (site no. 0). A continuous water supply was conducted through glass fibre wicks placed in pots and in specially constructed water reservoirs with volume ca. 8 l. Hence it was not necessary to check and water plants every week. The construction made it possible to locate plants at 130 cm height above ground at every site, so we obtained comparable results of plant response to air pollution by heavy metals.

### Specific leaf area (SLA) and relative water content (RWC) measurements

Specific leaf area is a parameter informing about leaf thickness. It is the ratio of leaf area to its dry matter weight:

$$\text{SLA} = \text{leaf area/leaf dry matter content (cm}^2 \cdot \text{mg}^{-1} \text{ d.m.)}$$

If the leaf is thicker, the value is lower. Leaf material was taken within the required time schedule – minimum 2-3 h after sunrise and maximum 3-4 h before sunset (GARNIER et AL. 2001). Leaves were weighed directly after sampling, and afterwards leaf

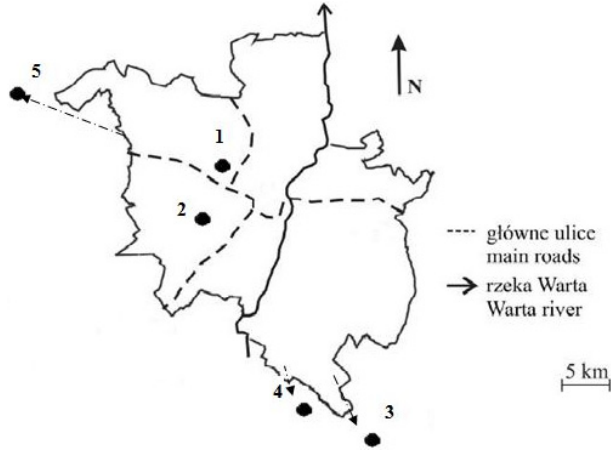


Fig. 1. Location of exposure sites  
Rys. 1. Lokalizacja stanowisk badawczych

area was measured with the aid of a handheld Ci-202 system (CID Bio-Science Inc., USA).

Relative water content was analysed based on the equation (GONZÁLEZ and GONZÁLEZ-VILAR 2001):

$$RWC = (FW - DW)/(TW - DW) \cdot 100 (\%)$$

where:

- FW – fresh weight (g),
- DW – dry weight (g),
- TW – full saturation weight (g).

### Statistical analysis

Results were analysed using one-way analysis of variance with exposure site as the treatment factor. Tukey's test was used to analyse the differences between measured parameters. For this purpose STATISTICA 9.1 software was employed.

## Results

### Trace element concentrations in leaves

One-way analysis of variance revealed a significant effect of exposure site to all measured heavy metals during all experimental series (Table 1). In the case of nickel the results varied. The lowest level was noted at the control site. Comparing exposure sites, the lowest levels were recorded at site no. 3 located in the agro-ecological landscape park (in the 1<sup>st</sup> and 4<sup>th</sup> series), suburban site no. 4 (3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> series), urban site no. 1 (2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> series), urban site no. 2 (2<sup>nd</sup> and 4<sup>th</sup> series) and rural site no. 5 (3<sup>rd</sup> series). The highest level of Ni throughout the entire experimental period was noted during the

Table 1. Results of one-way analysis of variance for SLA, RWC and heavy metals with exposure site influencing the investigated factor in certain exposure series ( $***\alpha \leq 0.001$ ,  $*\alpha \leq 0.05$ )Tabela 1. Wyniki jednoczynnikowej analizy wariancji dla SLA, RWC i metali ciężkich ze stanowiskiem ekspozycyjnym jako czynnikiem wpływającym na badany parametr w poszczególnych seriach ( $***\alpha \leq 0,001$ ,  $*\alpha \leq 0,05$ )

Series Seria	SLA	RWC	Ni	As	Cd	Pb
1	36.8***	10.8***	6.9***	16.6***	20.9***	41.0***
2	18.0***	24.8***	40.6***	569.6***	40.5***	50.4***
3	61.1***	26.0***	12.5***	3.8*	53.2***	31.4***
4	18.2***	38.3***	18.8***	12.2***	32.8***	28.5***
5	85.3***	44.2***	33.2***	52.1***	57.8***	132.0***

2<sup>nd</sup> series at the rural site (no. 5), suburban site (no. 4) and agro-ecological park (no. 3). There were no statistical differences between these sites. The highest level of arsenic was noted in the first three series in plants exposed at the agro-ecological landscape park (no. 3). A comparable level at exposure sites was recorded in the 4<sup>th</sup> series, while in the 5<sup>th</sup> series the highest level was observed in plants exposed at the rural site (no. 5). The cadmium concentrations were the lowest during the 1<sup>st</sup> exposure series. Afterwards higher levels were noted. The highest concentrations after the 2<sup>nd</sup> series were recorded in plants exposed at urban (no. 1), suburban (no. 4) and rural (no. 5) sites. A high level of cadmium was recorded at one of the urban sites (no. 2) in the 3<sup>rd</sup> and 4<sup>th</sup> site, and in the latter one also high levels were recorded at the other urban site (no. 1) and rural site (no. 5). The lowest level in the last series was recorded in the agro-ecological landscape park (site no. 3). Lead concentrations also varied in individual series. The lowest level in the first series was noted at the rural site (no. 5), and the highest in the landscape park and both urban sites, while in the second series almost the opposite situation was noted. The highest level of Pb was noted at the rural site during the 3<sup>rd</sup> series. In the last series the highest Pb concentrations throughout the entire experiment were observed at the rural site (no. 5) (Table 2).

### Relative water content (RWC)

A statistically significant effect of site was observed in all experimental series in RWC (Table 1). The lowest values of RWC were noted at the control site during the 1<sup>st</sup> series, while higher levels were observed at the urban site (no. 1). In the second series exposed plants revealed lower RWC values than control ones. The lowest levels were noted at both urban sites. In the third series results of RWC were higher at the exposure site than the control, while in the fourth series plants exposed at the suburban site and landscape park revealed the lowest RWC level. In the last series plant material taken from the rural site revealed the lowest level of this parameter (Fig. 2).

Table 2. Ni, As, Cd and Pb concentrations in Italian ryegrass leaves collected from the plants exposed on certain sites in five exposure series (means  $\pm$ standard error) ( $\text{mg}\cdot\text{kg}^{-1}$ )Tabela 2. Stężenia Ni, As, Cd i Pb w liściach życicy wielokwiatowej zebranych z roślin ekspozowanych na poszczególnych stanowiskach w pięciu seriach ekspozycyjnych (średnie  $\pm$ błąd standardowy) ( $\text{mg}\cdot\text{kg}^{-1}$ )

Site Stanowisko	Series Seria	Ni	As	Cd	Pb	Series Seria	Ni	As	Cd	Pb
0	1	0.493 $\pm 0.235$	0.011 $\pm 0.004$	0.030 $\pm 0.001$	0.090 $\pm 0.004$	4	0.277 $\pm 0.015$	0.011 $\pm 0.006$	0.030 $\pm 0.002$	0.019 $\pm 0.000$
1		2.835 $\pm 0.209$	0.204 $\pm 0.005$	0.145 $\pm 0.009$	0.975 $\pm 0.057$		2.091 $\pm 0.023$	0.160 $\pm 0.004$	0.348 $\pm 0.005$	1.022 $\pm 0.027$
2		2.950 $\pm 0.212$	0.195 $\pm 0.008$	0.115 $\pm 0.013$	0.952 $\pm 0.053$		2.209 $\pm 0.074$	0.135 $\pm 0.005$	0.323 $\pm 0.012$	1.099 $\pm 0.051$
3		2.281 $\pm 0.218$	0.644 $\pm 0.116$	0.064 $\pm 0.002$	0.783 $\pm 0.060$		2.062 $\pm 0.131$	0.134 $\pm 0.008$	0.248 $\pm 0.010$	0.963 $\pm 0.104$
4		2.646 $\pm 0.249$	0.172 $\pm 0.014$	0.064 $\pm 0.008$	0.729 $\pm 0.046$		2.190 $\pm 0.055$	0.183 $\pm 0.007$	0.208 $\pm 0.016$	0.969 $\pm 0.024$
5		2.413 $\pm 0.021$	0.128 $\pm 0.004$	0.071 $\pm 0.003$	0.545 $\pm 0.030$		2.490 $\pm 0.087$	0.161 $\pm 0.009$	0.314 $\pm 0.023$	0.911 $\pm 0.032$
0	2	0.493 $\pm 0.235$	0.012 $\pm 0.006$	0.030 $\pm 0.001$	0.046 $\pm 0.002$	5	0.493 $\pm 0.024$	0.010 $\pm 0.002$	0.093 $\pm 0.001$	0.020 $\pm 0.007$
1		2.498 $\pm 0.152$	0.080 $\pm 0.002$	0.248 $\pm 0.019$	0.672 $\pm 0.040$		3.261 $\pm 0.057$	0.151 $\pm 0.002$	0.250 $\pm 0.012$	1.002 $\pm 0.041$
2		2.681 $\pm 0.145$	0.065 $\pm 0.004$	0.488 $\pm 0.026$	0.722 $\pm 0.042$		2.278 $\pm 0.108$	0.138 $\pm 0.009$	0.287 $\pm 0.012$	0.921 $\pm 0.017$
3		4.263 $\pm 0.034$	1.179 $\pm 0.049$	0.358 $\pm 0.017$	0.803 $\pm 0.051$		2.199 $\pm 0.067$	0.135 $\pm 0.008$	0.184 $\pm 0.007$	0.784 $\pm 0.046$
4		4.802 $\pm 0.416$	0.121 $\pm 0.015$	0.523 $\pm 0.089$	1.060 $\pm 0.106$		1.994 $\pm 0.069$	0.144 $\pm 0.003$	0.252 $\pm 0.014$	0.901 $\pm 0.037$
5		4.691 $\pm 0.161$	0.122 $\pm 0.004$	0.528 $\pm 0.063$	1.166 $\pm 0.073$		3.361 $\pm 0.117$	0.236 $\pm 0.003$	0.283 $\pm 0.006$	1.681 $\pm 0.068$
0	3	0.577 $\pm 0.015$	0.011 $\pm 0.008$	0.030 $\pm 0.002$	0.020 $\pm 0.006$					
1		2.287 $\pm 0.119$	0.118 $\pm 0.002$	0.306 $\pm 0.012$	0.863 $\pm 0.024$					
2		2.462 $\pm 0.090$	0.100 $\pm 0.005$	0.412 $\pm 0.017$	0.892 $\pm 0.046$					
3		2.901 $\pm 0.266$	0.462 $\pm 0.180$	0.336 $\pm 0.021$	0.894 $\pm 0.055$					
4		1.931 $\pm 0.126$	0.205 $\pm 0.005$	0.216 $\pm 0.009$	0.863 $\pm 0.033$					
5		2.044 $\pm 0.000$	0.137 $\pm 0.007$	0.257 $\pm 0.000$	1.083 $\pm 0.058$					

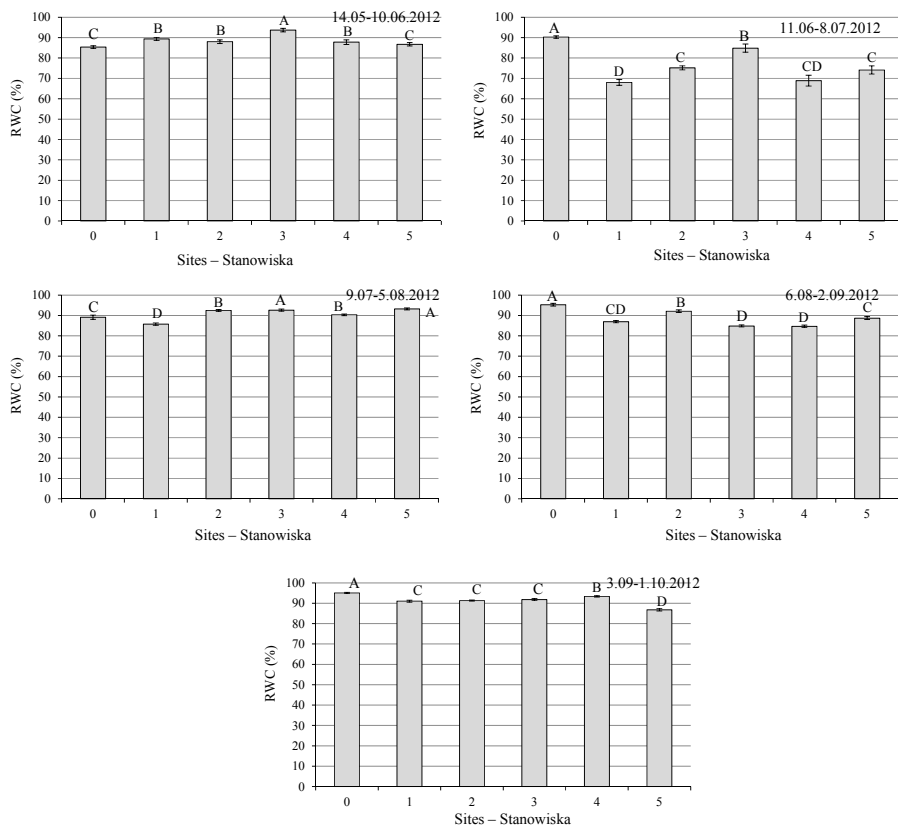


Fig. 2. Relative water content of plants exposed in different series and sites (means  $\pm$  standard error). Different letters denote statistically significant differences at the level of  $\alpha = 0.05$

Rys. 2. Względna zawartość wody w roślinach eksponowanych w różnych terminach i na różnych stanowiskach badawczych (średnie  $\pm$  błąd standardowy). Różne litery oznaczają różnice istotne statystycznie na poziomie  $\alpha = 0,05$

### Specific leaf area (SLA)

One-way analysis of variance revealed a statistically significant effect of exposure series on SLA (Table 1). The highest SLA values were always noted at the control site. The lowest levels of SLA were usually observed at one of the urban sites (no. 1), while the highest (excluding the control) were recorded at the other urban site (no. 2) (Fig. 3).

### Correlations between parameters and heavy metals

Analysis of linear correlations between heavy metals and SLA and RWC revealed almost in every series negative relations between all measured trace elements and both parameters. This means that high heavy metal concentrations are connected with thicker leaves and worsening of the water status of plants (Table 3).

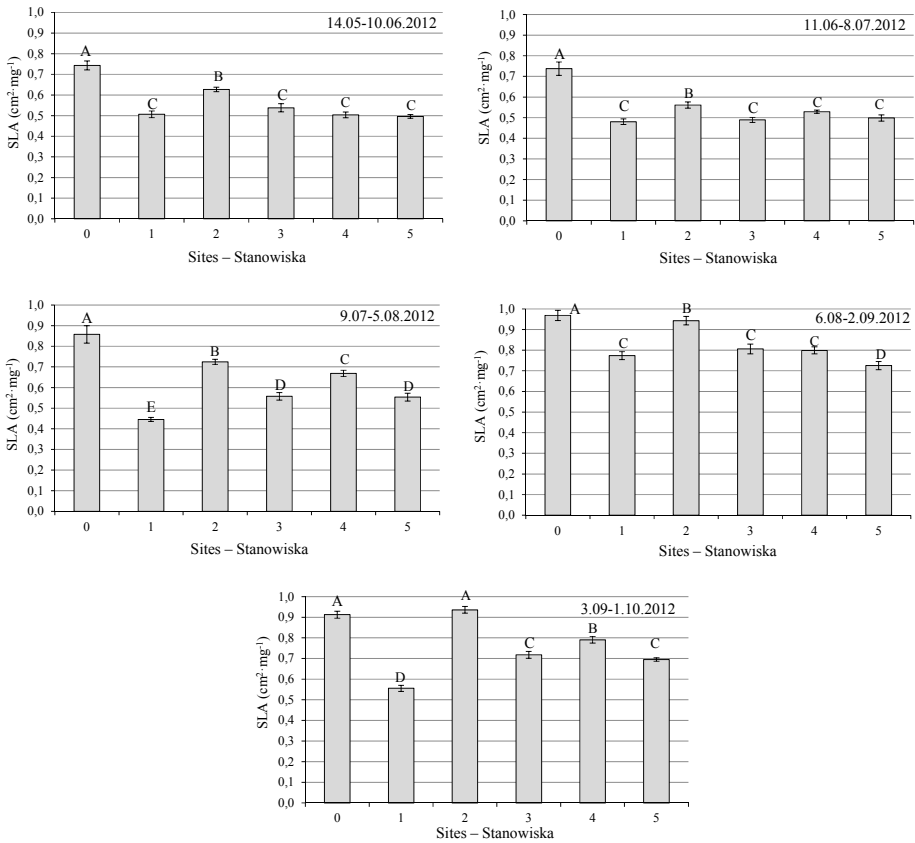


Fig. 3. Specific leaf area of plants exposed in different series and sites (means  $\pm$  standard error). Different letters denote statistically significant differences at the level of  $\alpha = 0.05$

Rys. 3. Specyficzna powierzchnia liści roślin eksponowanych w różnych terminach i na różnych stanowiskach badawczych (średnie  $\pm$  błąd standardowy). Różne litery oznaczają różnice istotne statystycznie na poziomie  $\alpha = 0,05$

Table 3. Linear correlation coefficients and significance levels between heavy metal concentrations in leaves and parameters measured in certain series

Tabela 3. Współczynniki korelacji liniowej i poziomy istotności pomiędzy stężeniami metali ciężkich w liściach a parametrami mierzonymi w poszczególnych seriach

Series Seria	Parameter Parametr	Ni	As	Cd	Pb
1	2	3	4	5	6
1	SLA	$r = -0.618$ $P \leq 0.001$	$r = -0.291$ $P = 0.006$	$r = -0.320$ $P = 0.002$	$r = -0.498$ $P \leq 0.001$
	RWC	$r = 0.039$ $P = 0.720$	$r = -0.315$ $P = 0.003$	$r = 0.108$ $P = 0.320$	$r = -0.017$ $P = 0.875$



Table 3 – cont. / Tabela 3 – cd.

1	2	3	4	5	6
2	SLA	r = -0.622 P ≤ 0.001	r = -0.298 P = 0.003	r = -0.578 P ≤ 0.001	r = -0.643 P ≤ 0.001
	RWC	r = -0.289 P = 0.017	r = 0.361 P = 0.002	r = -0.379 P = 0.001	r = -0.465 P = 0.000
3	SLA	r = -0.493 P ≤ 0.001	r = -0.346 P ≤ 0.001	r = -0.256 P = 0.059	r = -0.539 P ≤ 0.001
	RWC	r = 0.240 P = 0.075	r = 0.276 P = 0.039	r = 0.266 P = 0.047	r = 0.222 P = 0.099
4	SLA	r = -0.411 P ≤ 0.001	r = -0.527 P ≤ 0.001	r = -0.251 P = 0.047	r = -0.275 P = 0.029
	RWC	r = -0.501 P ≤ 0.001	r = -0.687 P ≤ 0.001	r = -0.195 P = 0.139	r = -0.436 P ≤ 0.001
5	SLA	r = -0.702 P ≤ 0.001	r = -0.497 P ≤ 0.001	r = -0.254 P = 0.018	r = -0.473 P ≤ 0.001
	RWC	r = -0.746 P ≤ 0.001	r = -0.771 P ≤ 0.001	r = -0.605 P ≤ 0.001	r = -0.794 P ≤ 0.001

## Discussion

Due to the negative effect of heavy metals on living organisms, their concentrations in ambient air are continuously monitored by many international institutions. There is also increasing concern about evaluation of heavy metal concentration using bioindicators, such as mosses, lichens or higher plants. One of the most interesting plants is Italian ryegrass (*Lolium multiflorum* Lam. ssp. *italicum* var. *Lema*), due to its several properties, such as fast growth and accumulation possibilities (KLUMPP et AL. 2009). This species is widely used for monitoring of highly polluted areas (WEISS et AL. 2004). The active biomonitoring method aims to achieve comparable results, while avoiding the effect of influencing factors, such as different water and fertilizer supply (VDI 3957 PART 2... 2003, WEISS et AL. 2004).

Results for all exposure series revealed the strongest relationship between SLA and Ni, which probably had the highest effect on this parameter. Moreover, an effect on SLA can be connected with particulate matter concentration, which was the lowest during the two last series (data not presented) and these series were characterised by the smallest differences of SLA at exposure sites and the control site. Particulate matter is a donor of heavy metals. There is still limited information about the effect of heavy metals on leaf blade thickness. In the case of barley (*Hordeum vulgare* L. cv. 'Hemus') applied with cadmium ions, an increase of SLA was noted in relation to control plants (VASSILEV et AL. 1998). BARCELÓ et AL. (1988) also found a similar relation for com-

mon bean (*Phaseolus vulgaris* L.). An effect of heavy metals might also be connected with plant age. In other bean species (*Phaseolus coccineus* L.), a decrease of SLA was observed during the first few days of the experiment, while afterwards an increase was noted (SKÓRZYŃSKA-POLIT and BASZYŃSKI 1995). The effect of heavy metals is usually related to their level; hence at lower levels sometimes a positive plant response (faster growth) was noted, while higher concentrations caused growth decrease in comparison to the control (GONZÁLEZ et AL. 2012). This may suggest that heavy metal concentrations in ambient air were not at a sufficient level to affect leaf blade thickness.

A negative heavy metal effect on water relations in plants has previously been widely described (KRZESŁOWSKA 2004, POSCHENRIEDER and BARCELÓ 2004). In the case of barley a decrease of RWC was observed after cadmium application in comparison to the control (VASSILEV et AL. 1998), as well as after application of other trace elements (GONZÁLEZ et AL. 2012). Also in common bean (*Phaseolus vulgaris* L. cv. 'Contender') treated with cadmium a decrease of RWC was noted (POSCHENRIEDER et AL. 1989). On the other hand, DIAS et AL. (2013) found no effect of cadmium application on RWC, and explained this situation as a result of increase of soluble sugar (due to stress conditions), which plays the role of an osmolyte to maintain cell turgor (ROLLAND et AL. 2006). VERNAY et AL. (2007) and HAAG-KERWER et AL. (1999) suggested that lower RWC was observed because plants suffered metal-induced stress regulate water loss through transpiration. Meanwhile HUSSAIN et AL. (2011) found a negative effect of cadmium on maize RWC only for higher concentrations, and they also found that Cd stress might have induced physiological drought in plants by disrupting the plant water relationships, as had been previously found by FAROUK et AL. (2011).

## Conclusions

Our results showed that trace elements in ambient air can cause an increase of leaf blade thickness, which might be a way of plant defence against a stress factor, and also that they were not at too high a level. On the other hand, in most of the exposure series a decrease of relative water content in exposed plants in comparison to control plants was noted, which may indicate a negative effect of heavy metals in ambient air on cell wall flexibility, lower turgor and further problems with cell elongation and plant growth. Overall, we can conclude that trace element concentrations in ambient air were high enough to affect *Lolium multiflorum* L. water relations, but too low to influence leaf blade thickness.

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## SPECYFICZNA POWIERZCHNIA LIŚCI I WZGLĘDNA ZAWARTOŚĆ WODY W LIŚCIACH ŻYCICY WIELOKWIATOWEJ EKSPONOWANEJ NA METALE CIĘŻKIE W WARUNKACH ZEWNĘTRZNYCH

**Streszczenie.** Metale ciężkie wpływają negatywnie na rośliny, powodując ich zmniejszony wzrost. To z kolei może być przyczyną zmniejszonego plonowania roślin uprawnych, jak również osłabienia funkcji ochronnych roślin w stosunku do zanieczyszczeń powietrza. Obecnie powszechnie się testuje biomonitoring pierwiastków śladowych. Jedną z roślin wykorzystywanych jako bioindykator metali ciężkich jest życica wielokwiatowa, która równocześnie może być stosowana jako wskaźnik wpływu tych zanieczyszczeń powietrza na różne reakcje roślin. W przedstawionych badaniach specyficzną powierzchnię liści oraz względną zawartość wody w roślinach. Rośliny eksponowano na pięciu stanowiskach różniących się warunkami środowiskowymi. Badania wykazały zwiększenie się grubości liści wraz ze wzrostem stężenia metali ciężkich w powietrzu. Zanotowano również spadek względnej zawartości wody przy większych stężeniach pierwiastków śladowych. Podsumowując, można stwierdzić, że stężenia metali ciężkich w powietrzu są na poziomie powodującym zaburzenia stosunków wodnych życicy wielokwiatowej, ale nie są odpowiednio duże, by wpłynąć na przyrost blaszki liściowej na grubość.

**Słowa kluczowe:** względna zawartość wody, specyficzna powierzchnia liści, ołów, arsen, kadm, nikiel, życica wielokwiatowa

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