Short Communication

The Growth and Reproductive Effort of *Betula pendula* Roth in a Heavy-Metals Polluted Area

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Abstract

Effects of environmental conditions on the distribution of resources between generative and vegetative reproduction were considered in relation to several theories. Our objective was to study the effects of heavy pollution-induced habitat deterioration on the growth and reproduction of *Betula pendula* Roth trees. The length of vegetative short shoots of the birch, the chlorophyll concentration in leaves, and the reproductive effort were studied for the most polluted site – a zinc-lead dump in Wełnowiec (the district of the city of Katowice) and the control site in the village of Smoleń near the town of Pilica. All the plant samples were collected from 10 microhabitats categorized on the basis of different levels of heavy metal concentrations in the topsoil. The length of the vegetative short shoots was greater than that of the trees growing at the control site. The same increasing tendency was observed in chlorophyll concentrations. The somatic cost of reproduction in *Betula pendula* was higher for the polluted site, presumably owing to both more intensive generative reproduction and resource limitations in the unfavorable environments.

Keywords: zinc-lead dump, *Betula pendula* Roth, germination capacity, generative reproduction

Introduction

Extreme levels of toxic contaminations generally cause adverse effects on whole ecosystems. Heavy metals such as Zn, Pb, and Cd can induce toxicity in all organisms if the soil levels of contaminants reach critical values. The plant accumulation in above-ground tissues can result in increased metal accumulation in the topsoil layer via leaf deposition, or can create an exposure pathway for the introduction of metals into the trophic chain. Plants living in metalliferous wastes can have exceptional properties that make them appropriate for phytoremediation [1]. Several authors have used plant and animal samples to illustrate heavy metals concentrations and their distribution trends in the investigated areas. Research on the speciation of metals, their toxicity accumulations, biomagnifications, and bioindications, conducted in the last decade, have proven the growing interest in these issues [2].

An integrated investigation of the growth and reproduction processes in species that survive in polluted environments is of practical importance in terms of restoration ecology. The effects of environmental conditions on the proportions between generative and vegetative reproduction have been considered in several models [3]. The chances of survival and reproduction depend on the plant biomass and the distribution of energy among fundamental living processes. Life strategies of plants can be treated as the result of a compromise between allocation of energy and the matter into the three main processes: growth, development and reproduction [4]. According to Grime's theory [5], the reproductive allocation should decrease proportion-

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ally to both an increase in the stress level and a decrease in environmental capacity. It means that generative reproduction investment should decrease along with an increase in pollution. Poor quality seeds will result in poor germination and low seedling survival.

Among deciduous trees, *Betula pendula* Roth is regarded as tolerant to the effects of industrial pollution. This species plays an important role in the reclamation of zinc-lead dump areas. Several studies have been reported on birch seed production [6]. However, information about the reproduction strategy and germination capacity of birch seeds collected from trees at a zinc-lead dump is insufficient. Several studies have reported that foliar chlorophyll content is a good indicator of plant stress and hence the potential for plant CO_2 uptake and growth [7]. Monitoring of chlorophyll contents may assist in the management of nutrition and forest health for optimal growth. The non-destructive chlorophyll meter (e.g. SPAD-502) has been used successfully in many species to estimate foliar chlorophyll [8, 9].

Vegetative short shoots of birch can be used as an indicator of plant stress, particularly in studies on pollution impact [10].

The objective of the present study was to determine the effects of the pollution-induced habitat deterioration on the length of short shoots and reproduction of silver birch trees. In addition, we have also measured the total chlorophyll in birch leaves, which is used as an indicator of plant stress.

Experimental Procedures

The study was performed at two sites: polluted and unpolluted. The heavilly polluted site represents the zinc-lead dump in the city of Katowice (the Upper Silesia region, Southern Poland). The neutral pH of the waste material and high concentrtions of heavy metals deserve some attention (Table 1). The unpolluted site is located in the village of Smoleń, 76 km from the city of Katowice. Nine microhabitats were selected on the zinc-lead dump site based on differences in heavy metal concentrations in the topsoil layer. The upolluted site is treated as a control mirohabitat. A number of branches from five 40-year-old birch trees per microhabitat were sampled in all directions in the area around each tree. The quality of birch seeds was tested in terms of the folowing characteristics: germination capacity (the percentage of seeds germinating within 14 days) and germination energy (within 21 days), according to the ISTA protocol. The seeds of the tress growing at the polluted and unpolluted sites had been collected in the vicinity of the sites four times druring the growing season of 2009 (June to September; 1200 seeds per site). 50 vegetative short shoots per birch tree were measured using an electronic slide caliper. 50 randomy selected leaves from vegetative short shoots per tree were tested for total chlorophyll concentrations using the SPAD-502 meter. Samples, which were entirely healthy, fully developed leaves of similar age and crown position, were collected in June and in

Table 1.Chracteristics of study sites.

Characteristic	Sites	
	Polluted	Unpolluted
Coordinates	19º01'13" E	19º40'38" E
	50º17'39" N	50º26'46" N
pH (H_2O)	7.30	4.50
pH (KCl)	6.20	3.99
C [%]	0.02	1.80
Exchangeable nutrients in soil 10-20 cm top layer [mg/kg dw]		
N-NO_3	0.94	132.57
N-NH_4	1.03	29.69
P	9.87	4.90
K	72.31	2.50
Mg	6.38	2.40
Pollution [mg/kg dw]		
Pb	1,468.60	51.86
Zn	40,046.60	76.46
Cd	146.20	0.764
Soil temp. [ºC]	10.3	11.2
Moisture [m^3/m^3]	0.094	0.168

Source of data: Regional Chemical and Agricultural Station in Gliwice.

August 2009. The SPAD-502 chlorophyll meter measures the absorption of light at 650 and 940 nm wavelenghs in order to estimate the chloropyll level. Measurements were taken during two sunny days (PAR=940 Emol/m^2s). The SPAD-502 meter calculates the chloropyll index and needs to be calibrated. For calibration, the same samples of leaves (5 leaf discs, each 6.45 mm in diameter) were used, and chlorophyll (a +b) was extracted using the method described by Arnon [11].

Differences between sampling sites in terms of short shoot lengh, germination energy, and capacity of birch seeds were evaluated by means of the t-Student test based on the data that had been transformed (log and arcsin) in order to obtain a normal distibution of features. The normality of the analyzed features was checked by means of the Shapiro-Wilk test and the homogenity of variance was analyzed using the Levenea test. On the basis of the data from the season of 2009 and from past years (2006-08), a developmental tendency model for germination capacity has been proposed, considering the percentage of germinating seeds from the data colected four times during each season each year. The model should have the following formula:

Table 2. Mean and SD of seed germination capacity and energy of silver birch (data for 2009 vegetative season, 1,200 seeds for each site).

Variables	Sites			
	Polluted	Unpolluted	t-Student	p-level
	Mean (SD)			
Germination capacity	17.23 (5.03)	43.20 (3.15)	9.07	<0.05
Germination energy	11.40 (0.80)	9.80 (0.90)	2.14	<0.05

$$Gc=b_o+b_1T+b_2x_2+b_3x_3+b_4x_4+e_t$$

...where: T – time variable, Gc – germination capacity value, x_i – 0, and 1 value;

- the value of 1 – where 1 is for an observation within a time period.
- the 0 value – for no such observation

Based on the above data, we have estimated the parameters for the regression function.

The two-way ANOVA/MANOVA analysis for the variable the chlorophyll index was performed, where the significance of the effects of two controlled factors on the experiment results was determined. Those factors were the sites and the calendar month in which the samples were taken. All the analyses were carried out by means of Statistica 7.0 PL software.

Results

The length of vegetative short shoots was significantly greater at polluted sites than of those growing at the unpolluted site (t-test; p<0.05). Fig. 1 A shows the means and SD of short shoot lengths for both sites. Table 2 shows the result of germination capacity and germination energy tests for seeds from the two types of sites. The results show a statistically significant difference between the two means for germination capacity for the polluted and unpolluted sites. A reverse relationship has been observed for the germination-energy variable. The results are presented for the whole germination test in 2009. The T-factor for the linear tendency in the regression analysis is positive, but statistically insignificant, which points to the existence of varied tendencies for specific time intervals. On the other hand, the positive b2 factor for the second time interval indicates a significantly increasing tendency. For the remaining time intervals, the tendency is decreasing (Table 3). The values of the chlorophyll index from the highly polluted site were significantly higher than indexes from the unpolluted site (Fig. 1 B). Table 4 shows the result of the two-way ANOVA/MANOVA analysis for the variable chlorophyll index, where the significance of all effects of the two factors is statistically high.

Discussion of the Results

Birch trees affected by pollution usually experience the stress and there are frequent reports on the adverse effects of toxic contamination on plant growth and reproduction. Short shoots' measurements from the highly polluted site were significantly longer than shoots from the unpolluted site (Student's t-test; p<0.05). The present results are comparable with [10] investigations, where the final conclusion was that vegetative short shoots can be used as a bioindicator of city pollution. Birch trees have two types of vegetative shoots: short and long. Within 2 or 3 years, short shoots become long shoots. In heavily polluted environments, the short shoot stage is considerably prolonged. This may be the result of contamination, drought, and photochemical

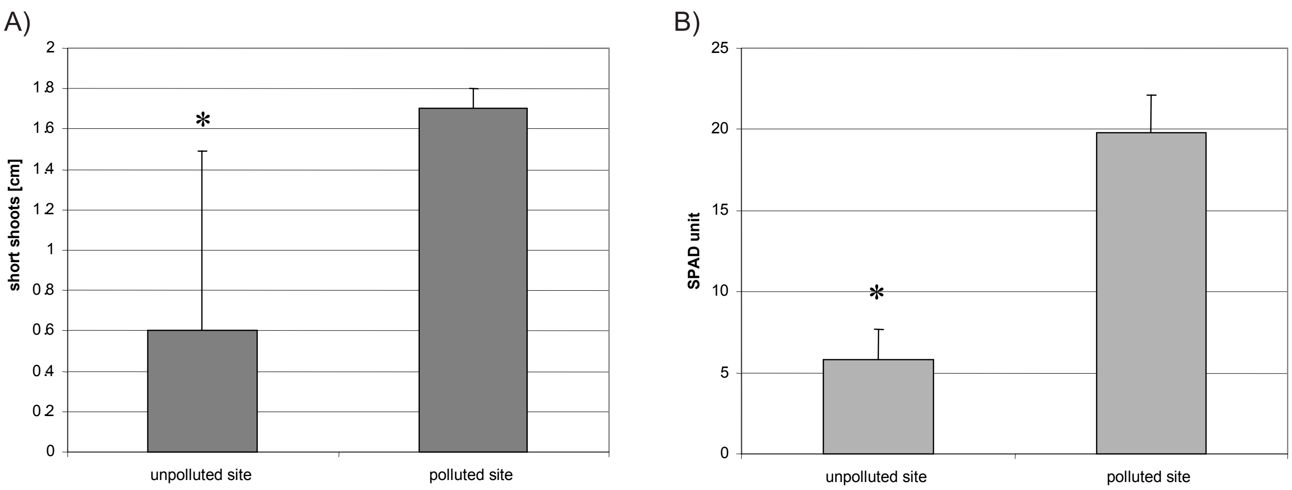

Fig. 1. Mean and SD of birch short shoots A, and SPAD unit B; *p-level <0.05.

Table 3. Parameters of developmental tendency model (explanation in text).

Germination capacity	b_1	b_2	b_3	b_4	Estimation error	R^2
Polluted site	0.34	10.11*	-0.50	-9.91*	3.81	0.781

*p-level <0.05

Table 4. Analysis of two way ANOVA/MANOVA for effects: site and months of collecting data June and August 2009.

Effects	df	MS	F	p-level
Site	3	771.55	109.28	0.001
Month	1	503.69	71.34	0.001
Site x month	3	51.81	7.33	0.001

stress. The long-lasting short shoots (and their increase in length) prevent regular long shoot development and cause tree crown transparency and, consequently, a decrease in longevity [10].

A decrease in total chlorophyll concentrations, proportional to the increase in the pollution load, was demonstrated for all birch trees on the zinc-lead dump in comparison with the unpolluted site. However, it is too early to conclude that chlorophyll concentrations in leaves depend on the levels of heavy metals in the topsoil layer. Environmental factors such as water deficit and high temperature are known to affect leaf morphology, which in turn affects the foliar optical properties and can be expected to influence the chlorophyll index. [12] found a strong correlation between the chlorophyll index and specific leaf weight, as well as the ratio of fresh area:dry mass. The differences for the chlorophyll index between the birch population from the polluted and unpolluted sites, observed in this research, may have been a function of differences in the adaptation to specific environments.

In heavily polluted microhabitats, generative reproduction is hampered by high soil toxicity [13]. In this situation, where the value of both vegetative and generative reproduction for plant populations is low, the benefit-cost model predicts the increased allocation of resources to generative reproduction, because seeds can disperse better in good habitat conditions' [14]. In the present results, the T-factor for the linear tendency in regression analysis is positive, which means the existence of varied tendencies for specific time intervals. Furthermore, the strong impact of pollution does not cause a decrease in seed vitality (germination capacity at 86% for the polluted site and 55% for the unpolluted area, with $p<0.001$ in July – unpublished data). The fitness of plants during colonization of new habitats may be manifested as an allocation of a greater amount of biomass to reproduction.

Conclusions

Short shoots' measurements from the highly polluted site were significantly longer than shoots from the unpolluted site.

The decrease in total chlorophyll concentrations proportional to the increase in pollution load was demonstrated for all birch trees on the zinc-lead dump in comparison with the unpolluted site.

The increased allocation of resources to generative reproduction in heavily polluted sites may indicate the plant strategy to disperse from contaminated areas.

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References

1. OSUMI K., SAKURAI S. The unstable fate of seedlings of the small-seeded pioneer tree species, *Betula maximowicziana.* For. Ecol. Manage. **160**, 85, **2002**.
2. SZYCZEWSKI P., SIEPAK J., NIEDZIELSKI P., SOBCZYŃSKI T. Research on heavy metals in Poland. Pol. J. Environ. Stud. **18**, (5), 755, **2009**.
3. LOEHLE C. Partitioning of reproductive effort for clonal plants: A benefit-cost model. Oikos **49**, 199, **1987**.
4. ERANEN J., KOZLOV M. Competition and facilitation in industrial barrens: Variation in Performance of mountain birch seedlings with distance from nurse plants. Chemosphere **67**, 1088, **2007**.
5. KOZLOV M., HAUKIOJA E. Performance of birch seedlings replanted in heavily polluted industrial barrens of the Kola Peninsula, Northwest Russia. Restor. Ecol. **7**, 145, **2007**.
6. GRIME J. Competitive exclusion in herbaceous vegetation. Nature **242**, 344, **1973**.
7. DATT B. A new reflectance index for remote sensing of chlorophyll content in higher plants: tests using *Eucalyptus* leaves. J. Plant Physiology **154**, 30, **1999**.
8. PINKARD E, PATEL V, MOHAMMED C. Chlorophyll and nitrogen determination for plantation-grown *Eucalyptus nitens* and *E. globulus* using non-destructive meter. For. Ecol. Manage. **223**, 211, **2006**.

9. CASTELLI F., CONTILLO R., MICELI F. Non-destructive determination of leaf chlorophyll content in four crop species. J. Agron. Crop. Sci. **177**, 275, **1996**.
10. SAMECKA-CYMERMAN A., KOLON K., KEMPERS A. Short shoots of *Betula pendula* Roth as bioindicators of urban environmental pollution in Wroclaw (Poland). Trees **23**, 923, **2009**.
11. ARNON D. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgari*s. Plant Physiology, **24**, 1, **1949**.
12. THOMSON J., SCHWEITZER L., NELSON R. Association of specific leaf weight, an estimate of chlorophyll, and chlorophyll concentration with apparent photosynthesis in soybean. Photosynth. Res. **49**, 1, **1996**.
13. DIMITRIOU I., ARONSSON P., WEIH M. Stress tolerance of five willow clones after irrigation with different amounts of landfill leachate. Biores. Technol. **97**, 150, **2006**.
14. VALKAMA J., KOZLOV M. Impact of climatic factors on the developmental stability of mountain birch growing in a contaminated area. J. App. Ecol. **38**, 665, **2001**.