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Pollen grain bioassay for environmental contamination biomonitoring

Biomonitoring polucije okolja z analizo poškodovanosti pelodnih zrn

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Abstract. Pollen grain bioassay near some highways was performed in 45 plant species for identification of environmental genotoxic impact on naturally growing plants due to environmental traffic contaminants. A relationship between the duration of heavy traffic on particular road sections and the degree of developmental and morphological changes of pollen grains was indicated. Increased frequency of pollen deformation after more than 20 years of traffic influence was established, indicating the applicability of pollen grain bioassay for genotoxic risk assessment of chronic low level contamination impact in natural habitats.

Key Words: naturally growing bioindicator plants, traffic contaminants, pollen grain deformation

Izvleček. V biomonitoringu onesnaženosti okolja zaradi prometa smo analizirali stopnjo poškodovanosti pelodnih zrn pri 45 vrstah rastlin z naravnih rastišč ob naših avtocestah. Ugotovili smo, da je stopnja poškodovanosti peloda na poskusnih lokalitetah povezana s trajanjem prometa na posameznih avtocestnih odsekih, povečan obseg poškodovanosti peloda je bil pri biondikatorskih rastlinah na rastiščih, obremenjenih z vplivi prometa več kot 20 let. Ti rezultati kažejo, da je analiza poškodovanosti pelodnih zrn uporabna za sledenje kroničnih vplivov nizkih nivojev zračne polucije z genotoksičnimi agensi in oceno ogroženosti rastlin na naravnih rastiščih.

Ključne besede: samonikle bioindikatorske rastline, onesnaževanje z izpušnimi plini, poškodovanost pelodnih zrn.

Introduction

Environmental contamination caused by highway traffic has been identified as a serious ecological problem. Air contaminants produced by motor traffic and released along the highways and main

road connections are particularly hazardous to public health and the environment. Low concentrations of many environmental pollutants are considered as harmless, but for the genotoxic contaminants there is no safe dose or concentration, and environmental hazard identification is required. Naturally growing plants are exposed to a changing mixture of environmental contaminants, and when used as bioindicators of long lasting exposure to complex pollution impact they have a key role in genotoxic risk evaluation of low contamination levels in natural habitats.

Our previous observation in plant natural habitats with elevated contamination load (PARADIŽ & LOVKA 1998, 1999a, 1999b, 2000) and literature data (KORMUTAK 1996, MIČIETA & MURIN 1996, MULCAHY 1981, MURIN 1995) suggest that with many different species, plant bioassay results could give a more complex information about the pollution threats in the environment. Using 45 species of naturally growing plants in pollen grain bioassay near some highways in Slovenia, we have performed screening of genotoxicity for risk identification of environmental impact of heavy traffic in natural habitats.

Material and Methods

Experimental sites near some highways in Slovenia, concerning the duration of traffic on the specific road sections were chosen for biomonitoring with pollen grain bioassay. At 8 localities (Bizovik, Arja vas, Vransko, Čebulovica, Divača, Dolenja vas, Zajčica, and Sela) plants in their habitats had been exposed to traffic influence for the period up to 10 years, at 2 localities (Povodje and Brdo) for 10 to 20 years, and at 5 localities (Gabrje, Ivanje selo, Ravnik, Trebnje and Žiče) for 20 to 30 years. Localities at Komen, Predmeja, Pugled, and Škofja Loka with no obvious traffic pollution sources were used for the comparison.

For pollen grain bioassay flower buds were sampled in 45 species of plants growing naturally at experimental and control sites (Tab. 1) on 18th, 24th, and 31st of March, 7th of April, 19th and 26th of May, 2nd, 9th, 17th and 23rd of June 1999. The samples were fixed in ethanol acetic acid mixture (3:1), then kept at –20°C in a refrigerator.

Microscopic slides of fully developed anthers were made by staining in aceto–carmine. Generally, five slides from five different flowers per bioindicator species at the site were examined. In at least 300 pollen grains per slide, stained (viable) and empty (sterile) grains, as well as pollen size and shape deformations were observed under an optical microscope. Deformation frequencies of pollen grains, as well as portions of morphological changes in relation to the duration of highway traffic for up to 10 years, for 10-20 years and for 20-30 years are presented.

Results

In naturally growing bioindicator plants at the control sites, over 95% of stained (fertile) pollen grains were observed. In plants at experimental sites near highways, deformation frequency of pollen grains increased with the duration of traffic influence. The frequency of empty (sterile) grains increased up to 10% after 30 years of traffic influence (Fig. 1). Smaller size (for about 1/3 of the normal one) as well as shape deformations were less frequent than empty not stained grains, however, their portion increased with the duration of highway traffic as well (Tab. 2).

Table 1. List of bioindicator species for pollen grain bioassay within sampling periods from March to June at experimental sites near highways and the control sites. Duration of traffic influence for particular road sections in brackets is stated.
Tabela 1. Seznam rastlinskih vrst vključenih v analizo poškodovanosti pelodnih zrn od marca do junija na poskusnih lokalitetah ob odsekih avtocest in kontrolnih lokalitetah. V oklepajih je navedeno trajanje prometa na posameznih odsekih avtocest.

Sampling	Bioindicator plants	Experimental and control sites
March	*Anemone nemorosa* L.	Bizovik (1), Arja vas (2) , Trebnje (30)
	Capsella bursa-pastoris (L.) Med.	Arja vas (2)
	Cornus mas L.	Komen, Žiče (23)
	Corydalis cava (L.) Schweigger & Koerte	Komen
	Corylus avellana L.	Povodje (14)
	Crocus albiflorus Kit. ex Schult.	Dolenja vas (4), Gabrje (25)
	Crocus napolitanus Mord. & Loisel.	Bizovik (1), Povodje (14), Brdo (18), Trebnje (30)
	Crocus variegatus Hoppe & Hornsch.	Komen
	Daphne mezereum L.	Trebnje (30)
	Erythronium dens-canis L.	Brdo (18)
	Galanthus nivalis L.	Komen
	Hacquetia epipactis (Scop.) DC.	Ravnik (27)
	Helleborus niger L.	Pugled
	Helleborus odorus W. & K. ex Willd.	Pugled, Dolenja vas (4)
	Lamium purpureum L.	Arja vas (2)
	Leucojum vernum L.	Povodje (14)
	Omphalodes verna Moench	Ravnik (27)
	Oxalis acetosella L.	Arja vas (2)
	Polygala chamaebuxus L.	Pugled
	Potentilla erecta (L.) Räuschel	Čebulovica (4)
	Pulmonaria officinalis L.	Povodje (14), Žiče (23), Ivanje selo (27)
	Symphytum officinale L.	Žiče (23)
	Tussilago farfara L.	Vransko (2), Divača (4)
	Vinca minor L.	Vransko (2)
April/May	*Campanula rapunculus* L.	Sela (7)
	Crocus napolitanus Mord. & Loisel.	Trebnje (30)
	Erythronium dens-canis L.	Sela (7), Povodje (14), Brdo (18)
	Lamium orvala L.	Povodje (14)
	Lamium purpureum L.	Bizovik (1), Povodje (14)
	Melittis melissophyllum L.	Pugled
	Myosotis sylvatica Ehrh. ex Hoffm.	Povodje (14), Brdo (18)
	Oxalis acetosella L.	Trebnje (30)
	Polygala chamaebuxus L.	Pugled
	Potentilla erecta (L.) Räuschel	Pugled
	Ranunculus acris L.	Bizovik (1)
	Ranunculus repens L.	Sela (7), Povodje (14)
	Symphytum officinale L.	Bizovik (1)
	Trifolium pratense L.	Sela (7)
	Trifolium repens L.	Bizovik (1)
	Vinca minor L.	Sela (7)
June	*Calamintha grandiflora* (L.) Moench	Ravnik (27)
	Campanula rapunculus L.	Škofja Loka
	Coronilla emeroides Boiss. & Sprun.	Vransko (2)
	Coronilla varia L.	Komen
	Dianthus sanguineus Vis.	Komen
	Erigeron annuus (L.) Pers.	Škofja Loka

Coronilla emeroides Boiss. & Sprun.	Vransko (2)
Coronilla varia L.	Komen
Dianthus sanguineus Vis.	Komen
Erigeron annuus (L.) Pers.	Škofja Loka
Filipendula vulgaris Moench	Komen
Geranium sylvaticum L.	Ravnik (27)
Lilium martagon L.	Gabrje (25)
Lysimachia nummularia L.	Škofja Loka
Muscari racemosum (L.)Mill. em. Lam. & DC.	Komen
Potentilla erecta (L.) Räuschel	Škofja Loka
Ranunculus acris L.	Predmeja, Škofja Loka, Arja vas (2)
Ranunculus bulbosus L.	Dolenja vas (4), Zajčica (4)
Ranunculus repens L.	Gabrje (25)
Sanicula europaea L.	Predmeja
Symphytum officinale L.	Arja vas (2)
Valeriana officinalis L.	Divača (4)

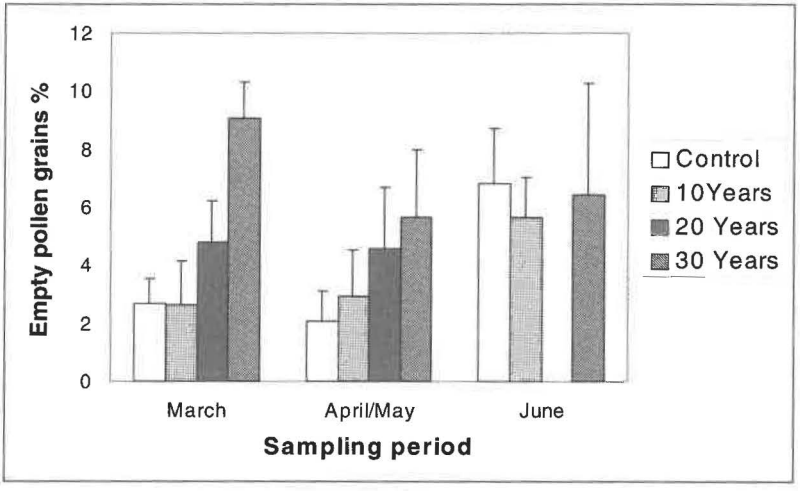

Figure 1. Pollen grain bioassay in naturally growing plants at experimental sites near highways and the control sites.
Slika 1. Poškodovanost peodnih zrn pri naravno rastočih rastlinah na poskusnih lokalitetah ob avtocestah in kontrolnih lokalitetah.

Table 2. Portion (%) of size (small) and shape deformations of pollen grains in naturally growing plants near highways and the control.
Tabela 2. Delež (%) malih in deformiranih pelodnih zrn pri naravno rastočih rastlinah ob avtocestah in kontroli.

Traffic influence	March			April/May			June		
	Size	Shape	Nr.	Size	Shape	Nr.	Size	Shape	Nr.
Control	7,2	0,1	7	4,2	0,0	3	3,4	0,1	11
10 Years	5,6	4,1	10	5,0	3,2	9	13,3	1,8	5
20 Years	9,5	2,0	5	4,5	11,7	5			
30 Years	8,7	11,8	9	1,5	1,7	2	4,6	6,3	4

Nr. – Number of bioindicator species used in the pollen grain bioassay

Discussion

The genotoxic risk of environmental contamination due to increasing traffic density and enlarged highway network in our country is of concern to both public health and the environment. Plants in their habitats are exposed to a broad spectrum of ecological and pollution risks, and they as bioindicators of long lasting exposure to genotoxic effects in the environment are used in biomonitoring pollution in view of environmental risk assessment (Bessonova & al. 1996, Gichner & Veleminsky 1999, Jabeen & Abraham 1996, Kordyum & Sidorenko 1997, Monarca & al. 1999, Müller & al. 1991, Paradiž & Lovka 1998, 1999a, 1999b, 2000).

In genotoxic risk identification, reduced pollen viability is one of the serious pollution impacts on reproductive capacity of plants that might link to ecological consequences. When the amount of stained pollen grains decrease less than 10%, pollen fertility of warm season species is not seriously affected (Tedesco & al. 2002), but under normal climatic conditions pollen abortion in plants is less than 5% (Murin 1995). This is in agreement with our results in bioindicator plants; the average frequency of 3% of empty (sterile) pollen grains at the control sites was established.

At experimental sites near highways, the frequency of empty pollen grains (generally due to irregularities during meiosis) in bioindicator plants increased with the duration of traffic to 10% after 30 years of traffic influence (Fig. 1), as well as the portion of size and shape deformations (Tab. 2). These results indicate that the genotoxic impact of long lasting environmental pollution might be related with traffic contaminants.

When naturally growing plants for biomonitoring genotoxicity of pollutants are used, synergy with other environmental factors, such as meteorological conditions could not be excluded. With successive sampling times at experimental sites near highways, the influence of extreme temperature or drought in summer on the results (Fig. 1) could be verified. But generally, prolonged sampling period for pollen grain bioassay is needed because of blooming season of various plant bioindicators.

On the basis of the present results, pollen grain bioassay for continuous biomonitoring could be suggested, in addition to screening of detailed cytogenetical damage in plants, as well as the routine measurements of atmospheric contaminants for risk assessment of traffic pollution impact in the environment.

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Povzetek

Ugotavljali smo vpliv škodljivih onesnaževalcev prometa na stopnjo poškodovanosti pelodnih zrn pri naravno rastočih rastlinah ob avtocestah. V raziskavo smo vključili 45 rastlinskih vrst in jemali cvetne popke od marca do julija za opazovanje sprememb v razvoju in obliki pelodnih zrn. Vzorčili smo na lokalitetah: Bizovik, Arja vas, Vransko, Čebulovica, Divača, Dolenja vas, Zajčica in Sela z vplivi prometa do 10 let; Povodje in Brdo z vplivi prometa 10 do 20 let; Gabrje, Ivanje selo, Ravnik, Žiče in Trebnje z vplivi prometa 20 do 30 let. Za primerjavo rezultatov smo uporabili lokalitete, oddaljene od glavnih prometnih cest (Komen, Predmeja, Pugled in Škofja Loka).

Na poskusnih lokalitetah ob posameznih odsekih avtocest, kjer se odvija promet več kot 20 let smo ugotovili povečano stopnjo poškodovanosti pelodnih zrn pri naravno rastočih rastlinah. To kaže na uporabnost analize poškodovanosti pelodnih zrn za ugotavljanje dolgotrajnega vpliva zračne polucije na rastline v naravnem okolju. Z nadaljevanjem tovrstnih analiz bi doprinesli rezultate o škodljivih vplivih nizkih nivojev kontaminacije za oceno stopnje ogroženosti rastlin obremenjenih rastišč.

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