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Active biomonitoring of heavy metal pollution using *Rosa rugosa* plants

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Rosa rugosa leaves and pollen are − to different extents − suitable monitoring tools for heavy metal pollution.

Abstract

The purpose of this work was to evaluate the quality of a rural area near Faenza (Italy) by using an active biomonitoring approach, i.e., by placing homogeneous individuals of the perennial shrub *Rosa rugosa* in different sites throughout the area. Further sites, within the city or its environs, were used for comparison. Soil and leaves of *R. rugosa* were analyzed for their heavy metal content. The total heavy metal pattern of leaves closely paralleled the pattern registered in soil, with the highest content (both in total and assimilable forms) at the site in the urban area, which is exposed to heavy traffic. Pollen quality (abortiveness and viability) was also tested as a potential indicator of pollution. Pollen abortiveness was strictly related to Pb levels in leaves, while viability was inversely related to leaf Cr content. Our results suggest that *R. rugosa* has the potential to be a good biomonitoring system.

Keywords: Biological monitoring; Trace elements; Pollen quality; *Rosa rugosa*

1. Introduction

Biological monitoring within a quality control programme involves the systematic use of living beings for obtaining quantitative information on changes in the environment, often due to anthropogenic activities (Bargagli, 1998). Biological responses can be considered more representative than data supplied by chemical or physical detectors, in that they are spatially and temporally extensive; moreover, they allow for estimating both the levels of pollutants and, even more important, the impact on biological receptors. Lower or higher plants can act as bioindicators, biomonitors and bioaccumulators (Knasmüller et al., 1998; Markert et al., 1999; Skelly, 2003; Chandra and Kulshreshtha, 2004). Generally, the responses of vegetative structures are examined at a variety of scale levels, from molecular to population or community rank (see for example Krupa et al., 1993; Antognoni et al., 1995; Cislaghi and Nimis, 1997; Citterio et al., 2002; Madejóng et al., 2004; Manning and Godzik, 2004; Tomašević et al., 2005). Nevertheless, a given pollution level may not be sufficient to elicit a response from the vegetative structures, while heavily affecting the overall reproductive potential of plants (Bergweiler and Manning, 1999). Indeed, pollen seems to be an exceptionally fine indicator of environmental pollutants. Pollen quality, which can be tested by several methods, is in fact controlled by a large number of genes and even

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any small deletion in virtually any part of the genome will induce pollen abortion (Mulcahy, 1981). However, the use of pollen, the male haploid generation of higher plants, is relatively less frequent (Bosac et al., 1993; Gottardini et al., 2004; Klumpp et al., 2004; Mišík et al., 2007).

Our study represents an initial investigation aimed at monitoring a rural area near the city of Faenza (Ravenna, Italy) called 'Oriolo dei Fichi' park. An active monitoring approach was tried, by placing plants of *Rosa rugosa* in four sites within the park, and in two other sites located in or near the urban area for comparative purposes. Among a number of species grown in public or private gardens of the territory, *R. rugosa* was chosen because of its plagiotropic-oriented leaves with quite large surface, thin cuticle layer, and prolonged flowering period. Moreover, it is easy to grow, pest- and drought-resistant. Furthermore, we took advantage of our previous experience in managing pollen of Rosaceae for either laboratory or open field trials (Calzoni et al., 1979; Calzoni and Speranza, 1998). Concentration of trace elements was assayed in both soil and *R. rugosa* leaves, in order to obtain information on the quality of the environment. Moreover, we investigated the possible relationship between heavy metals and *R. rugosa* pollen quality. We used the Alexander stain for a cytological characterization, i.e., to assess the presence or absence of cytoplasm as a measure of pollen abortiveness (Alexander, 1969; Ebel et al., 2004). Furthermore, by using the fluorochromatic reaction, we assayed a crucial feature of the grains, i.e., the integrity of plasmalemma in the vegetative cell (Heslop-Harrison and Heslop-Harrison, 1970). This characteristic gives information on pollen quality at the functional level also: indeed, this test is highly predictive of germination ability, that is, of whether the production of a pollen tube carrying the male gametes to the female partner for the fertilization process can occur (Heslop-Harrison et al., 1984). It should be underlined that quantitative or qualitative aspects of male function, and their changes due to pollution stress, are highly significant for environmental concerns, as they have an obvious impact on the reproductive success of plants.

2. Materials and methods

2.1. The study area and monitoring sites

Our study was performed within the context of an European Project (T.O.R.R.E., Organized Tourism Recover Rural Ecology) aimed at the environmental and touristic promotion of an area near the city of Faenza (Ravenna, Italy) called 'Oriolo dei Fichi'. Monitoring stations consisting of flowerbeds were set up in six different sites characterized by a loamy/silt–loamy soil: physical and chemical soil parameters are shown in Table 1. On the whole, the sites scattered in an area of about 50 km^2. Sites numbered 1–4 were inside the 'Oriolo dei Fichi' park, where many environment-friendly agritourism establishments and farmlands are present. Monitoring site no. 5 was located near the urban area, along (but about 40 m above) a heavily trafficked road. Monitoring site no. 6 was inside the urban area, at the crossroads between two heavily trafficked roads.

As for the bioclimatic condition, all the six sites lied in the transition area between the Central European Vegetation region and the Mediterranean one (Ellenberg, 1988). The climate diagram according to Walter and Lieth (1967) shows an arid period from May to July (113 mm as total rainfall, Fig. 1), while spring and autumn are the rainy seasons, with the highest rainfall (290 mm as a total, Fig. 1) in autumn.

Table 1
Physical and chemical soil characteristics of the six sites

Sites	Sand (%)	Silt (%)	Clay (%)	pH	Total limestone (%)	Cationic exchange capacity (meq/100 g)
1	28	56	16	8.13	16.0	26
2	26	63	11	8.09	15.8	29
3	28	50	22	7.95	8.5	31
4	29	60	11	8.44	19.0	24
5	33	48	19	7.89	8.0	29
6	30	57	13	8.34	11.7	36

2.2. Flowerbeds and plant material

Each flowerbed extended for about 16 m^2. The soil was broken up to a depth of about 40 cm, rototilled, and remixed with natural fertilizer, using the same procedure for each flowerbed. In February, the perennial shrub *Rosa rugosa* Thunb. was introduced into the flowerbeds; 16 individuals per site – which were randomly picked from a group of plants of near isogenic lines and at the same developmental stage – were planted in full sunlight. Afterwards, for the entire experiment time, no other care was given, except for the manual removal of weeds.

2.3. Heavy metal determination

At the time of planting and the following year, three distinct samples of soil from each flowerbed were randomly collected (0–30 cm depth) and independently processed for heavy metal content determination. After drying at 40 °C to a constant weight, samples were mineralized in order to determine trace elements except for Hg. To determine the total metal content, 1.5 g portions of the soil samples were digested at 95 °C (Digiprep, SCP Science) with aqua regia (3:1 HCl/HNO_3) and 30% H_2O_2, respectively, then filtered on Whatman 42 filter paper, diluted with demineralized water, and subjected to analysis. Cr and Ni were determined by an inductively coupled plasma atomic emission (ICP-AES) spectrophotometer (Vista-MPX, Varian) equipped with an autosampler (SPS-3, Varian). Pb and Cd were determined by Zeeman GTA-AAS in a Zeeman graphite furnace (SpectrAA220 Zeeman, Varian) equipped with an autosampler (PSD97/100, Varian). Hg was determined directly without acid digestion of samples by atomic absorption spectrometry using an Advanced Mercury Analyzer (AMA-254, Altec) equipped with an autosampler (ASS-254, Altec). In order to determine the available metal

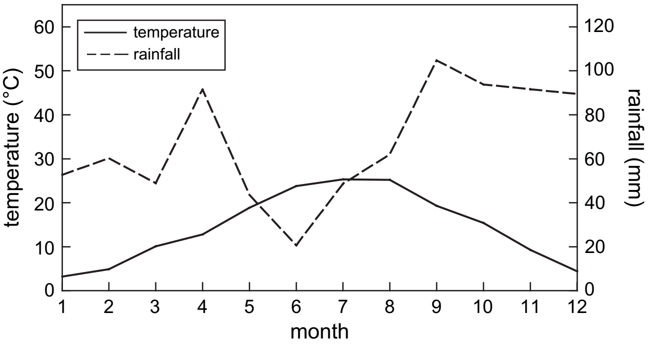

Fig. 1. Climate diagram over the period 2001–2005. Data referred to the left *Y* axis represent means from average °T/day of each month over the above period. Data referred to the right *Y* axis represent means from total rainfall per month over the above period.