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Rosa for the Environment

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ABSTRACT

Recently, increasing attention is being paid to the use of ornamental plants within the context of environmental technologies such as biomonitoring and phytoremediation. Different aspects concerning the potential of *Rosa* plants in this context have been reviewed here. The tolerance of a number of *Rosa* species towards critical environments and their usefulness as active biomonitors of some metals originated from either soil or air sources have recently been found. *Rosa rugosa* pollen was demonstrated to be an effective and more precocious indicator of environmental pollutants than vegetative plant portions. Moreover, *Rosa* flowers and seeds are efficient as biosorbents in removing metals from contaminated water, thus representing low cost biomasses available for an environmentally-friendly use. These studies demonstrate that a wide array of significant benefits may be derived from ornamental horticultural products, in addition to the fact that they represent a useful resource for people's psychological well-being and recovery from stress.

I am able to enumerate a Catalogue of native Plants, and such as are familiar to our Country and Clime, whose redolent and agreeable Emissions would even ravish our senses, as well as perfectly improve and meliorate the Aer about London (John Evelyn, 1620-1706)

Keywords: biomonitoring, bioremediation, heavy metals, ornamentals, rose

Abbreviations: Al, aluminium; **Ba**, barium; **Ca**, calcium; **Cd**, cadmium; **CN^{-1}**, cyanide ion; **Co**, cobalt; **Cr**, chromium; **Cu**, copper; **DNB**, 1,3-dinitrobenzene; **EPA**, Environmental Protection Agency; **Fe**, iron; **HCN**, hydrogen cyanide; **HF**, hydrofluoric acid; **Hg**, mercury; **Mg**, magnesium; **Mn**, manganese; **NH$_3$**, ammonia; **Ni**, nickel; **NO**, nitric oxide; **NO$_2$**, nitrogen dioxide; **NR**, nitrate reductase; **P**, phosphorus; **PAH**, polycyclic aromatic hydrocarbon; **Pb**, lead; **PCB**, polychlorinated biphenyl; **Si**, silicon; **SO$_2$**, sulfur dioxide; **TCDD**, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; **Zn**, zinc

CONTENTS

A SHORT HISTORY OF PLANTS AND ENVIRONMENTAL POLLUTION

Environmental pollution (air, soil and water) is an increasingly important issue, thus making the detection and implementation of possible remedies a stringent task for ecologists and environmental managers. Plants have a key role in the process of monitoring and remediation of environmental problems, which, unfortunately, is often overlooked or even ignored.

Environmental pollution dates back to when *Homo sapiens* L. lit a fire for the first time. Ever since, pollution has constantly spread and got increasingly worse. Its effects have evolved from the mostly domestic problem of cook stove smoke to pollution on a local and regional scale (early villages, towns and cities) and finally, in more recent times, to pollution on a global scale. Environmental pollution has accompanied the development of societies: with the first air and water pollution of variable extent, soils were damaged and species of plants and animals were extirpated (Borsos *et*

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al. 2003). Yet the effects were not so dramatic and only appeared at a regional level, causing no changes at a global level (Makra and Brimlecombe 2004).

Throughout time, the relationship between man and nature/the environment has always had an important role in conditioning historical events. Many major civilizations in different ages (including Sumerians, Assirians-Babilonians, Aegiptians, Chinese, Indians, Greeks, and Romans) saw the availability and mode of use of environmental resources as one of the main factors linked to their rise, development, decline and fall. As we have seen throughout history, when people do not live in harmony with the environment, the outcome can often be dramatic, causing both environmental and social stress. The decline and fall of the Roman Empire is an example of how environment-related events can contribute to bring major civilisations to an end. In addition to structural limits, due to the management of an increasingly boundless territory, at that time many unfavourable circumstances linked to the man-environment relationship had occurred: i) the deforestation of huge areas of Central- and Southern-Europe became more and more significant as large forests were cut down in order to obtain huge quantities of timber to build ships, bridges, and dwellings, but also to be used as firewood for heating and cooking; ii) in various towns of the Empire severe pollution was registered (for instance, in Rome the air was often described as unbreathable, and almost all parts of buildings, from the marble monuments to the inner walls of the humblest dwellings, were covered with thick layers of smoke and soot which represented the two major air pollutants at that time); and iii) extensive lead mining and the common use of lead devices in everyday life caused large-scale pollution and poisoning. In fact, lead was used for preserving food; cups, jugs, pots and frying pans were made of lead alloys; lead was used in the construction of water pipes, and moreover it was extensively used in ship and house building (Hong *et al.* 1994; Borsos *et al.* 2003; Makra and Brimblecombe 2004). According to some estimates, several hundred thousand people (over 140,000 per year) were exposed to either mine dust or emissions from forges and crucibles (Nriagu 1983). Many died from acute lead poisoning during mining and smelting processes. Therefore, the dramatic decrease in natural resources on the one hand, and the severe pollution in urban- and extra-urban areas on the other, had inevitably led to increasingly worse life conditions, the spread of diseases and poisoning, and, finally, a net decrease in the active population and, thus, to the decline of the Empire.

In more recent times, as for environmental pollution, it is interesting to recall the dramatic situation registered in the city of London long before the onset of the Industrial Revolution, as well as the consequent report and phytoremediation proposal that had already been carried out by John Evelyn in the mid-17th century (Evelyn 1661). In this brief report, air pollution was described as follows (Part I, p. 6):

”.... the City of *London* resembles the face rather of *Mount Ætna*, the *Court of Vulcan*, *Stromboli*, or the Suburbs of *Hell*, than an Assembly of Rational Creatures.... For when in all other places the *Aer* is most Serene and Pure, it is here Ecclipsed with such a Cloud of Sulphure, as the Sun itself, which gives day to all the World besides, is hardly able to penetrate and impart it here; and the weary *Traveller*, at many Miles distance, sooner smells, than sees the City to which he repairs. This is that pernicious Smoake which sullyes all her Glory, superinducing a sooty Crust or Fur upon all that it lights, spoyling the moveables, tarnishing the Plate, Gildings and Furniture, and corroding the very Iron-bars and hardest Stones with those piercing and acrimonious Spirits which accompany its Sulphure; and executing more in one year, than exposed to the pure *Aer* of the Country it could effect in some hundreds.”

Moreover, this horrid smoke:

“kills our *Bees* and *Flowers* abroad, suffering nothing in our Gardens to bud... *Fruits* never reach their defined maturity...”.

Only much later (and outside its intended context, i.e. the court of Charles II) has this piece been lauded for what Evelyn actually said about the nature of air, its pollution and phyto-remedies, and, as reported by Heidorn (1978), this was the first serious work on air pollution.

Again in London, in the early 20th century, Evelyn’s work on air pollution and phytoremediation was continued (Ruston 1921) and extended not only to the effects of pollution on plant life, but also to the ways in which the plant itself may be used as an index of the amount of that pollution. Some plants were described to be resistant, others to be particularly susceptible to smoke damage; in this latter case impurities due to smoke pollution not only get *on* the leaf but also *in* the leaf, as pollutants are absorbed (when stomatal openings are choked with a tarry deposit) through the epidermis, so, according to Ruston (1921), “the leaf perhaps is the safest index” for pollution.

This short overview on air pollution and plants shall be brought to an end by recalling that around the 1950s more and more extensive studies on this subject were carried out (Middleton *et al.* 1950; Haagen-Smit *et al.* 1952); it was at that time that the bases of modern biomonitoring and phytodepuration techniques were laid down.

In regard to this, we can emphasize that plant bioindicators and phytoremediation are relatively low-cost and require little maintenance; they can be applied to urban and rural areas, wide geographical regions, and remote areas where electrical power is unavailable.

Therefore, a significant aspect of environmental management is that this activity can be economically profitable. As a matter of fact, in the last few years, the evaluation of pollution and environment recovery are being developed into business and financial sectors. These sectors are beginning to elaborate strategies which aim to integrate the environment into their activities, thus following current legal and political moves to promote the ‘integration’ of environmental issues into economic activities (http://reports.eea.europa.eu/Technical_report_No_54/en).

In addition to representing a potential business, the design, implementation, and subsequent management of biomonitoring and/or phytodepuration stations can eventually have additional positive “side effects”. This is only possible if these activities actively involve the people living near environmentally crucial sites and let them assume responsebility (hopefully, at least in part). This represents an interesting compromise between business and social aspects, with the possibility of psycho-physical recovering of weak population groups, such as young and elderly people, but also disabled and other emarginated groups (Stoneham *et al.* 1995; Armstrong 2000; Sempik *et al.* 2005; Jiler 2006; Larson and Meyer 2006; Eames-Sheavly *et al.* 2007). Today, the level of pollution in large urban areas – and not only – has not changed to a significant extent if compared to what we recalled about both Imperial Rome and pre-industrial London. This being stated, we mention Evelyn’s work once again where a real phytodepuration technique is suggested (Part III, interestingly entitled: “*An Offer at the Improvement, and Melioration of the* Air *of* LONDON*, by way of Plantations, & c.*):

“... *Palisad’s* ...elegantly planted, diligently kept and supply’d, with such *Shrubs*, as yield the most fragrant and odoriferous *Flowers,* ...: Such as are (for instance amongst many others) the *Sweet-brier*, ...; the *Guelder-Rose*, the *Musk*, and all other *Roses*....”.

The contents of this chapter are conceptually linked to Evelyn’s proposal (1661) to use plants, and especially *Rosa*, in order to improve the quality of the environment. We indicate that *Rosa* can improve air quality, remove contaminants from soil, and treat wastewater. In the following paragraphs we are going to discuss various subjects, such as the relationship between *Rosa* and the environment in specific stress situations, due to either human activities or natural geological evolution as well as the use of *Rosa* for the biomonitoring of common soil and air pollutants. Furthermore, the use of *Rosa* in bioremediation is potentially very promising in terms of conventional techniques and innovative approaches, such as biosorption. Therefore, many significant benefits may derive from *Rosa*, largely beyond the fact that any ornamental plant is a useful resource for people's well-being and recovery from stress. *Rosa* is a wonderful group of plants which have accompanied the human adventure on earth for millions of years; with *Rosa* it seems that it is possible to remedy contaminated environments and, at the same time, make them more beautiful.

ROSA LIVING IN CONTAMINATED SITES

There are numerous contaminated sites in industrialized nations; contamination is often complex, resulting from a mixture of different pollutants. In spite of that, in several cases the areas are heavily vegetated. Therefore, it is interesting to study the indigenous plants, which were established there spontaneously, not only with the aim of investigating their physiological traits, but also to follow the structural evolution and the floristic composition of vegetation from an ecological point of view. This approach may provide useful information for sustainable management of polluted soils. A number of reports in this field indicate that some *Rosa* species or some Rosaceae family members have been naturally established in contaminated areas, thus indicating that they are tolerant of critical environments.

Pichtel *et al.* (2000) examined two heavily contaminated sites: a Superfund site and a battery dump located in the Midwest USA. "Superfund" is the common name for the environmental law (known as CERCLA) which was enacted by the US Congress on December 11, 1980 in response to the Love Canal disaster, to protect people, families, communities and others from heavily contaminated toxic waste sites. The Superfund site studied by Pichtel *et al.* (2000), in the close vicinity of homes, was contaminated over a 90-year period from foundry sand, secondary lead (Pb) smelting operations, and battery recycling, with battery casings and other debris still visible on the soil surface. The battery dump site also had battery casing visible on the surface, along with large blocks of plastics and foundry sand. Both sites exhibited an overall ground cover greater than 85% of the entire surface, though of different composition. The indigenous plant population at the Superfund site included annual grasses, legumes, and perennials; roots of all plants examined were stunted and did not penetrate beyond a depth of 5 cm. At the dump site, a mixed bottomland forest ecosystem predominated, with maple (*Acer* spp.) and boxelder (*Acer negundo* L.); among others, *Rosa multiflora* Thunb. appeared to be a common species. Soil samples and different portions of selected plant species from both sites were analyzed to assess levels of the major contaminants, i.e., Pb, cadmium (Cd), and barium (Ba). As for Pb, the total concentration in the soil averaged 55,480 mg/kg at the Superfund site, with some samples reaching 140,500 mg/kg; 29,400 mg/kg at the battery dump site, with maximum values of 112,500 mg/kg. It has to be considered that the total baseline level of Pb in natural surface soils is reported to be 20 mg/kg (Kabata-Pendias and Pendias 1992). As for Cd, it averaged 8.5 mg/kg in the Superfund site soil, and 3.9 mg/kg at the dump site. Ba reached up to 630 mg/kg (Superfund site) and 3,590 g/kg (battery dump); however, in all the plants studied, Ba was only taken up in minimal concentrations. In general, Pb uptake by plants was greater at the Superfund site, probably due to the greater percentage of the soluble Pb fraction in the soil, while similar Cd amounts were registered in plant tissues from both sites. In particular, *R. multiflora* living at the battery dump site accumulated 300.0 and 1,317.2 mg/kg Pb at the shoot and root level, respectively, as well as 6.4 and 4.2 mg/kg Cd in shoots and roots, respectively (Pichtel *et al.* 2000). The Pb uptake of *Rosa* shoots was significantly lower compared to that of *Ambrosia artemisiifolia* L., which was the best Pb accumulator (1,075.2 mg/kg) among the plants in the battery dump; conversely, *Rosa* roots took up Pb to a comparable extent with respect to *Ambrosia* roots (1,686.5 mg/kg Pb). The study by Pichtel *et al.* (2000) underlines that, even in heavily contaminated sites, several native plant species are able to take up a wide range of soil metals such as Pb and Cd, without being affected by excess of metals; their establishment and growth on the metal-enriched sites may have been due to a high frequency of tolerant genotypes in the population. Therefore, the study of the native vegetation of contaminated sites is a great help to identify the most promising species and genotypes for phytoremediation.

A negative impact on the presence of *Rosa* was an effect on vegetation diversity due to effluents from an oil sand industry near Fort McMurray, Alberta, Canada, as reported by Crowe *et al.* (2002). Wetlands are a low cost strategy for wastewater cleanup and maintenance; vegetation surveys conducted on and around this industrial site revealed that the constructed wetlands associated with the dyke drainage (effluent treated with phosphorus) and consolidated tails (effluent treated with gypsum) had low biodiversity compared to the five reference wetlands. The latter, all located 30 km from the company site, were largely dominated by aquatic macrophytes, trees, grasses, horsetails, legumes, ferns and woody shrubs that are common in northern Alberta: the woody shrubs included *Rosa* spp. and *Rubus spectabilis* Pursh. However, as well as other species, *Rosa* and *R. spectabilis* were not found in the constructed wetlands. The study of Crowe *et al.* (2002) also reported that the effluent exerted a strong inhibitory effect on seed germination of several plant species and reduced seedling fresh weight. This may account for the paucity of species in the vegetation of the oil sands impacted wetlands.

Furthermore, a significant contribution may derive from the analysis of plant communities living on naturally metal-enriched soils; for example, the peculiar vegetation of serpentine ecosystems. Serpentinized rocks are distributed all over the world; serpentine soils contain relatively large amounts of magnesium (Mg) and/or iron (Fe), but they are extremely rich in trace elements such as nickel (Ni), chromium (Cr), cobalt (Co), while they are poor in calcium (Ca), phosphorus (P), and other nutrients (Sequeira and Pinto da Silva 1991; Adriano 2001). Besides the excessive metals and scarce nutrients, other stress elements are drought and high light intensity. Plant species living on serpentine soils can be either serpentine-endemic (obligate), when they grow exclusively on that substrate and are not found on other areas, or serpentine-tolerant, when they are able to survive such extreme soil conditions but grow better elsewhere (Reeves *et al.* 1996; Dinelli *et al.* 1998). The ability of serpentinophytes to hyperaccumulate metals is well proven (Vergnano-Gambi *et al.* 1982; Reeves *et al.* 1996; Brooks 1998; Reeves *et al.* 1999). A study by Freitas *et al.* (2004) examined endemic and non-endemic plants growing on the serpentine area (about 8,000 ha) of Bragança, Portugal, to assess levels of different metals taken up from soil; soil samples were also collected and analyzed for the same metals. *Rosa canina* L. and a number of Rosaceae members (*Crataegus monogyna* Jacq., *Potentilla erecta* (L.) Raeusch., *Rubus ulmifolius* Schott, *Rubus caesius* L., *Agrimonia procera* Wallr.) were present among the non-serpentine species. Though levels of foliar Fe (124 mg/kg) and Mn (181 mg/kg) of *R. canina* were not negligible, the amounts of Ni, Cr, and Co recovered in different portions of *Rosa* (leaves, fruits, and twigs) were lower than those found in endemic species such as *Alyssum serpyllifolium* Desv. or *Linaria spartea* (L.) Chaz. (Freitas *et al.* 2004). Considering the great ability of these serpentine-endemic species to hyperaccumulate metals, *R. canina* can be defined as tolerant of the serpentine substrate. Nevertheless, as any other member of the local plant community, *Rosa* contributes to the interspecific diversity and thus plays a role in habitat conservation. This is a global priority, since serpentine habitats are declining drastically in many regions of the world (Freitas *et al.* 2004). Furthermore, a few *Rosa* species (*R. canina*, *R. gallica* L. and *R. agrestis* Savi) were also found in Italian ultramafic outcrops of northern Apennines. These *Rosa* species lie only on basalts and gabbros, i.e., on the geochemical types of soils with lower concentrations of Ni, Cr, and Co if compared to the very high metal levels characteristic of the serpentinites (Lombini *et al.* 2001).

BIOMONITORING THROUGH *ROSA*

In the last decade, great interest was paid to the effects of atmospheric particulates on human health, since this type of pollution has been demonstrated to have an important impact on respiratory diseases and death (Dockery and Pope 1994; Pope *et al.* 1995). In most cities, airborne metals are not monitored by automatic gauges, which are commonly used in pollution monitoring programs, owing to high costs and technical problems. This is a drawback, because vehicular traffic, which is a great source of fine particulate and airborne metals in urban environments (Cadle *et al.* 1997; Janssen *et al.* 1997), has increased dramatically in recent years.

To complement and complete information on trace element deposition obtained from automatic detectors, increasing attention has recently been paid to plants as biomonitors (Bargagli 1998; Markert *et al.* 2003). Plant responses have several important advantages over data supplied by automatic gauges, particularly because they give information over a longer period of time and a larger surface. Indeed, by providing a high density of sampling points, biomonitors make it possible to trace maps of airborne metal contamination in urban environments (Bargagli *et al.* 1997). Furthermore, biomonitors highlight the effects of pollutants on living organisms, thus providing additional information that cannot be easily obtained by only using traditional chemical and physical techniques for the analysis of soil, water and air (Monaci *et al.* 1997; Madejón *et al.* 2006). Rather than simply using the total contaminant level in the soil, this risk-based approach, which investigates the effect of contaminants on plants and ecosystems, is being increasingly adopted by regulatory authorities in different parts of the world to assess soil quality (Swartjes 1999; Tarazona *et al.* 2005).

Ornamental plants have been used as a successful urban pollution assessment system. In a three-year monitoring study carried out in Florence (Italy), the metal pattern in the inhalable fraction of particulate (PM_{10}; $d_p < 10$ µm) collected by air samplers at two sites was found to be similar to that in *Quercus ilex* leaves (Monaci *et al.* 2000); these leaves were found to accumulate airborne metals as a function of exposure time (i.e. leaf age). This study demonstrates that *Q. ilex* leaves are good biomonitors, able to provide quantitative information on urban environments. In a two-year study carried out to monitor the urban environment in Palermo (Italy), leaves from *Nerium oleander* L. plants were collected from six sampling sites corresponding to either areas of intense traffic and urbanization density, or areas far away from traffic. Concentrations of aluminium (Al), barium (Ba), Cr, copper (Cu), Fe, Pb, Mg, manganese (Mn), and zinc (Zn) were determined in leaf samples, and sampling sites were classed on the basis of the metal content in leaves. A good correlation was found between the metal content in leaves and the urbanization level of the sites, thus suggesting that the plant used can be considered a reliable tool for the assessment of urban pollution level (Mingorance and Rossini Oliva 2006).

Leaves from higher plants intercept pollutants from atmospheric deposition and accumulate metals from both the soil and atmosphere (Bargagli 1998). The deposited particles may be washed out by rain into the soil, resuspended or retained on the leaves. The degree of retention is affected by weather conditions, pollutant properties, plant surface characteristics, plant and leaf morphology (Harrison and Chirgawi 1989; Sardans *et al.* 2008). Large quantities of pollutants are taken up from the soil via the root system and translocated into vegetative and generative organs at various rates (Tiffin 1972; Fox and Guerinot 1998). Thus, the uptake of soil metals by plants growing on polluted sites has been the subject of many studies. Çelik *et al.* (2005), in a monitoring study aiming to evaluate the heavy metal contamination in Denizly (Turkey), found a positive correlation between the degree of metal contamination in the soil and levels of pollutants in the leaves of *Robinia pseudo-acacia* L., thus concluding that this plant does reflect the environmental changes accurately.

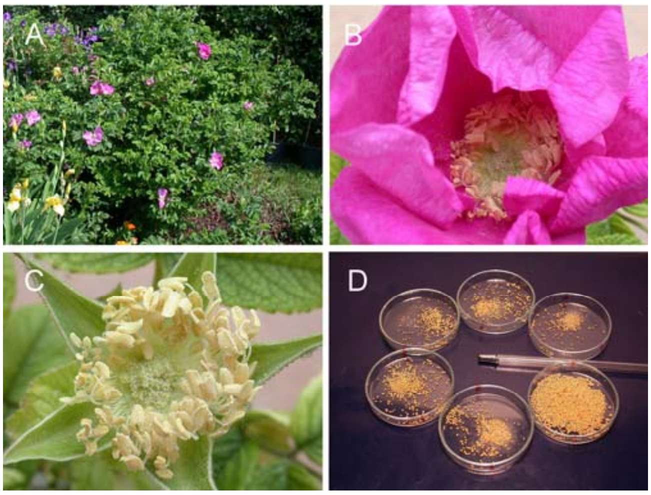

Fig. 1 *Rosa rugosa* (**A, B, C**) in a monitoring station set up in the context of the European Project T.O.R.R.E. (Organized Tourism Recover Rural Ecology) aimed at the environmental and tourist promotion of an area near the city of Faenza (Italy) described by Calzoni *et al.* (2007). (**D**) Anthers of freshly opened flowers from *Rosa* plants growing in the six monitoring sites were allowed to dehisce under laboratory conditions in order to obtain pure pollen. (**A-D**, original photographs by G.L. Calzoni)

Rosa rugosa Thunb. has been found to accumulate heavy metals in leaves (e.g. Fe, Cu, and Zn), trace elements (e.g. neodymium, lanthanum, and caesium; Kovàcs *et al.* 1982), and hydrogen sulphide (Syarheichyk and Sanko 1982). A great accumulation of mineral elements such as Fe, Zn, Ni, and Cu has been shown to occur in leaves of *R. rugosa*, particularly those associated with cynipid galls, and these high levels result in a modification of phloem tissues (Bagatto *et al.* 1991). Because heavy metals can be contributed by air pollutants, this plant has been suggested as a useful tool for the monitoring of environmental pollution in both air and soil. For this purpose, within the context of the European Project (Life-Environment Project), we performed a monitoring study to evaluate the quality of an area near Faenza (Italy) using *R. rugosa* plants as biomonitors (Calzoni *et al.* 2007; **Fig. 1**). Its plagiotropic-oriented leaves with a rather large surface and a thin cuticle layer make this plant suitable for that purpose. An active monitoring approach was used, by placing plants in six different sites throughout rural, urban, and suburban areas. Concentrations of heavy metals [Pb, Cr, Cd, Ni, and mercury (Hg)] were measured in both soil and *R. rugosa* leaves, in order to obtain information on soil quality. In each site, the total heavy metal pattern of *Rosa* leaves closely paralleled the total pattern registered in soil (Calzoni *et al.* 2007). In both total and available forms, top levels of Pb and Cr were found in the site located in the urban area and exposed to heavy traffic (site 6, **Fig. 2**). In particular, the pattern of Pb in *Rosa* leaves turned out to reflect that of soil, as shown in **Fig. 3**. Conversely, the accumulation of Pb and Cr did not differ significantly among the six sites in the leaves of *Iris germanica* L., another species tested as a possible biomonitor in our study (**Fig. 3**). Therefore, quite different responses in terms of metal bioaccumulation were obtained in the two species. At present, we are not able to identify the specific foliar characters which can explain the higher sensitivity of *R. rugosa* leaves. A large number of factors, in fact, control airborne pollutant interception by leaves: amongst these, leaf posture and physical orientation, leaf age, surface roughness, and epidermal characteristics have been reported (Bakker *et al.* 2000; Caini *et al.* 2003).

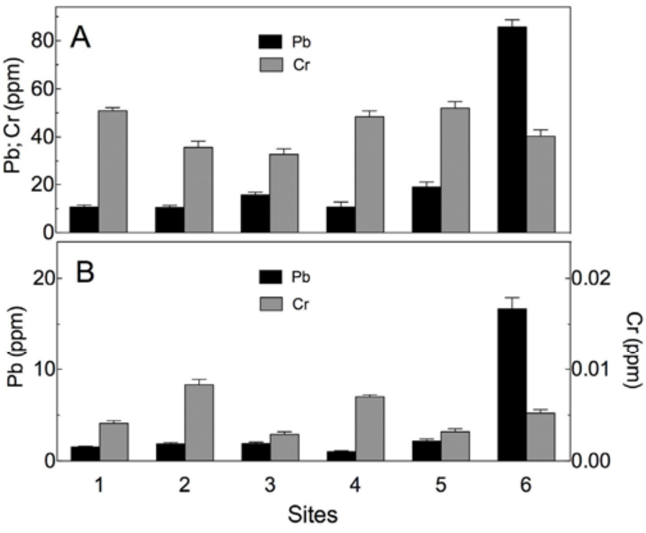

Fig. 2 Lead and chromium content in total (**A**) and available (**B**) soil fraction in the six stations of active biomonitoring with *Rosa rugosa* described by Calzoni *et al*. (2007). The metal values refer to the year after planting. (Modified from Calzoni *et al*. 2007)

The clear correlation observed between the leaf content and the total fraction of heavy metals in soil, more than the available fraction, suggests that leaf accumulation was due to atmospheric deposition more than soil uptake (Calzoni *et al*. 2007). Moreover, leaves of *Rosa* were shown to accumulate Cr, since the foliar content was much higher than that in the available soil fraction (**Fig. 3**). This accumulation of heavy metals does not cause any visible foliar injury in *R. rugosa*; however, it has a damaging effect on the reproductive potential of plants. Indeed, in the same work, the pollen of *R. rugosa* has been used as a fine indicator of environmental pollutants. The quality of pollen grains produced by plants grown in the six sites has been evaluated in relation to either normal cell structure, by using Alexander stain (**Figs. 4, 5**), and functional ability, by using the fluorochromatic reaction (**Fig. 6**). Aborted pollen grains, without cytoplasm, were most frequently found in urban (**Fig. 4**) or suburban sites exposed to heavy traffic, while the pollen of plants located far from the urban area was almost completely viable (**Fig. 6**). Moreover, the pollen of *R. rugosa* turned out to respond to environmental changes as a function of exposure time, since only one year after planting a clear effect on pollen quality was observed (**Fig. 5**). Interestingly, pollen abortiveness was strictly correlated to leaf (and soil) Pb levels, and pollen viability was inversely related to the Cr concentrations ascertained in leaves, according to a significant dose-response relationship. It is worth noticing that the levels of Pb and Cr found in leaves were far less than those which are reported to be toxic for plants. Thus, we can conclude that the bioassay based on *R. rugosa* pollen quality represents a potentially early, sensitive and effective monitoring test for environmental pollution.

Similar damage to the reproductive structures of plants exposed to air pollutants has been reported from both field and laboratory studies. In a study carried out in Vermont (USA) using *Apocynum androsaemifolium* L., a native bioindicator for ozone, Bergweiler and Manning (1999) reported that cumulative exposure to ozone is insufficient to elicit a leaf injury response, but it affects the normal development of reproductive structures. One possible explanation is that the exposure to ozone during flower development directly affects pollen formation and disrupts pollen germination or tube growth, as also hypothesized by Bosac *et al.* (1993). The sensitivity of the male gametophyte of higher plants to Cr toxicity was reported by Speranza *et al.* (2007). These authors found that both Cr(III) and Cr(VI) have deleterious effects on *in vitro* pollen germination and caused severe ultrastructural alterations at the morphological level, including chromatin condensation, swelling of mitochondria, cytoplasmic vacuolization, and perturbed ar-

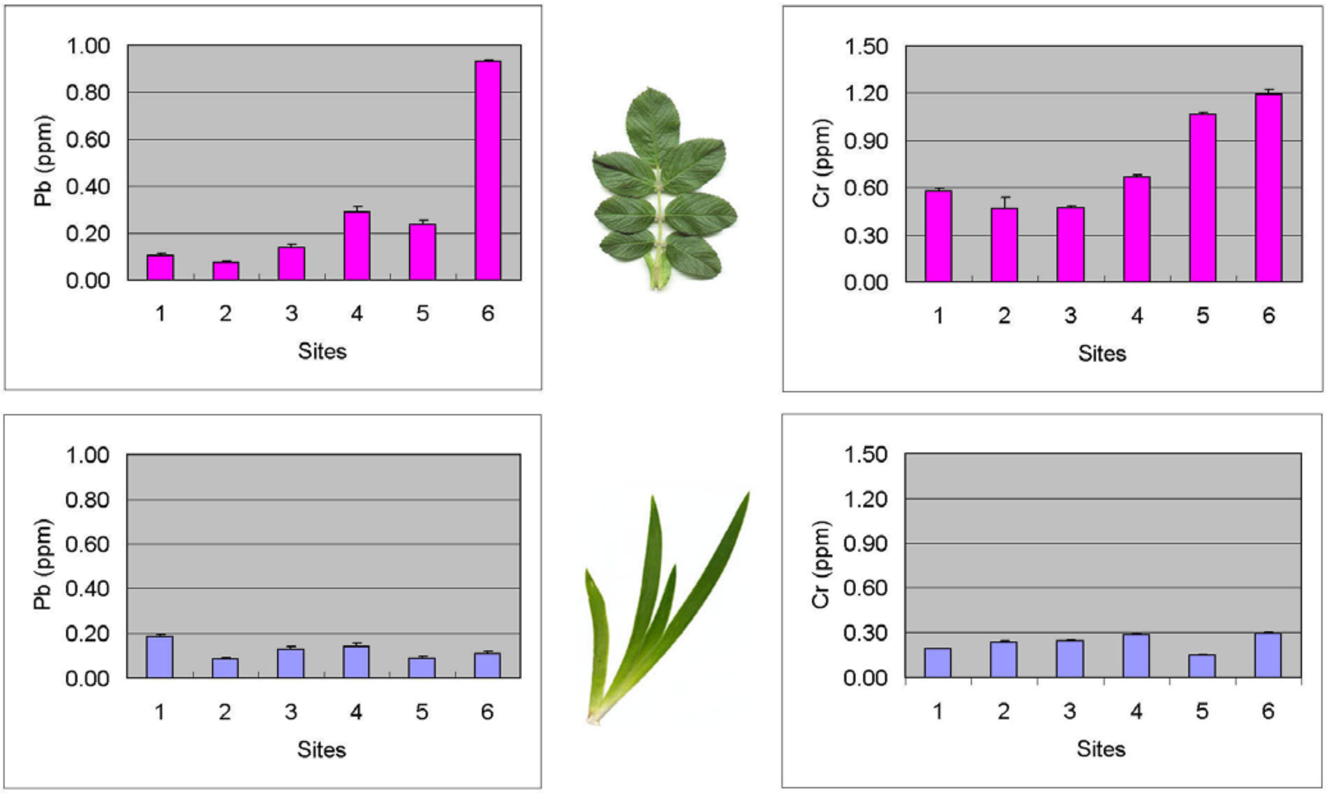

Fig. 3 Lead (Pb) and chromium (Cr) content in leaves of *Rosa rugosa* (upper panel) and *Iris germanica* (lower panel) growing at the biomonitoring sites described by Calzoni *et al*. (2007). The metal values refer to the year after planting. The leaf of *Rosa* exhibited a pattern with the highest Pb and Cr contents at site no. 6, which is located in an urban area. In particular, the Pearson's coefficient for the Pb content in soil and *Rosa* leaf was 0.96. In contrast, the leaf of *Iris* accumulated a smaller amount of metals, with no relationship to soil levels. (Modified from Calzoni *et al*. 2007).

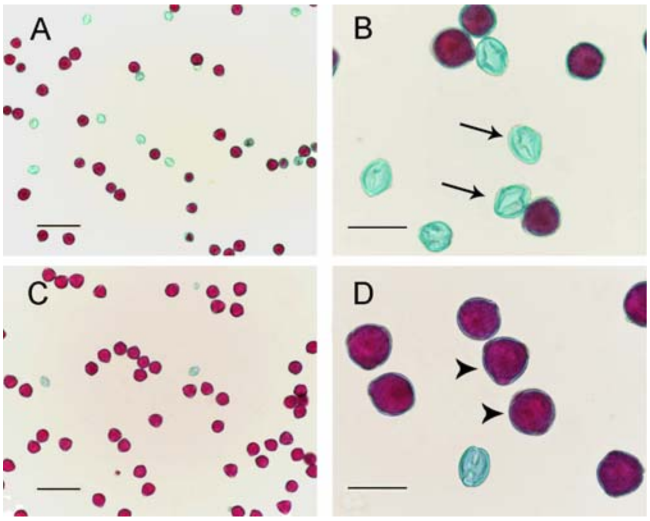

Fig. 4 Pollen grain quality of *Rosa rugosa* growing at the biomonitoring sites described by Calzoni *et al.* (2007) tested with the Alexander's staining (Alexander 1969). The stain contains both malachite green which stains pollen walls, and acid fuchsin which stains the cytoplasm. Aborted pollen (arrows), which did not develop a protoplasm, can be easily distinguished from regular, mature pollen grains (arrowheads). In the *R. rugosa* pollen population from the urban biomonitoring site (**A, B**) there was a significantly higher percentage of aborted grains if compared to rural area pollen (**C, D**). Bars = 100 µm (**A, C**) and = 50 µm (**B, D**). (**A-D**, original microphotographs by A. Speranza).

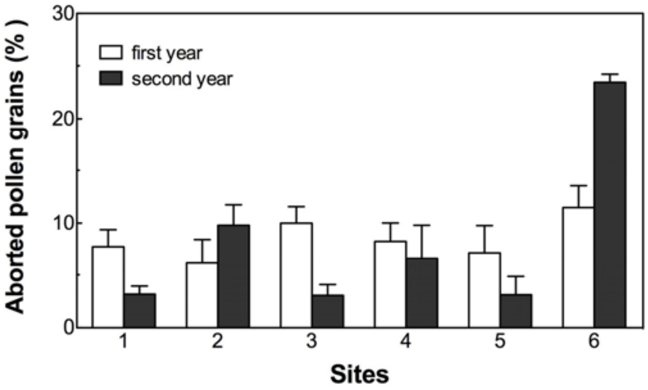

Fig. 5 Pollen quality of *Rosa rugosa* growing at the six biomonitoring sites described by Calzoni *et al.* (2007). The percentage of abortion of *R. rugosa* pollen grains was evaluated by the Alexander's test (1969) in both the same year of planting (first year) and one year later (second year). (Modified from Calzoni *et al.* 2007).

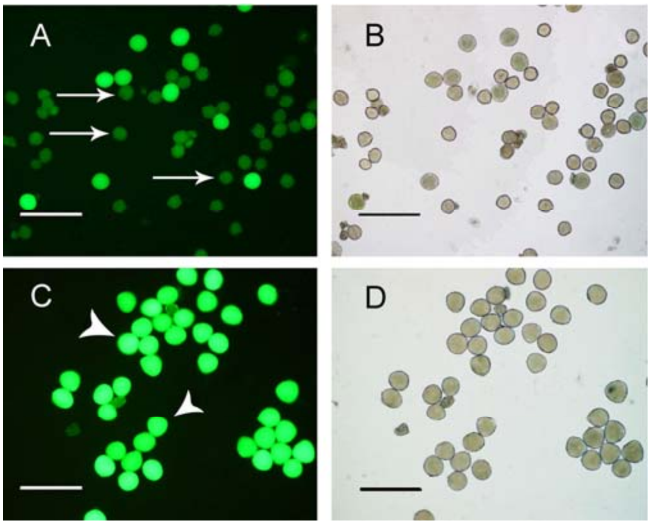

Fig. 6 Pollen grain quality of *Rosa rugosa* growing at the biomonitoring sites described by Calzoni *et al.* (2007) tested by using the fluorochromatic reaction with fluorescein diacetate, which assesses the integrity of plasmalemma in the vegetative cell (Heslop-Harrison and Heslop-Harrison 1970). Nonviable grains are poorly fluorescent (arrows), whereas viable pollen grains (arrowheads) show bright fluorescence due to the accumulation of fluorescein, since the intact plasmalemma does not allow this polar compound to exit through. In the *R. rugosa* pollen population from the urban biomonitoring site (**A**, epifluorescence; **B**, brightfield) there was a significantly higher percentage of non-viable grains if compared to rural area pollen (**C**, epifluorescence; **D**, brightfield). Bars = 100 µm. (**A-D**, original microphotographs by G.L. Calzoni)

rangement of endoplasmic reticulum cisternae. Thus, it seems that the impact of the two chromium forms on kiwifruit pollen may result in the severe compromise of both essential structures and functions of the male gametophyte.

In connection to the aspects treated in this section, some studies have considered the factors involved in the uptake of air pollutants by leaves (van Hove *et al.* 1989; van Hove and Adema 1996). In particular, the presence of an invisible layer of water on the leaf surface plays an important role in the dry deposition of gaseous pollutants from the atmosphere. For example, the absorption of sulfur dioxide (SO_2) and ammonia (NH_3) increases significantly with rising air humidity. The authors experimented with the dried leaves of some plant species: the leaves of *R. rugosa* (leaf area: 27.3 cm^2) had a considerable 89 mg increase in weight by passing from 20% to 95% relative humidity (R.H.), corresponding to a difference in water layer thickness of 17.9 µm. Unlike other species, the difference observed in *R. rugosa* was not linearly correlated with the leaf weight at 95% R.H. According to the authors' conclusion, not only are the water molecules associated with the cuticle, but also they form a water film in *continuum* with it; the cuticle behaves as a 'valve' between the inner and outer region of the leaf, controlled by the atmospheric R.H. (van Hove and Adema 1996). Moreover, the permeability of the cuticle to non-ionic and ionic species has recently been described; the polar paths of diffusion were identified with stomata, glandular trichomes, and their basal cells (Schreiber 2005).

As for SO_2, a number of Rosaceae were found to be sensitive to this gas (Krupa and Legge 1999): among these, the wild rose (*Rosa* spp.). The visible injury symptoms were observed both after acute SO_2 exposures in controlled environment studies and from field surveys in the vicinity of single or multiple, large stationary sources. They consist in unifacial, interveinal chlorosis and bifacial, interveinal necrosis: therefore, these species could serve as useful biological indicators of SO_2. Previous observations by Shu-Wen *et al.* (1990) had registered a severe decline of about 2,000 ha of forest since the 1980s, due to gaseous air pollutants such as SO_2 and hydrofluoric acid (HF) in the Sichuan province in China. The needles of the dominant species, *Pinus massoniana* Lamb., suffered various symptoms (tip necrosis, reduced length, premature abscission, etc.), and also the foliage of other species was affected; leaves of *Rosa chinensis*, for example, displayed necrotic lesions, both marginal and intravenous. Exposures between 50-64 days to nitric oxide (NO) or nitrogen dioxide (NO_2) of several plant species grown in containers showed different behaviours: some species were specifically sensitive to NO, others to NO_2, while the remaining were equally sensitive to any mixture dominated by either gas. *Rosa* 'Minimo Red' belongs to the latter group (Saxe 1994).

A heavy consequence of air pollution is ozone depletion in the stratosphere. This alteration increases exposure to UV-B (280-320 nm), which is detrimental to living beings because it induces DNA damage. In contrast, UV-A (320-400 nm) is not usually considered as harmful; in plants, it has been studied to a lesser extent. Leaves and petals of *Rosa hybrida* L. and *Fuchsia hybrida* hort. ex Siebold & Voss were used to investigate the response to supplemental

UV-A exposure, searching for changes in their levels of various antioxidant compounds such as ascorbate, glutathione, carotenoids, and flavonoids (Helsper *et al.* 2003). No morphological changes were observed. Petals hardly responded; major biochemical responses in leaves of either species concerned the levels of the flavonols, quercetin and kaempferol, thus suggesting that protection from UV-A exposure in *Rosa* and *Fuchsia* originates from the absorption of irradiation, and not from scavenging of reactive oxygen species.

A further point is that, paradoxically, some plants may contribute to air pollution. Many species emit volatile organic compounds, which react with anthropogenic sources of NO_x to produce ozone. However, biogenic hydrocarbons (e.g., isoprene, monoterpenes) are more reactive than those derived from gasoline combustion. The emission rate is species-specific and significantly varies with environmental factors such as light intensity and temperature (Benjiamin *et al.* 1996). Therefore, large-scale planting of trees or shrubs in urban areas has to take proper species selection into account. The ozone-forming potential was estimated in 308 plant species, while normalizing the data of biogenic emission for differences in biomass and photochemical reactivity and assuming meteorological conditions representative of a summer day in California (Benjamin and Winer 1998). Rose (*Rosa* spp.) and a number of Rosaceae members, mainly of the genus *Prunus*, were found not to produce ozone. Species producing more than 10 g ozone/day were ranked within the high-ozone-forming potential group. Nevertheless, the study by Benjamin and Winer (1998) emphasizes the overall benefits of urban vegetation to improve air quality.

Finally, one marginal but not negligible effect of air pollutants, is that they can affect plant-parasite interactions. Well known, for example, is the fungicide effect of SO_2 pollution on crops, quite similar to that obtained after sulphured anticryptogamic treatments. On the other hand, fumigation of *Vicia faba* L. with SO_2 or NO_2 for 7 days resulted in higher growth rates of the aphid *Aphis fabae* Scop. feeding on plants. Similarly, the growth of *Macrosiphon rosae* L. on rose (*Rosa* 'Nina Weibull') was increased by about 20% in Munich ambient summertime air (Dohmen 2003).

RE-STORATION: FROM PSYCHOLOGY TO ENVIRONMENTAL SCIENCES. THE BIOREMEDIATION POTENTIAL OF *ROSA*

Restorativeness is a term used by psychologists to indicate the ability to renew people's decreased psychological resources (ability to focus attention, to recover from stress and mental fatigue). It has been proven that natural elements - e.g. vegetation, including vegetation in cities - have a key role in the recovery of people in terms of their mind, spirit, and body: in other words, psychological restorativeness (Hernández and Hidalgo 2005; Scopelliti and Giuliani 2005; Hartig and Staats 2006; Berto 2007).

Similarly, an effective "therapeutic" action is exerted by certain plants in contaminated soil or waters; namely, this is phytoremediation, which aims to restore the original, natural environmental quality which was worn-out, mostly, due to anthropogenic activities. Recently, with the growth of contamination in urban areas, attention is being increasingly paid to the involvement of ornamental plants to remedy contaminated sites (reviewed in Teixeira da Silva 2006; Ivarez-Bernal *et al.* 2007; Liu *et al.* 2008a, 2008b, 2009).

This section presents early and recent studies dealing with the remediation ability of *Rosa* species. If compared to the extensive literature produced in this general field, specific studies on *Rosa* are quite scarce. However, the biotransformation ability of *Rosa* in a number of xenobiotics, for example, seems to be a well-established fact. The great potential of *Rosa* deserves to be put into effect.

An ecological appraisal of *Rosa* can be argued from a study on the re-vegetation of a reclaimed area of about 43 ha called 'Bosco delle Querce' (Sartori and Assini 2001). This area, located in the upper Po plane near Milan (Italy), features a wood, which was created after the Seveso chemical disaster of 1976. With the aim of restoring the most polluted zone, where TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) concentration in soil was higher than 50 μg/m^2, a surface layer of about 40 cm of upper ground was removed and replaced with 15 cm of fresh soil. Then, in 1984-85, a grassland was created and a first generation of trees was planted there. New plantings of trees and shrubs continued up to 1996, while mowings and irrigation were progressively reduced until complete cessation in 1992. The work of Sartori and Assini (2001) assessed the changes in vegetation in 1992-1993 and in 1998. The tree and high shrub layer increased due to the growth of the planted woody species, while the development of the low shrub layer was due to the spontaneous renewal of woody species. Among the young woody species, two rose species (*R. canina* and *R. rugosa*), and a number of Rosaceae were present (*Prunus avium* (L.) L., *P. domestica* L., *P. serotina* Ehrh., *P. spinosa* L.; *Rubus caesius*, *R. divaricatus* P. J. Müll., *R.* gr. *sylvatici* P. J. Müll., *R. hirtus* Waldst. & Kit., *R. ulmifolius*). That indicates a significant role of woody shrubs in the evolution of the vegetation structure.

Heavy metals

In another part of this review it has already been stressed that biological techniques to remediate contaminated sites have many advantages over physical or chemical methods. Phytoremediation – which uses plants to extract, contain, or immobilize contaminants from soil and water – may have variable success depending on the extent of the plant's ability to take up, translocate, and tolerate metals; an ability which is the function of the specific phenotype and genotype.

Six *Rosa* species were chosen for their common trait of high biomass yield among the 25 species which have been reported in Turkey (**Fig. 7**); they were studied in order to assess their potential for phytoextraction of heavy metals from moderately contaminated soils (Turan and Ercisli 2007). The experiment used plants of the same age of *R. villosa* L., *R. pulverulenta* M. Bieb., *R. dumalis* subsp. *boissieri* Crépin and subsp. *antalyensis* (Manden.), *R. pisiformis* Christ., and *R. canina*. They were allowed to grow under field conditions in the Erzurum province of Turkey from 1998 to 2005, then their hips were collected when ripe. These rose hips had ~40 seeds per fruit on average, thus representing 30-35% of the total fruit mass, while the remaining 65-70% is the fleshy hypanthium. Both fruit portions from the shrubs of the six *Rosa* species were separately tested for metal contents, together with soil samples from each experimental plot. The main metals detected in soil were Zn, Cu, Cd, Ni, and Al; their contents ranged from 8.7 to 30.1 mg kg^{-1} in the total fraction, and from 0.13 to 1.3 in the available fraction. Results on metal uptake by *Rosa* indicated that heavy metals such as Cd, Al, silicon (Si), and Ni accumulated to a significantly different extent in seeds if compared to the fleshy part of the fruit. In most cases, metal concentrations in seeds were 4-6 times higher than in the hypanthium. Among the species tested, *R. pulverulenta* was the most effective for the uptake of Cd, whose content in seeds approached 120 mg kg^{-1} dw; that indicates a high accumulation ability when considering the relatively lower Cd level in the available soil fraction (0.13 mg kg^{1}). The same *Rosa* species was also highly effective for Al and Si uptake, while *R. dumalis* subsp. *boissieri* was effective for Ni uptake. The results obtained by Turan and Ercisli (2007) indicate that certain *Rosa* species can both accumulate and tolerate heavy metals. Furthermore, their study indirectly demonstrates that metal translocation up to the reproductive structures occurs in *Rosa*.

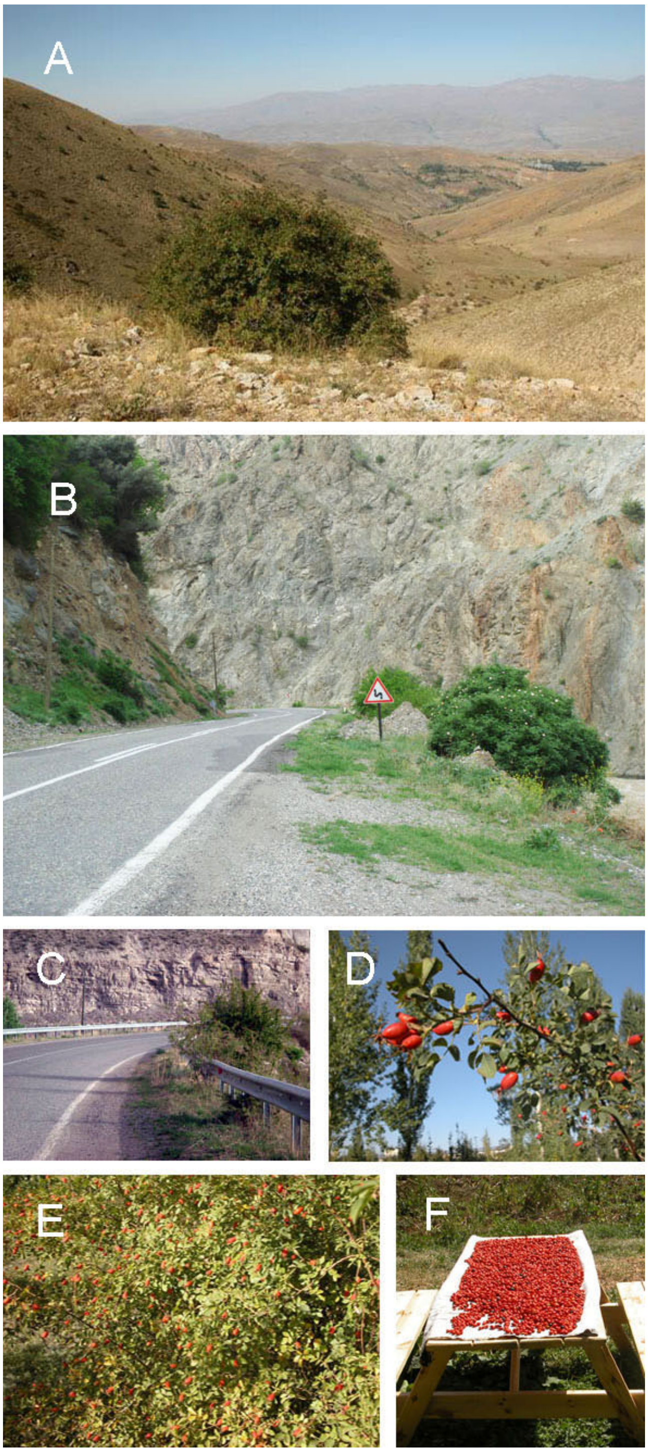

Fig. 7 (**A**) Wild shrub of *Rosa canina* at 1,536 m altitude in the North-eastern Anatolia region between Turkey and Georgia. This species grows between 30 to 1,700 m throughout Turkey, mainly on limestone. *Rosa canina* is used for erosion control in Turkey (Ercisli 2005). (**B, C**) Native *R. dumalis* shrubs alongside roads in Eastern Anatolia of Turkey. This species lives between 1,000-2,300 m on rocky slopes, hillsides, cliffs, as well as in scrubs and dry forest and meadows, often along water courses. Both *R. canina* and *R. dumalis* have a significant economic role. Their germplasm for genetic improvement programs is of high interest. Their use for medicinal purposes is also very important (Ercisli 2005). (**D-F**) fruits of *R. dumalis*. The rosehip is an excellent source of vitamins A, B3, C, D, and E, as well as flavonoids, citric acid, fructose, malic acid, tannins, and zinc. Fresh fruits are commonly used to make jam, marmalade, and fruit juice, while the dried fruits and roots are excellent for tea making (Ercisli 2005). (**A-F**, original photographs, courtesy Dr. Sezai Ercisli, Dept. Horticulture, Atatürk University, Turkey).

Organic molecules

In addition to heavy metals, the environment is increasingly contaminated by a huge array of organic molecules originating from several human activities. Both *in vitro* and field studies are crucial to: i) identify plant species able to metabolize contaminants, and then the genes and enzymes involved; ii) assess the metabolic fate of pollutants and the main metabolites formed which, afterwards, shall be compared with those in the intact plant and tested for any further toxicity. The present section contains information on the ability of rose to metabolize some widely diffused organic pollutants: it may be argued that *Rosa* has a clear potential for bioremediation, which emerges from cell and tissue culture experiments. In particular, as for *Rosa* 'Paul's Scarlet' cells, it has to be stressed that they have special enzymatic properties to metabolize recalcitrant xenobiotics such as polychlorinated biphenyls and fluoranthene at a high rate. The field testing of *Rosa*, however, is still missing.

Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are a class of compounds with 1 to 10 chlorine atoms linked to biphenyl, which consists of two benzene rings. There are up to 229 individual compounds, known as congeners. PCBs were used as coolants and lubricants in transformers, capacitors, and other electrical equipments; moreover, they were present in sealants, hydraulic oils, de-dusting agents, pesticide extenders, adhesives, carbonless copy paper, wood floor finishes and other products. Their manufacture was discontinued in the USA in 1977 because evidence was gathered that they build up in the environment and can cause harmful health effects (ATSDR 2001); similarly, at present, PCB production has been discontinued in most Western industrial countries. Nevertheless, PCBs remains among the most important pollutants since they are persistent (Palmer *et al.* 2008; Rudel *et al.* 2008). The sources of exposure may vary significantly; also the dietary exposure – especially through eating some crop products, or fish, and fish-eating wildlife from contaminated sites – has to be taken into consideration (ATSDR 2001; Biljana 2008). As an important consequence, today early-life exposure to PCBs is still worrying, as it is likely to be detrimental to neurodevelopment (Korrick and Sagiv 2008). The worldwide known "Belgian PCB/dioxin crisis" of 1999 was responsible for neurotoxic and behavioural effects in new-born babies, as well as an increase in the number of cancers (Covaci *et al.* 2008).

PCBs of soil are taken up by plant roots (Pal *et al.* 1980). Uptake by leaves is a further source of plant contamination (Ye *et al.* 1991). A recent study measured airborne PCBs accumulating in the epicuticular wax of pine needles; the results revealed that they are excellent, passive, non-destructive bioindicators for monitoring PCBs and other pollutants (Loganathan *et al.* 2008). As for PCB metabolism in plants, early studies focused on *Rosa* 'Paul's Scarlet' cell suspension cultures. In an initial study, a PCB isomer (2, 2', 4, 4'-tetrachlorobiphenyl), which is particularly resistant to microbial degradation, was used as a model compound: a metabolic rate of 45 pmoles PCB/h/DW was calculated. The *Rosa* metabolic activity was exerted between 14 and 18 days, the period of the culture cycle when neither cell division nor growth occurred; obviously, cultures were kept sterile (Fletcher *et al.* 1987). Then, four radiolabeled congeners of biphenyls with increasing chlorine content were provided to *Rosa* cultures for 4 days: it was found that the metabolizing capacity depended on the chlorine content, therefore 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl did not appear to be metabolized (Groeger and Flechter 1988). Furthermore, experiments on nineteen PCB congeners with chlorine content ranging from 2 to 6 indicated that *Rosa* cells in culture could metabolize eleven of them by >10%; oxidase inhibitors either stopped or severely reduced the biotransformation ability. The metabolic activity of *Rosa* appeared to be catalyzed by a cytochrome P-450- and/or P-

488-dependent enzyme system (Lee and Flechter 1992). The metabolites produced by *Rosa* were then partially identified as monohydroxylated PCBs, which are being glycosylated to more soluble compounds than the parent one (Butler *et al.* 1992). Interestingly, the physiology of *Rosa* 'Paul's Scarlet' cells used for these studies was found to closely resemble that of mature root cells; such a trait is highly promising for bioremediation with the intact plant, considering the large extension of whole root apparatus and its fine contact with soil fluids or particles (Flechter *et al.* 1987).

In spite of this clear evidence of the ability of *Rosa* to metabolize PCBs, subsequent researchers in this field only mentioned early studies on rose, but used different plant species (Wilken *et al.* 1995; Mackova *et al.* 1997; Kucerová *et al.* 2001; Rezek *et al.* 2007).

Fluoranthene

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants, resulting from incomplete combustion of organic materials. They are highly lipophilic compounds with two or more fused benzene rings. Anthropogenic emissions of PAHs are fossil fuel-burning, motor vehicles, waste incinerators, oil refining, coke and asphalt production, etc. (Srogi 2007). PAHs represent an increasing cause of concern for ecosystems, including human health, because some of them are mutagenic or even carcinogenic (Srogi 2007). PAHs are everywhere: in outdoor and indoor air, in waters, in urban and agricultural soil (Gevao *et al.* 2007; Morrillo *et al.* 2007; Sehili and Lammel 2007; Li *et al.* 2008). PAHs are taken up by plants and enter the food chain (García-Falcón and Simal-Gándara 2005; Fontcuberta *et al.* 2006; Aguinaga *et al.* 2007; Simon *et al.* 2007; Li *et al.* 2008); the dietary exposure to PAHs is more predominant than that due to inhalation (Suzuki and Yoshinaga 2007).

Fluoranthene is one of the most abundant PAHs, included in the EPA (Environmental Protection Agency, USA) priority list. Fluoranthene phytotoxicity has been studied in some plants (Oguntimehin *et al.* 2007; Kummerová *et al.* 2008). The metabolic fate of fluoranthene was investigated in 11 different plant species kept in cell suspension culture (Kolb and Harms 2000). Among these species, fed with radioactive fluoranthene during a 48 h incubation, an especially high rate of fluoranthene metabolism was exhibited by *Rosa* 'Paul's Scarlet' (50%), followed by *Lycopersicon esculentum* Mill. (15%). By doubling the incubation time, the accumulation of fluoranthene metabolites slightly increased in *Rosa* to 58.9% but significantly, to 26.7%, in tomato (Kolb and Harms 2000). The metabolic products of fluoranthene were further investigated only in *Rosa*, tomato, and *Triticum*; though quantitatively different depending on the plant species, they were all conjugated compounds with glucose and glucuronic acid, and the relative proportion of conjugation partners was again species-dependent. The formation of conjugates is not an uncommon way to metabolize toxic compounds; for example, it has been previously reported for plant metabolites of 4-*n*-nonylphenol (Bokern *et al.* 1996). Further analyses (gas chromatography-mass spectra and HPLC-diode array) identified *Rosa* and tomato metabolites as monohydroxylated derivatives of fluoranthene (1-, 3-, and 8- hydroxyfluoranthene). These same compounds identified in cell cultures were also formed by shoot and root tissues of intact tomato plants (Kolb and Harms 2000). Closely resembling the metabolites of *Rosa* and tomato plants, glucosylated 3-hydroxyfluoranthene is the major product of fluoranthene metabolism in the fungus *Cunninghamella elegans* Lendner: interestingly, the conjugated glucoside has no mutagenic effects (Pothuluri *et al.* 1990). Therefore, it seems worth taking into consideration the potential of fluoranthene bioremediation by plants such as *Rosa* and tomato.

Cyanide

Cyanide is a pollutant widely distributed in the environment (soil, water, and atmosphere) chiefly as a result of anthropogenic activity: mining operations are a major cyanide pollution source. Some years ago, the release of cyanide from water basins where it was accumulating from mining activities provoked more than one environmental disaster in river ecosystems (Harper 2005). Hydrogen cyanide (HCN) is a chemical used for a wide range of materials, such as plastics, cosmetics, adhesives, dyes, and others: its global production amounts to 1.4 million tons per year (Mudder and Botz 2001).

On the other hand, cyanide is known to represent a chemical defence tool in a large number of plant species and other organisms; moreover, all vascular plants are able to convert free cyanide into asparagine by means of the enzymes β-cyanoalanine synthase and β-cyanoalanine hydrolase (Castric *et al.* 1972); they also produce cyanide as a by-product in the synthesis of ethylene (Peiser *et al.* 1984). Nevertheless, plant sensitivity to exogenous free cyanide has been scarcely investigated. A study by Larsen and coworkers (2004) employed a garden rose 'Nordic Forever' among various other woody plants (willow, poplar, black elder, and birch) to evaluate their capacity to remove cyanide; rose and black elder are both known to be cyanogenic, that is, accumulate cyanogenic compounds for defence. In this study, detached leaves were exposed to 1 mg cyanide ion (CN^{-1}) solution in closed bottles: of all the leaves, willow showed the fastest removal of free cyanide (90% removal after 18 h), followed by elder and poplar (84 and 50% after 18 h, respectively). Rose leaves removed 75% of cyanide within 24 h, and petals only 19.4%; this delay in the uptake was probably due to the waxy surface of the epidermis, particularly in the case of petals. The results of Larsen *et al.* (2004) and those from other species (Trapp *et al.* 2003) clearly show that plants have a remarkable capacity to remove free cyanide. They also reported that in Denmark there are 150 waste sites (3,000 in the USA and Great Britain) of former gas plants which represent an important source of cyanide pollution. The selection of the most suitable plant as a 'bioreactor' for cyanide removal also depends on the climate and soil conditions at the mine site.

Chlorate

Chlorate is a chemically stable compound, which has strong oxidant properties (Urbansky 2002). Its presence in the environment is mostly anthropogenic in origin; for example, it is produced to obtain chlorine dioxide, a bleaching agent used in the pulp and paper industry. Brown algae exhibit an extraordinary sensitivity to chlorate of pulp mill effluents, while green algae and diatoms are unaffected (Rosemarin *et al.* 1994). Chlorate, moreover, is a by-product of drinking water disinfection with hypochlorite or chlorine dioxide (Narkis and Katz-Stoller 2002) and, as a result of ozonization when residual chlorine is present, its level in drinking waters may increase (Siddiqui 1996). The disinfection by-products became, in certain way, a new group of water contaminants (Moldoveanu and Bulea 2007). It was found that adverse pregnancy outcomes (in particular, premature births) are significantly associated with chlorate levels higher than 200 μg/l (Righi *et al.* 2003). The mechanism of chlorate toxicity was studied both *in vitro* and *in vivo* (Steffen and Wetzel 1993).

Chlorate is used in agriculture as an herbicide or as a defoliant; it also acts as an active product for flower induction in longan (*Dimocarpus longan* Lour.) in northern Thailand (Sutigoolabud *et al.* 2005). The lethal effect of chlorate on higher plants has long been recognized (Harvey 1931). Chlorate treatment at 28°C severely affects rice seedlings, inducing alterations in the chloroplast structure, increased lipid peroxidation at the leaf level, decrease of photochemical efficiency, and also damage to root tissues (Borges *et al.* 2004). The presence of nitrate reductase (NR) is necessary for the toxic effect of chlorate to be manifested: chlorate, acting as an analogue of nitrate, is a substrate of NR which reduces chlorate to chlorite. Higher plant mutants which are resistant to chlorate lack detectable NR, and do not grow on media containing nitrate as the sole nitrogen source (Müller and Grafe 1978; Mészáros and Pauk 2002). Surprisingly, chlorate resistant mutants of *Rosa damascena* Mill. Exhibited peculiar features (Murphy and Imbrie 1981). When strains of rose were selected for chlorate resistance, only a minor fraction (15%) did not grow on media containing nitrate as sole nitrogen source; these strains lacked the ability to reduce chlorate to chlorite. By contrast, the major fraction of the resistant strains (approximately 85%) grew on media containing nitrate as the sole nitrogen source. Therefore, the strategy adopted by these latter cells should have been different. Experiments using catalase, as an intracellular indicator for the presence of toxic products deriving from chlorate reduction, revealed that these strains lost catalase activity after chlorate treatment. Since these cells did not die, the loss of catalase did not depend on cell death. In other words, these strains have a mechanism for tolerating chlorate and its toxic reduction products, rather than avoiding them (Murphy and Imbrie 1981). These resistant strains of *R. damascena* were studied further; it was found that their resistance was greatly increased by the presence of glutamate in the medium. Cells took up chlorate and reduced it to chlorite, but the fraction that they reduced was smaller than that reduced by the wild type. The slower production of chlorite apparently accounted for their resistance to chlorate (Murphy *et al.* 1985).

1,3-dinitrobenzene

1,3-dinitrobenzene (DNB) and 1,3,5-trinitrobenzene are synthetic compounds used in explosives and ammunitions. Both compounds are air, soil, and water contaminants; their primary sources are waste discharge from Army ammunition plants. Soils in the vicinity of World War I ammunition plants are still contaminated (Haas *et al.* 2003). Exposure to high concentrations of DNB reduces the blood's ability to carry oxygen (Myers and Pinorini-Godly 2000); other effects on animal and human health include neurotoxicity (Tjalkens *et al.* 2000) and damages to the male reproductive system (Irimura *et al.* 2000; Sorenson and Brabec 2003).

As a further demonstration of the usefulness of plant tissue culture to evaluate plant/xenobiotic interactions, suspension cultures of *Rosa* 'Paul's Scarlet' cells were found to be able to metabolize DNB. As a matter of fact, in a 3-day period, 90% of the DNB supplied (96 nmol) was metabolized by 12 g (FW) approximately of rose cells; the primary end-product of DNB metabolism was recovered in the insoluble residue fraction of cell extracts (Wickliff and Flechter 1991). This metabolic ability of *Rosa* 'Paul's Scarlet' on ordnance compounds is a remarkable feature.

BIOSORPTION: AN UNCONVENTIONAL PROCESS OF BIOREMEDIATION

General concepts

All conventional techniques for the removal of heavy metals of environmental concern from contaminated waters have a number of disadvantages, such as incomplete metal removal, high reagent and energy requirements. Biosorption is a promising method of environmental control which offers an eco-friendly alternative for the treatment of heavy metal contaminated waters (Alluri *et al.* 2007; Wan Ngah and Hanafiaha 2008).

Biosorption is different from the more complex phenomenon of bioaccumulation, which is based on active metabolic transport. Biosorption has been defined as the property of certain biomolecules, which are present in certain types of biomasses, to bind and concentrate selected ions from aqueous solutions (Volesky 2007). In that, biosorption is passive, and even dead biomass can sustain the process. Biosorption is based on mechanisms such as electrostatic interaction (Aksu *et al.* 1992), ion exchange (Muraleedharan and Venkobachr 1990), and complexation (Aksu *et al.* 1992). More than one of these mechanisms may act simultaneously (Alluri *et al.* 2007).

The biosorption process involves a solid phase (the biosorbent) and a liquid phase, normally water, which contains a dissolved metal species to be sorbed (sorbate). Thanks to both the higher affinity of the sorbent for the sorbate, and the large preponderance of the sorbate molecules in the solution, these latter are attracted by the sorbent and bound to it (Alluri *et al.* 2007). The pH control in the system is important for both the configuration of the active ion-exchange sites (protonated or not) and the state of the sorbate in the solution (Volesky 2007). Many different types of biomasses are able to bind and concentrate various metal species thanks to appropriate chemical groups within their structures. The most important chemical groups involved in the metal binding are shown in **Table 1**.

The scheme of the biosorption process includes the selection of proper biomass to trap the metals, pre-treatment of the biosorbent to increase the efficiency of metal uptake, and its immobilization on inert matrices. Obviously, the manipulation of the natural biosorbent increases its cost (Holan and Volesky 1995; Volesky 2007). After metals are recovered in a concentrated form by the desorption process, the biosorbent can be regenerated and thus recycled for multiple uses. This is crucial to keep costs down (Alluri *et al.* 2007).

Since plant wastes are largely used as biosorbents, knowledge of the cell wall polymers is essential. Among these polymers, pectic substances are reported to increasingly bind heavy metals, in particular Pb(II), according to the reduction of the degree of esterification, i.e., making the negative charges of carboxyl groups available for covalent bonds with two valence metal ions (Kartel *et al.* 1999; Kho-

Table 1 Major binding groups for biosorption (from Volesky 2007, with modifications).

Binding group	HSAB classification	Ligand atom	Occurrence in biomolecules
hydroxyl	hard	O	PS, UA, SPS, AA
carbonyl	hard	O	peptide bond
carboxyl	hard	O	PS, UA, AA
sulfhydryl (thiol)	soft	S	AA
suphonate	hard	O	SPS
thioether	soft	S	AA
amine	int.	N	Cto, AA
secondary amine	int.	N	Cto, Pg, Peptide bond
amide	int.	N	AA
imine	int.	N	AA
imidazole	soft	N	AA
phosphonate	hard	O	PL
phosphodiester	hard	O	TA, LPS

HSAB = hard-soft acid/base principle; **PS** = polysaccharides; **UA** = uronic acids; **SPS** = sulphated PS; Cto = chitosan; **PG** = peptidoglycan; **AA** = amino acids; **TA** = teichoic acid; **PL** = phospholipids; **LPS** = lipoPS timchenko *et al.* 2007; Schiever and Patil 2008). On the other hand, dietary pectin with a low degree of esterification are being studied as perspective compounds to remove environmental lead from the body: a sort of biosorption within living organisms (Serguschenko *et al.* 2007). Waste materials from food processing industries or agricultural by-products provide large amounts of biomass for biosorption; they include exhausted coffee (Dakiky *et al.* 2002), wheat or rice bran, coconut husks (Alluri *et al.* 2007), leaves or leaf powder (Hanafiah *et al.* 2007; Sawalha *et al.* 2007), hardwood, cork biomass (Chubar *et al.* 2003), and many others (Wan Ngah and Hanafiah 2008). For example, fruit wastes (peels) derived from several citrus fruits, apple and grapes have good uptake capacity for Cd (Schiever and Patil 2008). Moreover, many organisms (bacteria, yeasts, fungi, and algae) represent abundant and good biosorbent masses. Some seaweeds are even specifically cultivated to be used for biosorption; brown algae, in particular, exhibit highly efficient biosorption ability, accumulating more than 30% biomass in dry weight in Pb and Cd (Volesky 1994; Regine and Volesky 2000; Romera *et al.* 2006).

Biosorption of heavy metals with *Rosa*

The use of flowers for biosorption is perhaps unique of rose. Not only are roses important shrubs cultivated throughout the world because of high market demand for ornamental (landscape, garden, cut flowers, hips, etc.), culinary, and medicinal uses, but also because they are the source of rose water and oil (Nasir *et al.* 2007). Rose oil, which is used in perfumes and fragrances, is obtained after distillation of rose petals from two major species cultivated for this purpose: *R. damascena* (Bulgaria, Turkey, Russia, India, and China) and *Rosa centifolia* L. (Morocco, France, and Egypt). A very large quantity of flowers is required, since it takes 4 kg of petals to obtain 1 g of oil. Therefore, rose waste becomes a problem due to high demand for the product: in 1998-99, the annual export of rose oil for production of cosmetics was >43 tons. *Rosa centifolia* petals have therefore been considered for their metal removing efficacy (Nasir *et al.* 2007). The sorption ability of Pb(II) and Zn(II) was found to be high, dependent on experimental parameters such as pH, concentration of metal, and contact time. Also native rose biomass can bind metals; however, the highest uptake capacity was reached after pre-treatment with NaOH. The functional groups likely involved in the sorption process are carboxyl, carbonyl, thioester, and sulfoxide (Nasir *et al.* 2007). Further research indicated that rose waste biomass was effective for removal of Pb(II) and Co(II) from contaminated water in the concentration range of 10 to 640 mg/l, exhibiting uptake capacities of 156 (Pb) and 27.15 (Co) mg/g (Javed *et al.* 2007).

In conclusion, biosorption offers several advantages over conventional procedures for treatment of metal-bearing industrial effluents. It can often be carried out on site, without transporting the toxic materials to treatment sites (Gavrilescu 2004). Other advantages include high efficiency, cost-effectiveness, minimization of chemical/biological sludge, and regeneration of sorbent with the possibility of metal recovery. When conventional methods results are either expensive or have low effectiveness, particularly in the treatment of large effluent volumes at low pollutant concentrations, biosorption is, on the contrary, effective. All these traits make biosorption an ideal method of water treatment also for countries with rapid industrial development and poor metal toxicity awareness (Alluri *et al.* 2007). In the particular case of roses in Turkey, it offers the concomitant advantage of exploiting the large petal waste which inevitably arises from distillation industries.

OTHER ENVIRONMENT-FRIENDLY USES OF *ROSA*

Activated carbon from rose waste

There is a considerable need for removal of dyes from wastewaters deriving from various types of industries (textiles, paper, carpet, leather, printing, etc.) to avoid contamination of natural aquatic bodies. Activated carbons exhibit adsorptive properties thanks to their extensive internal pore structure, which represents a high surface area available for the adsorption process (Gürses *et al.* 2006). Agricultural wastes are renewable and low cost sources of raw materials to obtain activated carbon: e.g., apricot, peach, and cherry stones; pecan, almond, and walnut shells; grape seeds; date pits, and many others. Chemical activation – generally consisting of impregnation of the raw material with phosphoric acid or zinc chloride – develops the pore structure (Khalili *et al.* 2000). Gürses and coworkers (2006) evaluated the use of *R. canina* seed wastes from a Turkish Herbal Tea Plant as the raw material to obtain activated carbon. As for the microstructure of the produced granular activated carbon, it presented both macropores and mesopores, with a porosity value of 43.5%. The maximum absorption capacity of a basic dye, methylene blue, onto the adsorbent from *Rosa* attained a value (47.2 mg/g) which was classified as 'normal'; it was much higher than that of other adsorbents such as those obtained from olive and apricot stones, or walnut and almond shells. Since *R. canina* seed wastes are found in abundance in Turkey, such a carbon cost is expected to be economical (Gürses *et al.* 2006). Furthermore, a valuable oil for medicinal use can be extracted from the waste material represented by rose achenes (Szentmihályi *et al.* 2002).

Biogas from rose waste

Biogas is a renewable source of energy with much lower environmental impact than conventional fossil fuels, since it reduces greenhouse gas emissions. It can be used to generate heat, hot water, and electricity at a relatively low cost. Biogas can be produced through anaerobic digestion of biomasses (such as manure in farm enterprises) obtaining 60-70% methane, 30-40% carbon dioxide, and other gases in traces. Anaerobic digestion is cost-competitive when compared to conventional waste treatments (EPA 2002). A Turkish research team has determined the characteristics of anaerobic digestion of residues derived from the rose oil industry in order to generate methane: the results showed that hydrolyzed rose residues produced slightly more methane than the original residue (Tosun *et al.* 2004).

Composting of rose waste

Composting is a biological treatment of solid organic wastes. The problem of large rose waste in Turkey has lead to the evaluation of the co-composting of rose processing waste and the organic fraction of municipal solid wastes. Mainly in the period from May to June, the rose oil industry in Isparta city produces two units of processing waste per one unit of rose flower milled on a wet weight basis (Tosun *et al.* 2008). Composting is the biological degradation of highly concentrated biodegradable organic wastes in the presence of oxygen to carbon dioxide and water; the final product of composting is a stable humus-like material which can be used as a soil conditioner or fertilizer (Zorpas *et al.* 2000; Tosun *et al.* 2008). The rose processing waste contains a high proportion of organic matter and plant nutrients without harmful components such as heavy metals and organic contaminants. The study of Tosun and coworkers (2008) has demonstrated the feasibility of the co-composting process at different mixture ratios and determined its kinetic features.

CONCLUSIONS

There is an enormous amount of literature dealing with plants within the context of environmental sciences or technologies such as biomonitoring and phytoremediation; in contrast, reports specifically focusing on *Rosa* are quite scarce. Nevertheless, from our survey it emerges that *Rosa* has great potential in this field and should be carefully considered. *Rosa* species are widespread geographically and are members of native vegetation established in critical environments. A number of experimental studies seem to confirm the ability of *Rosa* to accumulate heavy metals to a considerable extent and tolerate them. *Rosa rugosa* was shown to be highly promising for effective biomonitoring of some metals arising from either soil or air sources; this was true at both the vegetative and reproductive levels of the plant, though particularly pollen, the male gametophyte, exhibited a higher, more precocious sensitivity to environmental cues. Petals or seeds of *Rosa*, furthermore, represent abundant, low cost biomasses which are available for environment-friendly uses. Importantly, a number of *Rosa* species were successfully screened in culture for the ability to metabolize toxic compounds of anthropogenic origin. Therefore, it seems that the use of *Rosa* could offer many helpful opportunities towards progress in addressing the huge arrays of environmental concerns.

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