Environ Sci Pollut Res (2011) 18:842–856
DOI 10.1007/s11356-011-0498-0

REVIEW ARTICLE

Is phytoremediation a sustainable and reliable approach to clean-up contaminated water and soil in Alpine areas?

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Received: 12 November 2010 / Accepted: 16 March 2011 / Published online: 5 April 2011

Abstract
Background, aim and scope Phytoremediation does exploit natural plant physiological processes and can be used to decontaminate agricultural soils, industrial sites, brownfields, sediments and water containing inorganic and organic pollutants or to improve food chain safety by phytostabilisation of toxic elements. It is a low-cost and environment friendly technology targetting removal, degradation or immobilisation of contaminants. The aim of the present review is to highlight some recent advances in phytoremediation in the Alpine context.

Main features Case studies are presented where phytoremediation has been or can be successfully applied in Alpine areas to: (1) clean-up industrial wastewater containing sulphonated aromatic xenobiotics released by dye and textile industries; (2) remediate agricultural soils polluted by petroleum hydrocarbons; (3) improve food chain safety in soils contaminated with toxic trace elements (As, Co, Cr and Pb); and (4) treat soils impacted by modern agricultural activities with a special emphasis on phosphate fertilisation.
Conclusions, recommendations and perspectives Worlwide, including in Alpine areas, the controlled use of appropriate plants is destined to play a major role for remediation and restoration of polluted and degraded ecosystems, monitoring and assessment of environmental quality, prevention of landscape degradation and immobilisation of trace elements. Phytotechnologies do already offer promising approaches towards environmental remediation, human health, food safety and sustainable development for the 21st century in Alpine areas and elsewhere all over the world.

Keywords Phytoremediation · Alpine regions · Contaminated soils · Industrial wastewater · Petroleum hydrocarbons · Sulphonated aromatic compounds · Trace elements · Mycorrhizal fungi

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1 Background, aim and scope

Phytotechnologies can be defined as the use of plants to remediate, treat, stabilise or control contaminated substrates, and phytoremediation is one of these, dedicated to the removal or the destruction of contaminants. Phytoremediation does exploit natural plant physiological processes and can be used to decontaminate agricultural soils, industrial sites, brownfields, sediments and water containing metals and/or organic compounds. It is a low-cost and environment friendly technology targetting extraction, degradation or fixation of the contaminants (Schwitzguébel et al. 2009). Similar technologies of ecological engineering or ecoremediation can be used for revegetating degraded land (like quarries, road sides), as well as for removing excessive nutrient loads and cleaning-up wastewater (road runoff, municipal and industrial effluents, surface and seepage water) with soil–plant filters, buffer strips and constructed wetlands (Otto et al. 2008; Bulc and Slak 2009; Vymazal 2009; Borin et al. 2010). Plants represent a more environmentally compatible and less expensive method to site restoration compared to physico-chemical and engineering techniques, even though the time scale required to reach the fixed end-points can become a limiting factor for such ecoremediation approaches (Denys et al. 2006; Komives et al. 2009; Mench et al. 2009, 2010; Vangronsveld et al. 2009). Plants are already cleaning our environment constantly, everywhere, even if we do not recognise or know it. On the other hand, biomass produced on contaminated land can be used as renewable energy source, not competing with food supply and contributing to sustainable land use management (Van Ginneken et al. 2007; Meers et al. 2010). Since plants can also exclude metal or organic contaminants, and thus reduce their transfer to the food chain, phytotechnologies are also offering efficient tools to improve food safety.

The bioavailable fraction of soil contaminants should be considered as the most important one from an ecological, toxicological and health standpoint, and it is determined by the chemical properties of the metal or organic compound, soil and climatic characteristics, ageing processes and biota behaviour. Ageing usually decreases bioavailability, but root exudates, root-induced rhizosphere changes, mycorrhizal fungi and rhizospheric bacteria play a major role in the dynamics and the ability of contaminants to move from soils to plants; vertical and horizontal spreading of contaminants to the surroundings and groundwater will also be affected. The bioavailability of contaminants and their uptake by crop plants are essential parameters for establishing risk-based regulatory guidelines and enhancing food safety (Mench et al. 2009).

A significant part of agricultural land in Europe—including many Alpine territories—is contaminated with heavy metals and organic chemicals, some of which still in agricultural use. Food produced on those sites can pose human health risks: several important agro-ecosystem functions are impaired and such sites can be sources of food contamination and further pollution via re-spreading to the surroundings by wind and water erosion or leaching into groundwater. Due to more severe legislation in many countries, contaminated agricultural soils still under production and many additional areas, which until now have not been subject to regulation, will be taken out of food production and become marginalised. There are two alternatives to deal with such soils: they have to be set aside or cleaned. Conventional remediation methods like landfilling or excavation and extraction impose high costs, destroy soil structure, and diminish soil productivity. Sites like these need a sustainable plant cover to prevent re-entrainment of particulates and further contamination of more agricultural land as well as direct impact on local populations. Phytotechnologies can offer a cost-effective in situ alternative for low- or medium-contaminated soils resulting in increased soil fertility (Baker et al. 1994; Vangronsveld et al. 2009; Mench et al. 2010).

Brownfields—contaminated sites around former and present mines, abandoned old industrial sites or ash and slag dumps from coal-fuelled power plants, coal and gas plants, oil-refineries, ammunition plants, military bases, and pesticide tombs, are numerous in Europe, and their restoration for future safe use has become an important issue. An appropriate rehabilitation and sustainable management of contaminated brownfields is thus now a priority. Phytotechnologies are expected to play a major role in the restoration of former industrial areas, but the activities must also include site identification and characterisation, parallel soil treatability tests, as well as field-scale implementation and evaluation (French et al. 2006; Onwubuya et al. 2009; Vangronsveld et al. 2009; Mench et al. 2010). Green plants can also be used to treat freshly dredged polluted sediments, even if this approach is only at its infancy (Bert et al. 2009).

The most significant phytotreatments of soils, sites and brownfields are the following:

- Phytostabilisation, based on the immobilisation of organic and inorganic contaminants by the addition of appropriate soil amendments, the adsorption to plant roots or soil particles, and the precipitation in the root area, thus preventing their migration and decreasing erosion, runoff and leaching (Kumpiene et al. 2007). It also promotes restoration and biodiversity of ecosystems accounting for ecological benefits or the production of industrial crops producing essential oil or fibres. The most effective is the use of indigenous plant species, accustomed to the local climate and soils and not creating adaptation or invasion problems.
- Phytoextraction, based on the absorption of contaminants into roots, then translocation into shoots, followed by harvest and destruction of the plants. Depending on their market value, metals can be recovered from contaminated biomass or ash (Dickinson et al. 2009).
- Phytodegradation and phytotransformation of xenobiotic compounds, exploiting the huge potential and biodiversity

of plant secondary metabolism (Schwitzguébel et al. 2008).
- Phytostimulation: enhanced microbial metabolism of organic pollutants in plant rhizosphere; plant/microbial interactions are important for such a process (Gaskin and Bentham 2010).
- Hydraulic control of pollutants: the use of phreatophyte trees (poplar, willow and aspen) to transpire large amounts of water and thus limit the transport of groundwater pollutants (Liste and White 2008).

In constructed wetlands, plants are used as part of a managed ecosystem to remove contaminants from aqueous waste streams (Haberl et al. 2003; Imfeld et al. 2009; Vymazal 2009; Vymazal and Kröpfelovà 2009). Alternatively, hydroponic cultures or nutrient film techniques can be used in a process called rhizofiltration or phytofiltration (Schwitzguébel et al. 2008, 2011). Waters under consideration include industrial and domestic wastewater, groundwater and surface water as well as landfill leachates, containing biodegradable or recalcitrant organic compounds, toxic metals and/or radionuclides.

The aim of the present review paper is to highlight some recent advances and case studies where phytoremediation has been or can be successfully applied in the Alpine context to treat: (1) industrial wastewater containing sulphonated aromatic compounds released from synthetic dye and pigments production; (2) agricultural soils polluted by petroleum hydrocarbons; (3) soils contaminated with As, Co, Cr and Pb; and (4) soils polluted by modern agricultural activities with a special emphasis on phosphate fertilisation.

2 Toward the phytotreatment of wastewater from chemical industries

Due to the proximity and abundance of water and hydroelectric power plants, many industries have been established in Alpine regions, especially in the upper Rhone and Rhine valleys; among others, chemical industries producing fine chemicals like dyes and pigments. To treat effluents released by these industries, often containing recalcitrant compounds, classical wastewater treatment plants are not always efficient, and reliable alternatives are thus needed. More precisely, constructed wetlands and hydroponic systems (rhizofiltration or phytofiltration) are able to remove and degrade many organic pollutants from industrial wastewater (Haberl et al. 2003; Bulc and Ojstrsek 2008; Schwitzguébel et al. 2008, 2011). Both are based on the use of appropriate plant species and offer a low-cost, low-maintenance approach to treat recalcitrant xenobiotic compounds. Before, to be applicable at large scale, however, research and development are needed to choose the most efficient plant species, characterise the detoxification mechanisms, design and size the system and define the optimal operation conditions.

Such an approach has been applied to develop the phytotreatment of synthetic sulphonated aromatic compounds, the parent molecules for a large palette of dyes and an important starting material in their production. Dyes are intentionally designed to be resistant under typical usage conditions, making difficult the treatment of their by-products and of wastewater from production lines. Because they contain at least one sulphonic group and often also varying substitutions such as nitro groups, these chemicals are not uniformly susceptible to biodecolourisation and biodegradation. Effluents from dye, textile and detergent industries, but also leachates from landfills, are thus often contaminated with sulphonated aromatics, giving to these chemicals an actual impact on the environment, especially fresh water (Schwitzguébel et al. 2002). The removal and/or degradation of sulphonated xenobiotics from industrial wastewater thus remains a major challenge, not only because of the colour, but also of recalcitrance and toxicity. Over the last two decades, several physical or chemical treatments have been tested; however, they have major disadvantages, including high cost, low efficiency and inapplicability to a wide variety of dyes, as well as the formation of by-products, creating disposal problems of contaminated wastes. On the other hand, the microbial degradation of synthetic dyes including azo and anthraquinone derivatives often requires unusual catabolic properties rarely found in a single bacterial or fungal species, and the accumulation of dead-end products often occurs (Schwitzguébel et al. 2002). The limited ability of micro-organisms to degrade sulphonoaromatic compounds, and thus to cope with various mixtures of these xenobiotics, limits the efficiency and, therefore, the use of conventional wastewater treatment plants based on activated sludge.

In such a context, the ability of several plant species to remove sulphonated anthraquinones from synthetic wastewater has been tested in hydroponic systems (Aubert and Schwitzguébel 2004). As shown in Table 1, the most promising results were obtained with plants producing natural anthraquinones, like *Rheum rabarbarum* (rhubarb), especially the Valentine cultivar, or *Rumex hydrolapatum* and *Rumex acetosa* (Aubert 2003; Haberl et al. 2003; Aubert and Schwitzguébel 2004). However, the removal of a pollutant from a liquid medium does not mean that it is accumulated and degraded by the plant itself. The next step was thus to investigate any possible adsorption, uptake, metabolism and degradation by plants. As measured by capillary electrophoresis (Aubert and Schwitzguébel 2002), sulphonated anthraquinones were

Table 1 Removal of sulphonated anthraquinones by different plant species cultivated under hydroponic conditions

Plant species	AQ-1S (68.8 mg L^{-1})	AQ-2S (65.7 mg L^{-1})	AQ-1,5-SS (82.5 mg L^{-1})	AQ-1,8-SS (82.5 mg L^{-1})	AQ-2,8-SS (82.5 mg L^{-1})
Control (no plant, dark)	8–13	12–16	13–19	12–18	6–11
Rheum rabarbarum (Valentine)	78–89	84–94	79–89	79–89	70–82
Rheum rabarbarum (Sutton)	51–63	62–72	53–68	53–69	31–50
Rumex hydrolapatum	39–58	69–81	39–56	40–56	40–56
Rumex acetosa	44–53	70–75	31–53	39–53	38–47
Apium graveolens	53–70	56–69	32–42	31–42	30–40

The percentage of each sulphonated anthraquinone removed was measured 6 weeks after the simultaneous addition of each sulphonated anthraquinone in the liquid medium at the concentrations indicated and expressed as the minimal and maximal values of three replicates. Details of experimentation as previously described (Aubert 2003; Aubert and Schwitzguébel 2004)

found in leaves of rhubarb (Fig. 1) and *R. hydrolapatum* (Fig. 2), indicating their uptake and translocation by these plants. As compared to leaf extracts from plants cultivated without sulphonated anthraquinones, new metabolites were found in plants cultivated with these xenobiotics (Figs. 1 and 2), suggesting that at least some of them, if not all, were transformed by both plant species. Furthermore, the profile of metabolites produced depended on the plant used, highlighting the importance of a careful screening of plant species, ecotypes or cultivars before any application of phytoremediation. Enzymatic investigations have been performed to determine if sulphonated anthraquinones might be transformed by enzymes of the classical detoxification pathways in plants. Results obtained with glutathione S-transferases show that this class of enzymes is not significantly involved in the observed metabolism of sulphonated anthraquinones (Aubert 2003). In contrast, cytochrome P450 monooxygenases are involved in the detoxification of sulphonated anthraquinones (Page and Schwitzguébel 2009a, b) and glycosyl-transferases could also be involved in the next steps of the metabolism of synthetic sulphonated anthraquinones, with possible crosstalks with the metabolism of natural anthraquinones, often glycosylated as well.

Rhubarb is a hardy perennial plant and appears as a promising species in developing new phytotreatments to decontaminate effluents containing sulphonated aromatic compounds. However, before any industrial application, pilot-scale experimentation should be performed to assess the capacity of this plant and of other species producing natural anthraquinones to deal with real effluents at different concentrations and loading rates and to correctly design and size rhizofiltration treatment units.

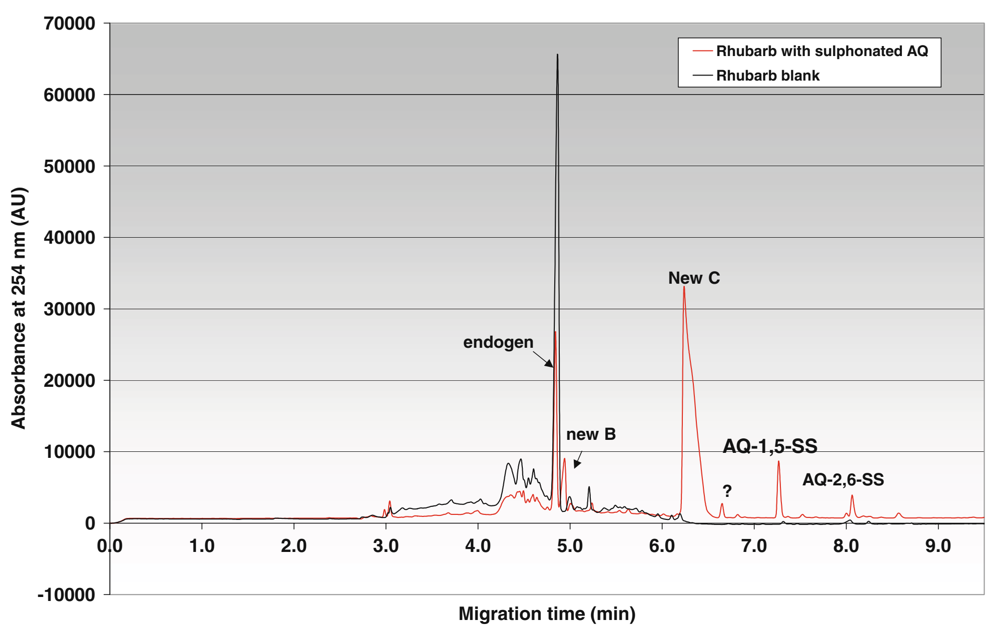

Fig. 1 Analysis by capillary electrophoresis of rhubarb leaf extracts. Rhubarb was cultivated with or without (*blank*) sulphonated anthraquinones

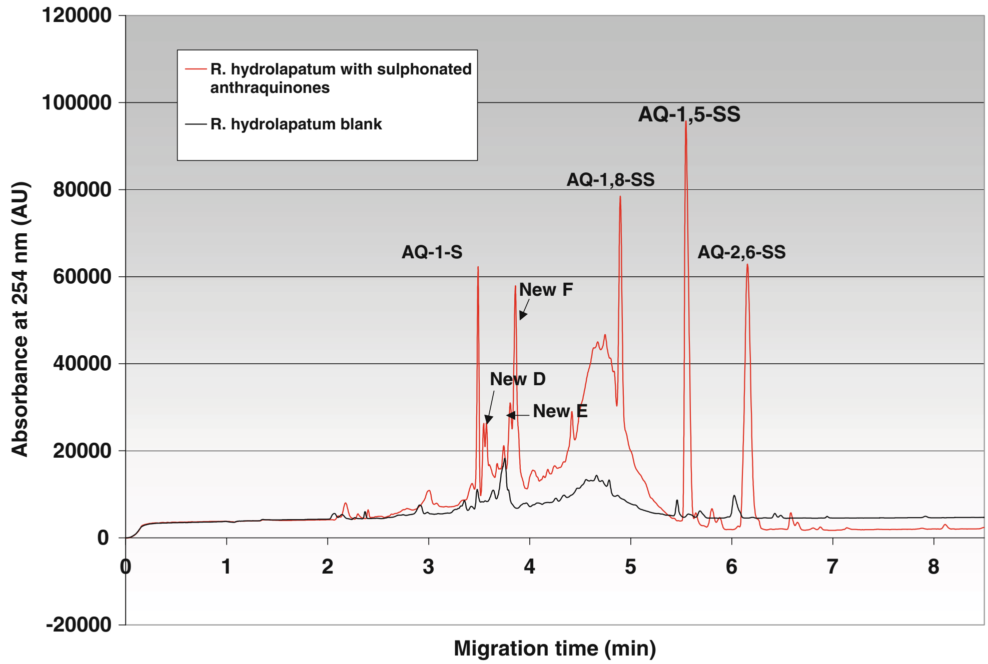

Fig. 2 Analysis by capillary electrophoresis of *Rumex hydrolapatum* leaf extracts. Rumex was cultivated with or without (*blank*) sulphonated anthraquinones

3 Phytoremediation of a soil polluted by petroleum hydrocarbons: a comprehensive field trial

A phytoremediation trial was conducted on agricultural soil polluted with petroleum hydrocarbons as a result of a land-based oil well blow-out in Northern Italy in 1994 (Fig. 3). Prior to this trial, the contaminated soil was extensively treated in a biopile to enhance hydrocarbon degradation and then spread back to its original location. The contaminated soil (15 hectares) was divided into parcels representing a modified Latin square design in which phytoremediation and landfarming were applied as replicates (Fig. 4). Local crops were used in the phytoremediation treatment.

The main objectives of the whole field trial were to assess the potential of phytotreatment for the removal of petroleum hydrocarbons under real conditions; compare landfarming treatment and natural attenuation versus phytoremediation in terms of soil remediation; reduce the pollutant's concentration to a level acceptable for an agricultural soil; evaluate the kinetics of the reduction of concentrations of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAH); select the crops with the highest ability to reduce crude petroleum hydrocarbons in contaminated soil; assess the potential of uptake and/or enhancement of microbial activities of each plant species in terms of remediation of contaminated soils. A complementary study was conducted in greenhouse to compare results obtained in the field and in a more controlled environment, since temperature, humidity and light parameters are set under greenhouse conditions (Zabłudowska et al. 2009). Here, we report on the first three growing seasons (summer 1998, winter 1998–99, and summer 1999), when 11 agricultural species were planted. Plants selected for summer seasons were alfalfa, fescue, clover, corn, ryegrass, sorghum and soya. Plants selected for the winter season were fescue, rape, ryegrass, rye, vetch and triticale. Landfarming treatment consisted of periodic tilling of parcels. Furthermore, the study included the assessment of natural attenuation (weedy areas) which consisted in weeds growing naturally between planted parcels (weed growth, no crops, no tilling).

Fig. 3 The spill of a land-based oil well in Northern Italy (March 1994)

From each parcel including landfarming and weedy areas, TPH were extracted using supercritical fluid extraction and analysed by gas chromatography coupled with flame ionisation detector. Accelerated solvent extraction was used to extract 35 individual PAH from soil, while for plants, Soxhlet extraction method was used. PAH in soils and in plants were analysed using gas chromatography

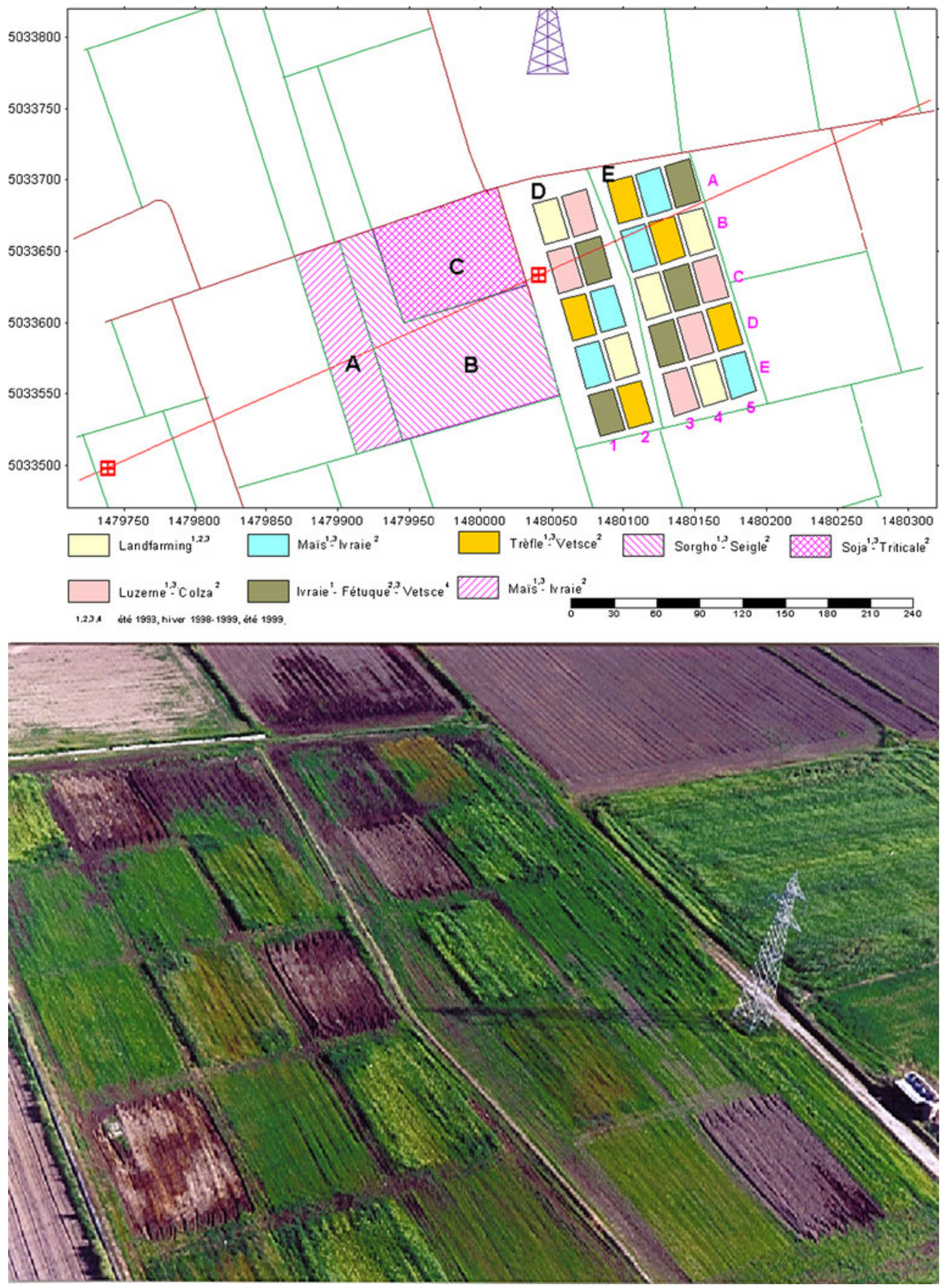

Fig. 4 Scheme and aerial picture of the contaminated soil divided according to the Latin square statistical model

coupled with mass spectrometry. Different statistical treatments were applied in this study; among them are analysis of variance (ANOVA), regression, linearity, parallelism, homogeneity of variance and Student tests. The lack of homogeneity of the soil in the field was also statistically established and coincided with the heterogeneity of plants growth in the same parcel. Details of the experimentation and of statistics are available elsewhere (Plata-Chebbah 2000).

Results obtained during the first summer season indicated that the start of the decontamination process was long and slow. Indeed, soil TPH and PAH concentrations decreased throughout planted, landfarmed parcels and natural attenuation areas, but significantly only with maize and red clover. Average reduction in soil TPH ranged from 721 to 2,849 kg ha^{-1} (Table 2). Reduction in soil PAH from the site was between 1.3 and 12 kg ha^{-1}. During this season, all the agricultural plants underwent stress and exhibited reduced growth, reduced size and yellow colour (Plata-Chebbah 2000).

During the winter season, the rate of soil TPH decrease was significantly greater in cultivated parcels and weedy areas than in landfarmed parcels (Table 2). Reduction in soil TPH from the site ranged from 1,644 (landfarming) to

Table 2 Removal of TPH and PAH from contaminated soil (mean values of 3–5 parcels sampling)

Season	Initial TPH (mg kg^{-1})	Final TPH (mg kg^{-1})	Removed TPH (kg ha^{-1})	Initial PAH (μg kg^{-1})	Final PAH (μg kg^{-1})	Removed PAH (kg ha^{-1})
Summer 98						
Alfalfa	3,308	2,405	2,168	6,329	4,960	3.3
Maize	3,235	2,227	2,419	6,680	4,017	12.0
Red clover	3,414	2,227	2,849	8,687	4,373	10.4
Ryegrass	3,751	3,451	721	8,351	6,655	4.1
Land farming	3,322	2,681	1,538	6,846	6,298	1.3
Winter 98–99						
Fescue	3,134	1,144	4,777	6,855	3,960	6.9
Rape	2,403	908	3,588	4,183	1,953	5.4
Rye	3,055	1,208	4,432	5,784	5,037	1.8
Ryegrass	2,687	1,081	3,854	6,258	2,785	8.3
Triticale	4,202	1,930	6,478	6,317	5,794	1.6
Vetch	2,152	765	3,330	5,224	2,916	5.9
Weed	2,440	701	4,173	5,213	1,692	8.5
Land farming	1,716	1,031	1,644	4,428	2,156	5.5
Summer 99						
Alfalfa	1,786	861	2,221	3,673	2,273	3.4
Fescue	2,055	826	2,950	3,861	2,408	3.5
Maize	2,932	561	5,692	4,100	1,927	5.2
Red clover	1,564	677	2,127	4,442	2,720	3.7
Sorghum	2,749	486	5,431	5,230	1,829	8.2
Soya	2,952	1,155	4,313	3,432	1,201	7.0
Weed	2,507	472	4,885	3,467	1,703	6.1
Landfarming	1,009	735	104	2,558	1,634	2.2

6,478 kg ha^{-1} (triticale). Soil PAH concentrations decreased in planted and landfarmed parcels, as well as in weedy areas. However, the smallest quantities of soil PAH removed were observed for rye and triticale, and the highest for ryegrass and weed. The growth of crop plants was less affected than during the first season (Plata-Chebbah 2000).

During the second summer season, the concentration of soil TPH and PAH only slightly decreased in landfarmed parcels (Table 2). The average removal of TPH was statistically significant for almost all other conditions and ranged from 2,127 (red clover) to 5,692 kg ha^{-1} (maize) in planted parcels, whereas it was only 104 kg ha^{-1} in landfarmed parcels. The removal of soil PAH ranged from 2.2 (landfarming) to 8.2 kg ha^{-1} (sorghum). The reduction in soil TPH concentration coincided with an increased plant growth, as compared to both previous seasons (Plata-Chebbah 2000).

In parallel alfalfa, clover and fescue (same seeds stock as that used on the contaminated site) were grown in greenhouse environment, in soils from the contaminated site. The results obtained showed that plants grown in greenhouse conditions had a more positive effect on the rate of soil TPH and PAH reduction and plant PAH uptake than those grown under field conditions (Plata-Chebbah 2000). Thus, any extrapolation of phytoremediation results obtained in a greenhouse (reduced scale) to the field (full scale) must be made cautiously, as all the environmental conditions that affect the outcome of field studies do not prevail in greenhouse conditions (Euliss et al. 2008; Zabłudowska et al. 2009).

During the three growing seasons, the very low plant PAH concentration (230–860 ng g^{-1} shoot DW) appeared to be a function of soil PAH concentrations. Plant PAH concentrations were thus the highest during the first season and lowest during the third season being however in the same order of magnitude as that of the control plants (not shown). Parallel determinations of PAH concentrations in the plants and PAH degradation rates in the soil indicated that the degradation of PAH (mostly 2–4 rings: naphthalene, phenanthrene, dibenzothiophene, pyrene, fluoranthene and chrysene) was due to rhizospheric bacteria, and that plants decisively improved their growth and working conditions, as already shown by other studies (Nedunuri et al. 2000; Huang et al. 2005; Denys et al. 2006; Liste and Prutz 2006; Palmroth et al. 2006; Rezek et al. 2008; Gurska et al. 2009; Gaskin and Bentham 2010). In contrast, the capacity of plant roots to take up PAH from soil appears to be limited (Gao and Ling 2006; Lin et al. 2007; Gao and Collins 2009; Xu et al. 2009).

When crop plants were cultivated on contaminated soils, TPH were degraded more rapidly than under landfarming conditions, which could be attributed to the positive effects of plants on rhizospheric micro-organisms and to the ploughing up of dirtier soils from lower soil layers (Huang et al. 2004, 2005; Keller et al. 2008). Phytoremediation treatment, especially in the case of maize and sorghum, was much more efficient than landfarming and better than natural attenuation as a facilitator of soil hydrocarbon degradation. The crop rotation maize/ryegrass was found to be the most efficient for the removal of petroleum hydrocarbon from soil (Plata-Chebbah 2000).

The phytoremediation trial began in 1998 and ended in 2004. Afterwards, the soil was clean enough to be reallocated to agriculture. It thus appears that crop plant cultivation and rotation combined with appropriate monitoring for less than a decade is a successful approach to the remediation of hydrocarbon-polluted agricultural soils, even under Alpine conditions.

4 Soil contamination by As, Co, Cr and Pb in an Alpine territory used for crop cultivation

Heavy metal contamination of soils is a worldwide concern, since forage plants and/or food crops are often still cultivated there (Nehnevajova et al. 2005; Quartacci et al. 2006; Kidd et al. 2007; Nehnevajova et al. 2007, 2009; Comino et al. 2009). When present in soil and water, metals can accumulate in living organisms, enter in food chain and affect human health, due to their toxicity. Phytomanagement of metal-contaminated agricultural land is nowadays an interesting approach to either extract or immobilise metals (Fitz and Wenzel 2002; Garcia et al. 2005; Remon et al. 2005; Grispen et al. 2006; Hartley and Lepp 2008; Hernandez-Allica et al. 2008; Verkleij 2008; Butcher 2009; Dickinson et al. 2009; Kidd et al. 2009; Marques et al. 2009a; Memon and Schröder 2009; Pedron et al. 2009; Robinson et al. 2009; Fässler et al. 2010).

A study was initiated because many soils utilised for agriculture, landfarming, grazing and green areas in Alpine areas are often contaminated by metal(loid)s, for example, in Northern Italy (Fig. 5). Many of them can be accumulated and eventually concentrated in the edible parts of crops, depending on their speciation, solubility and bioavailability and by the ability of a crop to take up the essential and non-essential elements and translocate them to the target organs (Burgos et al. 2008; Clemente et al. 2008; Dessureault-Rompre et al. 2008; Almendras et al. 2009; Quartacci et al. 2009). Metals can be absorbed by plants, wildlife and people through the food they eat. They can also be absorbed by drinking contaminated water. Some heavy metals can also be concentrated (biomagnification) when predator animals eat prey animals as part of the food chain.

The aim of the experimentation was to study the accumulation and translocation of arsenic (As), chromium (Cr), cobalt (Co) and lead (Pb) in plants used as grazing crops or as cover plants to create public open space and parkland. The target of this research was double: first to measure the amount of these elements accumulated in plants (food/feed safety), then to evaluate the capacity of plants to remove metals from soil (phytoextraction). From the results obtained, the risk of food chain contamination and the potential for soil phytoremediation could be assessed. Experimentation was done in laboratory by creating the conditions of a natural site.

The soil was collected at Monteu Roero, a suburban area, south west of Torino, Piedmont Region, Italy (point A in Fig. 5). Composite soil sample was collected from the surface to a depth of 20 cm, air dried, homogenised and analysed for granulometry. The textural analysis showed the following composition, 80.8% fine sand and 19.2% grit. The high level of fine sand indicated that this soil could be classified as sandy soil. The soil was analysed for basic chemical–physical properties and for the As, Cr, Co and Pb concentrations (Table 3).

The soil was spiked with four different concentrations of metal(oid)s (Table 4), whereas one soil sample did not receive any metal (control). Concentrations were 1×, 10×, 20× and 50× the Italian standard limit for discharging in superficial water bodies [Italian Environmental Code Dlgs 152/1999] because in this area, most of the water discharge is on soil surface.

Since it is always more appropriate to use local species for phytoremediation than exotic plants (Yoon et al. 2006; Antosiewicz et al. 2008; Barrutia et al. 2009; Marques et al. 2009b), seeds of plants characteristic of this area were chosen: *Medicago sativa* (alfalfa), *Trifolium incarnatum* (red clover) and a mix of seeds for forage. Alfalfa has the capacity to accumulate metal(oid)s above the tolerance levels of other plants and has the characteristics required by a plant for the extraction from contaminated water and soil (Peralta-Videa et al. 2001). Red clover was selected because of its use as a cover plant to create space/parkland and for its importance as a grazing crop. The mix for forage is made up of eight types of seeds: *Lolium perenne* Livree 26%; *Lolium multiflorum* L. 15%; *Trifolium pratensis* 13%; *Glomerata amba* 11%; *Festuca arundinacea* Demeter 11%; *Dactylis phleum* Pratense climax 10%; *Lotus corniculatus* 7%; and *Trifolium repens* Huia 7%. It was decided to analyse the capacity to absorb the metals from this composition because it is an ideal grazing/cover plants

Fig. 5 Polluted area where carried out the idea of the project and where the soil has been collected for the experimental test (Monteu Roero)

mix. On the other hand, co-planting has been reported to be at least as efficient as mono-cropping to remove Cd and Zn from contaminated soils (Jiang et al. 2010).

Details of the methodology used have been described elsewhere (Comino et al. 2009). To evaluate the phytoextraction potential of plants as well as the risk of food chain contamination, metal concentration in roots and shoots were measured; and the bioconcentration factor (BCF, the ratio of metal concentration in plant roots and in soil) as well as the translocation factor (TF, the ratio of metal concentration in plant shoots and in roots) were calculated (Table 5).

Table 3 Main characteristics of soil (Monteu Roero (CN), Italy)

Parameter	Sampled soil
pH (in water)	7.52
TDS [mg l^{-1}]	171.89
Na [g kg^{-1}]	0.004
K [g kg^{-1}]	0.064
NH_4^+ [g kg^{-1}]	0.007
Ca [g kg^{-1}]	0.146
Mg [g kg^{-1}]	0.024
F [g kg^{-1}]	0.001
Cl [g kg^{-1}]	0.391
NO_2^- [g kg^{-1}]	0.003
NO_3^- [g kg^{-1}]	0.011
PO_4^{3-} [g g^{-1}]	0.002
SO_4^{2-} [g kg^{-1}]	0.013
HCO_3^-[g kg^{-1}]	0.575
As [μg kg^{-1}]	0.006
Pb [μg kg^{-1}]	0.026
Co [μg kg^{-1}]	0.013
Cr [μg kg^{-1}]	0.083

At the end of the study period (6 months), the effect of metals on plants was visible. In fact, change in colour and a reduced development of the plant were the first indicators of the presence of As, Co, Cr and Pb, in agreement with previous studies concerning the effect of heavy metals on plants (Cheng 2003). The growth of *T. incarnatum* was reduced and the plant developed thick white spots on leaves; this phenomenon was also observed in *M. sativa* and in the mix.

In most cases, the concentrations of As, Cr, Co and Pb were low in the shoots and higher in the roots. In *M. sativa*, a low accumulation of As was observed, but only when 500 μg As L^{-1} were added, and TF values reached a value of 1 (Table 5). The concentration of Co in shoots ranged from 0.06 to 1.68 μg kg^{-1} DW, depending on the concentration of spiked Co, with an increased TF value as a function of added concentration. Chromium concentrations ranged from 0.7 to 1.5 μg kg^{-1} DW in shoots, and from 4.6 to 16 μg kg^{-1} DW in roots, with low TF values (Table 5). Low TF values were also observed for Pb.

Chromium was the metal that was the most accumulated by *T. incarnatum*, reaching values from 1.2 to 7 μg kg^{-1} DW in shoots and from 3.6 to 10.4 μg kg^{-1} DW in roots, with TF values above 1 for CII and CIII (Table 5). In *T. incarnatum*, As was more accumulated in shoots (0.67 μg kg^{-1} DW) than in roots (0.58 μg kg^{-1} DW), when added concentrations were CI or CII, showing TF values >1 in any case. The accumulation of Pb varied from 0.8 to 2.6 μg mg^{-1} DW for both shoots and roots, with TF values from 0.63 to 1.01. Cobalt accumulation varied from 0.27 to 4.16 μg kg^{-1} DW in shoots and from 1.05 to 3.8 μg kg^{-1} DW in roots, with TF usually higher than 1 (Table 5).

Plant species present in the mix for forage accumulated Cr in roots with concentrations up to 106.3 μg kg^{-1} DW, but seemed unable to accumulate significantly As, Co and Pb. In most cases, the translocation to shoots remained low (Table 5).

Arsenic, Co, Cr and Pb were accumulated mostly in roots, but the BCF remained very low for alfalfa and red clover for most concentrations tested, indicating that these elements did not exceed the respective soil level (Table 5). For the forage mix, the BCF remained low, except for Co, but the translocation of this element to shoots was low.

Even if soil concentrations of added As, Co, Cr and Pb were rather high (CIII, CIV), alfalfa, red clover and the mix for forage did not accumulate significantly these toxic elements. However, results need to be confirmed in the field, but all tested plants should be suitable for forage. This feature is important when considering the entire food chain from the soil where these forage crops are grown to animals and humans. In contrast, these plants did not appear to be suitable for phytoextraction of these elements, since concentrations in shoots remained low. This case study highlights that small scale tests are needed to check if and where pollutants are accumulated in plants before selecting the most appropriate species for food chain safety or phytoremediation.

5 Soil pollution by modern agricultural activities and the role of microbes association to improve soil quality

Over the last decades, the use of chemical fertilisers including phosphorus has significantly increased to enhance crops yield. This fertilisation has also led to the accumulation of inorganic elements in soils, often causing environmental concerns like eutrophication of surface water.

Table 4 Metal concentrations added in Rorison's solution to irrigate soil (CI=Italian standard limit for discharging in superficial water bodies [Dlgs 152/1999]

Metals	CI [μg L^{-1}]	CII [μg L^{-1}]	CIII [μg L^{-1}]	CIV [μg L^{-1}]
As	10	100	200	500
Pb	10	100	200	500
Co	50	500	1,000	2,500
Cr	50	500	1,000	2,500

Table 5 Bioconcentration factor (BCF) and translocation factor (TF) calculated for As, Co, Cr, Pb, [BCF=metal concentration ratio of plant roots to soil], [TF=metal concentration ratio of plant shoots to roots]

BCF					TF				
Alfalfa	As	Co	Cr	Pb	Alfalfa	As	Co	Cr	Pb
Control	0.04	0.06	0.08	0.04	Control	1.00	0.07	0.08	0.15
CI	0.04	0.13	0.08	0.04	CI	1.00	0.06	0.14	0.36
CII	0.04	0.13	0.07	0.02	CII	1.00	0.24	0.15	0.43
CIII	0.03	0.17	0.08	0.03	CIII	1.00	0.49	0.29	0.36
CIV	0.14	0.30	0.31	0.12	CIV	0.35	0.80	0.04	0.17
Red clover	As	Co	Cr	Pb	Red clover	As	Co	Cr	Pb
Control	0.03	0.01	0.02	0.01	Control	1.38	1.63	0.83	7.50
CI	0.04	0.10	0.07	0.04	CI	1.00	0.26	0.35	1.01
CII	0.03	0.12	0.07	0.06	CII	1.26	2.07	1.11	0.78
CIII	0.05	0.14	0.10	0.05	CIII	1.51	1.37	1.11	0.63
CIV	0.07	0.19	0.14	0.06	CIV	1.12	1.11	0.12	1.01
Forage mix	As	Co	Cr	Pb	Forage mix	As	Co	Cr	Pb
Control	0.04	0.22	0.36	0.01	Control	1.00	0.39	0.11	3.30
CI	0.06	1.70	0.30	0.07	CI	0.44	0.14	0.13	0.20
CII	0.16	0.52	0.58	0.16	CII	0.25	0.30	0.31	0.65
CIII	0.21	4.44	0.83	0.16	CIII	0.47	0.66	0.25	0.94
CIV	0.31	2.38	1.45	0.29	CIV	0.43	0.41	0.23	1.07

Due to the low phosphate (P) uptake by crops, farmers have repeatedly applied P to realise high crop yields regardless of the large amounts already present in soils. In some Western European countries, accumulation exceeds 4 kg Pha^{-1} y^{-1}, but this value swells up to 16 kg Pha^{-1} y^{-1}, when P applied as animal manure is also considered. Global supply of economically exploitable P present in rock, required for the manufacturing of fertilisers is limited and a non-renewable resource, future agricultural strategies should focus on maximising P use efficiency with minimum adverse environmental impact (Frossard et al. 2000). Values of extractable (by ammonium lactate) P accumulation as high as 400 to 700 mg kg^{-1} soil have been reported (Singh et al. 2005), while fertile soils usually contain about 100 mg Pkg^{-1}. Many farmers have built up P reserves in their soils and if mobilised, it would be sufficient for growing crops for decades without applying more P. Therefore, continued application of P fertiliser to soils already enriched with P is unsustainable and an economic waste. Evidently, there is a need to improve management of nutrients, particularly to enhance accessibility of the accumulated P by crops, leading to reduction of inputs, which will subsequently mitigate pollution of the environment. While P application is declining, concern is focused on P remaining in cultivated soils. Phosphorus not utilised by crops is either leached into groundwater or transported to surface water bodies by surface runoff. Mobilisation and transportation of nutrients from terrestrial systems to groundwater, rivers, lakes and marine environments causes deteriorating water quality and eutrophication. In all countries of Northern Europe, including Alpine areas, agriculture is estimated to be responsible for the greatest contribution of P to waters. Rahm and Danielsson (2007) have suggested that the only significant potential way to reduce the P load rests on reduction of diffuse (nonpoint source) emissions from agricultural land. Since P in soil is involved in both biological and chemical processes, losses from soils vary considerably over time and between fields. Furthermore, the complexity of these processes and their interactions make P even more difficult to control than N. Not only are efficient countermeasures and adequate strategies to drastically reduce P loss from agricultural soils still lacking, but the knowledge basis needed to implement appropriate measures is also rather limited.

Over the last 20 years, the use of plant growth-promoting rhizosphere micro-organisms for sustainable agriculture has tremendously increased in various parts of the world and especially in the EU (Khalid et al. 2004). Soil-born micro-organisms such as bacteria and mycorrhizae fungi, mostly those associated with plants rhizosphere, are able to exert a beneficial effect upon plant growth. Therefore, their use as control agent for agriculture and environment improvement has been a focus of research for some years (Glick 1995; Khalvati et al. 2005; Cozzolino et al. 2010). Higher concentrations of P-solubilising micro-organisms have been found in the rhizosphere in comparison with bulk soil, which can be of interest for biogeochemistry and the maintenance of soil health and quality (Jeffries et al. 2003). Phosphate uptake by plants and subsequent growth promotion in plant–soil systems inoculated with P-solubilising micro-organisms are more pronounced when co-inoculated with arbuscular mycorrhizal fungi (AMF). AMF are capable of sparingly mobilising soluble inorganic phosphate by the excretion of H^+ after the utilisation of ammonium ion by the hyphae (Yeo et al. 2001). Mycorrhizal roots can use sources of P in soil that are not available to non-mycorrhizal roots. The main contribution of AMF to the host plant is to reach and deliver P through their extracortical and extraradical hyphae (Fig. 6), penetrating as much as 5 to 9 cm into the soil (Khalvati 2005). This involves increased rates of solubilisation of inorganic P or hydrolysis of organic P and depends on localised alteration of pH, production of organic anions and of surface or soluble phosphatases. Consequently, it has been possible to calculate that the fungi contribute to about 70–80% of the P absorbed by mycorrhizal roots (Li et al. 1991; Tisserant et al. 1993).

In addition to AMF-mediated acquisition, P can also be very efficiently released by bacteria belonging to the genera *Rhizobium*, *Pseudomonas* and spore-forming *Bacillus* (Rodriguez and Fraga 1999). Such P-solubilising effects have been observed with *Sinorhizobium* and *Rhizobium* spp. for the benefit of leguminous plants (Khattak et al. 1991; Halder and Chakrabarty 1993) and for inoculation of soybean with *Pseudomonas* (Brockwell and Bottomley 1995; Gaind and Gaur 2002). However, there is some circumstantial evidence that development of activity is linked to the presence of arbuscules and transfer of P to the plant. Among the beneficial microbes the multiple interactions between bacteria and fungi may cause a further synergistic effect on the plant growth and fitness, as it has

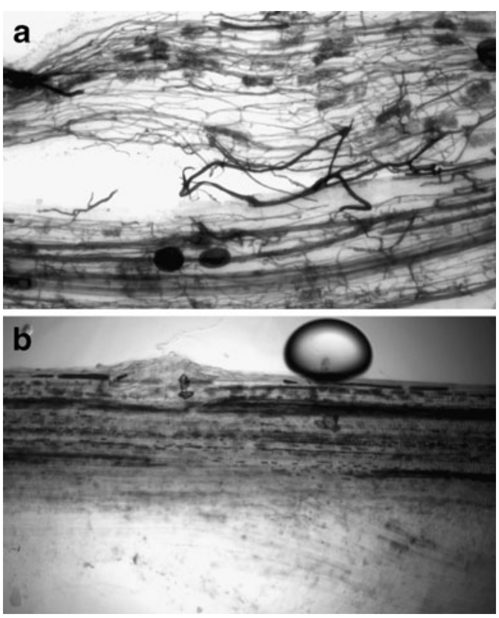

Fig. 6 Roots of barley (*Hordeum vulgaris* L. cv. Scarlett) inoculated (**a**) or not (**b**) with arbuscular mycorrhizal fungi (*Glomus intraradices*), as viewed under microscope (×100 magnification). For details of experimentation, see (Khalvati et al. 2005)

been demonstrated with combined *Azospirillum*, *Rhizobium* and mycorrhizal co-inoculation by Biro et al. (2000).

Mycorrhizae can also reduce the contact of plants with heavy metals and at the same time stimulate their growth. However, most experiments do not consider rhizosphere processes, e.g., the role of mycorrhizae. For example, glomalin-related soil protein (GRSP), a glycoprotein produced by AMF, contributes to the sequestering of Cu and Zn in the soil, and the microsite variation of other soil traits (pH, water-stable aggregates, soil organic carbon) affects the heavy metal sequestration by GRSP in polluted soils. The GRSP–Cu complex can be a substantial pool in the soil and can represent one of the main forms of immobilised Cu, contributing to the chemical stabilisation of contaminated soils through the deposition of enriched Cu particles. The accumulation of high quantities of GRSP may also contribute to the formation of soil aggregates, even under extreme conditions of acidity and heavy metals availability (Cornejo et al. 2008).

Mycorrhizal fungi do also influence the accumulation of heavy metals by plants (Vivas et al. 2006; Azcón et al. 2009; Pongrac et al. 2009). Plant-associated rhizospheric microorganisms thus play a major role in different biogeochemical processes and in the control of elements solubilisation, bioavailability and phytoextraction (Perriguey et al. 2008; Turnau et al. 2008; Becerra-Castro et al. 2009; Luster et al. 2009; Martinez-Alcala et al. 2009; Wenzel 2009).

At the present time, however, their study and utilisation under real field conditions in Alpine areas are scarce and should be developed, especially in regions impacted by human activities. In the future, such an approach would help improving the quality of Alpine soils containing too much phosphate or toxic metals and cultivating healthy plants for both phytoremediation and food chain safety purposes.

6 Conclusions, recommendations and perspectives

Worlwide, including Alpine areas, the controlled use of appropriate plants is destined to play a major role for remediation and restoration of polluted and degraded ecosystems, monitoring and assessment of environmental quality, prevention of landscape degradation and improvement of food quality. The different case studies and approaches mentioned above are promising enough to offer efficient and environment friendly tools to clean up contaminated soils, brownfields and wastewater in the very specific environment and climate of the Alps.

However, each of these goals requires a sound understanding of how plants specifically accumulate or exclude essential elements, toxic metals, phosphate and organic pollutants. Basic knowledge is thus required on the concentration and toxicity of trace elements and xenobiotics in the environment, their bioavailability in the rhizosphere, their uptake by roots and translocation to shoot, their detoxification, metabolism and storage. For such a purpose, significant efforts have been carried out over the last decade in different mountainous countries.

One of the most important challenges is now to use basic scientific knowledge to improve the efficiency of phytotechnologies in the field. The dissemination of results, risk assessment, public awareness and acceptance of this green technology, as well as the promotion of networking between scientists, environmental engineers, industrials, stakeholders, end-users, non-governmental organisations and local authorities are major issues that must be tackled to ensure that phytoremediation programmes are correctly and successfully implemented, more precisely under Alpine conditions. It is clear that phytotechnologies do offer promising and sustainable approaches towards environmental remediation, human health and a sustainable development for the 21st century, in Alpine areas and elsewhere all over the world.

Acknowledgments The authors acknowledge the support of the European project Alps Bio Cluster (Biotech and Medtech in Alpine Space), http://www.alpsbiocluster.eu.

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