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ORIGINAL PAPER

Plant colonization of brownfield soil and post-washing sludge: effect of organic amendment and environmental conditions

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Abstract This paper analyses the effects of substrate properties and environmental conditions on spontaneous vegetation of soil and sludges from a dismantled steel plant moderately polluted by heavy metals and polycyclic aromatic hydrocarbons. Plant colonization was monitored in the presence or absence of acidic peat for 5 years both inside the degraded brownfield site and after transferral into a nearby Oak Park environment. Overall, 57 plant species grew healthily on the substrates, with peat enhancing plant growth in the unfavourable brownfield site. Most of the species were found in the park (91 %), showing plant colonization was mainly affected by the immediate environment rather than by substrate properties. Restricted metal uptake and tissue accumulation by selected plants were measured, with only *Daucus carota* showing a higher ability to translocate metals to shoots (shoot/root metal concentration quotient >1 with peat). Phytostabilization with native plants represents an economically more realistic and cost-effective option than excavation, soil washing and sludge disposal, especially for vast industrial sites. Addition of organic matter and planting strategically selected vegetation islands could facilitate the spontaneous recovery of such highly degraded environments.

Keywords Phytoremediation · Heavy metals · Polycyclic aromatic hydrocarbons · Vascular flora · Peat

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Introduction

Soil pollution is a growing threat to the environment and poses serious risks for biota and human health. In brownfield sites, generally experiencing multi-component contamination and located in urban or peri-urban zones, these risks are of particular interest to city-planning authorities who have to select the appropriate technologies and procedures to achieve optimum site remediation (Thornton et al. 2007). Soil washing is one of the most promising approaches which reduces the initial volume of contaminated soil by separating the smaller soil particles, binding pollutants, from the larger ones (Dermont et al. 2008). Although it is a cost-effective approach, it produces highly polluted fine products (sludges) suited only to landfill disposal or, for organic pollutants, to thermal destruction, with consequent environmental impacts. Alternative treatments are clearly required for soil as well for post-washing sludge materials for the purpose of on-site reuse. In this context, the use of "green" technologies, based on low-cost agricultural practices rather than earth-moving equipment, is suitable when soil ecosystem disruption is not great enough to trigger government action or the economic costs for remediation are too high (Cunningham and Berti 1993).

Phytoremediation includes many uses of plants to achieve remediation of soil risks (Marques et al. 2009). Phytostabilization obtained by seeding native plant species or by spontaneous revegetation uses plants to re-establish vegetative cover at sites where natural vegetation is lacking. This rehabilitation strategy prevents dispersion of contaminated particles by water or wind erosion through physical stabilization of soils and/or reduces the mobility of contaminants in soil, through absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone (Bargagli 1998). Compared to seeding techniques, revegetation by spontaneous plant species is a cheaper and sustainable practice, since it has the advantage of directly selecting the best-suited flora for the particular polluted soils in reclamation environments (Córdova et al. 2011).

In disused industrial areas, however, several constraints may slow or prevent the process of spontaneous revegetation. Such environments often consist of huge areas in which very little or no vegetation is found over several hectares. Consequently, seed rain from surrounding vegetated areas is often too slow to allow quick seedling establishment, precluding the development of a sufficiently diversified flora. Furthermore, micro-environmental conditions in such areas are usually too extreme for plants to survive over a long time. If, in addition, the level of soil pollution is so high as to negatively affect plant growth, the process of revegetation could actually risk being ineffective to prevent pollutant dispersion unless contaminant solubility/availability is originally low or reduced by soil amendments (Madejón et al. 2006; Conesa et al. 2007).

Thus, in-depth understanding of the role played by soil pollution and environmental conditions on plant successions on dismantled industrial areas is essential to achieve effective results in reclamation projects. In this work, we tried to analyse separately the effects of substrate and environment on spontaneous vegetation. Plant colonization was therefore monitored on industrial polluted soil and post-washing sludge with or without natural organic matter (peat) addition, either on-site or after transfer to a more natural environment. The main objectives of the work were as follows: (1) to monitor colonization by spontaneous vascular plants of polluted soil and post-washing sludges in the presence or in the absence of acidic peat as organic amendment; (2) to ascertain whether the plant colonization dynamic changes depending on environmental conditions around substrates, particularly the presence or absence of surrounding natural vegetation; (3) to measure the extent of metal accumulation by selected plants. We also assessed the potential risks or benefits of using plants to remediate heavy metal polluted substrates, in terms of plant-induced changes in substrate chemical properties and in mobility/bioavailability of metals. These data will be described in a separate paper.

The present research was conducted from 2006 to 2011 in two locations of Campania region, southern Italy.

Materials and methods

The Bagnoli brownfield site

The Bagnoli brownfield site (latitude 40°48.570′N, longitude 14°10.557′E, 2–10 m above sea level), with an area of approximately 300 ha located in the western urban sector of Naples (southern Italy), is one of the largest Italian industrial areas, classified as a SNI (Site of National Interest) by the Italian Parliament (2000) and abandoned by the Italian steel-producing company Italsider in the 1990s. The geological setting of the area is the result of volcanic activity in the Phlegrean Fields, which gave rise to the parent soil material. Within the site, the variability in past industrial activities and material deposition has greatly affected the geopedological status of the area, producing a patchy distribution of soil morphological and pollution features (Buondonno et al. 1998; Adamo et al. 2002; De Vivo and Lima 2008). Both potentially toxic metals (mainly As, Cd, Cu, Co, Cr, Pb, Zn and Ni) and organic pollutants (primarily polycyclic aromatic hydrocarbons, PAHs) occur in soils well above the Italian Ministry of the Environment's clean-up criteria (Italian Parliament 2006). In accordance with the results of isotopic investigations carried out by Tarzia et al. (2002), hydrothermal fluids other than anthropogenic sources contribute to increase the high concentrations of heavy metals and As found in soils and waters of the brownfield area. Speciation of metals and determination of the different chemical pools in the soil allowed us to determine a low risk of mobility of the metals both largely trapped in the mineralogical structure of oxides and silicates and occluded in easily reducible manganese or iron oxides. With the aim of recovering and reusing the site, a remediation project funded by the Italian government started in 1994 (CIPE 1994). Reclamation was mainly based on excavation and soil-washing techniques and did not take into account either the internal mosaic of soil pollution or metal geochemical fractionation. The washing process is undertaken in a small area of the brownfield site, where the excavated soils, first separated into metal parts and gravels, the washed soil and the sludge fraction (i.e. the fine soil particles adsorbing contaminants and separated by washing from bulk soil), are temporarily stocked in trenches in view, respectively, of their use in soil reconstruction inside the area or disposal in a licensed landfill. Only scatter vegetation is found inside the industrial area, mainly constituted by cosmopolitan or Mediterranean species (Buondonno et al. 1998). In the surrounding areas, the land is covered by Mediterranean plant species (Motti and Ricciardi 2005). The *Città della Scienza* (City of Science) complex of Naples, which went up in smoke on 4 March 2013, is located in Bagnoli site in proximity to the industrial area.

The Royal "Gussone Park" of Portici

The Royal Park of Portici (latitude 40°48.860′N, longitude 14°20.851′E) covers an area of about 60 hectares and overlooks the Bay of Naples from the south-western foothills of Mt. Vesuvius, between 20 and 90 m above sea level. Morphologically it appears as a long, roughly rectangular area, sloping regularly NE–SW towards the coast. This area is mainly covered by holm oak trees (*Quercus ilex* L. subsp. *ilex*), one of the most common plant species of Mediterranean evergreen broadleaf forests. The remaining part is occupied by experimental fields of the Department of Food Science and Agriculture of Naples University, uncultivated areas, grassy places and surfaces variously altered by human activities. The vascular flora of the park amounts to 551 species (Stinca and Motti 2009; Motti and Stinca 2011; Stinca et al. 2012), indicating the high biodiversity of this site (over 60 % of the flora of the whole Somma-Vesuvius volcanic complex).

Climatic features of experimental sites

The sites of Portici and Bagnoli present very similar macroclimatic features, since both are characterized by a typical Mediterranean climate with a summer drought, which lasts from June to August, an average monthly temperature of 18 °C and total annual rainfall of 930 mm. By contrast, the two sites differ greatly in their biotic environment. Bagnoli presents the typical scenario of a disused industrial area, with extensive tracts not covered by natural vegetation, affected by the passing of heavy vehicles and frequent dusty winds; on the contrary, Portici is strongly conditioned by the micro-environmental influence of the rich surrounding plant community.

Experimental design

A factorial experiment with 3 factors (substrate, amendment, site) and 2 levels for each factor was carried out, testing two substrates [pre-washing soil (S) and fine products or sludges from post-washing plant (F)], an amendment treatment [acidic *Sphagnum* peat (p) versus untreated control], in two different sites of the Naples urban area (inside the Bagnoli brownfield site and in the experimental fields of the Department of Agricultural Sciences of Naples University).

In 2006, pre-washing soil (S) and the post-washing sludges (F) were collected from the stoking trenches in the area of the brownfield site devoted to soil washing. In the same area, they were mixed with acidic *Sphagnum* peat (p), thereby obtaining the Sp and Fp substrates. The four substrates (S, F, Sp and Fp) were transferred in triplicate into individual 0.5-m^3 pots. Two series of twelve pots (3 with S, 3 with F, 3 with Sp and 3 with Fp) were prepared. One series was left inside the brownfield site; the other was taken to the experimental fields at the Department of Agricultural Sciences, Naples University, where all pots were freely exposed to the atmosphere and left to vegetate naturally.

Substrate analysis

Before plant growth, each substrate was analysed for its chemical properties and pollutant levels. Field-moist samples were sieved at 2 mm to determine moisture content, pH-H_2O (1:2.5 soil/water ratio), particle size distribution (laser diffraction by Sedigraph 5100 instruments), organic carbon (Walkley and Black method, 1934) and carbonates (calcimeter method).

Analysis of metallic elements (Na, K, Ca, Mg, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Ti, V, Zn) was performed by XRF. Samples of soils were ground in a mill with input of silicon carbide to reach graining below 63 µm, and 2 g of sample with binder was then tableted under a pressure of 2 t/cm^2. Finally, direct XRF analysis with a Panalytical AXIOS instrument was carried out.

Analysis of polycyclic aromatic hydrocarbons (PAHs), both single compounds and total, was performed at CHELAB laboratories (Methods EPA 3550 C 2007 + EPA 8270 D 2007). As required by EPA Method 8270, the recovery of the surrogates ranged between 70 and 130 %. Detection limits are 0.02 mg/kg for each PAH.

Plant colonization monitoring

From December 2006 to July 2011, plant colonization was monitored at regular intervals by listing all the species and evaluating their relative abundance, expressed as per cent cover on each of the replicate pots. The plant specimens were directly identified in the field except for dubious cases, which were later identified at the Herbarium Porticense (PORUN) according to Pignatti (1982) and Tutin et al. (1964–1980, 1993). The nomenclature follows Conti et al. (2005, 2007).

For each species found in the study areas, the following information is provided: Raunkiaer (1934) plant life form, verified by field observations and according to Pignatti (1982), and chorotype according to Pignatti (1982) or derived from other sources.

Plant analysis

Samples of plant species (*B. bituminosa, D. carota* and *D. glomerata*) were taken from plots F and Fp, both in Portici and in Bagnoli. Plants were carefully washed with distilled water and then dried at 65 °C for 72 h. Shoots (stem and leaves) and roots (root and rhizome) were separated prior to grinding. Metal (Cu, Pb, Zn, Mn, Fe, As, V and Sn) content of plant material was measured after wet digestion in a Milestone Microwave system using concentrated high purity 60 % HNO_3 (Ultrapur®, Merck, Darmstadt, Germany) and 30 % H_2O_2 (Suprapur®, Merck). All the metal

Table 1 Properties and pollutant content (mean ± standard deviation, $n = 3$) of the brownfield soil and post-washing sludges used in the experiment (*S* soil, *Sp* soil + peat, *F* sludges, *Fp* sludges + peat)

		S	Sp	F	Fp	Limit
Sand (2–0.02 mm)	%	65.56	65.43	30.82	39.09	
Silt (0.02–0.002 mm)	"	32.54	33.07	61.68	56.77	
Clay (<0.002 mm)	"	1.90	1.50	7.50	4.14	
pH-H_2O		8.8 ± 0.2	8.0 ± 0.1	8.6 ± 0.1	8.1 ± 0.1	
Carbonates	g kg^{-1}	83 ± 48	76 ± 46	69 ± 41	65 ± 38	
Organic C	"	3.5 ± 0.5	25.0 ± 0.8	8.7 ± 0.3	32.3 ± 0.3	
Al	"	73.1 ± 9.6	68.8 ± 13.0	76.1 ± 10.0	85.1 ± 4.5	
Fe	"	73.9 ± 1.7	67.0 ± 1.8	58.2 ± 8.1	55.1 ± 6.6	
Mn	"	3.02 ± 0.14	2.48 ± 0.14	2.04 ± 0.25	1.88 ± 0.21	
As	mg kg^{-1}	*39.6 ± 3.7*	*35.5 ± 3.6*	*45.9 ± 3.0*	*43.4 ± 0.8*	20
Ba	"	767 ± 53.3	694 ± 23.4	638 ± 64.8	625 ± 31.1	
Cd	"	0.7 ± 0.6	1.0 ± 0.4	1.6 ± 0.8	1.7 ± 0.3	2
Co	"	10.3 ± 5.6	11.1 ± 4.3	8.0 ± 1.8	8.0 ± 3.0	20
Cr	"	80.1 ± 19.1	82.4 ± 3.3	29.9 ± 9.5	28.8 ± 12.9	150
Cu	"	47.4 ± 1.2	46.2 ± 7.5	55.5 ± 14.3	51.1 ± 7.1	120
Mo	"	3.7 ± 0.2	3.7 ± 0.1	4.6 ± 0.9	4.3 ± 0.7	
Ni	"	41.3 ± 3.9	47.3 ± 3.0	18.8 ± 4.1	19.0 ± 5.9	120
Pb	"	*159 ± 1*	*154 ± 2*	*286 ± 8*	*273 ± 16*	100
Sb	"	5.2 ± 1.5	4.1 ± 2.6	9.7 ± 0.3	9.5 ± 1.9	10
Sn	"	*8.9 ± 0.1*	*9.3 ± 0.5*	*14.7 ± 1.3*	*13.8 ± 1.8*	1
Sr	"	508 ± 6.6	465 ± 12.9	386 ± 4.1	375 ± 16.9	
V	"	*119 ± 20.2*	*98 ± 5.7*	77 ± 3.4	71 ± 4.6	90
Zn	"	*333 ± 41.9*	*293 ± 11.6*	*1,069 ± 0.2*	*958 ± 30.0*	150
Benzo(a)anthracene	"	*1.46 ± 0.02*	*1.29 ± 0.08*	*1.68 ± 0.12*	*1.76 ± 0.07*	0.5
Benzo(a)pyrene	"	*1.44 ± 0.03*	*1.33 ± 0.02*	*1.74 ± 0.06*	*1.792 ± 0.03*	0.1
Benzo(b)fluoranthene	"	*2.69 ± 0.35*	*2.31 ± 0.01*	*3.23 ± 0.05*	*2.96 ± 0.14*	0.5
Benzo(k)fluoranthene	"	*1.08 ± 0.09*	*0.98 ± 0.07*	*1.26 ± 0.09*	*1.35 ± 0.04*	0.5
Benzo(ghi)perylene	"	*1.52 ± 0.05*	*1.33 ± 0.02*	*2.07 ± 0.10*	*2.07 ± 0.03*	0.1
Chrysene	"	1.34 ± 0.04	1.25 ± 0.16	1.66 ± 0.05	1.75 ± 0.03	5
Dibenzo(a.e)pyrene	"	*0.42 ± 0.07*	*0.33 ± 0.05*	*0.52 ± 0.01*	*0.57 ± 0.02*	0.1
Dibenzo(a.i)pyrene	"	*0.12 ± 0.02*	*0.11 ± 0.07*	*0.17 ± 0.00*	*0.16 ± 0.01*	0.1
Dibenzo(a.l)pyrene	"	*0.76 ± 0.01*	*0.61 ± 0.02*	*0.92 ± 0.10*	*0.99 ± 0.01*	0.1
Dibenzo(a.h)pyrene	"	0.05 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.09 ± 0.00	0.1
Dibenzo(a.h)anthracene	"	*0.56 ± 0.02*	*0.47 ± 0.03*	*0.63 ± 0.03*	*0.67 ± 0.02*	0.1
Indeno(1.2.3-cd)pyrene	"	*1.40 ± 0.06*	*1.22 ± 0.01*	*1.83 ± 0.11*	*1.85 ± 0.05*	0.1
Pyrene	"	*1.94 ± 0.04*	*1.61 ± 0.08*	*2.60 ± 0.23*	*2.57 ± 0.06*	5
ΣPAHs	"	*14.77 ± 0.25*	*12.90 ± 0.37*	*18.37 ± 0.98*	*18.58 ± 0.12*	10

In italics values above the legal limits established for soils of public, residential and private areas by the Italian Ministry of Environment (Italian Parliament 2006)

PAHs polycyclic aromatic hydrocarbons

solution concentrations were measured using ICP-MS spectroscopy. Procedural blanks were usually below detection limits, and the accuracy of digestion and analytical procedures was checked by routine element determination in standard reference materials (CTA-VTL-2 and CRM 482).

Statistical analysis

Since the first exploratory analysis revealed the effect of time on the plant community to be quite fluctuating, plant cover values for the 5 years of observation were averaged for each replicate pot before performing the whole analysis.

Data from plant colonization monitoring were analysed in two steps: first, an ANOVA model was applied to total plant cover and the number of species per replicate pot, with site, substrate and amendment as main factors; then, multivariate analysis was applied to only the plant species occurring with a frequency no lower than 10 % (see Table 3 in "Appendix"). In both analyses, % cover values were first arcsine transformed to achieve normality. Cluster analysis was conducted with average link (UPGMA) as agglomerative criteria and chord distance as dissimilarity index. PCA was conducted on standardized values. The SPSS 13.0 package was employed to perform variance analysis. Multivariate analyses were performed by using the Syntax 2000 package (Podani 2001). An assessment of plant contamination by soil particles was performed by calculating the enrichment factor (EF), defined as the ratio: $EF = (Cx_p/CAl_p)/(Cx_s/CAl_s)$, where Cx_p and CAl_p are the content of a metal and of Al in the plant; Cx_s and CAl_s are the content of the same metal and Al in the soil. Aluminium was chosen as the reference element due to its low metabolic significance.

Results and discussion

Substrate properties

The main physical and chemical properties of the brownfield soil, S, and post-washing sludges, F, used in the revegetation experiment, are given in Table 1. The soil is characterized by a sandy texture, while, as normal, the sludge is mostly loamy. For both substrates, pH is alkaline due to the presence of carbonates. Organic matter content is low, particularly in S. As expected, acidic peat addition significantly increases the content of organic matter and reduces the pH of the substrates, persisting in their alkalinity.

The main mineralogical constituents of both soil and sludges are as follows: (1) volcanic materials, such as mica, analcime, K-feldspars and pyroxenes, commonly occurring in soils developed on Phlegrean products; (2) quartz and calcite, used as soldering flux; (3) iron minerals, namely haematite, magnetite and goethite, likely deriving from steel processing.

Both soil and sludges show levels of As, Pb, Sn and Zn well above the maximum concentrations (As: 20, Pb: 100, Sn: 1, Zn: 150 mg kg^{-1}) established for soils of public, residential and private areas by the Italian Ministry of the Environment (Italian Parliament 2006). Concentrations of these metals are much higher in sludges than in soil, demonstrating the usefulness of the soil-washing treatment. Vanadium content is also above the legal limit (V: 90 mg kg^{-1}), but only in soil. Peat contains metallic pollutants, but at a very low amount compared to soil and sludge (As: 2, Pb: 9, Sn: 0,1, Zn: 16, V: 6 mg kg^{-1}). Hence, its addition slightly dilutes and therefore reduces the total metal contents in the substrate, but never below legal limits.

Contents of Co, Cr, Fe, Mn, Ni and Sr, although never above the legal limits, are more enhanced in soil than in sludges, suggesting preferential association of these elements with coarse particles; the opposite holds for Al, Cd, Cu, Mo and Sb likely to be more tightly bound to fine particles.

We analysed polycyclic aromatic hydrocarbons (PAHs), both single compounds and total, listed by the Italian Ministry of the Environment (Italian Parliament 2006) as priority pollutants that are typically found in contaminated soils (Table 1). As expected, the values of sludges are slightly higher than those of soil. The total concentration of the 13 PAHs detected in F (ΣPAHs) shows a mean value of 18.4 ± 0.25 mg kg^{-1}, against 14.8 ± 0.98 mg kg^{-1}, for S. These data are in good agreement with those reported in the literature for the Bagnoli brownfield site (De Vivo and Lima 2008; Albanese et al. 2010) exceeding the limits established under Italian Law (Italian Parliament 2006) for soils of public, residential and private areas. This condition also holds for the majority of the single PAH compounds, well above the single legal limits, with the exception of chrysene, dibenzo(a,h)pyrene and pyrene.

Although none of the PAHs found in soil and sludge were detected in peat (HC < 12 content always below mean detection limits of 5 mg kg^{-1}), organic amendment apparently increases organic pollution.

According to De Vivo and Lima (2008), except for As (mostly introduced into the environment by volcanic thermal springs in the Phlegrean Fields), metallic elements and organic compounds (PAHs) occurring in Bagnoli soils in concentrations above the legal limits are a direct consequence of past industrial activities on the site for almost 100 years. Potential sources of anthropogenic pollution in the area include dust, ash, scum, slag, carbon coke residues, minerals used to produce cast iron and steel, coal used as a source of energy in smelting furnaces, heavy oils, hydrocarbons and combustion residues. Geochemical mapping and geostatistical analyses allowed De Vivo and Lima (2008) and Albanese et al. (2010) to identify the fossil fuel and oil by-product deposit areas as the main sources of PAH contaminants distributed in different spots across the brownfield site, released from these areas to the underlying groundwater and ultimately found in marine sediments.

Microscope analyses, sequential chemical extractions and leachability tests proved that scum, slag, Fe minerals, steelwork mud and soils were geochemically very stable relative to their metal contents at the pH and local conditions existing in the area (Adamo et al. 2002; Tarzia et al.

2002). As observed in numerous investigations carried out on industrial steelwork waste in the European Community, heavy elements are mainly trapped in the lattice of relatively insoluble mineral phases, and their leaching is unlikely to contribute metallic pollution to local groundwater, on a reasonable human timescale (Sierra et al. 2013; Jordanova et al. 2013). Therefore, the presence of a natural contribution to soil metal contamination (i.e. hydrothermal activity associated with quiescent volcanism introducing into the environment high quantities of heavy metals) and the non-bioavailability of metal pollutants make the heavy metal remediation of soils in the Bagnoli brownfield area of little use; the real pollution to be remediated in the area is the occurrence of polycyclic aromatic hydrocarbons (PAHs). According to De Vivo and Lima (2008), hydrothermal fluids associated with the Phlegraean fields-active volcanism, an area where fumaroles and hydrothermal springs are quite abundant, provide significant contribution to the metals present at this site. The natural contamination due to the upwelling of geothermal fluids (enriched in heavy and potentially toxic metals such as As, Cu, Pb and Hg) is confirmed by the high concentrations of heavy metals found in the thermal springs (spas) located at the margins of the brownfield site of Bagnoli (e.g. the Terme di Bagnoli, Dazio, the Terme Puteolane, the Stufe di Nerone) and the nearby Island of Ischia (Daniele 2000; Lima et al. 2001, 2003). Values at the Stufe di Nerone reach up to 8,000 ppb, while Ischia values are >1,500 ppb.

In order to assess the potential ecological risk to the environment and organisms due to contamination, a generic hazard quotient (HQ) (measured pollutant concentration divided by a reference value representing the "no effect concentration") was calculated for metallic and organic pollutants in soil and sludges. This approach is in line with the ecological risk assessment proposed by Khairy et al. (2009) evaluating the possibility and extent of occurrence of adverse ecological effects due to environmental contamination. The legal limits established for soil by Italian law (Italian Parliament 2006) and reported in Table 1 were selected as "no effect concentrations". The output revealed that adverse ecological effects are expected to occur in both soil and sludge samples always characterized by HQ >1 up to considerable mean values of 21 and 16, respectively, for benzo(ghi)perylene and Sn in substrates F and Fp.

Plant colonization

Overall, 57 different plant species were recorded on the studied substrates. Most of these species were found in Portici, where 52 species colonized the experimental pots, of which 37 were found only in this site. Only 20 species were found in Bagnoli, 5 of which were exclusive to this site. Both plant cover and species richness varied significantly depending on the site, with Gussone Park showing significantly higher values than Bagnoli: the average number of species per pot was about 20 % higher ($F_{1,16} = 12.4$; $P < 0.01$) and plant cover almost double ($F_{1,16} = 32.3$; $P < 0.001$) in Gussone Park compared to Bagnoli. By contrast, no significant differences in species number and coverage were found among treatments. Thus, as a general pattern, environmental effects rather than soil contamination levels appeared to be the main factors constraining the expression of plant diversity and abundance.

Raunkier types did not differ greatly between the two sites, with a marked dominance of annual species, as is generally found in the earlier stages of plant succession in Mediterranean environments. Some small amounts of phanerophyte seedlings and geophytes, however, were found in Portici, whereas no such types were observed in Bagnoli, signalling a fairly different effect of seed rain from the surrounding vegetation in the two areas. Chorological types were quite similar in the two sites, with a majority of species characterized by a Mediterranean (sensu lato) distribution (45–50 %), followed by cosmopolitan and subcosmopolitan species (25–27 %). In both sites, some invasive alien species were found (about 10 %), probably stemming from their higher ability to promptly capture available nutrients and space resources in the more disturbed substrates.

Multivariate analysis was conducted on the 20 most frequent species (see Table 3 in "Appendix" for species list). Principal component analysis strongly evidenced the effects of environment on plant composition: the two sites were distinctly separated along the main axis (Fig. 1a), explaining about 44 % of total variability. Indeed, all the four treatments of Gussone Park were dispersed on the left-hand side of the graph, together with the plant species that most frequently occurred in this site (Fig. 1b), whereas Bagnoli treatments and their dominant species were found on the right-hand side. In contrast, the second axis mainly responded to the effects of substrate features on plant composition (24 % of variability explained), with sludges (F) found on the lower and soil (S) on the upper side of the graph. The spread among soil and sludge treatments was rather more evident on the right than on the left-hand side of the diagram, signalling a stronger effect of soil contamination level at the unfavourable site of Bagnoli compared to Gussone Park. Only minor evidence of amendment effects was visible in the third and fourth axis: also in this case, the spread among treatments was higher for plants growing in Bagnoli (data not shown).

The similarities among plant response patterns were evidenced by cluster analysis applied to plant species (Fig. 1c; see also Table 3 in "Appendix"). Two main groups of species were found, cluster *g* with the plant taxa that chiefly occurred in Gussone Park and cluster *b* with the

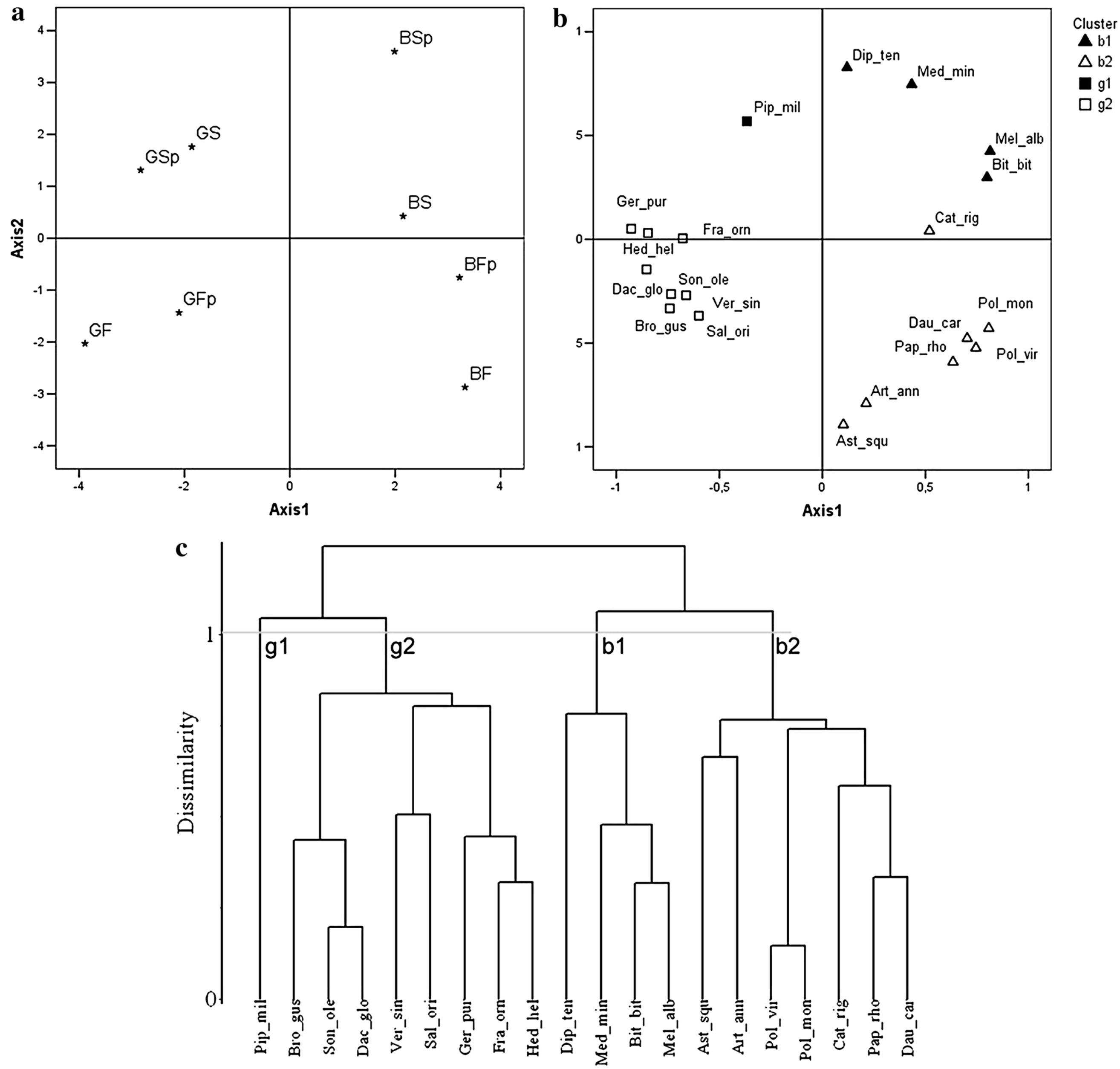

Fig. 1 Principal component analysis (***a***, ***b***) and cluster analysis (***c***) applied to the 20 most frequent species found during a 5-year spontaneous revegetation of reclaimed soils. In the PCA, the scores of treatments and species are reported on the left and right box, respectively. *B* Bagnoli, *G* Gussone Park, *S* soils, *F* fine sludge fraction, *p* with peat amendment. See Table 3 in "Appendix" for species label legend

species that were more abundant in Bagnoli. A more detailed analysis conducted on species cover values allowed us to identify the main characters of each cluster (Fig. 2). Among the cluster *b* species, two subclusters were identified, one (*b1*) mainly comprising leguminous species, with a clear tendency to escape sludges, particularly if not amended with peat (Fig. 2); and the other (*b2*) mainly constituted by tolerant species that showed a higher cover in sludges than in soil. Most of the species belonging to cluster *g* attained a relatively higher cover in sludges than in soil (subcluster *g2*), whereas one only species, *Piptatherum miliaceum*, showed the opposite trend (separated as subcluster *g1*).

Three leguminous species belonging to cluster *b1* proved quite sensitive to the higher metal concentration found in sludges. This could be partly related to some

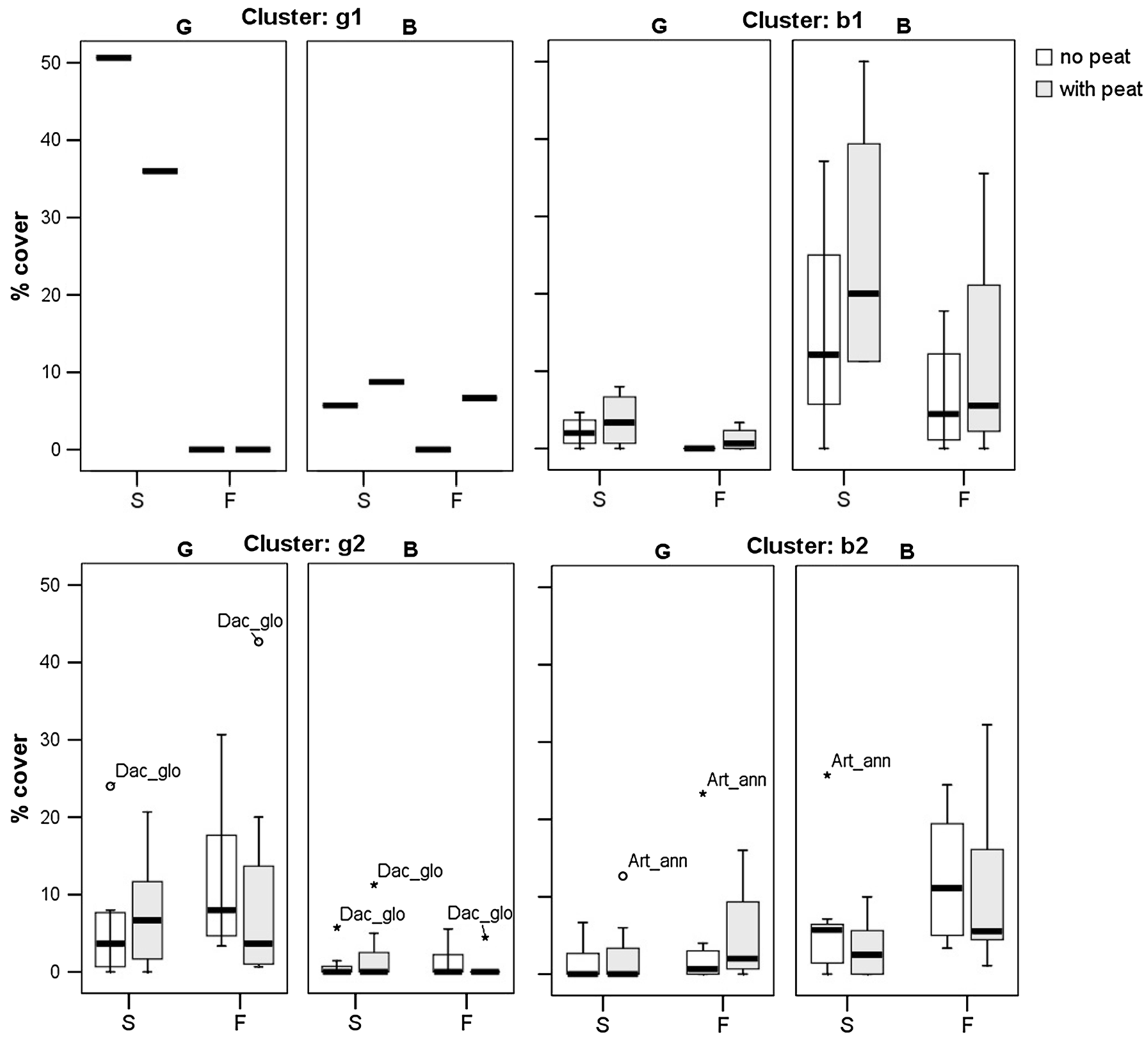

Fig. 2 *Box plots* reporting the cover values assumed by plant species in each of the four clusters identified with multivariate analysis (see Fig. 1), either at Bagnoli (B) or at Gussone (G) site, grown on soils (S) or fine sludges (F), with peat (*shaded bars*) or without peat (*empty bars*). *Box plots* (Tukey 1970, 1977; McGill et al. 1978) provide a synthetic summary of the basic information about a data distribution: the *thick horizontal lines* represent median values; *grey bars* fill the whole range from 25th to 75th percentile; *vertical lines* that end in horizontal strokes ("whiskers") represent the range from lower to upper extremes of the distribution, excluding outside values, if any; outliers (from 1.5 to 3 box lengths above the 75th percentile) and extreme values (>3 box lengths above the 75th percentile) are represented by *open circle* and *stars*, respectively. See Table 3 in "Appendix" for species label legend

detrimental effects of metals on the symbiotic bacterial flora of leguminous plants. The effects of metals on micro-organisms have been documented elsewhere (Giller et al. 2009). Clones of *Rhizobium* are known to be able to withstand high heavy metal concentrations, but they tend to lose their ability to fix atmospheric nitrogen; in general, the presence of heavy elements at high concentrations, particularly Zn, seems to induce a decrease in total microbial mass (Giller et al. 2009; Zaidi et al. 2012). Since nitrogen-fixing species may play a crucial role in facilitating plant colonization of extreme substrates, even enhancing rhizosphere ability to transform and/or degrade pollutants (Zaidi et al. 2012), care should be taken to favour the spread of these species in reclamation strategies. Interestingly, peat treatment partly released the growth constraint found on such species, pointing to the positive role of organic amendments as a tool to reduce substrate toxicity (Brown, Gill and Allen 2000) and allow establishment of the more sensitive plant species (Kumpiene et al. 2007).

By contrast, a vast group of species seemed to tolerate the higher toxicity of sludges quite well, achieving even higher cover values on this substrate. These tolerant species may well have taken advantage of the higher water retention and availability of the more finely textured and carbon-enriched sludges (Nasta et al. 2009). It is on these taxa that

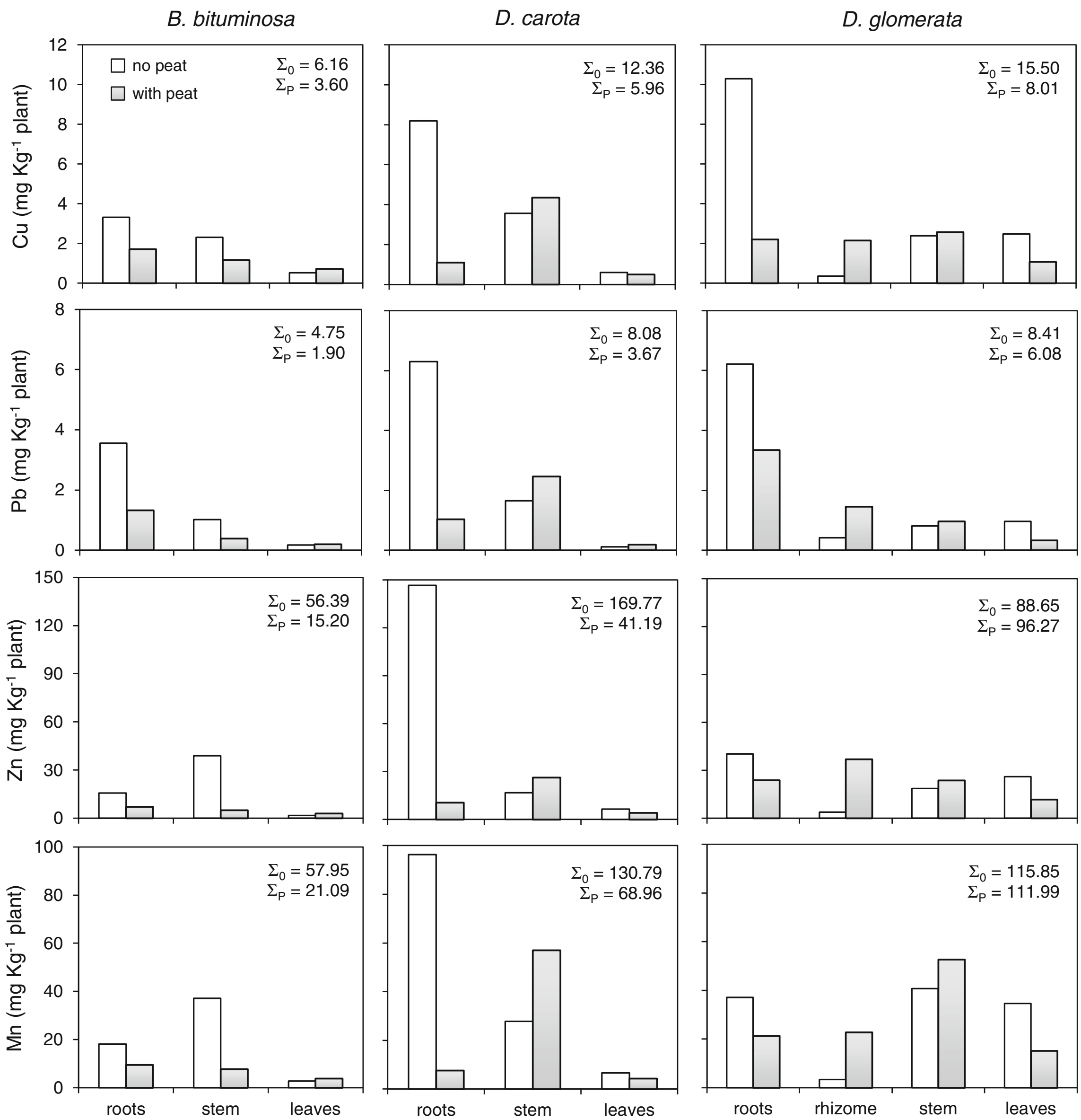

Fig. 3 Metal concentration (Cu, Pb, Zn, Mn) in plant tissues of *B. bituminosa*, *D. carota* and *D. glomerata* grown on sludge substrate with peat (*shaded bars*) or without peat (*empty bars*). The overall concentration for the whole plant is reported both for non-amended (Σ_0) and peat-amended substrates (Σ_P). Samples were kept in Bagnoli site, with the exception of *D. glomerata* that was sampled in Portici site

research should focus to identify possible phytoextractive or phytostabilizing plant species.

Plant biomass and tissue metal concentrations

In order to evaluate plant ability to uptake and translocate metal pollutants, plant analysis was restricted to sludge due to the higher potential phytotoxicity of this substrate. Three species were chosen as they best represented the main clusters found in multivariate analysis. Metal concentrations in plant tissues of *Bituminaria bituminosa* and *Daucus carota* grown in substrates F and Fp in Bagnoli and of *Dactylis glomerata* grown in substrates F and Fp in Portici are shown in Figs. 3 and 4.

In both sites organic amendment enhanced plant growth. In Bagnoli, total dry matter production doubled in the presence of peat (16 vs 31 g plant^{-1} for *B. bituminosa* and 3 vs 6 g plant^{-1} for *D. carota*), and in Portici, it increased sixfold (12 vs 59 g plant^{-1} for *D. glomerata*). Distribution of dry matter between root rhizome and stem leaves was not changed by organic amendment in *B. bituminosa* (34 and 66 % without peat vs 35 and 65 % with

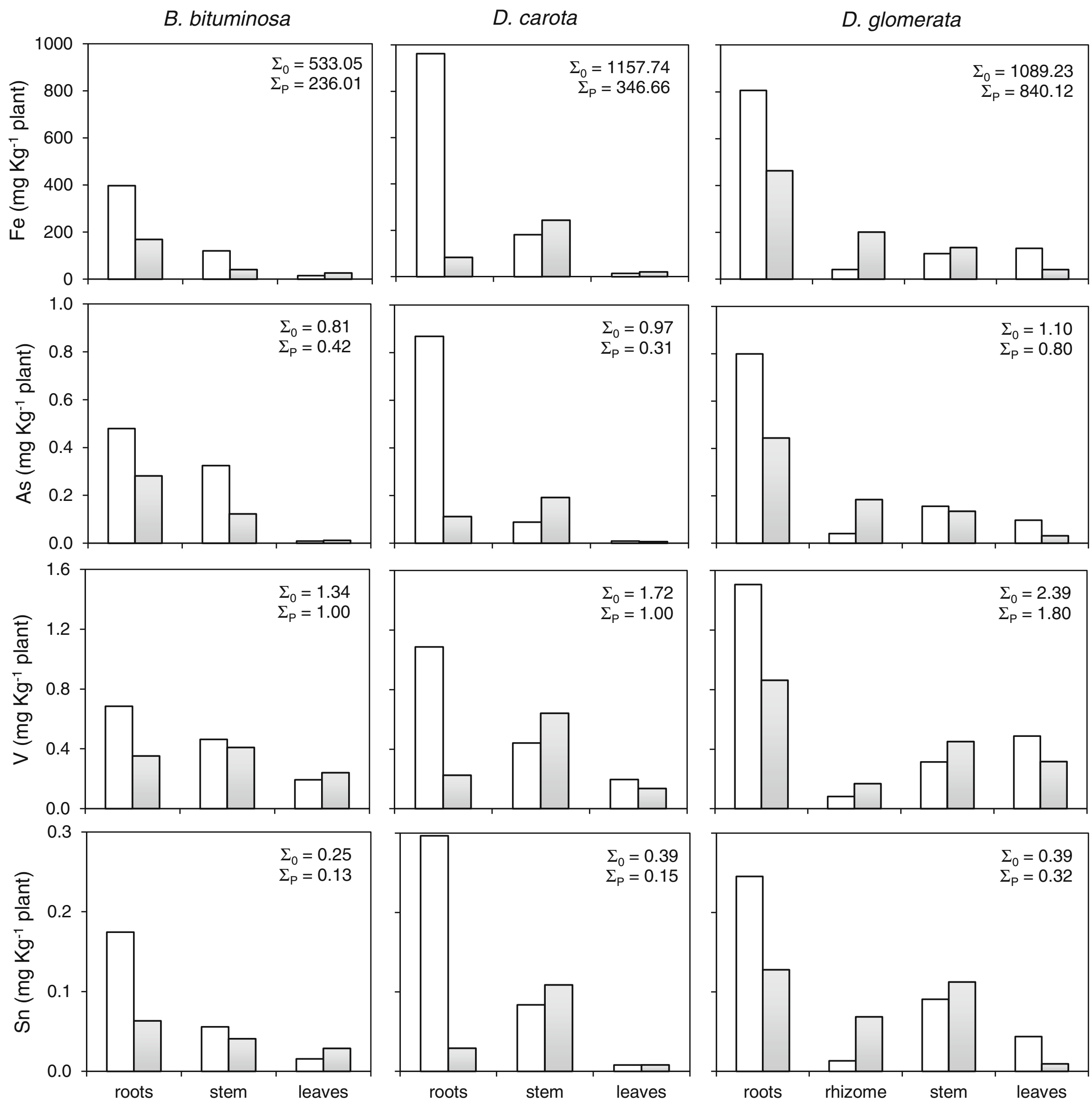

Fig. 4 Metal concentration (Fe, As, V, Sn) in plant tissues of *B. bituminosa*, *D. carota* and *D. glomerata.* Captions as in Fig. 3

peat) and *D. glomerata* (13 and 80 vs 17 and 77 %). A relative increase of stem and leaves dry matter with peat is observed in *D. carota* (36 and 64 vs 22 and 78 %). Peat is known to improve soil quality and reduce mobility and availability of metals and polar organic molecules by specific adsorption on polar functional groups of its major lignin and humic acid constituents (Brown et al. 2000; Madejón et al. 2006). This ability is probably the cause of the general restricted metal uptake and tissue accumulation in plants grown in the presence of peat (a total of 279 vs 661 mg of metals per kg of plant for *B. bituminosa*; 468 vs 1,482 mg kg^{-1} for *D. carota*; 1,065 vs 1,322 mg kg^{-1} for *D. glomerata*) (Figs. 3 and 4). In relative terms, however, peat effect on metal uptake was more effective in Bagnoli than in Gussone Park: in the first site, plant biomass doubled in the presence of peat against a 50 % reduction in plant tissue metal content; in the second site, only a 20 % reduction in metal concentration in plants occurred after peat treatment, in spite of a sixfold biomass increment.

None of the three species analysed shows metal concentrations higher than the normal levels for plants (Kabata-Pendias and Pendias 1984), and none can be considered as an accumulator according to the bioaccu-

Table 2 Bioaccumulation factor (BF = $[metal]_{plant}/[metal]_{soil}$) and shoot/root metal concentration quotients (MS/MR) in plant species grown on post-washing sludges (*F* sludges, *Fp* sludges + peat) in Bagnoli (*B. bituminosa* and *D. carota*) and Portici (*D. glomerata*) sites

	B. bituminosa		*D. carota*		*D. glomerata*	
	BF	MS/MR	BF	MS/MR	BF	MS/MR
Cu						
F	0.13	0.85	0.26	0.51	0.33	0.46
Fp	0.08	1.10	0.13	4.41	0.16	0.84
Pb						
F	0.02	0.33	0.03	0.28	0.03	0.27
Fp	0.01	0.44	0.01	2.57	0.02	0.27
Zn						
F	0.05	2.61	0.16	0.16	0.09	1.02
Fp	0.01	1.12	0.04	2.88	0.09	0.58
Mn						
F	0.03	2.20	0.06	0.36	0.05	1.86
Fp	0.01	1.22	0.03	8.05	0.05	1.54
Fe						
F	0.01	0.34	0.02	0.20	0.02	0.28
Fp	0.00	0.40	0.01	3.18	0.01	0.27
As						
F	0.02	0.70	0.02	0.11	0.03	0.30
Fp	0.01	0.48	0.01	1.77	0.02	0.27
V						
F	0.02	0.96	0.02	0.59	0.03	0.50
Fp	0.01	1.84	0.01	3.46	0.02	0.74
Sn						
F	0.02	0.41	0.02	0.31	0.03	0.52
Fp	0.01	1.10	0.01	4.01	0.02	0.62

mulation factors always much lower than one (Bargagli 1998) (Table 2).

Looking at Figs. 3 and 4, the majority of cumulative plant metal content is found in roots for *B. bituminosa* (on average 55 and 54 % with and without peat, respectively), followed by the stem (39 and 30 %) and leaves (6 and 16 %). Similar behaviour is observed for *D. glomerata* (65 and 64 % of total metals in root rhizome, 18 and 27 % in stems, 17 and 9 % in leaves, with and without peat, respectively). For *D. carota*, this distribution is found only in plants grown without peat addition (on average 77 % of cumulative total metal content in root, 19 % in stems and 4 % in leaves), while in plants grown in the presence of peat most, metal content is localized in stems (70 %), followed by roots (23 %) and leaves (7 %).

The higher concentration of most metals in plant roots indicates an important restriction of the internal transport of metals from the roots towards stems and leaves. Such metal immobilization in root cells is emphasized by *MS/MR* (shoot/root metal concentration quotient) <1 (Table 2) and is related to an exclusion strategy (Baker 1981). The translocation of metals from roots to shoots is minimal in *B. bituminosa* and *D. glomerata*, indicating metal immobilization by the roots. Hence, the latter two species could be useful in the phytostabilization of soils. By contrast, the behaviour of *D. carota* in the presence of peat with respect to all metals is characterized by *M*S/*M*R quotients >1, indicating the plant's ability to translocate metal contaminants to aerial plant parts. Nevertheless, despite this ability, *D. carota* under our experimental conditions does not appear suitable for phytoremediation in contaminated soils because both shoot biomass production and metal uptake are very low.

Assuming Al as the reference element, the enrichment factor (EF) for elements retained in plant tissues with respect to their concentration in substrates F and Fp was calculated (averaged values for F and FT in Fig. 5—"Appendix"). In the roots of analysed plant species, the majority of the metals (Fe, Pb, As, Sn, Mn and V) have EF values approximating unity; only those of Cu (up to 16, 22 and 16 in *B. bituminosa, D. carota* and *D. glomerata,* respectively) and of Zn in *D. carota* (12) suggest active uptake other than soil particles (Sardans and Peñuelas 2005). In plant leaves, the highest EF values are recorded, with Cu ranging from 77 (in *B. bituminosa*) to 99 (in *D. glomerata*), and Zn from 17 (in *B. bituminosa*) to 39 (in *D. glomerata*). The trend of Zn in *B. bituminosa* differs sensibly from that of *D. carota* and *D. glomerata*, with a relatively higher stem EF in the former species compared with the corresponding EF in leaves. This probably depends on the different functional role assumed by stem tissues in the three species at the stage where they were sampled: mainly mechanical support for *B. bituminosa,* reproductive allocation for the other two species.

Conclusion

The results of this work show that an acceptably healthy vegetation cover on polluted soils from the Bagnoli brownfield site as well as on post-washing sludges could be achieved by the proposed natural revegetation approach. In addition, organic amendment, in the form of peat addition, can help plant growth, probably improving soil physical properties as well reducing pollutant availability and hence phytotoxicity. Plant growth, in terms of number of species and biomass production, is enhanced in the more natural and less disturbed Gussone Park than inside the disused industrial area. This is because in Bagnoli, the severity of environmental degradation rather than the contamination level of substrates affects the plant colonization process. In this context, it is hardly surprising that the ameliorative effect of peat on soil quality and plant growth is more evident in Bagnoli than in Gussone Park and significant for the valuable but more sensitive leguminous species.

Brownfield sites are often host to harsh conditions which have to be overcome to establish vegetation successfully and sustainably. Some sites may lack soil resources completely and require the use of soil-forming materials to simulate soil conditions. Others may pose restrictions on plant growth through the presence of large dusty open spaces. A key to creating effective and sustainable vegetation may be to plan for and create the right site conditions to enable vegetation to reach maturity. The spontaneous process of recovery could be facilitated at low cost, for instance, by just planting strategically selected small vegetation islands, aimed at “nursing” the subsequent stages of plant succession. There is considerable evidence to support the idea that nurse plants may be adopted as strategic tools for facilitating the ecological recovery of degraded environments (Gomez-Aparicio 2009). Further research should focus on how nurse vegetation might be established in brownfield sites prior to implementing recolonization programmes in order to achieve healthier plants and support engraftment.

The revegetation approach could represent an economically more realistic and cost-effective option than excavation, soil washing and in situ or off site sludge disposal, especially for vast industrial sites such as the ex ILVA of Bagnoli or the yet active ILVA plant of Taranto. This technique could also be suited to face extreme contamination of agricultural lands, as found in the most polluted sites of the so called “Land of Fires”, an area of Campania Region widely known for illegal waste discharge and dumping or burning. It is desirable that further regulations concerning soil pollution take into account the mobility/bioavailability of pollutants besides the absolute level of pollution, in order to properly differentiate remediation actions: revegetation techniques could be preferred whenever bioavailability is sufficiently low to allow natural plant growth and safety for population.

It can be anticipated that further development of the vegetation could increase the extent of depletion of metal pools by plant uptake, resulting in a change in metal distribution, mobility and availability in soil (Hammer and Keller 2002; Arienzo et al. 2004). In addition, with time an enhanced degradation and dissipation of resistant high molecular weight organic pollutants by revegetation may give cause for concern and should be monitored (Sun et al. 2010). The results support further investigation on the possibility of using such soils for profitable non-food crops.

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Appendix

See Fig. 5 and Table 3.

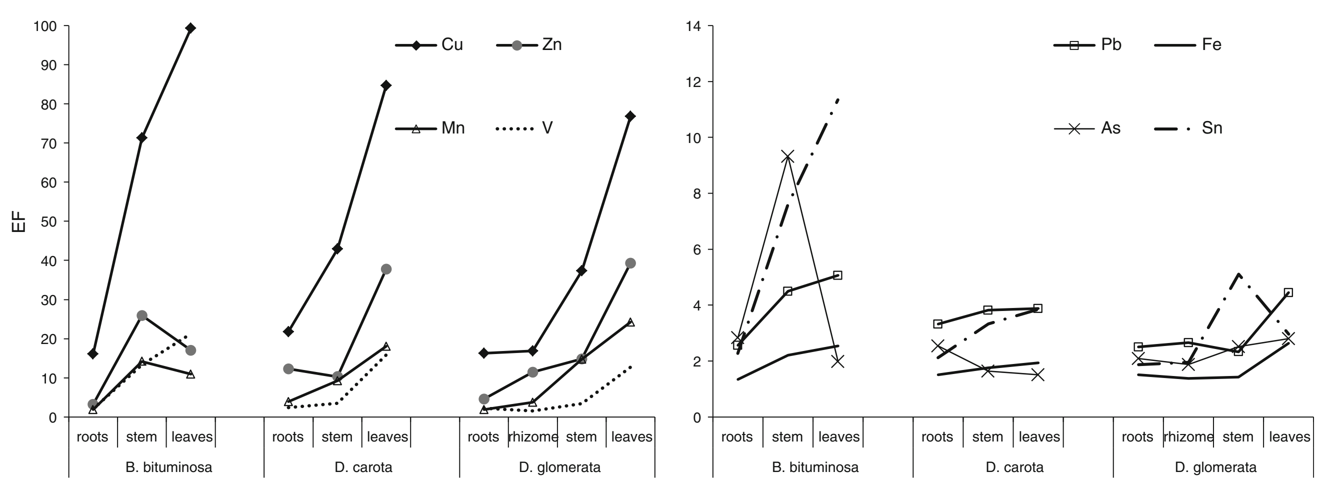

Fig. 5 Enrichment factor (EF) of elements in tissues of plants grown on post-washing sludges (*F* sludges, *Fp* sludges + peat) in Bagnoli (*B. bituminosa* and *D. carota*) and Portici (*D. glomerata*) sites (average EF values for F and Fp)

Table 3 Label legend of the species reported in Figs. 1 and 2

Species	Label	Cluster
Piptatherum miliaceum	Pip_mil	g1
Bromus gussonei	Bro_gus	g2
Dactylis glomerata	Dac_glo	
Fraxinus ornus	Fra_orn	
Geranium purpureum	Ger_pur	
Hedera helix	Hed_hel	
Salpichroa origanifolia	Sal_ori	
Sonchus oleraceus	Son_ole	
Verbascum sinuatum	Ver_sin	
Bituminaria bituminosa	Bit_bit	g3
Diplotaxis tenuifolia	Dip_ten	
Medicago minima	Med_min	
Melilotus albus	Mel_alb	
Artemisia annua	Art_ann	g4
Aster squamatus	Ast_squ	
Catapodium rigidum	Cat_rig	
Daucus carota	Dau_car	
Papaver rhoeas	Pap_rho	
Polypogon monspeliensis	Pol_mon	
Polypogon viridis	Pol_vir	

The clusters to which each species was assigned is reported in the third columns

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