

Clonal variation in growth, arsenic and heavy metal uptakes of hybrid *Eucalyptus* clones in a Mediterranean environment

Giovanni Mughini · Francesco Alianiello ·
Anna Benedetti · Lapo Mughini Gras ·
Maria A. Gras · Luca Salvati

Received: 6 July 2012 / Accepted: 16 January 2013 / Published online: 24 January 2013
© Springer Science+Business Media Dordrecht 2013

Abstract Variation in growth, arsenic and heavy metal uptakes by aboveground tissues (leaves, stems and branches) of 13 hybrid *Eucalyptus* clones selected for biomass production in a Mediterranean environment (*E. camaldulensis* × *E. viminalis*; *E. camaldulensis* × *E. grandis*; *E. camaldulensis* × *E. globulus* subsp. *bicostata*) was investigated on agricultural soils field-contaminated with arsenic (As), cadmium (Cd), chrome (Cr), lead (Pb), copper (Cu) and zinc (Zn) in an *ex situ* nursery experiment in central Italy. Large variation in growth and contaminant uptake amongst the tested clones was observed. All plants survived and 12 clones grew better than the control (*E. camaldulensis*). All clones accumulated the contaminants to which they were exposed: As, Cu, Pb and Zn concentrations were significantly higher in leaves than in stems and branches,

supporting the potential for phytoremediation of these contaminants by *Eucalyptus* short rotation woody crops (SRWC). Significant positive correlations between the average contents of Cd–Pb, Cd–Cu, Cd–Zn, Pb–Cu, Pb–Zn and Cu–Zn in the aboveground tissues were detected. Clones revealed better phytoextraction performance than that of the control. Four promising clones for biomass production and phytoremediation were identified for prospective use in SRWC on contaminated soils in Mediterranean environments.

Keywords Soil phytoremediation · *Eucalyptus* · Trace element · Mediterranean environment · Central Italy

Introduction

Arsenic (As) and heavy metal (HM) contamination of soil and groundwater is a major public health concern in many parts of the world. Naturally occurring As is commonly present in volcanic rocks as a result of geochemical evolution, alluvium and weathering of As-rich parent materials (Welch et al. 1988; Purdy and Smart 2008). Anthropogenic sources of As and HMs involve metal mining, refining and smelting activities, long-term waste disposals, urban and industrial expansion, and intensive applications of old pesticides, herbicides and wood preservatives (Alloway 1995), all of which can ultimately exacerbate an original geogenic contamination.

G. Mughini · M. A. Gras
Consiglio per la Ricerca e la Sperimentazione in
Agricoltura, Unità di Ricerca per le Produzioni Legnose
Fuori Foresta (CRA-PLF), Rome, Italy

F. Alianiello · A. Benedetti · L. Salvati
Consiglio per la Ricerca e la Sperimentazione in
Agricoltura, Centro di Ricerca per lo Studio delle
Relazioni tra Pianta e Suolo (CRA-RPS), Rome, Italy

L. Mughini Gras (✉)
Dipartimento di Sanità Pubblica Veterinaria e Sicurezza
Alimentare, Istituto Superiore di Sanità (ISS), Reparto
di Epidemiologia ed Analisi del Rischio,
Viale regina Elena 299, 00161 Rome, Italy
e-mail: lapo.mughinigras2@unibo.it;
lapomugras@libero.it

Italy's law establishes strict concentration limits of tolerance for As in soils of residential (20 mg/kg) and commercial or industrial areas (50 mg/kg). In agricultural soils, As should not be detected or at least not in an available form. Additionally, As should not exceed 10 µg/L in drinking water and 20 µg/L in irrigation water. Regrettably, these limits have frequently been exceeded either in Italy or in other countries (Pigna et al. 2009).

In several areas of central and southern Italy, both geogenic and anthropogenic factors have resulted in extensive As contamination of agricultural soil and groundwater (Preziosi et al. 2010). For instance, in the Latium region, As exceeded 10 µg/L in 37 % of the sampled water wells, 12 % of which exceeded the legal limits more than three times (Achene et al. 2010). Furthermore, in the Tuscany region, sediments of the Pecora River revealed an extremely high As concentration of 665 mg/kg that was largely caused by pyrite intensive mining activities perpetuated since Etruscan times (Tanelli et al. 2003; Castagliola et al. 2008; Benvenuti et al. 2009). Exceptionally high cadmium (Cd), chrome (Cr), lead (Pb), copper (Cu) and zinc (Zn) concentrations were also found in various industrial areas and neighbouring arable lands in central and southern Italy (GLOBSI 2004; ISPRA-SINANET 2009).

The phytoremediation of As- and HM-contaminated areas using short rotation woody crops (SRWC) for biomass production would allow for both their long-term reclamation and an economically valuable bioenergy production (Pulford et al. 2002; Rockwood et al. 2003, 2004), providing an attractive solution for exploiting degraded lands while preserving the environment. Although most woody species used for biomass production (e.g. *Salix* spp., *Populus* spp. and *Eucalyptus* spp.) are not considered as hyperaccumulators of As and HMs (Rockwood et al. 2003, 2004), selected genotypes of poplar and willow have been used for phytoremediation just because of their fast growth, elevated water and nutrient usage and extensive root system (e.g. French et al. 2006; Zalesny and Bauer 2007; Castiglione et al. 2009). Moreover, compared with hyperaccumulators, most non-hyperaccumulating woody species can accumulate and remove comparable, or even greater, quantity of contaminants just because of their higher biomass production (Purdy and Smart 2008).

In recent years, there have been numerous studies on As and HM tolerance and uptake by plants. However, much of this research has been focused on hyperaccumulators and selected ranges of other non-

hyperaccumulating plants, including trees such as poplars, willows and birches, for which there is a wealth of information in the literature. In contrast, exhaustive information on the phytoremediation potential of *Eucalyptus* spp. is scarce, and many aspects of *Eucalyptus* biology in contaminated areas are still poorly understood (Assareh et al. 2008). *Eucalyptus* spp. are globally diffused in different environments: from deserts to rainforests and alpine environments (King et al. 2008). They are therefore greatly adapted to varying environmental conditions and are characterised by high genetic variability and economic value, thereby increasing their attractiveness for phytoremediation purposes as well.

Some *Eucalyptus* species have been cultivated on As- and HM-contaminated soils in many parts of the world (Hartley 1977; Grant et al. 2002; Saliba et al. 2002; Ding et al. 2007; King et al. 2008; Assareh et al. 2008; Melo et al. 2010), as well as on soils contaminated with radionuclides (Madruga et al. 2001) and with high salinity (Tossell et al. 2000). These studies obtained interesting but heterogeneous results. Moreover, comprehensive information on the growth and contaminant-specific uptake ability of biomass-producing hybrid *Eucalyptus* clones is lacking with regard to the Mediterranean environment.

In Mediterranean environments, *Eucalyptus* spp. have long been recognised as appropriate trees to be utilised in SRWC for biomass production, and several sources suggest that *Eucalyptus camaldulensis* Dehnh of Lake Albacutya provenance is the best species for this purpose (Gemignani 1968; Lacaze 1970; Mughini 1991). *E. camaldulensis* proved to be highly adaptable to some possible local environmental difficulties, such as drought periods, high ambient temperatures and clayey soils, revealing fast-growing abilities which are better than *E. gomphocephala* DC and *E. occidentalis* Endl (Mughini 1991).

In this study, we investigated the variation in growth and contaminant uptake ability of 13 hybrid *Eucalyptus* clones selected for high biomass production in central and southern Italy, using agricultural soils field-contaminated with As, Cd, Cr, Pb, Cu and Zn. The aims of this study were to: (1) evaluate the growth of these clones on As- and HM-contaminated soils; (2) determine the As and HM average concentrations in their aboveground tissues (leaves, stems and branches); and (3) estimate the As and HM average contents in their aboveground tissues. Such

information allowed us to characterise the potential for establishing *Eucalyptus* genotypes on As- and HM-contaminated agricultural soils and to identify those genotypes that are promising for both biomass production and phytoremediation in future SRWC in Mediterranean environments.

Materials and methods

Clones and soil

This study was carried out at the Research Unit for Wood Production Outside Forest (CRA-PLF) plant nursery of Casalotti, Rome, central Italy (41°54'N lat.; 12°40'E long.). Thirteen hybrid *Eucalyptus* clones selected for high biomass production at CRA-PLF based on their current utilisation, growth and adaptability in the Mediterranean landscape of central and southern Italy, were cultivated outdoor during 8 months (March–October 2009) in 20 L-volume pots. Details of the clones are reported in Table 1. In every pot, a 15 cm rooted cutting of 6 months of age was placed. Five equally aged ramets of each clone were used. The experiment was based on a completely randomised design with five replications. The elementary plot was represented by one ramet. Five seedlings of *E. camaldulensis* of 6 months of age were used as control.

The pots were filled with an agricultural soil contaminated with As and HMs taken from one of Italy's most notoriously contaminated areas located in

the Scarlino municipality, Tuscany region (42°54'N lat.; 10°51'E long.), where several studies related to soil contamination have previously been conducted (e.g. Tanelli et al. 2003; Castagliola et al. 2008; Benvenuti et al. 2009; Pigna et al. 2010). In this area, both natural (volcanic origin) and anthropogenic causes (mining activities and chemical industries) are responsible for the soil contamination. A total of approximately 2 m³ of soil was excavated at a depth of 0–50 cm from multiple points and carefully mixed and transported in airtight containers to the CRA-PLF plant nursery of Casalotti, where the pots were filled. Soil analyses were performed using official protocols of soil chemical analyses provided by the Italian Ministry of Agriculture and Forestry (Violante 2000). Texture, pH and cation concentrations of the soil are reported in Table 2. The soil was a clay-loam limestone, sub-alkaline with medium organic C content. The C/N ratio was rather low (9.4), K was abundant, whereas available P was low. As expected in limestone soils, CSC was medium and pH was slightly alkaline, meaning that As and HM availability was lower than in a neutral or acidic soil.

After 8 months of regular irrigation, individual trees were harvested and separated into leaf and stem + branch components.

Plant growth

To assess differences in aboveground biomass production amongst the clones/control, the dry aboveground biomass (g/plant) was calculated as: (1) total

Table 1 Hybrid *Eucalyptus* clones and the control

Abbreviation	Species and hybrid clones
Velino ex 7	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
8	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
14	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
20	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
29	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
44	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
45	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
330	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
Viglio ex 358	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
13A	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
7IMP	<i>E. camaldulensis</i> × <i>E. globulus</i> subsp. <i>bicostata</i>
65	<i>E. camaldulensis</i> × <i>E. grandis</i>
81	<i>E. camaldulensis</i> × <i>E. viminalis</i>
Control	<i>E. camaldulensis</i>

Table 2 Texture, pH and cation concentrations in the soil taken from the Scarlino area, Tuscany region, central Italy (0–50 cm underground)

Parameter	Value	Parameter	Value
Sand ^a	28 %	Available P ⁱ	1.00 mg/kg
Silt ^a	44 %	Total As ^h	208.00 mg/kg
Clay ^a	28 %	Total B ^h	67.00 mg/kg
Texture ^b	Clay-loam	Total Cd ^h	<1.00 mg/kg
pH ^c	7.7	Total Cr ^h	98.00 mg/kg
Total limestone ^d	14 %	Total Cu ^h	52.00 mg/kg
Total organic C ^e	11.90 g/kg	Total Hg ^h	<0.50 mg/kg
Total N ^f	1.26 g/kg	Total Mo ^h	<2.00 mg/kg
Total P ^g	0.60 g/kg	Total Ni ^h	51.00 mg/kg
Total K ^h	17.50 g/kg	Total Pb ^h	43.00 mg/kg
Total S ^h	1.20 g/kg	Total Sb ^h	<10.00 mg/kg
Total Al ^h	44.90 g/kg	Total Se ^h	<10.00 mg/kg
Total Fe ^h	43.60 g/kg	Total Sn ^h	<8.00 mg/kg
Total Ca ^h	54.90 g/kg	Total Zn ^h	167.00 mg/kg
Total Mg ^h	7.30 g/kg	CEC ^j	16.50 meq/100 g

Analyses were performed on the soil taken from the same study area as that of Tanelli et al. (2003), Castagliola et al. (2008) and Pigna et al. (2010)

^a Densimetric method

^b According to USDA soil texture classification

^c Measured in deionized water (solution ratio 1:2.5)

^d Volumetric method

^e Springer-Klee's method

^f Kjeldahl's distillation

^g Spectrophotometric method

^h Extraction with aqua regia and determination with ICP-OES

ⁱ Olsen's method

^j Cation exchange capacity, extraction with ammonium acetate

dry biomass = leaves + stems + branches (LSB); (2) leaves (L); and (3) stems + branches (SB). All these tissues were washed with distilled water, blotted dry and then dried using a thermostatic oven at 105 °C. Dryness was confirmed by weighing consecutively until constant weight. Leaves of each plant were dried separately from their stems and branches.

Contaminant concentration

For each clone/control, As, Cd, Cr, Pb, Cu and Zn average concentrations (mg/kg plant) in leaves and in stems + branches were measured in a compound sample obtained by pooling the plant materials from the five replicates. Samples were weighed and dried at 40 °C and the residual moisture was measured in

thermostatic oven at 105 °C. Dry material was milled to 0.02 cm and extracted by aqua regia and H₂O₂. As, Cd, Cr, Pb, Cu and Zn concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) using an IRIS Dual-View (Thermo Jarrell Ash, Franklin, USA) spectrometer. Simultaneous analyses of certified standards (111355 ICP multi-element standard solution IV, Merck KGaA®, Darmstadt, Germany) with known element concentration confirmed the procedure correctness. The following data were obtained: (1) contaminant concentrations in leaves (L_c); and (2) contaminant concentrations in stems + branches (SB_c). Hereafter, the chemical symbol of the contaminant will precede the character specifying the plant tissue (e.g. As-L_c = Arsenic concentration in leaves).

Contaminant content

For each clone/control, As, Cd, Cr, Pb, Cu and Zn average contents (mg) in leaves and in stems + branches were calculated by multiplying their average concentrations by the corresponding mean biomass produced. The following data were obtained: (1) average contaminant contents in leaves (L_{cc}); (2) average contaminant contents in stems + branches; (SB_{cc}); (3) average contaminant contents in stems + branches + leaves (LSB_{cc}).

Statistical analysis

Analysis of variance (ANOVA) and Duncan's post hoc test were used to test for differences in means of L, SB and LSB between the clones/control. For each contaminant, differences between L_c and SB_c , and between L_{cc} and SB_{cc} were tested using the Mann–Whitney's U test. The Spearman's rank correlation test was used to assess pair-wise correlations between the biomass production (L and SB) and the corresponding average contaminant concentrations, and between the average contents of the different contaminants (LSB_{cc}).

A hierarchical cluster analysis was performed for grouping clones/control of similar LSB_{cc} into related phytoextraction performance groups. The tree clustering

was based on Euclidean distance as similarity measure and unweighted pair-group method with arithmetic mean (UPGMA) as linkage rule.

Data analyses were performed using Statistica® 6.0 software (StatSoft, Inc. Tulsa, OK, USA) and, when applicable, the statistical significance was set to $P < 0.05$.

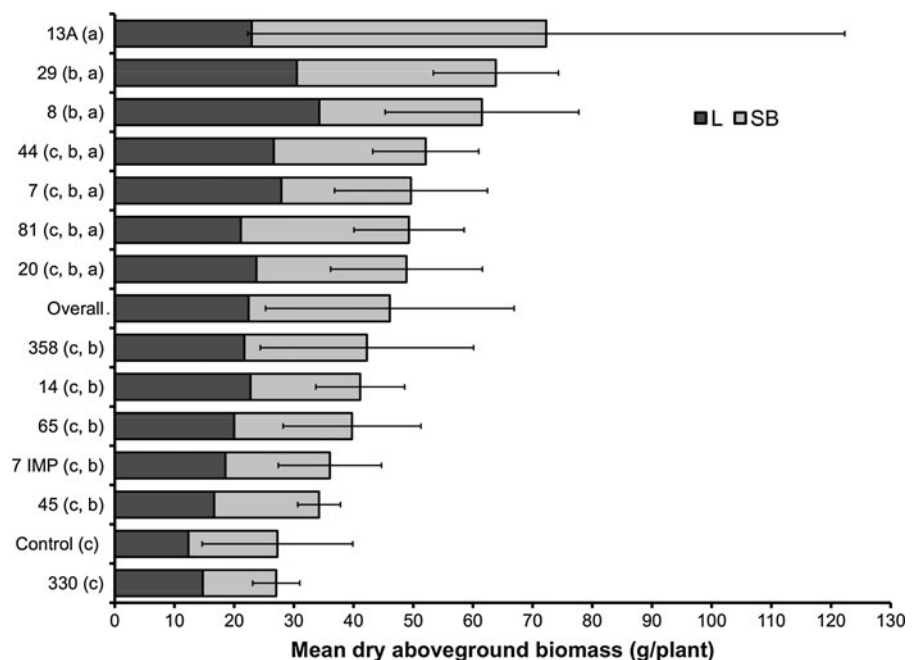
Results and discussion

Plant growth

For L, differences amongst the clones/control were highly significant ($P < 0.01$) and those for SB and LSB were significant ($P < 0.05$) (Fig. 1). Leaves varied from 12.34 g/plant (control) to 34.27 g/plant (clone 8), with an overall mean of 22.42 g/plant. SB ranged from 12.30 g/plant (330) to 49.32 g/plant (13A), with an overall mean of 23.66 g/plant, and the control was the second lowest (14.91 g/plant). LSB ranged from 27.04 g/plant (330) to 72.27 g/plant (13A), with an overall mean of 45.62 g/plant.

Mean values of L and SB were similar (22.42 and 23.66 g/plant, respectively). This was probably due to the early age of the tested plants. Values of L in older trees (2–3 years of age) are indeed considerably lower than those of SB, accounting for approximately 15 %

Fig. 1 Mean dry biomass (g/plant) of leaves (L) and stems + branches (SB) $\pm$ standard deviation in the 13 hybrid *Eucalyptus* clones and in the control. Clones/control with the same letter in parenthesis have no statistically significant differences between them (Duncan's post hoc test after ANOVA, $P > 0.05$)



of the total dry aboveground biomass (Mughini et al. 1996). As *Eucalyptus* spp. are evergreen trees, the entire aboveground biomass, including leaves, can be easily removed at the harvesting time in SRWC. *Eucalyptus* leaves can therefore be considered as part of the obtainable biomass, although the fact that *Eucalyptus* spp. shed leaves with age and in response to drought stress should be taken into account.

Despite the early age of the tested plants, LSB values of the clones were always higher than those of the control, except for clone 330 that seemed to be particularly affected by the contaminated soil. Also in previous clonal field trials conducted on uncontaminated soils (Mughini et al. 1996), all the clones tested here showed a better growth performance than that of the control, particularly the clones Velino *ex* 7 and Viglio *ex* 358 which also proved to be the most tolerant clones to gall inducer insects (e.g. *Leptocybe invasa* and *Ophelymus maskelli*) that have recently been introduced accidentally in most Mediterranean countries, including Italy (Mughini et al. 2007; Bella and Lo Verde 2002). For both these two clones, the application for Community Plant Variety Right to the European Union Community Plant Variety Office (CPVO) was presented.

Trees that are not especially selected for contaminant tolerance can survive in contaminated soil through facultative tolerance such as redistribution of roots to less contaminated zones, albeit usually with poor or highly variable growth performances (Dickinson et al. 1992). As genetic stability of tolerance can be both induced and lost in trees, their ability to acclimatise to fluctuating stress due to contamination is very important for survival and growth of trees (Dickinson et al. 1992; Pulford and Watson 2003). The high variation in biomass production observed in clone 13A may therefore reflect an intrinsic high sensitivity of this clone to acclimation to the soil environment and pollution stress, similarly to what has been reported for *Salix* clones by Pulford and Watson (2003).

Although As may delay the growth of *Eucalyptus* spp. (King et al. 2008; Melo et al. 2010), and HMs may affect the survival of many plant species (Cheng 2003), all plants tested have survived and grown well, indicating tolerance to the contaminant concentrations to which they were exposed, at least for the lifetime of this experiment, which, however, covered one of the most sensitive periods in plant lifespan. These findings

are in line with previous studies (Grant et al. 2002; Ding et al. 2007; King et al. 2008; Assareh et al. 2008) in which a number of *Eucalyptus* species have normally been cultivated on either acid or alkaline As- and HM-contaminated soils. Results also suggest that, except for clone 330, the contaminated soil did not affect the superiority in biomass production of the tested clones compared to the control.

Contaminant concentration

As- L_c , Cu- L_c , Pb- L_c and Zn- L_c values were significantly higher than those of SB $_c$ (Table 3). Conversely, Cd-SB $_c$ and Cr-SB $_c$ values were significantly higher than those of L_c . Except for Cd- L_c , Cu- L_c and Pb- L_c , all other leaf concentrations were lower in the control than the overall mean. The highest variation amongst the clones/control, expressed as percent variation coefficient (VC %), was found for As- L_c , Cd- L_c , and Cr-SB $_c$, with VC % values exceeding 60 % (As- L_c 65 %, Cd- L_c 76 % and Cr-SB $_c$ 68 %). In all other cases, VC % ranged from 23 % (Cd-SB $_c$) to 33 % (Pb- L_c). Such variability levels strongly encourage further clonal selections for these characters. Cd- L_c and Pb- L_c values in clone Velino *ex* 7, and those of Cr-SB $_c$ and Zn-SB $_c$ in clone Viglio *ex* 358, were never lower than the overall mean, and always higher than the control with the exception of As.

No significant pair-wise correlations ($P > 0.05$) between biomass production and contaminant concentrations were detected, suggesting that these may be two unrelated characters. Therefore, it is unlikely that the best performing clone regarding aboveground biomass production would also be the best clone regarding contaminant uptakes, and vice versa.

Bioavailability of contaminants to trees and their accumulation in tree tissues can vary hugely according to the source of contamination and site conditions (Pulford and Watson 2003). As contaminants are sparingly soluble under alkaline conditions, our soil pH of 7.7 resulted in suboptimal conditions for uptake. However, this was exactly the soil pH as measured in the field and did not differ significantly from that found in previous studies conducted in the same area where the soil was taken (Tanelli et al. 2003; Castagliola et al. 2008; Pigna et al. 2010). Therefore, although this pH does not provide optimal contaminant solubility, it reflects the real situation in the field where the clones should eventually be planted and

Table 3 Average concentrations (mg/kg plant) of As, Cd, Cr, Cu, Pb and Zn in leaves (L_c) and stems + branches (SB_c) of the 13 *Eucalyptus* clones and the control, together with their overallmean, standard deviation (SD), variation coefficient in percentage (VC %) and the *P* value from the Mann–Whitney *U* test comparing contaminant concentrations between L_c and SB_c

Clone/control	Average concentrations (mg/kg plant)											
	As		Cd		Cr		Cu		Pb		Zn	
	L_c	SB_c	L_c	SB_c	L_c	SB_c	L_c	SB_c	L_c	SB_c	L_c	SB_c
Velino <i>ex</i> 7	1.25	1.19	0.22	0.38	0.50	3.15	11.60	9.50	2.38	0.40	38.74	13.50
8	1.35	0.57	0.10	0.39	1.18	6.00	11.80	8.50	1.07	0.65	35.41	33.00
14	2.49	1.45	0.10	0.38	1.41	1.45	15.14	12.00	1.51	0.45	43.24	17.50
20	5.85	1.20	0.09	0.38	1.63	3.85	10.18	10.00	1.73	0.27	37.92	12.00
29	3.10	2.25	0.11	0.55	1.40	2.01	20.00	17.20	1.29	0.60	50.43	19.00
44	2.89	1.35	0.12	0.39	1.93	3.30	14.99	11.50	1.29	0.55	50.32	25.30
45	3.01	2.00	0.06	0.43	1.40	3.05	17.22	13.00	1.45	0.29	66.74	20.50
65	2.80	1.50	0.02	0.39	1.08	8.50	9.16	8.00	1.19	0.47	35.56	30.50
81	7.78	1.25	0.13	0.44	1.35	1.94	11.88	8.00	1.30	0.60	46.44	20.00
330	3.46	1.80	0.03	0.75	1.08	1.66	15.15	11.50	2.27	0.55	55.20	26.50
Viglio <i>ex</i> 358	1.40	1.25	0.14	0.40	1.30	7.00	10.04	8.50	1.30	0.50	32.40	27.00
13A	1.30	1.07	0.16	0.42	1.04	1.45	9.23	9.00	2.58	0.65	33.26	27.50
7IMP	2.20	1.87	0.42	0.50	1.61	1.87	15.05	13.50	2.04	0.60	51.61	25.00
Control	1.45	1.08	0.31	0.45	1.29	1.50	16.20	9.00	2.80	0.45	26.00	12.94
Overall mean	2.88	1.42	0.14	0.45	1.30	3.34	13.40	10.66	1.73	0.50	43.09	22.16
Overall SD	1.87	0.44	0.11	0.10	0.33	2.27	3.31	2.64	0.57	0.12	10.96	6.67
Overall VC %	65.0	31.0	75.8	22.5	25.7	67.9	24.7	24.7	33.2	24.2	25.4	30.1
<i>P</i> value	<0.01		<0.001		<0.001		<0.05		<0.001		<0.001	

allowed to grow. Findings should thus be interpreted based on the given conditions which are particularly widespread in Mediterranean environments.

Most contaminants were more concentrated in leaves than in stems + branches, suggesting that the use of *Eucalyptus* spp. for phytoremediation could be more efficient if the entire aboveground biomass, including leaves, is removed. As mentioned above, this is easily feasible in *Eucalyptus* SRWC because they are evergreen trees. In contrast, this is more complicated with deciduous trees frequently used for biomass production, such as poplars and willows, for which Rockwood and Alker (2000) suggested to anticipate the harvesting time before leaves fall.

Findings concerning contaminant concentration and compartmentalization in aboveground tissues should be compared cautiously with those present in the literature. Differences in plant age and genetics, contaminant concentrations and availability in soils, soil characteristics themselves, climatic and environmental conditions, timing and experimental designs,

as well as applied analytical methodologies, are the major causes of the differences in the results from the different studies. It is worth mentioning that our clones showed higher As concentrations than those recorded in 5-year-old *Eucalyptus* species grown on arsenical gold mine tailings in western Victoria, Australia (King et al. 2008), but that the As accumulation partitioning in their aboveground tissues was very similar, with As concentrations significantly higher in leaves than in stems + branches. This suggests that our results based on young plants should not change drastically in adult trees. To our knowledge, the only case in which As– SB_c was significantly higher than As– L_c was reported by Tossell et al. (2000) for *E. camaldulensis* in a laboratory experiment.

There was large variation in As uptake amongst the tested clones. Interestingly, the only clone with *E. camaldulensis* and *E. viminalis* as parents showed the highest As– L_c . To improve As uptake in this specific hybrid, further studies are needed. In accordance with previous studies (Ding et al. 2007, Assareh

et al. 2008), Zn- L_c and Pb- L_c were higher than those of SB_c , but our results regarding Cr ($SB_c > L_c$) and Cu ($L_c > SB_c$) were in contrast with Ding et al. (2007) and Assareh et al. (2008), respectively. These differences could only partially be attributed to selective absorption and compartmentalization of HMs by plants (Cheng 2003), as several influencing factors differed greatly between these studies.

As contaminant concentrations were determined in a single compound sample, it was not possible to provide detailed information on the within-clone variation of As and HM uptakes. While this may have limited our understanding of the uptake capabilities of individual clones, each compound sample reflected the average uptake capability of the respective clone as all plant replicates contributed equally to that sample.

Contaminant content

Values of L_{cc} and SB_{cc} for each contaminant are reported in Table 4, while those of LSB_{cc} are reported

Table 4 Average contents (mg/plant) of AS, Cd, Cr, Cu, Pb and Zn in leaves (L_{cc}) and stems + branches (SB_{cc}) of the 13 *Eucalyptus* clones and the control, together with their overall

in Fig. 2 together with the cluster analysis dendrogram grouping those clones/control with similar LSB_{cc} performances. As- L_{cc} , Pb- L_{cc} and Zn- L_{cc} values were significantly higher than those of SB_{cc} as found in their concentrations. Cu- L_{cc} was higher than Cu- SB_{cc} , but this difference was not significant ($P > 0.05$) in contrast to that found in their concentrations. Cd- SB_{cc} and Cr- SB_{cc} values were significantly higher than those of L_c . Except for Cd- L_{cc} , all other contaminant contents were lower in the control than the overall mean. The highest VC % was measured for As- L_{cc} , Cd- L_{cc} and Cr- SB_{cc} , with values exceeding 65 % (As- L_{cc} 67 %, Cd- L_{cc} 67 % and Cr- SB_{cc} 65 %). In all other cases, VC % ranged from 32 % (Zn- L_{cc}) to 60 % (Pb- SB_{cc}).

Significant pair-wise correlations were detected between Cd- LSB_{cc} and Pb- LSB_{cc} ($r_s = 0.84$, $P < 0.001$), Cd- LSB_{cc} and Cu- LSB_{cc} ($r_s = 0.58$, $P < 0.05$), Cd- LSB_{cc} and Zn- LSB_{cc} ($r_s = 0.64$, $P = 0.01$), Pb- LSB_{cc} and Cu- LSB_{cc} ($r_s = 0.70$, $P < 0.01$), Pb- LSB_{cc} and Zn- LSB_{cc} ($r_s = 0.57$, $P < 0.05$), and Cu- LSB_{cc} and Zn- LSB_{cc}

mean, standard deviation (SD), variation coefficient in percentage (VC %), and the P value from the Mann–Whitney U test comparing contaminant contents between L_{cc} and SB_{cc}

Clone/control	Average contents (mg/plant)											
	As		Cd		Cr		Cu		Pb		Zn	
	L_{cc}	SB_{cc}	L_{cc}	SB_{cc}	L_{cc}	SB_{cc}	L_{cc}	SB_{cc}	L_{cc}	SB_{cc}	L_{cc}	SB_{cc}
Velino <i>ex</i> 7	0.0350	0.0270	0.0063	0.0085	0.0140	0.0685	0.3235	0.2060	0.0663	0.0085	1.0808	0.2890
8	0.0462	0.0154	0.0032	0.0106	0.0404	0.1634	0.4044	0.2316	0.0366	0.0178	1.2132	0.8986
14	0.0565	0.0265	0.0020	0.0070	0.0318	0.0265	0.3440	0.2203	0.0345	0.0083	0.9838	0.3218
20	0.1386	0.0300	0.0018	0.0094	0.0388	0.0968	0.2416	0.2514	0.0412	0.0068	0.9002	0.3020
29	0.0945	0.0750	0.0035	0.0185	0.0425	0.0670	0.5240	0.4665	0.0390	0.0200	1.5385	0.6335
44	0.0772	0.0344	0.0034	0.0100	0.0512	0.0838	0.3994	0.2928	0.0344	0.0140	1.3404	0.6440
45	0.0503	0.0353	0.0010	0.0075	0.0235	0.0535	0.2868	0.2285	0.0243	0.0053	1.1110	0.3600
65	0.0558	0.0298	0.0003	0.0078	0.0215	0.1678	0.1833	0.1580	0.0238	0.0093	0.7113	0.6018
81	0.1642	0.0352	0.0026	0.0124	0.0274	0.0548	0.2506	0.2252	0.0274	0.0170	0.9808	0.5632
330	0.0508	0.0220	0.0005	0.0095	0.0158	0.0205	0.2235	0.1415	0.0333	0.0065	0.8138	0.3260
Viglio <i>ex</i> 358	0.0304	0.0258	0.0032	0.0082	0.0280	0.1438	0.2180	0.1746	0.0280	0.0102	0.7032	0.5542
13A	0.0300	0.0526	0.0036	0.0208	0.0240	0.0716	0.2118	0.4438	0.0590	0.0322	0.7634	1.3560
7IMP	0.0408	0.0318	0.0078	0.0088	0.0298	0.0326	0.2792	0.2360	0.0380	0.0104	0.9568	0.4376
Control	0.0178	0.0158	0.0040	0.0066	0.0162	0.0226	0.1998	0.1342	0.0344	0.0066	0.3206	0.1930
Overall mean	0.0634	0.0326	0.0031	0.0104	0.0289	0.0767	0.2921	0.2436	0.0371	0.0123	0.9584	0.5343
Overall SD	0.0423	0.0153	0.0021	0.0042	0.0111	0.0500	0.0973	0.0998	0.0121	0.0074	0.3015	0.3031
Overall VC %	66.784	46.874	66.842	40.616	38.239	65.217	33.307	40.964	32.647	59.800	31.463	56.732
P value	<0.01		<0.001		<0.01		ns		<0.001		<0.001	

ns not significant ($P > 0.05$)

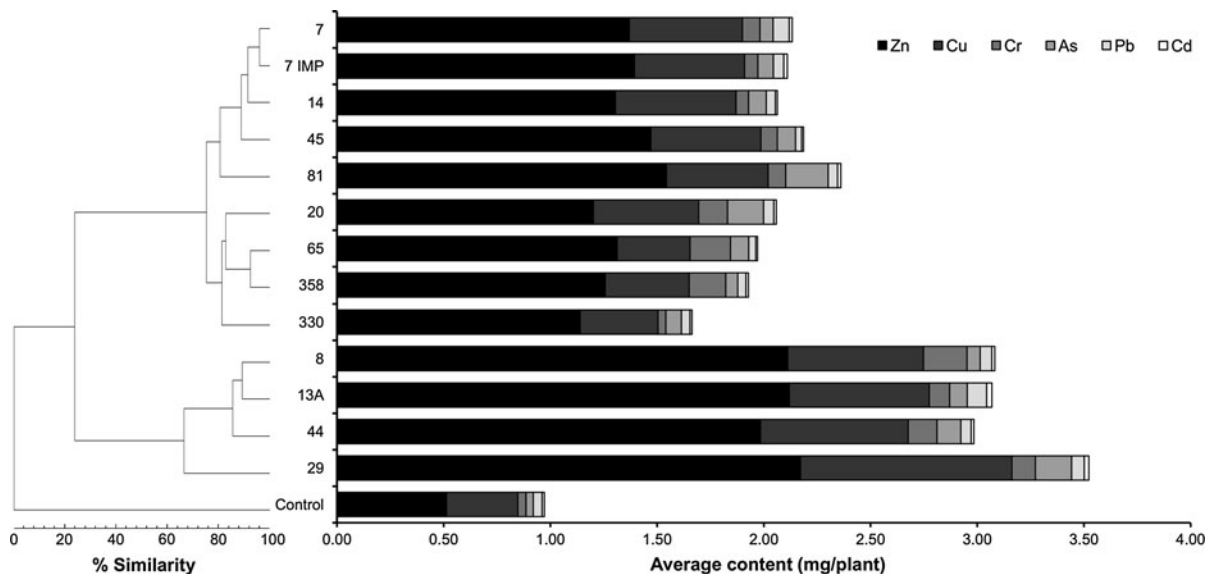


Fig. 2 Average contents (mg/plant) of Zn, Cu, Cr, As, Pb and Cd in leaves + stems + branches (LSB_{cc}) of the 13 hybrid *Eucalyptus* clones and the control, together with the cluster analysis dendrogram grouping the clones/control with similar LSB_{cc} performances

($r_s = 0.80$, $P = 0.001$). These correlations suggest that clonal selections based on these contaminant uptakes may simultaneously improve the uptakes of the other positively correlated contaminants.

The cluster analysis dendrogram revealed that the control clustered alone and showed the worst phytoextraction performance (Fig. 2). All clones clustered together at 23 % similarity. A cluster including four clones (8, 13A, 44 and 29) with almost 65 % similarity showed the best phytoextraction performances. A cluster including the remaining clones (Velino *ex* 7, 7IMP, 14, 45, 81, 20, 65, Viglio *ex* 358 and 330) with almost 75 % similarity showed intermediate phytoextraction performances. Assuming that such performances measured in young plants are preserved over years, the phytoremediation potential of the tested clones is much more promising than that of *E. camaldulensis*, which incidentally represents the most diffused *Eucalyptus* species in Italy. Among the many potential sources of variation in contaminant uptake by plants, genetic variability plays an important role in determining varying levels of accumulation (Meharg 2003; Tlustoš et al. 2007; King et al. 2008). However, given our experimental design, we can exclude the possibility that the variability in contaminant uptake we found was due to non-genetic factors.

Comparisons between L_{cc} and SB_{cc} (Table 4) were in line with those between L_c and SB_c (Table 3), with only one exception (Cu), suggesting that the produced biomass did not influence significantly the proportions of contaminants that can be removed by leaves and by stems + branches. This is probably because the leaf biomass in young trees is almost 50 % of the total aboveground biomass. Although this percentage can change in older trees, leaves remain an important part of the biomass obtainable from *Eucalyptus* trees. LSB_{cc} therefore represents the most appropriate indicator for the comparative evaluation of phytoextraction performances of our clones/control, as it takes into account both the contaminant concentration and the total aboveground biomass production.

The high biomass production and the large water and nutrient demand of fast-growing trees are characteristics which can be exploited for phytoremediation success, providing diversification of land-use into non-food commodity crops. This topic has recently been explored by several authors worldwide using mostly woody crops other than *Eucalyptus* spp. Given the scarcity of information on the use of *Eucalyptus* for phytoremediation, this study may contribute to current knowledge on soil-reclaiming *Eucalyptus* SRWC in Mediterranean-type environments. Although *Eucalyptus* spp. are exotic trees for the Mediterranean

basin, they have extensively been planted Mediterranean-wide and have never become invasive over the about 200 years since their former introduction in Italy (Agostini 1953). Moreover, the impact of *Eucalyptus* spp. on native soil-growing bryophyte and lichen diversity claimed by Zedda et al. (2010) is relatively negligible in the context of SRWC on contaminated arable lands. Further studies should be focused on testing the selected clones in field conditions, not only in the area where the soil was taken, but also in other contaminated sites with different climatic and soil characteristics in order to further explore the potential of such clones. It is also necessary to identify the inter-specific hybridisations (i.e. parent species) that maximise both biomass production and contaminant uptake. This can be carried out by planning ad hoc tree-breeding programmes.

Conclusions

This study pointed out the potential for phytoremediation of As, Cd, Cu, Pb, Cr and Zn by a selection of 13 hybrid *Eucalyptus* clones. The higher biomass production of 12 of these clones seemed to have not been affected by the contaminated soil, suggesting that tree-cloning may be the correct way to follow for improving both biomass production and phytoremediation capabilities in this tree genus. In summary, it was concluded that:

- With large variation amongst clones, all plants survived and grew well, tolerating the levels of contaminants to which they were exposed.
- As, Cu, Pb and Zn were more concentrated in leaves than in stems + branches, suggesting that the removal of the entire aboveground biomass, including leaves, would enhance phytoremediation of these contaminants using *Eucalyptus* spp. This is much more feasible with such evergreen trees than with deciduous trees such as poplars and willows more commonly used for biomass production.
- Significant positive pair-wise correlations between average contents of Cd and Pb, Cd and Cu, Cd and Zn, Pb and Cu, Pb and Zn, and Cu and Zn in aboveground tissues were detected, suggesting that clonal selections based on the uptake of these contaminants may simultaneously improve the uptake of the other positively correlated contaminants.
- All clones showed better phytoextraction performance than that of the control. Four promising genotypes (8, 13A, 44 and 29 clones) were identified to improve the phytoremediation potential of *E. camaldulensis*, which is the species traditionally used in Italian plantations.
- Clones Velino *ex* 7 and Viglio *ex* 358 showed the best growth performances in previous field trials conducted on uncontaminated soils (Mughini et al. 1996), but they showed intermediate phytoextraction performances (higher than the control but lower than the four promising genotypes) in this study.

In conclusion, there is some evidence that hybrid *Eucalyptus* clones represent a promising resource for both biomass production and phytoremediation in Mediterranean-type environments, and that further clonal selections can significantly improve their performances, with benefits proportional to the quality of these selections.

Acknowledgments We are grateful to Prof. G. Tanelli (Florence University), Dr. L. Agati (ARPA Toscana) and Dr. G. Meloni (Nuova Solmine) for their collaboration in supplying the contaminated soil, and to Dr. M. Micozzi for the helpful proofreading of the manuscript. Thanks are also extended to Dr. R. Aromolo and Dr. M.G. Mascia for supporting chemical analyses, and to Mr. M. Giagnoli, Mr. A. Pedemonti, Mr. M. Riccardi and Mr. C. Emery for supporting data collection.

References

- Achene L, Ferretti E, Lucentini L, Pettine P, Veschetti E, Ottaviani M (2010) Arsenic content in drinking-water supplies of an important volcanic aquifer in central Italy. *J Toxicol Environ Chem* 92:509–520
- Agostini R (1953) Cenni storici sulla introduzione degli eucalitti in Italia. *Ital J For Mt Environ* 8:117–122
- Alloway BJ (1995) Heavy metals in soils. Blackie Academic and Professional, Glasgow
- Assareh MH, Shariat A, Ghamari-Zare A (2008) Seedling response of three *Eucalyptus* species to copper and zinc toxic concentrations. *Casp J Environ Sci* 6:97–103
- Bella S, Lo Verde G (2002) Presenza nell'Italia continentale e in Sicilia di *Ophelimus* prope *eucalypti* (Gahan) e *Aprostocetus* sr, galligeni degli eucalitti (Hymenoptera Eulophidae). *Naturalista Sicil* 26:191–197
- Benvenuti MG, Benvenuti M, Castagliola P, Tanelli G (2009) Quaternary evolution of the Pecora River (southern Tuscany, Italy): paleohydrography and sediments provenance. *Ital J Geosci* 128:61–72
- Castagliola P, Benvenuti M, Chiarantini L, Bianchi S, Di Benedetto F, Paolieri M, Rossato L (2008) Impact of

- ancient metal smelting on arsenic pollution in the Pecora River Valley, Southern Tuscany, Italy. *Appl Geochem* 23: 1241–1259
- Castiglione S, Todeschini V, Franchin C, Torrigiani P, Gastaldi D, Cicatelli A, Rinaudo C, Berta G, Biondi S, Lingua G (2009) Clonal differences in survival capacity, copper and zinc accumulation, and correlation with leaf polyamine levels in poplar: a large-scale field trial on heavily polluted soil. *Environ Pollut* 157:2108–2117
- Cheng S (2003) Heavy metals in plants and phytoremediation. *Environ Sci Pollut Res* 10:335–340
- Dickinson NM, Turner AP, Watmough SA, Lepp NW (1992) Acclimation of trees to pollution stress: cellular metal tolerance traits. *Ann Bot* 70:569–572
- Ding A, Cheng L, Liu P, Carpenter PJ, Teng YP (2007) Plant response to metal contamination at an oil shale tailing site in maoming, South China. *Ground Water Monit Remediat* 27:111–117
- French CJ, Dickinson NM, Putwain PD (2006) Woody biomass phytoremediation of contaminated brownfield land. *Environ Pollut* 141:387–395
- Gemignani G (1968) Preliminary observation on the Australian origins of *Eucalyptus camaldulensis*. Committee on the Coordination of Mediterranean Forestry Research, Third Session. Report No FO-SCM/FR/68/8A. Food and Agriculture Organization (FAO), Rome
- GLOBSI - Gruppo di Lavoro Obiettivo Bonifica Siti Inquinati (2004) Bonifica dei siti inquinati nella programmazione dei fondi strutturali 2000–2006: analisi delle problematiche, valutazioni e suggerimenti. Ministero dell'Ambiente e della Tutela del Territorio e del Mare e Ministero dello Sviluppo Economico, Rome
- Grant CD, Campbell CJ, Charnock NR (2002) Selection of species suitable for derelict mine site rehabilitation in New South Wales, Australia. *Water Air Soil Poll* 139:215–235
- Hartley A (1977) The establishment of *Eucalyptus tereticornis* on tailings from the Bougainville copper mine, Papua-New-Guinea. *Commonw Forest Rev* 56:239–245
- ISPRA-SINANET—Istituto Superiore per la Protezione e la Ricerca Ambientale, Sistema Informativo Nazionale Ambientale (2009) Siti contaminati WGS-84. <http://www.mais.sinanet.ispraambiente.it/ost/>. Accessed 10 Sept 2010
- King DJ, Doronila AI, Feenstra C, Baker AJ, Woodrow IE (2008) Phytostabilisation of arsenical gold mine tailings using four *Eucalyptus* species: growth, arsenic uptake and availability after 5 years. *Sci Total Environ* 406:35–42
- Lacaze JF (1970) Study on the ecological adaptation of *Eucalyptus*. Committee on the Coordination of Mediterranean Forestry Research, Fourth Session. Report No FO-SCMI/FR 70/2/10. Food and Agriculture Organization (FAO), Rome
- Madrugá MJ, Brogueira A, Alberto G, Cardoso F (2001) 226Ra bioavailability to plants at the Urgeiriça uranium mill tailings site. *J Environ Radioact* 54:175–188
- Meharg AA (2003) Variation in arsenic accumulation–hyper-accumulation in ferns and their allies. *New Phytol* 157: 25–31
- Melo RF, Dias LE, Mello JWV, de Oliveira JA (2010) Behavior of *Eucalyptus grandis* and *E. cloeziana* seedlings grown in arsenic-contaminated soil. *Rev Bras Ciênc Solo* 34: 985–992
- Mughini G (1991) Comportamento di alcune specie di eucalitto in tre prove in Italia meridionale. *Cellul Carta* 42:2–7
- Mughini G, Facciotto G, Sperandi G, Confalonieri P, Gras MA, Giorelli A, Allegro G (1996) Ricerche sulla selvicoltura a breve rotazione a scopo energetico. Ente Nazionale per l'Energia Elettrica—Centro di Ricerca Ambiente e Materiali, Milan
- Mughini G, Gras MA, Facciotto G (2007) *Eucalyptus* clones selection in central-south Italy for biomass production. In: Proceedings of the fifteenth European biomass conference and exhibition, Berlin, 7–11 May 2007, p 3
- Pigna M, Cozzolino V, Violante A, Meharg AA (2009) Influence of phosphate on the arsenic uptake by wheat (*Triticum durum* L.) irrigated with arsenic solutions at three different concentrations. *Water Air Soil Pollut* 197:371–380
- Pigna M, Cozzolino V, Giandonato Caporale A, Mora ML, Di Meo V, Jara AA, Violante A (2010) Effects of phosphorus fertilization on arsenic uptake by wheat grown in polluted soils. *J Soil Sci Plant Nutr* 10:428–442
- Preziosi E, Giuliano G, Vivona R (2010) Natural background levels and threshold values derivation for naturally As, V and F rich groundwater bodies: a methodological case study in Central Italy. *Environ Earth Sci* 61:885–897
- Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees: a review. *Environ Int* 29: 529–540
- Pulford ID, Riddell-Black D, Stewart C (2002) Heavy metal uptake by willow clones from sewage sludge-treated soil: the potential for phytoremediation. *Int J Phytorem* 4:59–72
- Purdy JJ, Smart LB (2008) Hydroponic screening of shrub willow (*Salix* spp.) for arsenic tolerance and uptake. *Int J Phytorem* 10:515–528
- Rockwood DL, Alker GR (2000) Phytoremediation of heavy metal contaminated groundwater using cottonwood trees. University of Florida, Gainesville
- Rockwood DL, Cardellino R, Alker G, Lin C, Brown N, Spriggs T, Tsangaris S, Isebrands J, Hall R, Lange R, Nwokie B (2003) Fast-growing trees for heavy metal and chlorinated solvent phytoremediation. In: Magar VS, Kelley ME (eds) 7th international in situ and on site bioremediation symposium. Battelle Press, Columbus, 2–5 Jun 2003, p F-12
- Rockwood DL, Naidu CV, Carter DR, Rahmani M, Spriggs TA, Lin C, Alker GR, Isebrands JG, Segrestet SA (2004) Short-rotation woody crops and phytoremediation: opportunities for agroforestry? *Agroforest Syst* 61:51–63
- Saliba R, Gauthier H, Gauthier R, Petit-Ramel M (2002) The use of *Eucalyptus* barks for the adsorption of heavy metal ions and dyes. *Adsorpt Sci Technol* 20:119–129
- Tanelli G, Benvenuti M, Costagliola P, Mascaro I, Lascialfari S, Rossato L (2003) Studio della dispersione dell'arsenico nella Piana di Scarlino (GR). Florence University, Florence
- Trustoš P, Ji Száková, Vysloužilová M, Pavlíková D, Weger J, Javorská H (2007) Variation in the uptake of arsenic, cadmium, lead, and zinc by different species of willows *Salix* spp. grown in contaminated soils. *Cent Eur J Chem Biol* 2:254–275
- Tossell RW, Binard K, Rafferty MT (2000) Uptake of arsenic by tamarisk and eucalyptus under saline conditions. In: Wickramanayake GB, Gavaskar AR, Meman BC, Magar VC (eds) Bioremediation and Phytoremediation of Chlorinated and Recalcitrant Compounds. Battelle Press, Columbus, pp 485–492

- Violante P (2000) Metodi ufficiali di analisi chimica del suolo. Ministero delle Politiche Agricole e Forestali, Rome. Gazzetta Ufficiale 248/21-10-99:5–222
- Welch AH, Lico MS, Hughes JL (1988) Arsenic in ground water of the Western United States. *Ground Water* 26:333–347
- Zalesny RS, Bauer EO (2007) Evaluation of *Populus* and *Salix* continuously irrigated with landfill leachate I. Genotype-specific elemental phytoremediation. *Int J Phytorem* 9:281–306
- Zedda L, Cogoni A, Flore F, Brundu G (2010) Impacts of alien plants and man-made disturbance on soil-growing bryophyte and lichen diversity in coastal areas of Sardinia (Italy). *Plant Biosystems* 144:547–562