Comparison of arsenic resistance in Mediterranean woody shrubs used in restoration activities

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Abstract

Myrtus communis, Arbutus unedo and Retama sphaerocarpa are Mediterranean shrubs widely used in revegetation of semiarid degraded soils. The aim of this work is to study the resistance of these plants to arsenic under controlled conditions, in order to evaluate their potential use in revegetation and/or phytoremediation of As-polluted soils. R. sphaerocarpa showed higher resistance to As than M. communis or A. unedo according to its higher EC_{50}, P status and P/As molar ratio in both, roots and shoots, and the lower increases in lipid peroxidation and decrease of chlorophyll levels in response to arsenic, while the highest arsenate sensitivity was obtained for A. unedo. Arsenic was mainly retained in roots, and, although M. communis accumulated higher arsenic amounts than the other two species, R. sphaerocarpa showed the highest root to shoot transfer. Most of the studied parameters (chlorophylls, MDA and total thiols) showed significant correlation with arsenic concentration in roots and leaves of plants, so they can be useful indexes in the diagnosis of arsenic toxicity in these species. According to our results, both M. communis and R. sphaerocarpa could be used in the revegetation of moderately arsenic contaminated sites.

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1. Introduction

Arsenic is an environmental pollutant widely distributed, with toxic effects in plants. Levels of As in soil have been elevated by mining, industrial and agricultural activities (Adriano, 2001), arsenate being the most abundant soluble species in upland soil (Carbonell-Barrachina et al., 1998). Moreover, As levels in soils are directly reflected in arsenic in crops and are one of the major sources of arsenic in drinking water (Zhang et al., 2002). Plants can stabilize and extract As from soils, alleviating the environmental risk (Fitz and Wenzel, 2002). Arsenate is taken up by plants via phosphate transporters at the plasma membrane of root cells and is rapidly reduced to arsenite once inside the cytoplasm. Due to the analogy arsenate/phosphate, arsenate toxicity is clearly linked to phosphorus nutrition and high levels of phosphate can alleviate arsenate toxicity (Esteban et al., 2003). Arsenic can induce reactive oxygen species (ROS) in plants, which cause increases in lipid peroxidation and oxidative stress (Hartley-Whitaker et al., 2001; Mascher et al., 2002; Srivastava et al., 2005). Arsenate also affects negatively chlorophyll levels in leaves (Mascher et al., 2002), though sometimes an increase has been reported (Päivöke and Simola, 2001). The pool of thiols in plant tissues plays an important role on arsenic detoxification (Pickering et al., 2000; Vázquez et al., 2005).

Effects of arsenic on plant behaviour have been traditionally focused on grasses, agricultural species or ferns, i.e. Holcus lanatus, Lupinus albus, Triticum aestivum or Pteris vittata (Hartley-Whitaker et al., 2001; Vázquez et al., 2005; Srivastava et al., 2005; Geng et al., 2006). Despite the potential of native woody plants to be used in phytoremediation of polluted lands (Lepp and Dickinson, 1998), little information is available about trace element
effects on Mediterranean woody species, which are commonly used for ecological restoration (Fuentes et al., 2007). Myrtus communis L. (myrtle), Arbutus unedo L. (dwarf strawberry tree) and Retama sphaerocarpa L. (retama) are evergreen Mediterranean shrubs which have been used for revegetation of semiarid degraded lands (García et al., 2005; Rodríguez-Echeverría and Pérez-Fernández, 2005). The three plant species are able to establish mycorrhizal associations with arbuscular mycorrhizal (AM) species (Maremmani et al., 2003; Alguacil et al., 2004). R. sphaerocarpa is also able to fix N2 in symbiotic association with rhizobium (Valladares et al., 2002). Plant-microbial associations can increase plant survival at contaminated sites. Thus, these plant species could be useful as a tool for restoration, pursuing a long-term plantation without artificial aid and to allow primary succession (Singh et al., 2002). Moreover, as sources as mining, industry or agriculture have given rise to pollution in Mediterranean lands, as in Aznalcóllar (Spain), where arsenic remains as a pollutant after removal of pyritic sludge (Taggart et al., 2004). Recently, there has been increasing interest in the use of vascular plants for environmental monitoring and assessment (Wang and Freeman, 1995), thus, some physiological parameters could be analysed as biomarkers of trace element pollution (Prasad, 2003).

The aim of this work is to study the behaviour and resistance of M. communis, A. unedo and R. sphaerocarpa plants when arsenic is applied under controlled conditions, in order to evaluate their potential use in the restoration and/or phytoremediation of semiarid degraded lands contaminated with As. Moreover, their resistance to arsenic toxicity has been tested using several stress indicators.

2. Materials and methods

2.1. Plant culture

One-year-old plants of M. communis L., A. unedo L. and R. sphaerocarpa L. (San Jerónimo Greenhouse, Andalusian Government) from seedling trays (15–20 cm long; 13–16 g FW) were selected for this experiment; this is the size used when transferring these species to soils. After washing thoroughly the roots with water, each plant was transferred to one pot (20 l) containing 2 l of nutrient solution and 17 l of perlite as substrate. Nutrient solution (pH 6.5) was renewed every 9 days and its composition was: 1.5 mM KNO3, 1.5 mM Ca(NO3)2, 1 mM KH2PO4, 15 kg) of perlite as substrate. Nutrient solution (pH 6.5) was 466–473

2.2. Harvesting procedure

Four plants of each species were harvest before As supply, in order to determine root and shoot weight at the beginning of the treatment. The As-treated plants of M. communis, A. unedo and R. sphaerocarpa were harvested and divided into root, stem and leaves (green stems of R. sphaerocarpa), which were thoroughly washed with tap water and then rinsed with distilled water. All plant material was weighted (total fresh weight). Then, a sample was dried at 60 °C during 72 h and milled for mineral analysis; the rest of the material was frozen in liquid N2 and stored at –20 °C.

2.3. Analytical determinations

For acid mineralization of plant organs, 10 ml of mili-Q water, 3 ml of HNO3 and 2 ml of H2O2 were added to 500 mg dry weight (DW) of plant tissue and digested at 1.5 kPas and 125 °C in autoclave (Lozano-Rodríguez et al., 1995). The solution was filtered and diluted to 25 ml for measurement of As and nutrients by ICP-MS, P was measured spectrophotometrically according to MAPA (1994).

Chlorophylls were extracted by homogenizing 0.5 g fresh weight (FW) of leaf tissue in 80% acetone. After filtering and diluting to 50 ml with acetone, absorbance was determined at 4645 and 663 nm. Chlorophyll content was estimated according to Wellburn (1994).

Acid soluble thiols were extracted from roots and leaves and determined by the method of Jocelyn (1987): 0.1 g FW was extracted with 0.4 ml of NaOH (0.1 M) + NaBH4 (25 mg ml–1) and 0.2 ml of distilled water and centrifuged at 11000 g for 5 min. Supernatant (0.5 ml) was diluted with 0.2 ml of HCl (35%) and centrifuged at 11000 g for 5 min. Then 0.5 ml of 300 μM DTNB in 0.5 M phosphate buffer (pH 7.5) was added to 0.5 ml of supernatant and heated at 30 °C for 2 min. Absorbance was determined at 412 nm. For quantification, GSH standards were used.

Lipid peroxidation as malondialdehyde (MDA) concentration in root and leaf tissues was determined as described by Heath and Packer (1968). Fresh tissue (0.1 g) were extracted with 1 ml of colorimetric reactive TCA (15%–TBA (0.37%–HCl (0.25 M), heated at 90 °C for 30 min and then cooled. After centrifugation at 11000 g for 10 min, the absorbance of supernatant was measured at 532 nm and 600 nm. An extinction coefficient of 1.56 × 105 M–1 cm–1 was used.

2.4. Data analysis

Effective accumulation (%) of As = 100 × mgAsNS/mgAsNS, where mg AsNS represents the total amount of As added to plants...
significant differences in shoot As concentrations among other plant species for the 50 μM As dose. Roots of Retama sphaerocarpa showed the highest effective accumulation at the two lower As doses, but stored a higher amount of As in the shoot than A. unedo, for all treatments, and a higher amount than M. communis for the As dose of 250 μM.

3. Results

3.1. Arsenic concentration and accumulation in plants

Arsenic concentration in M. communis, A. unedo and Retama sphaerocarpa plants increased with increasing As doses in the nutrient solution (Table 1). Significant differences in arsenic concentration among plant species were observed but also among treatments and species * treatment interaction (P < 0.001). Roots of A. unedo accumulated significantly higher concentrations of As than the roots of the other plant species for the 50 μM As dose. There were no significant differences in shoot As concentrations among plant species until the 250 μM As dose. Significant differences in the shoot:root ratio for arsenic concentration were observed among plant species, among treatments and for the species * treatment interaction (P < 0.001). R. sphaerocarpa reached the highest values of the shoot:to-root ratio and A. unedo the lowest ones. In agreement, the shoot As content (mg As plant⁻¹) in shoot was also higher for R. sphaerocarpa, followed by M. communis and A. unedo (Fig. 1). The percentage of As in the shoots ranged between 3–6% for A. unedo, 7–11% for M. communis and 11–25% for R. sphaerocarpa.

Significant differences in arsenic effective accumulation were observed among plant species, among treatments and for the species * treatment interaction (P < 0.001). M. communis plants showed the highest effective accumulation of As from nutrient solution (Table 1), although differences are only significant (P < 0.05) for the 5 μM dose, and also stored more As than the other two species (Fig. 1). On the contrary, R. sphaerocarpa plants showed lower values of effective accumulation at the two lower As doses, but stored a higher amount of As in the shoot than A. unedo, for all treatments, and a higher amount than M. communis for the As dose of 250 μM.

3.2. The P concentration and the P/As molar ratio

P concentration progressively decreased as the As concentration in the nutrient solution increased (Table 2), both for roots and shoots. Significant differences in P status and P/As molar ratio were observed among plant species, treatments and species * treatment interaction (P < 0.001). R. sphaerocarpa showed the highest P concentration of the three species tested, as well as the lowest reduction in P concentration for the highest As doses. The sharpest decrease in P concentration was obtained for A. unedo shoots. P/As molar ratio was significantly decreased for all treatments and plant species, with the highest reductions in shoots. P/As molar ratio in shoots was higher than 95 for R. sphaerocarpa and M. communis except for the highest As dose, while A. unedo showed values lower than 70 from the 50 μM As dose. Roots of R. sphaerocarpa showed the highest values for the P/As molar ratio.

3.3. Plant growth

Relative growth increment (RGI) decreased in the three plant species for 50 and 250 μM As doses (Table 3), while

### Table 1

<table>
<thead>
<tr>
<th>As (μM)</th>
<th>μg As g⁻¹ DW</th>
<th>Shoot:Root Ratio</th>
<th>Eff. Acc. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td></td>
</tr>
<tr>
<td>Myrtus communis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.4 ± 0.1 a</td>
<td>0.26 ± 0.04 a</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>24.8 ± 2.2 a</td>
<td>1.27 ± 0.02 a</td>
<td>0.051 ± 0.004 b</td>
</tr>
<tr>
<td>50</td>
<td>138 ± 6 b</td>
<td>6.23 ± 0.53 b</td>
<td>0.058 ± 0.004 bc</td>
</tr>
<tr>
<td>250</td>
<td>235 ± 7 d</td>
<td>16.9 ± 1.3 c</td>
<td>0.072 ± 0.003 c</td>
</tr>
<tr>
<td>Arbutus unedo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.27 ± 0.10 a</td>
<td>0.03 ± 0.02 a</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>36.3 ± 4.6 a</td>
<td>0.40 ± 0.03 a</td>
<td>0.011 ± 0.002 a</td>
</tr>
<tr>
<td>50</td>
<td>185 ± 21 b</td>
<td>5.15 ± 0.25 b</td>
<td>0.028 ± 0.002 a</td>
</tr>
<tr>
<td>250</td>
<td>208 ± 9 d</td>
<td>5.83 ± 0.28 b</td>
<td>0.028 ± 0.002 a</td>
</tr>
<tr>
<td>Retama sphaerocarpa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.9 ± 0.2 b</td>
<td>0.24 ± 0.03 a</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>11.1 ± 1.3 a</td>
<td>1.57 ± 0.25 a</td>
<td>0.140 ± 0.009 d</td>
</tr>
<tr>
<td>50</td>
<td>90.7 ± 15.9 b</td>
<td>5.89 ± 0.47 b</td>
<td>0.065 ± 0.008 bc</td>
</tr>
<tr>
<td>250</td>
<td>208 ± 16 d</td>
<td>23.9 ± 1.7 d</td>
<td>0.123 ± 0.010 d</td>
</tr>
</tbody>
</table>

Significant differences among treatments * species are indicated by different letters (P < 0.05).
An increase was observed for *M. communis* affected by 5 μM As. The decrease was associated to a decrease in the water content of the three species for the highest As dose (data not shown). *A. unedo* plants showed the highest growth reductions caused by As. EC50 values for *A. unedo* were much lower in both, shoots and roots, than for the other two species, while the highest values were obtained for *R. sphaerocarpa*. For the three species, EC50 in root was lower than in shoot.

### 3.4. Stress indexes

Chlorophyll levels significantly decreased in leaves of *M. communis, A. unedo* and *R. sphaerocarpa* as the As supply increased ($P < 0.001$); the results for chlorophyll *a* are shown in Fig. 2. Although the three plant species were affected, the sharpest decreases corresponded to *A. unedo* for the 50 μM dose and to *M. communis* for the 250 μM dose.

Supplying arsenic induced significant increments of MDA concentration in roots and leaves of *M. communis, A. unedo* ($P < 0.001$) and *R. sphaerocarpa* ($P < 0.05$), mainly for the highest doses (Table 4). Significant increases in MDA levels were observed for *A. unedo* even for the 5 μM As dose, while for *M. communis* and *R. sphaerocarpa* the increments took place at the 50 and 250 μM As doses.
respectively. Percentage of MDA increment in comparison to the control plants was higher for *A. unedo* than for the others, both in roots and leaves.

A significant increase in total thiols concentration was observed in roots and leaves of the three plant species in response to the increasing As supply in nutrient solution (Fig. 3; \( P < 0.001 \)). Thiols concentration was higher in leaves than in roots, but the increase in total thiols concentration after As supply was higher in roots than in leaves.

There was a clear increase in \( \sim \text{SH} \) compounds in roots and leaves of *M. communis*, as well as in *A. unedo* roots. However, a slight response to As was observed in leaves of *A. unedo* and in *R. sphaerocarpa* leaves and roots.

To test the accuracy of the biomarkers measured in our study for *M. communis*, *A. unedo* and *R. sphaerocarpa* (chlorophyll \( a \), MDA and total thiols), linear correlations between these parameters and As concentration in leaves or roots were calculated (Table 5). Correlations showed significant \( R^2 \) values \((P < 0.01)\), being higher than 0.5 for most cases (except for MDA vs. [As] for leaves of *M. communis*). No significant correlation was obtained for MDA in *R. sphaerocarpa* roots.

### 4. Discussion

Plant growth in *M. communis, A. unedo* and *R. sphaerocarpa* was reduced when arsenic concentration increased in plant organs. Despite that, positive effects in *M. communis* were observed for the 5 \( \mu \text{M} \) As dose, in agreement with results reported for other plant species affected by low As doses (Carbonell-Barrachina et al., 1998; Mascher et al., 2002).

*A. unedo* showed stronger excluder behaviour, while *M. communis* accumulated arsenic more efficiently than the other species. Arsenic was mainly accumulated in roots of all the plants tested. In this sense, at least 94% of the total arsenic in plant was stored in roots of *A. unedo* and around 90% in roots of *M. communis*, but the percentage of the total As found in shoots reached 25% for *R. sphaerocarpa*. In agreement to our results, plants of pea, lupin, *H. lanatus*, turnip and red clover concentrated As mainly in

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**Table 4**

<table>
<thead>
<tr>
<th>As (( \mu \text{M} ))</th>
<th>MDA Concentration (nmol MDA g(^{-1}) FW, mean ± SE)</th>
<th>Increment of MDA in relation to control plants (in parenthesis,%) in roots and leaves of <em>M. communis</em>, <em>A. unedo</em> and <em>R. sphaerocarpa</em> after culture with different As doses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myrtus communis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16.0 ± 1.1 a</td>
<td>21.4 ± 1.3 a</td>
</tr>
<tr>
<td>5</td>
<td>16.8 ± 1.7 a (4%)</td>
<td>21.7 ± 1.4 a (0%)</td>
</tr>
<tr>
<td>50</td>
<td>21.7 ± 1.4 b (35%)</td>
<td>26.4 ± 0.4 b (23%)</td>
</tr>
<tr>
<td>250</td>
<td>25.1 ± 1.2 c (56%)</td>
<td>25.0 ± 0.7 b (17%)</td>
</tr>
<tr>
<td><strong>Arbutus unedo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.9 ± 0.3 a</td>
<td>10.0 ± 0.5 a</td>
</tr>
<tr>
<td>5</td>
<td>8.2 ± 1.2 b (67%)</td>
<td>11.7 ± 1.4 a (17%)</td>
</tr>
<tr>
<td>50</td>
<td>9.2 ± 0.8 bc (88%)</td>
<td>14.2 ± 1.8 ab (42%)</td>
</tr>
<tr>
<td>250</td>
<td>12.5 ± 1.4 c (155%)</td>
<td>19.8 ± 0.6 b (99%)</td>
</tr>
<tr>
<td><strong>Retama sphaerocarpa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>23.9 ± 0.5 a</td>
<td>24.7 ± 0.5 a</td>
</tr>
<tr>
<td>5</td>
<td>24.6 ± 0.4 a (2%)</td>
<td>24.6 ± 2.4 a (0%)</td>
</tr>
<tr>
<td>50</td>
<td>24.4 ± 1.1 a (1%)</td>
<td>24.5 ± 2.1 a (0%)</td>
</tr>
<tr>
<td>250</td>
<td>30.4 ± 3.0 b (27%)</td>
<td>27.0 ± 1.1 b (9%)</td>
</tr>
</tbody>
</table>

Different letters indicate significant differences \((P < 0.05)\) between treatments.
roots, storing in roots more than 95% of the total As in the plant (Carbonell-Barrachina et al., 1999; Hartley-Whitaker et al., 2001; Päivöke and Simola, 2001; Mascher et al., 2002; Vázquez et al., 2005). Only hyperaccumulators, as *P. vittata*, showed higher As concentration in shoot than in roots and more than 90% of As stored in shoots (Zhang et al., 2002). Thus, As allocation to aboveground plant organs is usually low, as shoot-to-root ratios are, i.e. <0.1 in *Brassica juncea* and <0.03 in lupin, 0.03–0.09 in wheat, 0.03–0.07 in *Arabidopsis thaliana* and <0.02 in tomato and rice (Pickering et al., 2000; Vázquez et al., 2005; Geng et al., 2006). These values are in the range observed in our study, well below the ratios observed for hyperaccumulator *P. vittata* (Srivastava et al., 2005).

Arsenate uptake is related to P nutrition, as this toxic oxianion is a phosphate analogue. Arsenate influx via phosphate transporters in plant roots is well established, so that As competes with P for in plant uptake, causing a depletion of P status (Meherg and Macnair, 1992; Esteban et al., 2003). Hence, P concentration in plants was decreased by As supply in pea and lupin (Päivöke and Simola, 2001; Vázquez et al., 2005). In the same way, phosphorus concentration was decreased by 50 and 250 μM As doses in plants of *M. communis, A. unedo* and *R. sphaerocarpa*. Arsenic sensitivity is intimately linked to phosphate nutrition, and As toxicity in plants can be partially explained by the substitution of P by As in biochemical processes. Therefore the P/As ratio in organs was evaluated, as it could be an indicator of the effects of As on plants (Tu and Ma, 2005). The P/As molar ratio showed the same tendency as plant growth and MDA concentration, and indicate that *R. sphaerocarpa* was the most resistant species. On the other hand, a P/As molar ratio <1 in fronds has been reported as a threshold value for normal growth of the hyperaccumulator *P. vittata* (Tu and Ma, 2005). However, in *M. communis, A. unedo* and *R. sphaerocarpa* critical values seem to be higher: P/As values <90 in shoots or <15 in roots were associated to toxicity (growth inhibition, oxidative stress, chlorophyll decrease) in our experiment. In agreement with our results, P/As values much higher than 1 caused plant growth inhibition in non-hyperaccumulator plants, as *Brassica napus*, pea or lupin (Cox and Kvar, 2001; Päivöke and Simola, 2001; Vázquez et al., 2005).

Supplying arsenic in the nutrient solution induced an increment of total thiols concentrations in root and leaves of *M. communis, A. unedo* and *R. sphaerocarpa*. Levels of total thiols were also increased in tissues of *H. lanatus, P. vittata* and *L. albus* in response to increments of As in nutrient solution, indicating the role of −SH groups in metalloid detoxification. In this sense, As complexation by phytochelatins has been demonstrated in many plant species (Schmöger et al., 2000; Vázquez et al., 2005), but GSH also protect against oxidative stress upon As toxicity, complexing it as arsenite-tris-thiolate (Pickering et al., 2000). Despite that, the arsenic accumulated in *M. communis, A. unedo* and *R. sphaerocarpa* plants could not be completely detoxificated by the pool of thiols, as a decrease in chlorophyll levels and an increase in oxidative stress were observed. Arsenic could induce ROS (reactive oxygen species) in plant tissues as a short-term effect, but plants can develop defence mechanisms against oxidative stress such as increased enzymatic activity, which can alleviate As-toxicity. In agreement with the decrease of chlorophyll concentration in the Mediterranean shrubs used in our experiment, a decrease in chlorophylls levels has been previously reported for arsenate (Mascher et al., 2002), due to the inhibition of pigment biosynthesis (Jain and Gadre, 1997). Chlorophyll *a* suffered a small decrease of a 23% in *R. sphaerocarpa* for the highest As level, while in *A. unedo* decreased up to 32% from the 50 μM As dose. Significant positive correlation between As concentration in roots and leaves of plants was found for MDA and total thiols, while significant negative correlation was obtained for chlorophylls, so they can be useful indexes in the diagnosis of arsenic toxicity in *M. communis, A. unedo* and *R. sphaerocarpa*.

The EC$_{50}$ in roots was lower than in shoots for the three species. In the same way, higher increases on lipid peroxidation and thiol biosynthesis in comparison to control plants was observed in roots, relative to leaves. Hence, all parameters indicate that the roots of these Mediterranean shrubs were more sensitive to the As treatments than were the shoots, as most of the As was retained in roots. Greater growth inhibition and toxicity in roots, compared to shoots, has been reported as a common effect of arsenic supply in other plant species (Carbonell-Barrachina et al., 1999; Abedin and Meharg, 2002; Vázquez et al., 2005). However, for maize, shoots were more affected than roots (Requejo, 2004).

*R. sphaerocarpa* showed higher resistance to As than *M. communis or A. unedo*, as shown by its highest EC$_{50}$ in both, roots and shoots, and the lower increases in lipid peroxidation and decrease of chlorophyll levels in response to

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**Table 5**

Pearson’s correlation coefficient ($r^2$) between As concentration and biomarkers concentration both in a FW basis ($**P < 0.001; ***P < 0.01; *P < 0.05$)

<table>
<thead>
<tr>
<th></th>
<th>Leaves</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Chl] vs. [As]</td>
<td>[MDA] vs. [As]</td>
</tr>
<tr>
<td><em>M. communis</em></td>
<td>0.830*** (-)</td>
<td>0.252** (+)</td>
</tr>
<tr>
<td><em>A. unedo</em></td>
<td>0.835*** (-)</td>
<td>0.567** (+)</td>
</tr>
<tr>
<td><em>R. sphaerocarpa</em></td>
<td>0.678*** (-)</td>
<td>0.519** (+)</td>
</tr>
</tbody>
</table>

In parenthesis, direction of significant correlations.

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than annual cultivars. On the other hand, it maintain As phytostabilisation for longer time intervals (Wenzel, 2002) and also because it is a N₂-fixing legume which showed a high As-resistance, could improve soil quality by acting as a phytostabiliser species (Fitz and Wenzel, 2002). It is also an evergreen shrub, so it could ing it more interesting for phytoimmobilisation (Fitz and Wenzel, 2002). It is also a phytostabilisation for longer time intervals than annual cultivars. On the other hand, R. sphaerocarpa, which showed a high As-resistance, could improve soil quality by acting as a phytostabiliser species (Fitz and Wenzel, 2002) and also because it is a N₂-fixing legume and increases microbial activity in soils (García et al., 2005). Moreover, R. sphaerocarpa has a notable ecological role due to the formation of “islands of fertility” where the growth of a number of annuals and other woody species is favoured (Valladares et al., 2002).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2007.10.030.

References


