An Investigation on Cd Accumulation Potential in *Myrtus communis* and *Pinus brutia*

Asghar Mosleh Arany1*, Mehri Khosravi2, Hamid Reza Azimzadeh3, Hamid Sovdaeizadeh4, Asghar Sapahvand5

1. Associate Professor, Faculty of Natural Resources, University of Yazd, Yazd, Iran. (amosleh@yazd.ac.ir)
2. MS Student in Forestry, Faculty of Natural Resources, University of Yazd, Yazd, Iran. (khosravimehri@yahoo.com)
3. Associate Professor, Faculty of Natural Resources, University of Yazd, Yazd, Iran. (hrazimzadeh@yahoo.com)
4. Assistant professor, Faculty of Natural Resources, University of Yazd, Yazd, Iran. (hsodaie@yazd.ac.ir)
5. Ph.D in Forestry, Natural Resources Office, Lorestan Province, Iran. (sapahvand@yahoo.com)

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**Introduction**

Heavy metal contamination has disastrous effects on plant productivity and threatens human and animal health. Among metals, cadmium (Cd) is commonly released into the environment from industrial processes. Cd is known to cause cellular damage in plants by producing oxidative stress via the over-production of reactive oxygen species (ROS). To combat oxidative damage, plants have developed a defense system consisting of a variety of antioxidant enzymes which can neutralize, convert, and scavenge the ROS. Proline accumulates heavily in several plants under stress providing the plants protection against damage by the ROS. When translocated to the shoots, Cd causes strong oxidative stress and the inhibition of plant metabolism, including photosynthesis by direct and indirect mechanisms.

To reduce the environmental risks of cadmium contamination, phytoremediation has been considered as the most promising and a relative new method for cleanup of polluted environments. This study investigated Cd uptake by root through *Myrtus communis* and *Pinus brutia*. The objectives of this study were 1. to compare the Cd accumulation of *Myrtus communis* and *Pinus brutia* and 2. to provide evidence for the role of proline and soluble sugar in lead tolerance of the studied species.

**Materials and methods**

Seeds of *Myrtus communis* and *Pinus brutia* sown into the pots with a diameter of 0.10 cm and a depth of 20 cm, each filled with 2 kg of soil. After germination, the samples of both plants were kept in greenhouse for two year. The plants of the same size were collected and used for the experiment. The experiment was done in triplicates by a random design. Cd in different concentrations of 0, 200, 500, and 1000 were made and poured in pots. After 70 days, the samples of roots and leaves were collected, washed and their proline, soluble sugar, and Cd accumulation were measured. Proline content was measured according to the previously described method by Bate (1973). Fresh seedlings (0.5 g) were ground in 3% (w/v) aqueous sulphosalicylic acid. The proline content was then estimated by ninhydrin reagent. The absorbance of the fraction with toluene aspired from the liquid phase was read at 520 nm. The proline concentration was determined by a calibration curve method that is expressed in mmol/g fresh weight. Soluble sugar content was measured according to the method described by Kochert (1978). Before the Cd analysis, the dried plant and soil sample were ground using a ball mill. The 2 g leaves and 1 g root and 2 g soil powder were digested with concentrated HNO₃/HClO₄ solution for determination of the total Cd concentration. The Cd concentration was then determined using an atomic absorption novAA300.

**Results and discussion**

The results presented in Table 1 show that proline was almost doubled when the Cd concentration increased to 500 ppm in *Pinus brutia*. A number of studies showed that proline can play an important protective role against heavy metal stress. It has been demonstrated that the free proline could chelate with the Cd ion in the plants and form a nontoxic Cd-proline complex. In contrast, increasing Cd concentration did not increase the proline
content in the leaves of *Myrtus communis* as can be understood from Fig. 1. Thus, it could be suggested that the free proline might play an important protective role against the Cd stress and *Pinus brutia* had the stronger self-protection ability than *Myrtus communis*. Similar to the observations on *Myrtus communis*, the application of the Cd did not change the level of the free proline in the roots of *Solanum melongena*.

Table 1. The effects of Cd on soluble sugar and proline, and accumulation amount in the roots and leaves of *Myrtus* and *Pinus*.

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Soluble sugar (mg/gdw)</th>
<th>Proline (mg/gfw)</th>
<th>Accumulation in root (ppm)</th>
<th>Accumulation in leaves (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Myrtus communis</em></td>
<td><em>Pinus brutia</em></td>
<td><em>Myrtus communis</em></td>
<td><em>Pinus brutia</em></td>
</tr>
<tr>
<td>Control</td>
<td>110^a</td>
<td>4.9^a</td>
<td>0.4^a</td>
<td>0.4^a</td>
</tr>
<tr>
<td>200</td>
<td>122^a</td>
<td>5.7^a</td>
<td>3.3^a</td>
<td>4.9^a</td>
</tr>
<tr>
<td>500</td>
<td>132^a</td>
<td>5.1^a</td>
<td>41^a</td>
<td>15.8^a</td>
</tr>
<tr>
<td>1000</td>
<td>140^a</td>
<td>5.7^a</td>
<td>480^a</td>
<td>178^a</td>
</tr>
</tbody>
</table>

Figure 1. The effects of Cd concentration on the amounts of soluble sugar (a), proline (b), and accumulation amount in the leaves (c) and roots (d) of *Myrtus* and *Pinus*. Comparison within each plant.

Soluble sugar was also doubled in *Myrtus communis* when the Cd concentration increased to 1000 ppm. In contrast, the soluble sugar did not change in *Pinus brutia* with an increase in the Cd concentration. Accumulation of the soluble sugar in response to heavy metal exposure seems to be seen in some plants. It is known that soluble sugar play an important role in osmotic adjustment in plants. Exposure to heavy metal, especially Cd, is known to deteriorate the plant water balance. With respect to the possible role of soluble sugar, the increase of
An Investigation on Cd Accumulation ...
Asghar Mosleh Arany, et al.

this solute may increase tolerance of the studied plants. The highest Cd accumulation in roots of *Pinus brutia* was equal to 480 ppm, of this only 29 ppm (6%) was transferred from the roots to the leaves. The highest Cd accumulation in the roots of *Myrtus communis* was equal to 593.9 ppm, of this only 177.97 ppm (30%) was transferred from the roots to the leaves. The results also showed that an increase in the Cd concentration in soil increased the Cd accumulation in the roots and leaves of the plants. It is important to note that of the total amount of ions associated with the roots, a part of it is only absorbed into the cells. A significant ion fraction is physically adsorbed at the extracellular negatively charged sites (COO-) of the root cell walls. The cell wall-bound fraction cannot be translocated to the shoots and, therefore, cannot be removed by harvesting shoot biomass through phytoextraction. Thus, it is possible for a plant exhibiting significant metal accumulation into the root to express a limited capacity for phytoextraction. Binding to the cell wall is not the only plant mechanism responsible for metal immobilization into the roots and the subsequent inhibition of ion translocation to the shoot. Metals can also be complexed and sequestered in cellular structures (e.g., vacuole), becoming unavailable for translocation to the shoot. Uptake of the metals into root cells is a step of major importance for the process of phytoextraction. However, for phytoextraction to occur, the metals must also be transported from the root to the shoot. Our result showed the Cd translocation in *Myrtus communis* is more than *Pinus brutia*. It is also concluded that for a high Cd accumulation in the leaves of *Myrtus communis*, this plant can introduce as a super accumulator and can use as a phytoextraction.

**Keywords**: cadmium, *Myrtus communis*, phytoremediation, *Pinus brutia*, proline