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Copper Tolerance to Germination in Mesquite, a Potential Tree Species for Restoring Mined-lands in Oman

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Abstract

Prosopis juliflora (Sw.) DC. (Fabaceae - Mimosoideae), commonly known as Ghaf Bahri or Velvet Mesquite, an introduced exotic tree species in Oman is considered as a noxious weed. Efforts to contain this tree are labor intensive and expensive. Therefore, an efficient use of this species for socioeconomic and environmental benefits in Oman must be considered seriously. This study initiates a feasibility investigation of using *P. juliflora* for the restoration of copper mined lands in Oman by evaluating copper tolerance to seed germination in the laboratory. Continuous exposure experiments showed the ability of *P. juliflora* to germinate in copper concentrations ranging from 10-1280 ppm. The adverse effect of copper on percentage seed germination was more pronounced in the first two days. From 3-8 days, *P. juliflora* showed a remarkable ability for recovery. Seedling morphology showed the adverse effect of copper exposure from 80 –1280 ppm and seedling height decreased exponentially with the increase in copper concentrations. The relationship between copper concentrations and the average copper content of the seedlings fits a non-linear power model. Thus the germinating seeds of *P. juliflora* also seem to serve as a sink for copper in contaminated soils. These *ex situ* germination experiments suggest that *P. juliflora* is a suitable species for use in the restoration of copper mined-lands. Using known facts, identifying both ecosystem and socioeconomic factors, a SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis is provided here to assist in recommending management strategies and policy decisions.

Introduction

Prosopis juliflora (Sw.) DC. (Fabaceae - Mimosoideae), commonly known as Ghaf Bahri or Velvet Mesquite, is an exotic tree species that was introduced into Oman, southern Arabia nearly three decades ago as a fast growing ornamental in landscape planting (Al Rawahy et al., 2003). It is considered as an invasive alien in many tropical countries where it was introduced from its native southwest United States and northwest Mexico (Shiferaw et al., 2004). It is widespread in both southern and northern Oman and has also started taking hold in some parts of the oil exploration areas in central Oman. *P. juliflora* in Oman is considered as a threat to the environment, although its impacts, especially on biodiversity are not yet documented (Ghazanfar, 1996; Robinson 2003). An intensive eradication program for this species has been in operation in the Dhofar region, southern Oman since 1998, but it is regenerating and recolonizing the coastal plains including saline soils with phenomenal rapidity. Experience of the pan tropical world where *P. juliflora* has established itself shows that it is hard to eradicate (Tiwari, 1999). Efforts to contain this tree is labor intensive, expensive and may not be feasible in many developing countries. Therefore, as has been done elsewhere, an efficient use of this species for socioeconomic and environmental benefits in Oman must be considered seriously (Varshney, 1996; Tewari et al., 2001). This study evaluates copper tolerance to the seed germination of *P. juliflora* in the laboratory and provides a SWOT analysis for its sustainable use in the restoration of copper-mined-lands in Oman.

Materials and methods

Sample collection and pretreatment

Seeds were collected from mature *Prosopis juliflora* trees in the Botanical Garden of Sultan Qaboos University, Muscat. Healthy dry pods were collected whilst still on trees to minimize insect infestation. Pods were further sun-dried in the laboratory for seven days and seeds were manually extracted. Seeds, immediately washed with de-ionized water were air-dried at room temperature (24 - 25° C) and then stored in sterile, closed polyethylene containers for a period of 6 – 7 days before used in experiments. Healthy seeds were scarified before germination trials. Copper solutions of eight different concentrations (10, 20, 40, 80, 160, 320, 640 and 1280 ppm) for tolerance testing were prepared in de-ionized water using hydrated copper nitrate ($Cu\ (NO)_2.3H_2O$).

Tolerance studies

Tolerance testing on germination, defined here as the emergence of the radicle, was conducted using the continuous exposure technique. Scarified seeds were transferred to a petridish (diameter 9.0 cm) containing two, circular Whatman No.1 filter papers soaked with 10 ml of the test solution. Preliminary experiments suggested that scarification was necessary to reduce 'noise levels' in data and to ensure the effective exposure of seeds to copper in solution during the germination process. This also offered protection against Type II error that could possibly result due to physical dormancy. Each petridish contained 10 randomly chosen seeds and each treatment was replicated 10 times. All petridishes irrespective of test concentrations were placed in an experimental chamber with constant light and a temperature range of 24 ± 1°C following completely randomized design. The filter papers were changed daily and irrigated with the same amount of appropriate test solution.

Seed germination in each petridish was observed and recorded for eight days. Germination data obtained after two, three and eight day's exposure was used in analyses. The first three days showed the immediate germination response to copper exposure, while after eight days there were no further changes in the numbers of seeds germinating. Percentage germination was considered adequate as a measurable parameter and the use of special indices for calculating germination rates (Khan and Unger, 1984; El-Keblawy and Al-Rawai, 2005) was considered unnecessary. At the end of experiments, seedling heights were measured to the accuracy of the nearest mm for three random seedlings chosen from each petridish. To show the differences in growth morphology, typical seedlings from each exposure concentration were photographed.

Digestion procedure and ICP – AES study

Whole seedlings were digested using the procedure described in detail by Pillay et al. (2005). A Perkin Elmer 3300 instrument was used for analysis. It was computer controlled and equipped with radial and axial configurations, and sophisticated software for facile operation. The instrument was calibrated with aqueous reference standards (from Perkin Elmer), and precautions were taken against spectral interferences (Abbu, et al., 2000). The consistency of the analytical procedure was examined using suitable 'working' references of plant material (Pillay et al., 2003). Measurements of these 'working' references produced uncertainties of less than 5%, which was considered acceptable. ICP-AES was ideal for the concentration ranges encountered in this work, and is sufficiently sensitive to assess levels of tolerance, particularly heavy metals at trace levels (Baird, 2000).

Data analyses

All statistical analyses were performed using Microsoft Excel and SPSS Version 11.5. Graphs were plotted using SigmaPlot Version 9. SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis (Miser, 1995) is used here to consider the feasibility of using *P. juliflora* for the restoration of copper mined-lands in Oman.

RESULTS

Continuous exposure experiments

Mean percentages of germination after two, three and eight days in continuous exposure experiments are shown in Table 1. ANOVA showed significant differences in mean germination in all three durations (F for 2 days = 15.46; for 3 days = 4.97; for 8 days = 5.54; $P<0.0001$). Tukey tests as *a posteriori* comparisons indicated variable results.

Table 1. Summary of percentage germination in continuous exposure experiments at different concentrations of copper after 2, 3 and 8 days; N/E = number per experiment; means denoted by same letters are not significantly different (Tukey tests after ANOVA; $P > 0.05$); * variability non-normal, not used in ANOVA

Cu (ppm)	Replication N/E.	Percentage germination/ duration, Mean ± SE		
		2-days	3-days	8-days
Control	10	86.0 ± 5.5 **a**	94.0 ± 4.1 **d, e**	96.5 ± 3.7 **g, h, I**
10	10	22.2 ± 5.2 **b**	93.5 ± 7.0 **d, e**	99.5 ± 3.9 **g, h**
20	10	16.6 ± 5.8 **b, c**	93.0 ± 8.9 **d, e**	99.4 ± 4.5 **g, h, I**
40	10	29.8 ± 4.2 **b**	96.5 ± 3.7 **d**	100.0 ± 0.0 **g**
80	10	40.2 ± 3.9 **b**	96.7 ± 4.4 **d**	100.0 ± 0.0 **g**
160	10	43.7 ± 5.9 **b**	88.7 ± 3.4 **d, e, f**	96.5 ± 3.7 **g, h, I**
320	10	8.3 ± 4.0 **c**	79.7 ± 3.8 **d, e, f**	86.4 ± 4.0 **i**
640	10	8.6 ± 3.2 **c**	63.5 ± 2.4 **e, f**	86.5 ± 4.1 **i**
1280	10	0.9 *	57.3 ± 4.5 **f**	88.0 ± 5.8 **h, i**

After two days exposure, the mean germination in control was significantly higher than those of all other copper concentrations ranging from 10 – 640 ppm. The lowest germination, 0.9 % was recorded in 1280 ppm, but the variability indicated non-normal data distribution thus warranting its exclusion from parametric analyses. Germination percentages were not significantly different in 10 – 160 ppm ($P>0.05$), while 320 and 640 ppm with low and similar germination rates were significantly lower than that of all concentrations ($P< 0.01$) except 20 ppm.

After three days, high mean germination was recorded in 40 and 80 ppm, but these were significantly different only from 640 and 1280 ppm ($P<0.01$). The mean germination in control was not significantly different from those in 10 – 640 ppm ($P>0.05$), while means of 160 – 1280 ppm also were not different from each other ($P>0.05$).

After eight days, the mean germination in control and all other copper concentrations were high (86 – 100%). The control was not significantly higher than those of other concentrations ($P> 0.05$). The mean germination in 40 and 80 ppm were significantly higher than those in 320, 640 and 1280 ppm ($P < 0.01$), but not different from control, 20 and 160 ppm ($P>0.05$).

Seedling morphology

Figure 1 shows the typical morphology of seedlings grown in control and in the eight experimental concentrations, 10, 20, 40, 80, 160, 320, 640 and 1280 ppm during continuous exposure experiments for eight days. It was obvious that seedlings grown in concentrations 80 - 1280 ppm were stunted. Figure 2 shows the non-linear relationship between copper concentrations and the height of seedlings. The exponential decay model best fits this relationship and is described by the equation, seedling height = $5.4840\ e^{-0.0084x}$ ($r^2 = 0.9749$; ANOVA, $F = 136.40$; $P< 0.0001$).

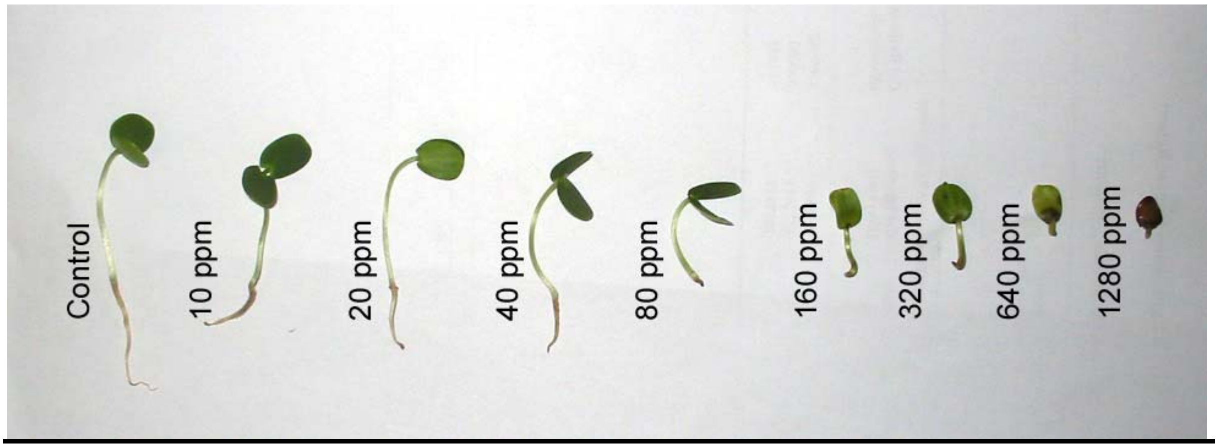

Fig.1 Morphology of typical seedlings grown in different copper concentrations in continuous exposure experiments

Copper content in seedlings

Seedlings obtained from some sets of continuous exposure experiments were processed for the estimation of copper content using ICP-AES study. Seedlings grown in control conditions (0 ppm) had an average copper content of 2.4 ppm. Figure 3 shows the non-linear relationship between copper concentrations in the experimental media and the average copper content of the seedlings (n =2 / concentration). The power model for two factors best fits this relationship and is described by the equation, copper content in seedlings = $2.66\ E\text{-}007x^{3.18}$ ($r^2 = 0.99$; ANOVA, $F=331.20$; $P< 0.0001$).

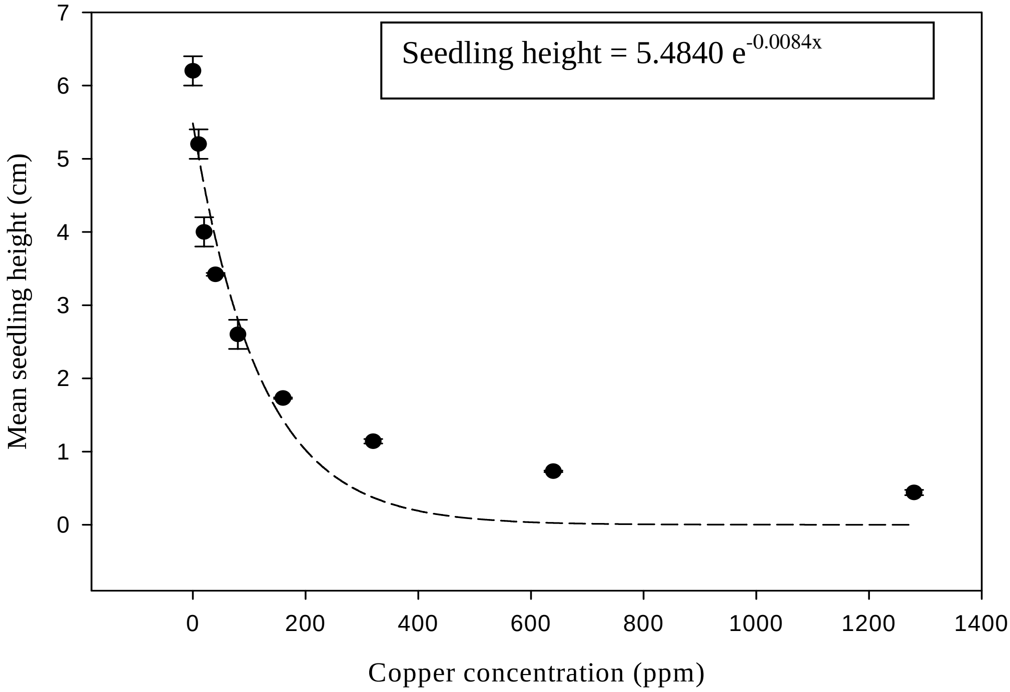

Fig.2. Relationship between Copper concentrations and mean seedling heights in continuous exposure experiments; n =3 /concentration, dots are actual data and bars are standard deviation; dashed line is best fit

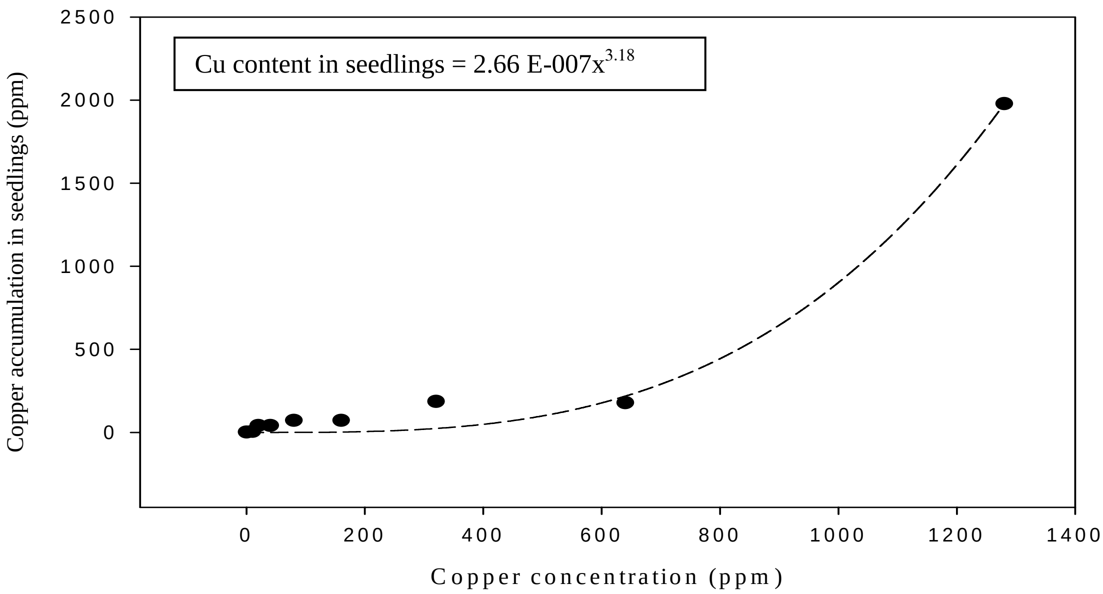

Fig.3. Relationship between copper concentrations in the media and copper accumulation in seedlings; dots show actual data, dashed line is best fit.

SWOT Analysis

Using *P. juliflora* as a tool in the restoration of copper mined-lands is a management decision with a much wider scope extending beyond the experimental results of this study. Table 2 presents the main components of a SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis to assist in recommending management strategies and policy decisions. After a field visit in March 2006 to survey the environment of the mined-lands in the Wadi Jizzi area of Oman, the components of the SWOT were scripted using known facts contributing to logical deductions. The two basic issues identified here are ecosystem and socioeconomic factors specifically addressing the recovery of copper mined-lands through the sustainable use of *P. juliflora*.

Table 2. SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis for the sustainable use of *Prosopis juliflora* in the recovery of copper mined-lands in Oman

Strengths

- The species is readily available for use.
- Hardy species capable of surviving and propagating in severe arid environments.
- Seems to tolerate high level of copper concentrations in the environment.
- Seedlings accumulate high concentrations of copper.
- Potential for copper removal from mined soil both as plants and as germinating seeds.
- Cost effective propagation.
- Fast stabilization and recovery of mined soil.

Weaknesses

- An introduced exotic.
- No history of use for restoration of mined-lands in the country or in the region.
- Performance levels in terms of copper tolerance to germination, seedling survival and bioaccumulation have not been compared with other native plant species.
- No field trials to prove *in situ* effectiveness in copper removal.
- Legislation concerning use and propagation is confusing, contradictory and vague.

Opportunities

- Ecological benefits of restored lands such as development of natural communities and stabilization of the physical environment.
- Economic benefits of restored lands for other sustainable land use activities.
- Cheap alternative fuel in the form of wood and charcoal.
- Use of pods as cheap fodder for goats and camels.
- Use of pods for development of flour for human consumption.
- Species with potential for the reclamation of saline soils in the area.

Threats

- An invasive species that may out-compete other native species and threaten ecological integrity.
- Uncertainties in exploiting opportunities offered by the species.
- Lack of management strategies and legislative framework for monitoring and control.

DISCUSSION

Prosopis juliflora has an extraordinary ecologic amplitude and tolerance for a variety of elements (Nagaraju and Prasad, 1998). Some studies have shown that *P. juliflora* is very effective in reclaiming highly sodic soils by restoring productivity and fertility (Bhojvaid et al., 1991). Despite being an introduced invasive, it has been recognized as one of the promising species for the rehabilitation of soils with salinity regimes equivalent to that of seawater and reclamation of copper mined areas (Singh and Singh, 1993; Lima et al., 2003).

Studies on copper toxicity in plants are many (Chen et al., 2000; Sheldon and Menzies, 2004), although investigations exploring toxicity thresholds in species used for ecological restoration are only a few (Pasche and Redente, 2002). Similarly, there have been studies on the effects of environmental factors on the seed germination of *P. juliflora* in arid regions (El Keblawy and Al-Rawai, 2005), but specific studies linking copper tolerance to seed germination are not known. Tolerance and toxicity testing in plants by adding copper in solution culture or to the test soils has been criticized because the plant response to elevated copper level in natural conditions is likely to be influenced by many factors interacting to restrict the actual bioavailability (Ginocchio and Narvaez, 2002). The source of copper used in experimental conditions also requires careful consideration. For example, copper sulfate is a known herbicide and its effect on seed germination is likely to be more severe when compared to copper nitrate that is intentionally used in this study, because of the copper-plant nutrient combination. However, screening the potential plants for tolerance to copper by *ex situ* laboratory experiments is an essential and cost effective exercise that should precede field trials for restoring copper mined-lands. This study provides the results of such screening and goes a step further in linking these to other known data on *P. juliflora* to develop a SWOT analysis that would be useful in planning management strategies for restoring copper-mined-lands in Oman.

The results of this study (Table 1) showed that the adverse effect of copper on percentage seed germination was more pronounced in the first two days, especially in concentrations ranging from 320-1280 ppm. After 3 days, the percentage germination in all concentrations except 640 and 1280 ppm were significantly similar to that of control, and after 8 days there was further improvement in similarities among all concentrations and the control. Statistical tests for significant differences in mean percentage germination among test concentrations showed anomalies, probably due to inter-seed variability but the elevated copper levels had an effect in the initial stages (2 days), and *P. juliflora* had the remarkable ability for subsequent recovery (3-8 days) by germinating in concentrations ranging from 10-1280 ppm possibly due to the presence of nitrate as nutrient.

High germination rates do not necessarily imply high seedling survival or viability. Seedling morphology (Fig.1) and the relationship between copper concentrations and seedling heights (Fig.2) suggested that seedling survival would be low in exposure concentrations of 80-1280 ppm. These seedlings despite showing morphological symptoms of toxicity still accumulated high levels of copper (Fig.3). Thus the germinating seeds of *P. juliflora* also seem to serve as a sink for copper in contaminated soils.

It is strongly argued that restoration strategies for the recovery of mined-lands should use native tree species, but practical difficulties such as low survival and low regeneration rates of these species in soils affected by mining residues may pose problems. As has been shown in the case of northeast Brazil, some non-endemic exotics show good potential for use in the recovery of mined-lands because of their fast growth and high survival rates (Lima et al., 2003). The survey of the copper mined areas during this study showed the poor recolonization and regeneration potential of *Acacia tortilis,* the most common native tree species in this region. Here, the germination experiments demonstrated the tolerance of *P. juliflora* to a wide range of copper concentrations and the ability of seedlings to accumulate copper, so that its choice as a suitable species for the recovery of copper mined areas should be considered. However, such recommendations are not enough to make sound management decisions on using *P. juliflora* for the restoration of copper-mined-lands unless all critical aspects of its use are analyzed. Once introduced intentionally, *P. juliflora*, because of its biological characteristics, is likely to invade the new area rapidly (Shiferaw at al., 2004) and establish itself as the dominant species.

SWOT analysis for the sustainable use of *P. juliflora* in the recovery of copper mined lands (Table 2) lists more strengths and opportunities (62%) than weaknesses and threats (38%). These results are qualitative and value-laden, but are based on facts obtained in this experimental study and also from available literature (Sharma, 1981; Lima et al. 2003). Many introduced species like *P. juliflora* in their non-native environments are not going to be eliminated that easily, but in due course will be integrated into local plant communities. Therefore, instead of trying to eradicate these species, redirecting efforts and resources for their efficient, beneficial and sustainable utilization seem wise.

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