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Original Research Article

Influence of copper and lead on germination of three Mimosoideae plant species

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Abstract

Contamination with heavy metals is a critical problem facing large areas of agricultural soils. Seed, a developmental stage, is considered highly protective against environmental stresses. This study aimed to examine the influence of two heavy metals; copper and lead, on the germination of *Acacia tortilis*, *A. raddiana*, and *Prosopis juliflora*.

Seeds were exposed to different concentrations of copper and lead including control, low (1000 ppm) and high (2000 ppm) copper or lead, low mix (500 ppm of copper and lead), or high mix (1000 ppm of copper and lead).

High copper and high mix had highly negative effects on germination of *A. tortilis*. Germination of *A. raddiana* was slightly affected under stress. While all stress treatment showed significantly negative effects on germination rate of *Prosopis juliflora*. Both Acacia species were not significantly affected after recovery, while the germination of stressed seeds of *Prosopis juliflora* has been induced after recovery. The 1st germination day was greatly affected with treatments, especially for *Prosopis juliflora*, where 1st germination day delayed about eight days under high mix treatment compared to control.

The results suggested that heavy metals had negative impacts on germination rate. *Prosopis juliflora* was more sensitive to heavy metal stress compared to Acacia species.

Keywords: Copper; Lead; Germination; *Acacia tortilis*; *Prosopis juliflora*

Introduction

Nowadays, large areas of agricultural soils are facing a critical problem of contamination with heavy metals. The environmental contamination is due to different sources such as wastewater, sewage sludge, metallurgy and manufacturing. This contamination has deleterious impacts on the ecosystems and human beings themselves (Lombardi and Sebastiani, 2005; Yuswir et al., 2015). Some plant species can spread where the vegetation is limited by environmental factors such as heavy metal contamination (Yang et al., 2007; Mateos-Naranjo et al., 2011). Over the last 10 years, increasing attention was received to the efficiency of using trees as a suitable vegetation cover in heavy metal-contaminated soils (EPA, 2000). Tree species have been suggested as a sustainable, eco-friendly, and low-cost solution to remediate heavy metal-contaminated soils (Dickinson, 2000).

Some heavy metals are essential micronutrients for plants, such as copper, zinc and nickle, however these metals have toxic effects on organisms at high levels (Munzuroglu and Geckil, 2002). On the other hand, some other toxic heavy metals are non-essential including Hg^{+2}, Cd^{+2}, and Pb^{+2}. These metals are naturally accumulated in water and soil or as contaminations due to human activities. Among toxic heavy metals, Cu and Pb are pollutants presented mostly in free ions form (Cu^{+2} and Pb^{+2}). Copper (Cu) is essential nutrition for plant with important role in plant metabolism, enzyme activity, and detoxification (Taiz and Zeiger, 2002). Copper is transported through the xylem in bounding with amino acids (Reichman, 2002). The excess accumulation of Cu can cause toxic effects such as suppression of photosynthesis and metabolism as well as chromosome injury (Påhlsson, 1989). In contrast, lead (Pb) is not essential to plant function. Extensive studies focused on soil contamination with Pb in agricultural, rural, and urban regions (Markus and McBratney, 2001). Lead contamination occurs as a result of air pollution from motor traffic in addition to industrial materials (Bogdanov, 2005), so certain species that grow near to roadsides accumulate such metal (Price et al., 1974). On the List of Comprehensive Environmental Response, Compensation, and Liability Act Priorities, lead is listed the second hazardous substance (US EPA, 2011). Lead cause a wide range of toxic effects on plants, whether morphological, physiological, and/or biochemical effects. Lead has negative impacts on plant growth, root development, germination, transpiration, photosynthetic tissues, cell division, and protein content (Krzesłowska et al., 2009; Maestri et al., 2010; Pourrut et al., 2011). The toxic effects depend on lead content, period of exposure, and phase of plant development. Fortunately, plants develop various mechanism to cope the toxic metal effects including internal detoxification, excretion, complication by specific glands, and compartmentalization (Gupta et al., 2009; Jiang and Liu, 2010; Maestri et al., 2010). Recently, several studies investigated the molecular mechanism underlying heavy metal uptake and transport (Song et al., 2003). Further investigation is necessary to explore the effect of essential and non-essential metals on plant during different growth stages.

Acacia tortilis is geographically distributed in the Northern Africa including the Sahel, Eastern and Southern Africa, and Arabia regions (Brenan, 1983; Fagg and Barnes, 1990; Barnes et al., 1996). Brenan (1983) distinguishes four subspecies; *raddiana*, *spirocarpa*, *heteracantha*, and *tortilis*. *Raddiana* and *tortilis* are found in Northern Africa and parts of the Arabic peninsula, whereas *spirocarpa* and *heteracantha* are found in Eastern Africa and Southern Africa, respectively. *Acacia tortilis* and *A. raddiana* are recommended in semiarid areas that face severe shortage of forage, particularly where soil erosion, desertification, and/or sand stabilization are problems. *Prosopis juliflora* was considered among the top 100 least wanted species worldwide in the rating of Invasive Species Specialist Group of (IUCN, 2004). It characterizes with rapid growth, great rooting system, effective uptake of nutrients, high evapotranspiration rate, great biomass production, and pronounced clone specific capacity for heavy metal uptake (Nasr et al., 2012), which makes it a very effective tree for phytoremediation purposes (Pegado et al., 2006). *P. juliflora* was introduced to Egypt intentionally into Gabel Elba National Park during the late 80s by local people of Old-Hala'ib village for agro-forestry purposes. After eight years, local people alike showed a problematic and noxious of *P. juliflora* tree due to its strongly aggressive invasion ability (Abbas et al., 2016). In Egypt, *P. juliflora* spread into new areas of invasion, including populations in different dry (ephemeral) riverbeds such as wades in South Egypt where Acacias widely spread, and where many factories causing environment pollution with heavy metals, specially copper and lead.

This study was carried out to assess the effect of copper, lead, and/or their combination on germination of the invasive tree *P. juliflora* and two native *Acacia tortilis* and *Acacia tortilis* subspecies *raddiana* as these are the key events for the establishment of plants under any prevailing environment. We hypothesized that a high proportion of *Prosopis* and *Acacia* seeds would not survive when treating with the heavy metals, at the same time that their germination would be depressed.

Materials and Methods

Fruit collection

Fruits of three species belonging to the subfamily Mimosoideae, family Fabaceae; *Acacia tortilis*, *Acacia tortilis* subspecies *raddiana* (*A. raddiana*, herafter), and *Prosopis juliflora*, were collected randomly from multiple mature individuals in May 2016 from Wadi Merikwan, Wadi Rahaba, Wadi Khoda, and Gebel Elba National Park located in the southeastern part of Egypt (22°14′2″N – 36°36′30″E. Collected fruits were stored in dry and dark conditions in the laboratory at 25 ºC until using.

Seed germination

In the end of August 2016, seeds were subjected to copper (Cu) and lead (Pb) in seven groups of

concentrations; 1) control (distilled water); 2) low Cu (1000 ppm); 3) high Cu (2000 ppm); 4) low Pb (1000 ppm); 5) high Pb (2000 ppm); 6) low mix (500 ppm Cu + 500 ppm Pb); and 7) high mix (1000 ppm Cu + 1000 ppm Pb). Seeds were disinfected before treatments by soaking in a solution of 1% sodium hypochlorite for two minutes and completely washed with sterile water before the germination treatment (Mancilla-Leytón et al., 2011).
In petri dishes, seeds were sowed on a 9 cm filter paper (10 seeds per dish and 4 replicated dishes per treatment). Dishes were placed in a germinator after wrapping with parafilm (Lab-Line Biotronette, Instruments, INC, Meleose, Park, ILL, USA) for 25 days under 12 h of light (at 25 ºC, 35 µmol $m^{-2}s^{-1}$ of 400–700 nm wavelength) and 12 h of darkness (at 20 ºC). This is the normal condition at the end of autumn in the South of Egypt, where these species are presented (El-Keblawy and Al-Rawai, 2005). The germinated seeds were counted daily and the seed was considered as germinated when its radicle emerged (Abbas et al., 2012). Final germination rate, first germination time, and mean time to germination (MTG) were calculated. MTG was calculating using the equation:

$$\mathrm{MTG} = \frac{\Sigma ini \times di}{N}$$

where *n* is the number of germinated seeds at day *i*, *d* the incubation period in days and *N* the total number of germinated seeds in the treatment (Brenchley and Probert, 1998).

Recovery experiment

Recovery test was conducted to determine if high heavy metals concentration inhibited or caused damage to the seeds. After continuous exposure to different treatments, non-germinated seeds were washed using distilled water, and submerged in new Petri dishes with 5 ml of sterile water under same conditions as previous for twenty days. The three germination parameters were recorded; final rate of germination, first germination time, and MTG.

Viability test

The viability of the embryo was determined after the germination experiment using the tetrazolium test to non-geminated seeds (MacKay, 1972; Cui et al., 2007). Seeds were maintained in water for 16 h at a 25° C, and then in darkness for one day in 1% aqueous solution of 2,3,5-triphenyl- tetrazolium chloride, pH 7. The seeds were dissected and through a magnifying glass, the embryo was analyzed (Bradbeer, 1998).

Statistical analysis

Statistical analysis was carried out using JMP software (version 4.0; SAS institute, Cary, NC, USA). The statistical differences were analyzed by ANOVA test. Differences in germination rate parameters among treatments were detected using Tukey's Honest Significant Difference (HSD) test. A factorial experiment was set up base on complete randomized design incorporating four replications.

Results

Analysis of variance (ANOVA) showed that copper (Cu) and lead (Pb) treatments significantly affected on germination rate of all species (Table 1). For *Acacia tortilis*, the increments of germination rate differed among treatments. High Cu and high mix (Cu+Pb) treatments had the least germination rate compared to other treatments. The differences were obviously appeared after eight days of treatments. For *A. raddiana*, germination rate slightly increased with time. High Cu and Pb concentrations showed slightly lower germination rate compared to control and low Cu and Pb concentrations. On the other hand, germination rate of *Prosopis juliflora* decreased significantly under Cu and Pb treatments compared to control, and this was significantly evident on the tenth day of treatments and beyond (Figure 1).
After 20 days of treatments, non-geminated seeds were successively washed and treated with distilled water for recovery. Germination rate significantly differed for *Prosopis juliflora* after recovery compared to before recovery under Cu and Pb treatments, but not under control conditions. The differences among treatments were significant at 22 day, and the differences have decreased over time to become insignificant starting from the 28th day. The other species, *A. raddiana* and *A. tortilis*, showed no significant changes in germination rate after recovery compared to before recovery (Table 1 and Figure 1).

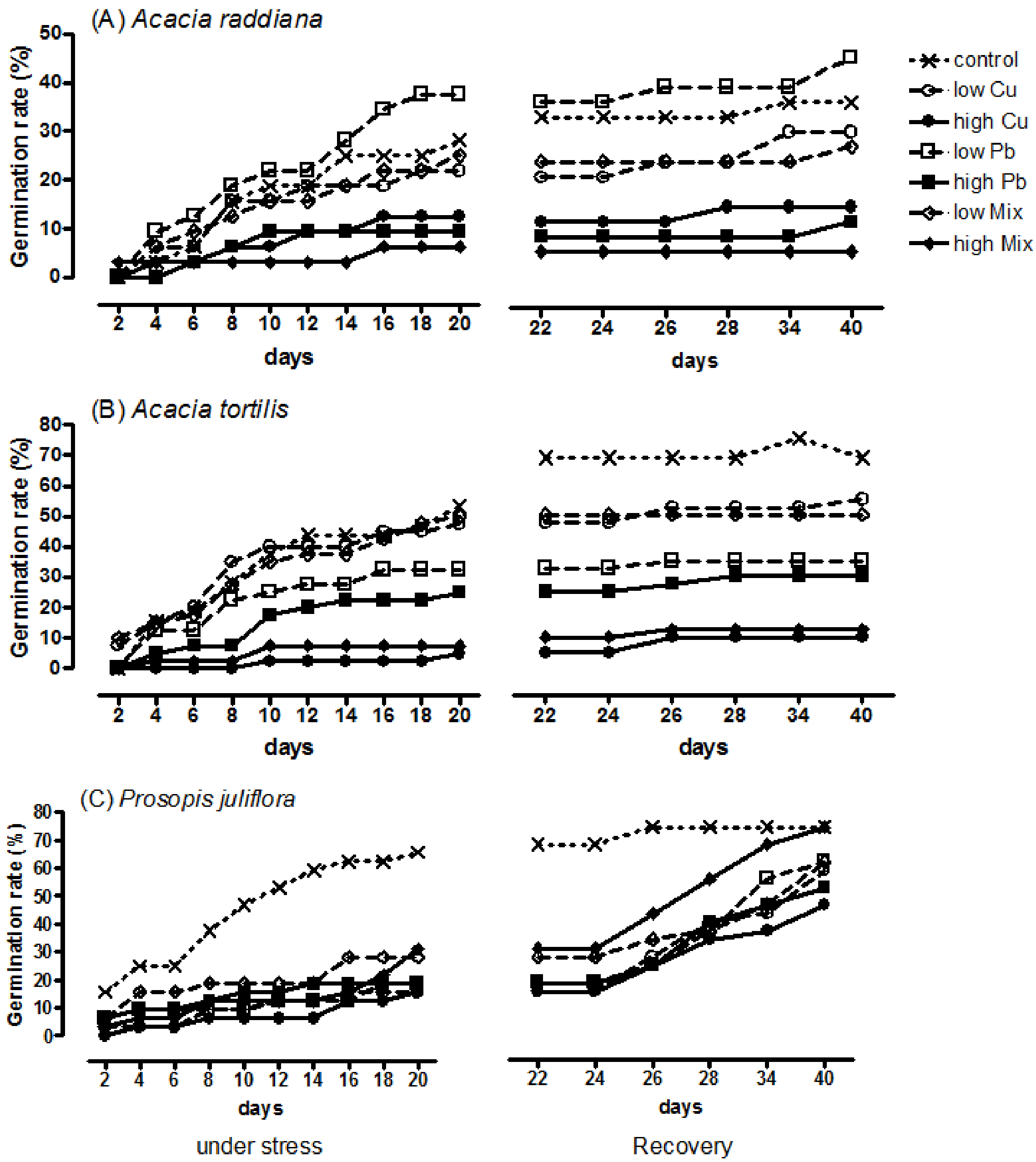

Fig. – 1: Germination rate of *Acacia tortilis*, *A. raddiana*, and *Prosopis juliflora* under copper (Cu) and lead (Pb) treatments; low Cu or Pb (1000 ppm), high Cu or Pb (2000 ppm), low mix (500 ppm of both Cu and Pb), and high mix (1000 ppm of both cu and Pb) as well as after recovery (treatment with distilled water).

The 1st germination day differed greatly among species and among treatments. The 1st germination day was during the second day for *Prosopis juliflora*, during the fourth day for *A. tortilis*, and during the sixth day for *A. raddiana* under control conditions (Table 2). For *Prosopis juliflora*, the 1st germination day ranged between the second day under control and the 14th day under high mix treatment. For *A. tortils* and *A. raddiana*, the earliest germination was under low Cu treatment and the latest germination was under high mix (10th, and 9th day, respectively). Mean time of germination (MTG) showed slight differences among treatments for *A. tortilis* and *A. raddiana*, and the differences were obviously clear for *Prosopis juliflora* (Table 2). For *Prosopis juliflora*, the highest MTG was under high mix treatments (1.84).

The Viability test showed that heavy metal treatment did not show clear effects on the viability of seeds. Few numbers of seeds were dead which were not related to specific treatment (Table 2).

Table - 1: Analysis of Variance (F value) of germination rate under different copper and Lead treatments as well as under recovery of the three species; *Acacia tortilis, A. raddiana,* and *Prosopis juliflora*

	A. tortilis	*A. raddiana*	*P. juliflora*
Under stress			
Treatments	30.2***	13.33***	34.9***
days	11.2***	9.99***	7.49***
Recovery			
Treatments	63.5***	19.0***	23.8***
days	0.70	0.40	19.1***

*** Significant at P = 0.001.

Table - 2: Number of days to first germination, mean time to germination (MTG) and viability under control, low Cu, high Cu, low Pb, high Pb, low mix and high mix for the invasive *Prosopis juliflora* and two native; *A. tortilis, A. raddiana* from Southeast Egypt.

Treatment	1st germination	MTG (d)	Viability (%)
	P. juliflora		
Control	1.75±0.48	0.97±0.11	96.9±3.1
Low Cu	2.50±0.50	0.94±0.15	100±0.0
High Cu	8.67±3.53	1.38±0.52	100±0.0
Low Pb	8.00±3.67	1.26±0.48	100±0.0
High Pb	2.00±1.00	0.75±0.22	100±0.0
Low mix	4.75±3.09	1.00±0.28	100±0.0
High mix	13.75±2.9	1.84±0.42	100±0.0
	A. tortilis		
Control	4.00±2.03	1.19±0.13	90.6±6.0[ab]
Low Cu	1.00±1.25	3.25±0.15	97.5±2.5[a]
High Cu	9.00±0.00	1.50±0.38	100±0.0[ab]
Low Pb	4.38±1.30	0.70±0.04	100±0.0[ab]
High Pb	7.63±2.75	1.05±0.23	97.5±2.5[ab]
Low mix	4.75±2.14	0.95±0.22	100±0.0[ab]
High mix	10.00±3.8	1.10±0.35	95.0±5.0[b]
	A. raddiana		
Control	6.00±2.08	1.25±0.18	93.8±6.0
Low Cu	5.50±2.02	1.04±0.26	96.9±3.0
High Cu	7.33±2.03	1.17±0.31	100±0.0
Low Pb	6.00±1.58	1.19±0.25	100±0.0
High Pb	7.67±1.45	0.96±0.27	100±0.0
Low mix	5.33±2.33	1.25±0.31	100±0.0
High mix	9.00±7.00	1.13±0.48	100±0.0

Discussion

Seed, a developmental stage, is considered highly protective against external environmental stresses. In contrast, vegetative development stage is generally sensitive to stress (Li et al., 2005). The toxicity of metals on *Arabidopsis* seeds depended on the physiological state of seeds and the metal ions selective permeation through tissues surrounding the embryo (Li et al., 2005). Among common pollutants, plants frequently encountered the toxicity of copper and lead (Påhlsson, 1989; Cecchi et al., 2008; Shahid et al. 2011). Exposure of plants to lead has negative effects on germination and growth even at micro-molar levels (Islam et al., 2007).

In this study, heavy metal treatments showed significant decreases in germination rate (Table 1). The germination rate under high stress treatments was lower than that under control and low stress treatments (Figure 1). These results showed the negative impacts of both Cu and Pb on germination rate especially under high concentrations. This is consistent with previous findings in general. High concentration of copper (Cu) has negatively affected on germination rate of wheat and rice (Mahmood et al., 2007), alfalfa (Aydinalp and Marinova, 2009), *Vigna mungi* (Solanki et al., 2011), and Crambe (Hu et al., 2015). Also, there are several reports about lead inhibition of species seed germination including *Brassica penkinensis*, *Elsholtzia argyi*, *Hordeum vulgare*, *Oryza sativa*, *Pinus halepensis*, *Spartina alterniflora*, and *Zea mays* (Islam et al., 2007; Sengar et al., 2009). In this study, Germination rate ranged between 6-40% for *A. raddiana* at 20 day of stress treatments, to be between 6-47% after recovery. For *A. tortilis*, the germination rate ranged between 7-55% at 20 day of stress, and between 10-70% after recovery. On the other hand, germination rate of *Prosopis juliflora* ranged between 15-66% at 20 day of stress, and 45-75% after recovery. These results indicated the negative impact of heavy metals, Cu and Pb, on germination especially for *Prosopis juliflora*, which showed significant increases in germination rate after recovery for stressed seeds. These results suggested that *Prosopis juliflora* was more sensitive to both Cu and Pb stress. On the other hand, *Acacia tortilis* was more sensitive to Cu rather than Pb. *A. raddiana* was the least affected to Cu and Pb stress compared to others species.

In this study, the 1st germination day affected significantly with heavy metal treatments (Table 2). *Prosopis juliflora* was the most affected species compared to others. The earliest germination was during the second day under control, and the latest germination was during the 14th day under high mix (1000 ppm Cu+1000 ppm Pb) treatment. The 1st germination day for *A. tortilis* and *A. raddiana* were during the 4th day and 6th day, respectively, under control. The latest germination was under high mix treatment for both *A. tortilis* and *A. raddiana* (10th, and 9th day, respectively). The mean time of germination and viability test showed no clear effects on all species. These results suggested the role of heavy metal absorption during the germination stage on slowdown of germination, but not on viability of seeds.

From the results of this study, it is concluded that heavy metals had negative impacts on germination especially for *Prosopis juliflora* species which showed sensitivity to heavy metal stress compared to Acacia species. The mean time of germination, MTG, and seed viability were not significantly affected by heavy metals which reflect the role of heavy metal absorption on slowdown of germination, but not on viability of seeds.

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