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EFFECT OF LEAD ON THE GERMINATION OF *MORINGA OLEIFERA L.* SEEDS

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Abstract

Lead is one of the most toxic and widely used metals by humans and it has any biological advantage, its presence at high doses causes several disturbances in the plant. *Moringa oleifera* L. is a vegetable species that grows in Asia and Africa; it has significant potential for water purification and resistance to salt stress. This work consists to determine the effect of lead in the form of lead nitrate ($Pb(NO_3)_2$) on the germination rate of *Moringa oleifera L.* seeds, as well as the growth of the radicle and tigella. The seeds were germinated in Petri dishes soaked in increasing concentrations of lead at 0, 3, 5, 7 and 10 mM applied at five repetitions for each treatment, in the dark in an oven set at 27°C for fifteen days. The effect of lead on the germination behaviour of *Moringa oleifera* L. is reflected in a decrease in the germination rate, which reaches 100% for the control. The results obtained show that the lengths of the rootlets and tigelles of the seedling decrease with the increasing application of lead doses.

***Keywords*:** Lead, *Moringa oleifera* L., germination, Stress.

Introduction

One of the major environmental problems is the contamination of atmosphere, water and soil by many toxic elements and compounds such as lead (Brunet, 2008). Lead is one of the most widespread contaminants in the environment (Hernández-Ochoa *et al.,* 2005; Jarup, 2003). Toxic or harmful, even at low concentrations to many living organisms (CITEPA, 2009). In addition, it is not biodegradable, persists and accumulates in soils (Seregin and Ivanov, 2001). Once it has successfully penetrated the plant, it can affect many of its physiological processes (Cecchi, 2008) and cause cell death at higher doses (Seregin and Ivanov, 2001).

The solution to this problem is to treat contaminated soils to remove metallic elements or, at least, reduce their concentration to levels acceptable for the viability of the ecosystem. One of these treatment methods, which appeared in the early 1990s as a remediation alternative, consists of using plants capable of growing on soils with a high metal content and capable of mobilizing or absorbing a significant quantity of metals. This technique, called phytoremediation (Kumar *et al*., 1995), is promising because it is inexpensive and more environmentally friendly than conventional, physico-chemical methods (Chaney *et al*., 1997, Kupper *et al*., 1999).

In general, plants are more sensitive to germination and emergence stages than to maturity stages (Ashraf and Harris, 2004). Germination is a physiological phase during which the seed passes from the slowed down state of life to the active state of life (Caboche *et al*., 1998) However, a good progress of germination processes depends on the environment close to the seed, it is strongly influenced by temperature, water and oxygen levels and soil structure (Munns, 2002; Maaouia-Houimli *et al*., 2011).

Moringa oleifera L. still called "tree of life" (Besse, 1996; fuglie, 2001) is a fast-growing plant of the Moringacea family, native to India but widespread throughout the world and especially in tropical and subtropical countries (Louni, 2009). Its introduction into East Africa took place at the beginning of the 20th century through trade and maritime exchanges (Foidl *et al*., 2001). *Moringa oleifera* L. is a tree with multiple medicinal, nutritional and industrial properties. In addition, the seeds can be used for clarification and purification of wastewater (Louni, 2009).

In this perspective, the evolution of the germination potential of seeds in a lead-contaminated environment is fundamental because germination is the starting point for the growth and development of the plant in this environment. The objective of this study is to study the effect of lead at different concentrations on the germination rate of seeds of this species, as well as the growth of the radicle and tigelle.

Materials and Methods

Plant Material: The seeds used are of Algerian origin, harvested in 2017 from Oued el Alleug wilaya of Blida in Algeria.

Germination of *Moringa oleifera L.* seeds: The germination test was carried out at the laboratory of biodiversity and conservation of water and soils of Abdelhamid Ibn Badis University, Mostaganem (Algeria). The seeds are sorted and disinfected by washing with sodium hypochlorite at 8° for five minutes to eliminate any fungal contamination, then rinsed three times with distilled water in order to remove all traces of sodium hypochlorite. To facilitate and homogenize their germination, the seeds are soaked in distilled water for 24 hours, and put to germinate in 9 cm diameter plastic Petri dishes containing two layers of filter paper at a rate of 10 seeds per box and 5 repetitions per treatment. Each Petri dish receives 10 ml of distilled water for the control seeds (0 mM) and the same volume of different lead concentrations (3, 5, 7, and 10 mM) for the treated seeds, then the Petri dishes were placed in the dark in the oven at a temperature of 27 °C, and followed every 24 hours for 15 days.

Parameters analyzed

Final germination rate: A seed was considered germinated when the radicle pierced the envelope and became visible to the naked eye, as defined by **Come (1970).** And the final germination rate was calculated according to the following ratio:

$$TG\% = \frac{Gx}{Gt} \times 100$$

Where, TG: Final germination rate; Gx: number of germinated seeds; Gt: total number of seeds put to germinate.

Kinetics of germination: It is expressed as the percentage of seeds germinated each day in relation to the total number of seeds per Petri dish (%) (Mazliak, 1981), during fifteen days.

Indeed, the germination rate is calculated according to the following formula:

$$TG\ (\%) = Gx/Gt*100$$

Where: TG: Average germination rate in (%), Gx: Number of germinated seeds, Gt: Total number of seeds put to germinate.

Radicle and tigelle length It was measured with a graduated ruler, of six seeds per box every two days for fifteen days to assess the plant's growth in response to stress. Measurements of this parameter are made from the 4th day of the experiment until the end of the test (15th day).

Statistical analyses: The results obtained were statistically analyzed by software R version 3.5.2 (2018-12-20).

Results and Discussion

Effect of lead on the germination rate of *Moringa oleifera L.*: The results obtained show an insignificant decrease or probability is equal to 0.1 ($P > 0.05$) in the germination rate of *Moringa oleifera* L. with values of 98, 94, 92 and 96% respectively for doses 3, 5, 7 and 10 mM of Pb compared to the control where the germination rate is 100% (figure 01).

Effect of lead on the kinetics of the germination rate of *Moringa oleifera* L: The results obtained show that the seeds start to germinate from the 4th day, where the germination rate of the seeds is more than 80% in the presence and absence of lead. Indeed, control seeds and seeds treated with 3 mM lead germinate from day 4 and reach 96% of the germination rate for both 0 and 3 mM doses of Pb, germination ends on day 5 with germination rates of 100 and 98% respectively for control and treated seeds at 3 mM Pb. Seeds treated at 5mM from Pb evolve slowly until the 7th day to reach a final germination rate of 94%. On the other hand, seeds treated with 7 and 10 mM doses of Pb reach a final germination rate of 92 and 96% respectively for 7 and 10 mM doses, after 6 days of germination, it is noted that the germination rate remains constant until the 15th day (Figure 02).

Statistical analysis of the effect of lead on the kinetics of the germination rate of *Moringa oleifera* L. reveals an insignificant decrease in the germination rate with a probability of 0.8 ($P > 0.05$).

In several plant species, the integument provides very high protection against abiotic stresses, and the strong interspecific variations in the morphologies of these integuments can affect their permeability to metals (Moise *et al*., 2005). Even at low doses, lead inhibits the germination of grains that have permeable envelopes such as peas *Pisum sativum* (Seregin and Ivanov, 2001). On the other hand, high doses lead to total inhibition of germination in beans (*Phaseolus vulgaris*), whose seed coat does not sufficiently limit the penetration of these ions (Wixrzbicka and obidzinska, 1998). However, the impermeability of seeds coats of some species offer them a high resistance to lead.

Analysis of the effect of lead on the germination capacity of seeds shows a decrease in the germination rate from control to treated plants; however, the inhibitory effect is minimal and the percentages obtained are still high (92% at 7 mM from Pb). These results are identical to those obtained by many researchers testing the impact of different lead concentrations on the germination of different plants. Rouibi (2012) recorded a reduction in the germination rate of *Triticum durum* seeds (62%) to 10^{-2} M Pb concentration; however the concentration of 0.3 g/L inhibited completely the germination of the three varieties of *Triticum durum* (Nedjah, 2015). While at 103.6 g/l and 207.2 g/l, there is a very significant reduction in germination parameters of different wheat varieties (Hamoum, 2003). Verma and Dubey (2003) showed a decrease in germination from 14 to 30% in *Oryza sativa* seeds at high lead concentrations and the concentration of 1000 µg /ml reduced to 43.33% the percentage of germination of *Brassica pekinensis* (Xiong, 1997) and 1000 mg/l affects the radish plant (*Raphanus sativus* L) by growth inhibition, a decrease in germination rate (75%) (Aoumeur, 2012).

The inhibitory effects of high concentrations could be largely explained by metal inhibitory action on enzymes responsible for restoring life and building a new plant, and/or by inhibition of hydrolytic activities during germination of seeds exposed to heavy metals (Chugh and Sawhney, 1999; Bansal *et al*., 2001).

Effect of lead on the radicle length of *Moringa oleifera* L.: The results obtained show a very highly significant decrease ($P < 0.001$) in rootlet elongation kinetics as the applied lead dose increases (Figure 03) with lengths 107, 11.8, 7, 4.9, and 2.8 mm for 0, 3, 5, 7 and 10 mM Pb doses respectively. Maximum lengths are recorded in the absence of lead. The lengthening continues until the tenth day and stabilizes around the twelfth day for all treatments.

Roots are the most sensitive to heavy metals compared to other plant organs because they are the first targets for the passage and accumulation of these metals (Seregin and Ivanov, 2001), particularly primary roots which are more sensitive than lateral roots (Obrouscheva *et al*., 1998). Exposure to a low concentration of lead leads to the development of shorter but more compact roots (Boutchiche, 2017). A high lead content inhibits their growth (Obroucheva *et al*., 1998). Over long periods of time, lead treatments, even at non-lethal doses, can lead to the development of necrosis in the root apexes (Khan and Frankland, 1983; Liu *et al*., 2000).

The results obtained show that lead had a negative effect on the lengths of the *Moringa oleifera* L. rootlets, where there was a significant decrease in the latter for the different doses compared to the control and even the density of the secondary roots, where there was a total absence of secondary roots for doses of 10 mM Pb, and the appearance of necrosis at the root ends from 5 mM Pb. These results are

consistent with other work done by Kopittke *et al*, (2007) in a study on cornille (*Vigna unguiculata*), which showed that roots are more sensitive than aerial parts to lead exposure. In addition, root biomass production is inhibited from the minimum threshold of 0.1µM lead in the solution, with symptoms visible on the roots from 1.5µM Sereguine and Ivanov (1998) also observed a 50% inhibition of root growth of lead-treated corn (*Zea mays*), and Aoumeur (2012) also showed a 50% inhibition of root growth of lead-treated radish. Kranner and Colville (2011), also confirmed the inhibition of root growth in more than 15 plant species in the presence of increasing lead concentrations.

Therefore, lead seems to affect the lengthwise growth of plant roots, with necrosis appearing in the root apexes. Inhibition of cell division and elongation are the most frequently reported phenomena to explain these lead effects (Seregin and Ivanov, 2001; Malkwoski *et al*., 2002; Patra *et al*., 2004; Kopittke *et al*., 2007). This had already been demonstrated nearly 84 years ago by Hammett (1929) who showed a decrease in the mitotic index in maize (*Zea mays*) and onion (*Allium cepa* L.) roots, caused by the presence of $Pb(NO_3)_2$, and which he had explained by the binding of lead to the -SH groups of proteins.

Effect of lead on the length of *Moringa oleifera* L. tigelles: The results obtained show that the maximum elongations are recorded in the absence of lead in the control plants. The elongation continues until the tenth day and stabilizes on the twelfth day for all lead treatments. Lead reduces tigelle growth and this reduction is greater for the different doses compared to the control where the followig lengths were recorded: 174.6, 131.8, 108.7, 70.5 and 23.4 mm with 0, 3, 5, 7 and 10 mM Pb doses respectively (Figure 04).

It should be noted that the effect of lead on the elongation of tigelles, in fact, the closer the amplitude of the curves is to the X axis, the greater the effect of Pb.

Statistical analysis shows that the length of *Moringa oleifera* L. tigelles decreases very significantly ($P < 0.001$) as a function of the increase in lead doses applied (figure 04).

Lead affects the growth and morphogenesis of plants, disrupting many physiological mechanisms. Exposure of plants to lead leads to a reduction in growth, in the biomass produced, when stress is too high, leads to the appearance of root and leaf necrosis, chlorosis, or even death of the plant (Seregin and Ivanov, 2001; Sharma and Dubey, 2005; Cecchi, 2008).

The results from this study are consistent with those reported by Rooney *et al* (1999), where they showed that for EDTA extractable concentrations in soil up to 800 mg Kg^{-1}, the growth of Ray-grass (*Lolium perenne* L.) was not affected. However, Päivöke, (2002) shows that lead has adverse effects on the growth of pea plants (*Pisum sativum* L.) at concentrations below 500 mg.Kg^{-1} of Pb. Work carried out by Liu *et al*., 2003 on different rice cultivars (*Oryza sativa* L.) has shown growth and development delays caused by 800 mg.Kg^{-1} of Pb. Malkowski *et al*., 2002 showed that the growth of maize (*Zea mays*) was inhibited by the presence of 10 µM Pb in the culture solution. Kopittke *et al*., 2007 in a study on the cornilla (a plant of the Fabaceae family, close to the bean) show that roots are more sensitive than aerial parts to lead exposure. In addition, root biomass production is inhibited from the minimum threshold of 0.1µM lead in the solution, with symptoms visible on the roots from 1.5µM Inhibition of biomass of the aerial parts manifesting itself from 0.3 µM Depending on environmental conditions; plants can absorb some of the lead in the soil. Pb^{+2} ions diffuse in the root, but are blocked by the physical barrier of the endoderm, which strongly limits their translocation to the aerial parts (Cecchi, 2008). Inhibition of cell division and elongation are the most frequently reported phenomena to explain these lead effects (Seregin and Ivanov, 2001; Malkwoski *et al*., 2002; Patra et al., 2004; Kopittke *et al*., 2007).

Conclusion

The high concentrations of lead act negatively on all germinative parameters *of Moringa oleifera* L, resulting in a decrease in the final germination rate compared to the control, and a reduction in the lengthwise growth of the seedlings and both the size of the roots and the density of the absorbent hairs which leads to the development of shorter but more compact roots. Germination at the applied doses (0, 3, 5, 7 and 10 mM) shows the strong potential of *Moringa oleifera L.* which could have the characteristics of a model plant to explain the biochemical and molecular strategies involved in the phenomenon of lead tolerance of seeds.

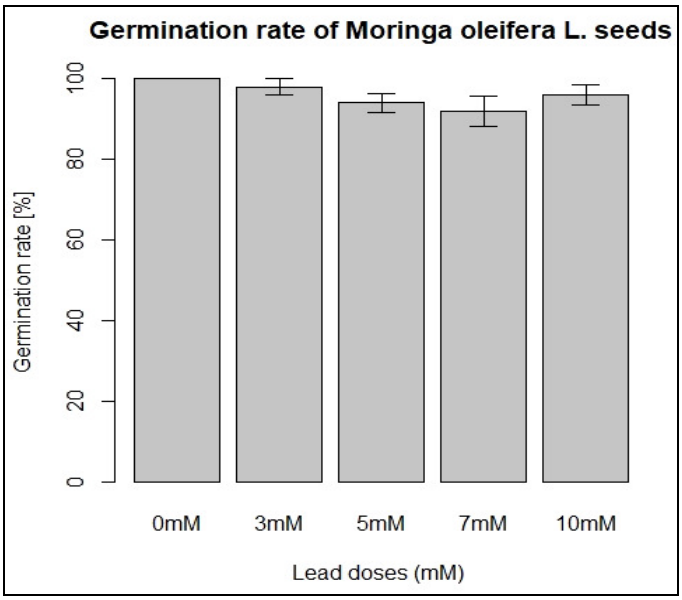

Fig. 1 : Effect of lead on the germination rate of *Moringa oleifera L.* seeds.

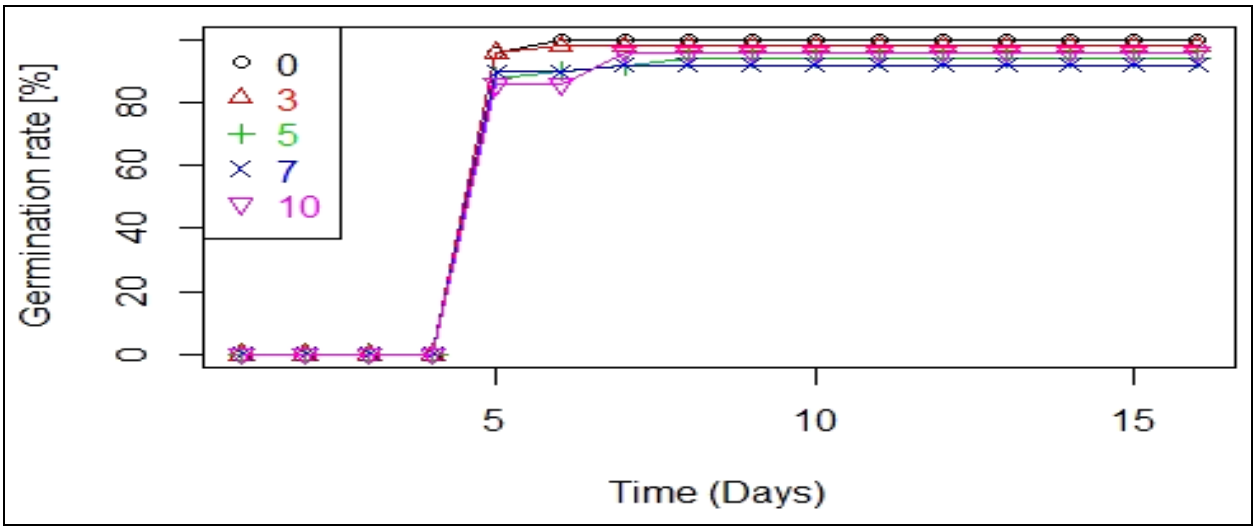

Fig. 2 : Effect of lead on the germination kinetics of *Moringa oleifera* L seeds.

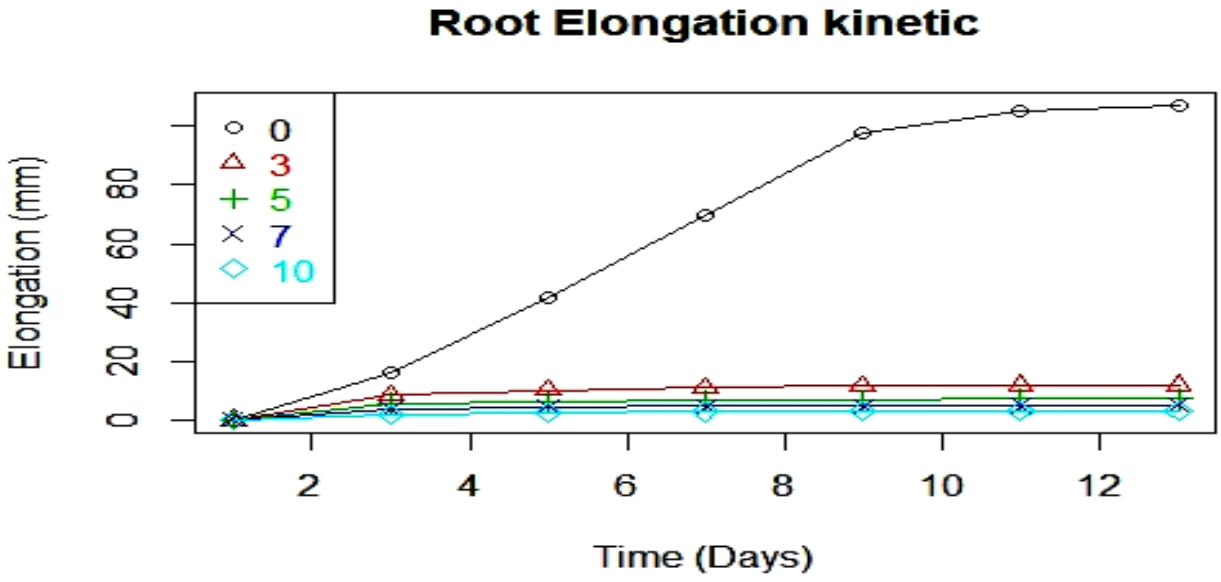

Fig. 3 : Effect of lead on the kinetics of rootlet lengths of *Moringa oleifera L.*

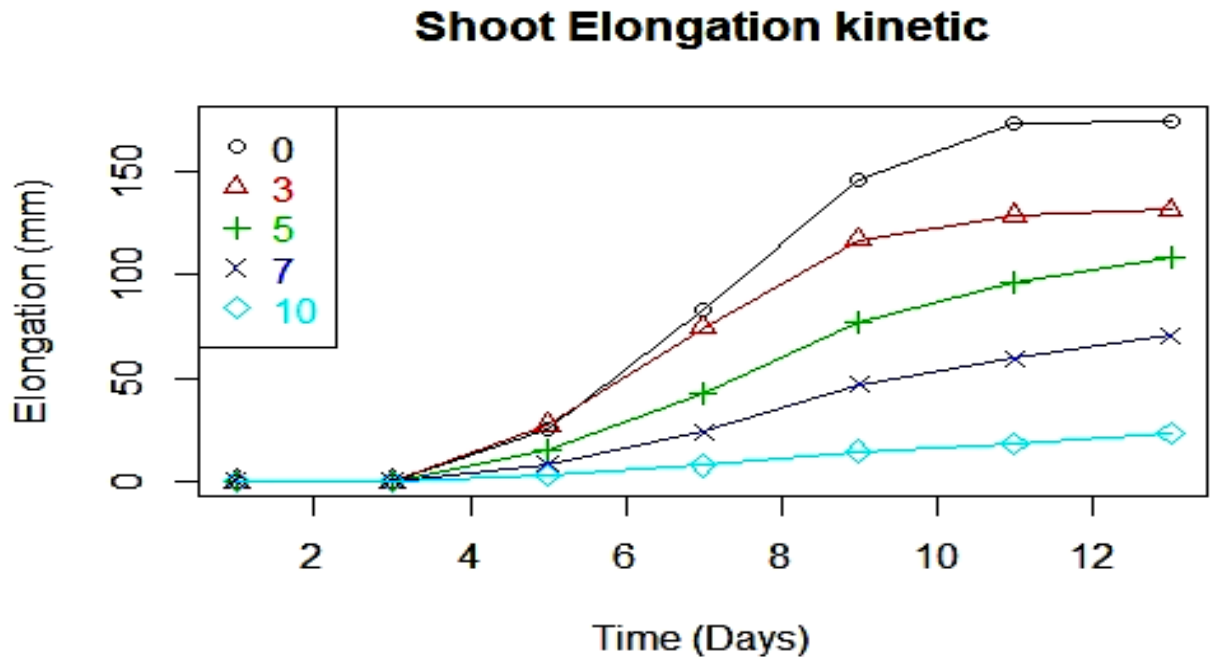

Fig. 4 : Effect of lead on the kinetics of tiger lengths of *Moringa oleifera L.*

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