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EFFECT OF LEAD ON SEED GERMINATION AND SEEDLING GROWTH OF *CLEOME AMBLYOCARPA* BARR. & MURB.

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Abstract

The inappropriate treatment of special waste of industrial and agricultural origin and poor management of natural resources, make environment vulnerable to heavy metals contamination. Lead (Pb) is a metal non-biodegradable utilized by humans for many thousands of years which is freely present in the soil. Some plants have developed detoxification mechanisms to accumulate large amounts of heavy metals. Pb accumulation in the plants causes a various of health hazards. Our purpose is to study the effect of six concentrations of lead nitrate (0.50, 100, 150, 200 and 250ppm) at five repetitions on seeds of *Cleome amblyocarpa* Barr. & Murb stressed after 15 days of germination under laboratory conditions in the dark in an oven set at 26°C. The germination rate and seedling growth were analyzed. The seed germination rate decreases insignificantly relative to the control. The tigelles and radicles length of the seedlings and tolerance index decreases significantly with increasing levels of lead. The increase of Pb concentrations increases significantly the percent phytotoxicity on radicles length of *Cleome amblyocarpa* Barr. & Murb. The effects of Pb on germination show alteration depending to concentration, duration of exposure and seedling organs.

Key words: Lead (Pb), Metallic stress, *Cleome amblyocarpa* Barr. & Murb, Germination.

Introduction

Growing industrial sector and related anthropogenic activities are producing a lot of wastes in terms of solid and effluents (Aktar *et al*., 2009; Rajendiran *et al*., 2015; Solanki *et al*., 2018), which are having huge water potential and meagre amount of plant nutrients. In developing countries most of household waste merging in industrial waste and reach to farmers fields (Meena *et al*., 2015; Meena *et al*., 2019a). On the other hand, soil contains a range of heavy metals, which are carcinogenic and adversely affects the soil-plant nutrient dynamics and soil health (Rajendiran *et al*., 2018). Most common heavy metals are chromium (Cr), lead (Pb), arsenic (As), selenium (Se), mercury (Hg), cadmium (Cd), iron (Fe) and nickel (Ni). Among heavy metals, Pb has occupies a prominent place and is mainly contributed by paint, petroleum combustion, battery-based industries. As per international research organizations, lead is categorized as a potential pollutant and found to easily accumulate in soil and sediments containing significant amounts of clay or organic matter (Dotaniya *et al*., 2017a).

A number of heavy metals amputation technologies including ultrafiltration, chemical precipitation, ion-exchange, adsorption, electrodialysis, coagulation-flocculation, reverse osmosis and flotation are generally bring into play. These technologies are too expensive, unfavourable and unsafe to do away with heavy metals from contaminated sites. Exploiting micro-organisms and plant systems for remediation intentions is therefore a potential way out for pollution due to heavy metal in view of the fact that it includes sustainable decontamination methods to repair and restore the normal state of the rhizosphere and top soil. (Jagetiya and Purohit, 2006; Jagetiya and Porwal, 2019; Jagetiya and Sharma, 2013; Jagetiya *et al*., 2011; Yadav *et al*., 2017).

Cleome is the largest genus from Cleomaceae family, with over 200 species distributed in drier areas of the tropics and subtropics (Iltis, 1960). It consists mainly of annual or perennial herbaceous plants and, rarely, shrubs. Several species of Cleome are used in traditional medicine and many of them have been subject of pharmacological and phytochemical studies (Aparadh *et al.*, 2012). *Cleome amblyocarpa* Barr. & Murb is a species endemic to northern Sahara, which is mainly found in sandy-bottomed wadi beds, where it can colonize large surfaces. It is a perennial plant, of the *Capparidaceae* family branched, yellowish green, 10 to 40cm high, with odour foul and unpleasant. Straightened rods, trifoliolate leaves. Leaflets lanceolate, flowers purple and large number of fruits in elongated and hairy capsules (Ozenda, 1991; Quezel and Santa, 1963).

Heavy metals have been shown to affect different processes of plant functions and clearly have a negative impact on seed germination (Singh and Thakur, 2014). The prevention of sprouting and maturation of seedling is a general consequence of heavy metal exposure in plants (Radic *et al*., 2010). Seed germination and root elongation are two important physiological indicators in the early stage of plant growth to assess plant tolerance (Jing *et al*., 2018). In this context, this study aims to evaluate the effects of lead nitrate [$Pb(NO_3)_2$] of some germination parameters (final germination rate, kinetics of germination, radicles and tigelles length, tolerance index and percent phytotoxicity) of *Cleome amblyocarpa* Barr. & Murb.

Materials and Methods

Plant material

• *Cleome amblyocarpa* seeds: The seeds used have been harvested since September 2019, from a site traced by a national road RN6, in EL-BAYADH district located at (33°54′54.21″ N, 0°12′20′59″ E, South-East Algeria), in the high steppe plains of South-West Algeria (Fig. 1).

Seed treatment

Seed treatment is carried out during the month of February, 2020 at the biodiversity and soil and water conservation laboratory of Mostaganem University (UMAB), Algeria. The seeds are rinsed with water and then plunged for fifteen minutes in a solution of hypochlorite of sodium at 8°C. They are then washed thoroughly with distilled water and soaked in distilled water for 24 hours and placed in plastic Petri dishes (20 seeds per dish) on Whatman paper. And they are treated with 10 ml solution of 50, 100, 150, 200 and 250 ppm lead nitrate with five repetitions per treatment. Distilled water is used for the control seeds treatments (0 ppm) with the same volume.

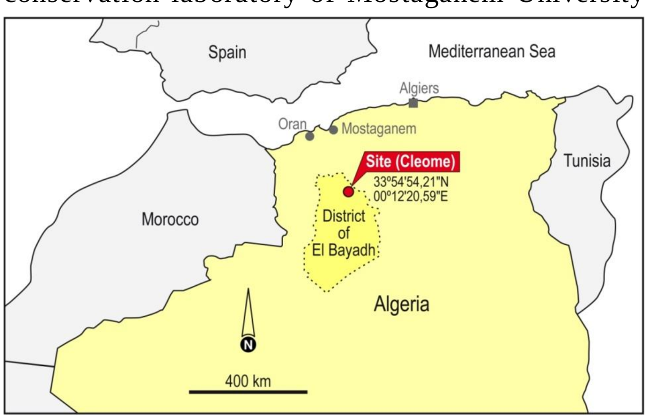

Fig. 1: Seed collection site.

Germination of seeds

Cleome seeds have been put to germinate in the dark in an oven set at 26°C, the duration of germination is fifteen days, each day the seeds are removed, counted to determine the seeds having germinated, then returned to germinate again in the oven. The radicle breakthrough criterion was adopted to evaluate seed germination. A seed is considered germinated when the white radicle fate out of the integument. These counts are repeated daily during throughout the germination period.

Parameters analyzed

• Final germination rate: A seed was considered germinated when the radicle pierced the envelope and became visible to the naked eye. And the final germination rate was calculated as given by Tanveer *et al*., (2010):

$$GR(\%) = \frac{\text{Germination seeds}}{\text{Total seeds}} \times 100$$

• Kinetics of germination: It is expressed as a daily percentage of seeds germinated in relation to the total number of seeds per Petri dish (%) (Mazliak, 1982), during fifteen days.

• Relative germination rate (RGR) and the radicle and tigelle length was measured, as well as the ratio between them.

$$RGR = \frac{\text{Germination percentage in metal concentration}}{\text{Germination percentage in the control}}$$

• 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 3rd day of the experiment until the end of the test.

• Tolerance indice (T.I.): was calculated using the formula given by Iqbal and Rahmati, (1992).

$$T.I. = \frac{\text{Mean root length in metal solution}}{\text{Mean root length in control}} \times 100$$

• Percent phytotoxicity (P.P.): was calculated according to Chou and Lin (1976) and Ray and Banerjee, (1981).

$$PP = \frac{\text{Radicle length of control - Radicle length of the test}}{\text{Radicle length of control}} \times 100$$

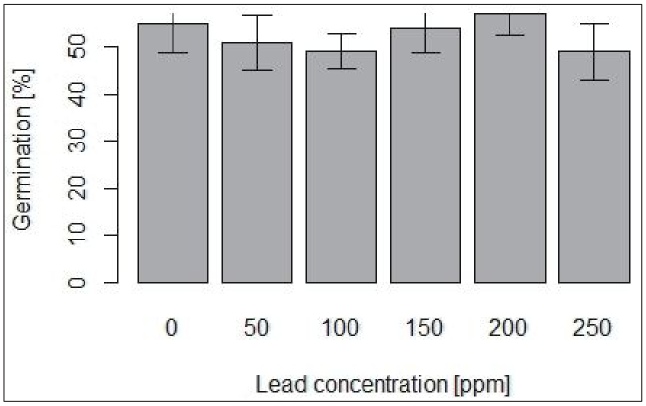

Fig. 2: Effect of lead on the germination rate of *Cleome amblyocarpa* Barr. & Murb.

• Statistical analysis: The results obtained were statistically analyzed using software R version 3.5.2 (2018-12-20), an ANOVA analysis of variance is carried out to study the possible effect of lead on the seed germination.

Results

Effect of lead on the germination rate of *Cleome amblyocarba* seeds

The results obtained show that the germination rate of *Cleome amblyocarba* seeds is higher than 45% for all $Pb(NO_3)_2$ doses applied (0, 50, 100, 150, 200 and 250 ppm). The highest values of germination rate of *Cleome amblyocarba* seeds are recorded in control seeds with a rate of 55% and seeds treated with 200 ppm with a rate of 57%. Then, the germination rate decreased in a non-significant way (F=0.396; P=0.846) where the following values were recorded: 51, 49, 54 and 49% respectively with the $Pb(NO_3)_2$ rates applied 50, 100, 150 and 250 ppm (Fig. 2).

Effect of lead on the germination kinetics of the *Cleoma amblyocarpa*

The germination rate index shows that the

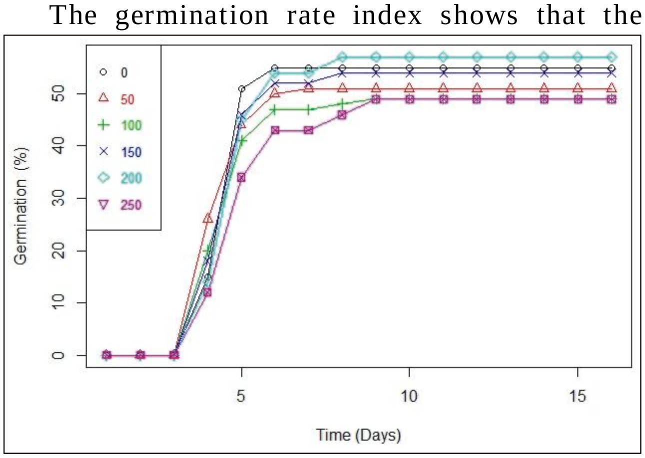

Fig. 3: Effect of lead on the germination kinetics of *Cleome amblyocarpa* Barr. & Murb seeds.

germination rate was significantly decreased compared to the control (F=2.375; P=0.043). Germination starts from the 3rd day with a germination rate is more than 12% for all seeds. Seeds treated at 0, 150 and 200 ppm Pb, germinated faster and reached a final germination rate on day 7 with values of 55%, 54% and 57% respectively with the applied rates, while slower germination was recorded in *Cleome amblyocarpa* seeds treated at rates of 50, 100 and 250 ppm Pb with values of 51%, 49% and 49% respectively with the applied rates of Pb. Constant germination was noted up to day 15, after seven days of germination (Fig. 3).

Effect of lead on relative germination rate (RGR)

The results corresponding to the RGR show that the effect of lead at different concentrations: 50, 100, 150, 200 and 250 ppm, with values 1.04, 0.9, 1, 1.14 and 0.98 respectively, does not significantly reduce the relative rate germination of *Cleome amblyocarpa*, even with an increase in concentrations (F=0.199; P=0.936) (Fig. 4).

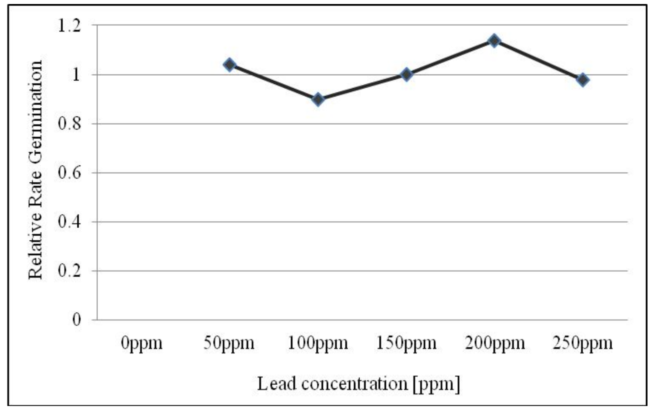

Fig. 4: Effect of lead on relative germination rate of *Cleome amblyocarpa* Barr. & Murb.

Effect of lead on the radicles length of *Cleome amblyocarpa*

After 15 days of exposure of *Cleome amblyocarpa* seeds to increasing concentrations of Pb, the results statistically analysed of radicles lengths reveals there is

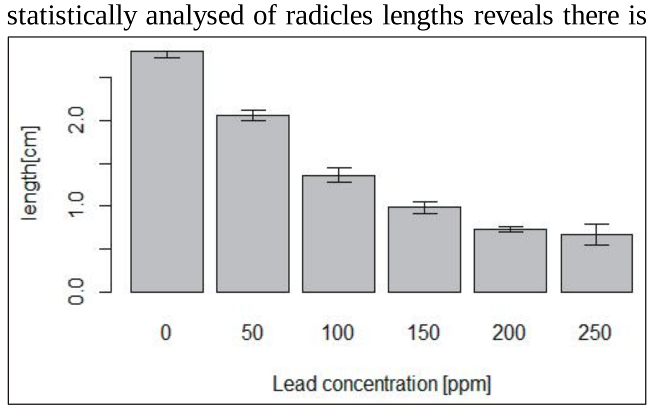

Fig. 5: Effect of lead on the tigelles length of *Cleome amblyocarpa* Barr. & Murb.

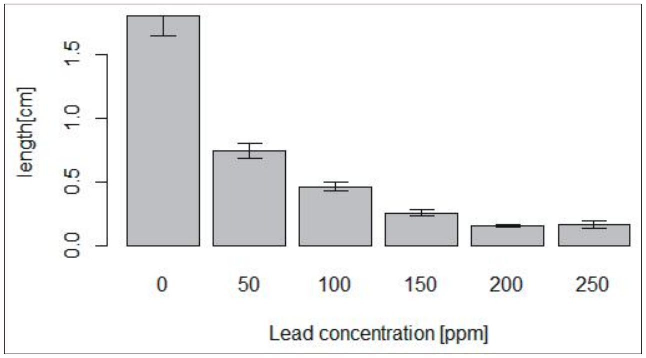

Fig. 6: Effect of lead on the radicles length of *Cleome amblyocarpa* seedlings.

a very significant decrease (F=79.47; P=0.000) for doses of 50, 100, 150, 200 and 250ppm of Pb, the root lengths are evaluated respectively at 0.75, 0.46, 0.25, 0.15 and 0.16 cm compared to the control where the maximal rootlet length is estimated at 1.80cm (Fig. 6).

Effect of lead on the tigelles length of *Cleome amblyocarpa*

Compared to the control, where tigelles length is estimated at 2.81 cm, *Cleome amblyocarpa* seedlings show a decrease in tigelles length; after 15 days of exposure to different concentrations of Pb at: 50, 100, 150, 200 and 250 ppm with 2.06, 1.36, 0.99, 0.73 and 0.67 cm respectively. These results show that metallic stress exerts an effect on the growth of *Cleome amblyocarpa* seedlings, resulting in a highly significant decrease (F=115.1; P<0.001) in the length of the aerial part as a function of the increase in Pb concentrations (Fig. 5).

Effect of lead on the radicle/tigelle ration of *Cleome amblyocarpa*

According to the results shown in fig. 7, *Cleome amblyocarpa* treated at different concentrations (0 to 250 ppm) of Pb, have a highly significant decrease of this parameter by concentration increment (F=29.07; P=0.000).

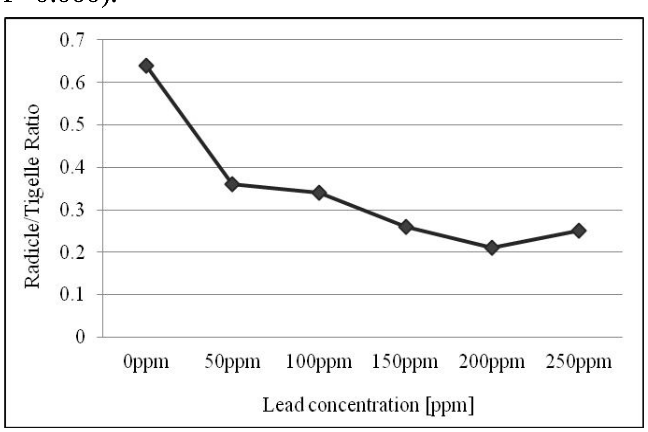

Fig. 7: Effect of lead on the radical/tigelle ration of *Cleome amblyocarpa* Barr. & Murb.

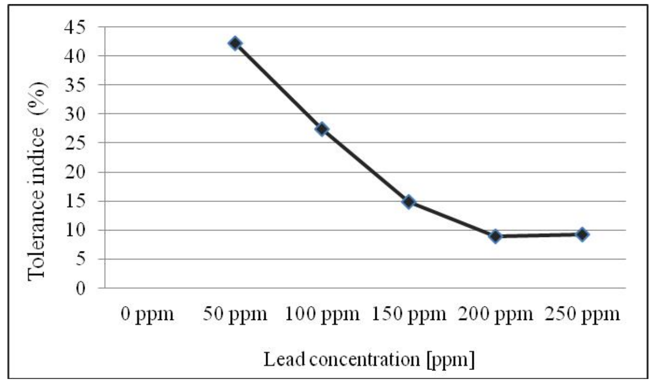

Fig. 8: Effect of lead on the tolerance index (T.I) of *Cleome amblyocarpa* Barr. & Murb.

Effect of lead on the tolerance indice (T.I) of *Cleome amblyocarpa*

The results in fig. 8 show that the presence of Pb at different concentrations have a positive impact on the tolerance index (T.I) of *Cleome amblyocarpa*, resulting in a very significant decrease (F=19.61; P=0.000). The highest tolerance index of Cleome is obtained in seeds treated at 50 and 100 ppm with values of 42.25, 27.42% respectively and the decrease of this parameter is recorded in seeds stressed by 150, 200 and 250 ppm with values of 14.83, 8.95 and 9.23% respectively with the doses compared to the control.

Effect of lead on the Percent phytotoxicity (P.P) of *Cleome amblyocarpa*

The results of the effect of different lead concentrations (50 to 250 ppm) on phytotoxicity on radicle length of Cleome fig. 9 show a highly significant increase of the percent phytotoxicity on radicles length by levels Pb increment (F=19.61; P=0.000). The values observed respectively (57.75, 72.58, 85.17, 91.05 and 90.77%).

Discussion

The study of the effect of Pb on the percentage of germination shows an insignificant inhibition on seeds germination of *Cleome amblyocarpa* Barr. & Murb.

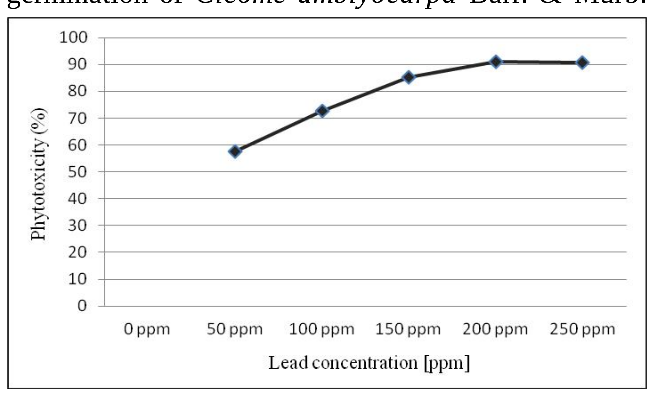

Fig. 9: Effect of lead on the percent phytotoxicity (P.P) of *Cleome amblyocarpa* Barr. & Murb.

However, the cleome seeds germination seemed to be more tolerant to Pb even at high Pb concentrations. The negative effect of metals on seed germination was reported by different authors (Mathur *et al.*, 1987; Iqbal and Mehmood, 1991; Athar and Masood, 2002; Li *et al.*, 2005; Datta *et al.*, 2011). Lead has adverse effects on germination and maturation even at micromolar level (Kopittke *et al.*, 2007). Muhammad *et al.*, (2008) recorded toxic effect on seed germination in *L. leucocephala* of various treatments of lead at 25, 50, 75 and 100 ppm. Kalimuthu and Siva, (1990) found reduced in seed germination in corn treated with 20, 50, 100 and 200 $\mu g.mL^{-1}$ lead acetate. Treatment of wheat with lead at 1, 2, 5, 10 and 20 mM reduced the germination process showing gradual reduction in germination with increase in concentration (Hasnain *et al.*, 1995). Lead has an inhibitory effect in the seed germination process of many plant species (Pandey *et al.*, 2007). The major effect of Pb is seen on the inhibition of germination enzymes such as protease and amylase which is synthesized during the germination. Pandey *et al.*, (2007) reported that amylase and protease activities decreased seed germination of *Catharanthus roseus* under high concentration of Pb. Lamhamdi *et al.,* (2011) observed that amylase activity in wheat seeds was reduced with increasing level of Pb. This inhibition might show that lead ions replace the calcium ions which are essential for the activities of these enzymes (Lamhamdi *et al.,* 2011).

Lead restrains the growth of both underground and above-ground parts of plants at low level (Islam *et al.*, 2007; Kopittke *et al*., 2007; Gupta *et al.,* 2010), but root is more affected as it accumulates higher amounts of lead (Liu *et al*., 2008). Morphological effect of Pb toxicity exhibits distended inclined and stumpy roots, profuse secondary roots per unit root length and cessation of root elongation (Kopittke *et al*., 2007; Arias *et al.,* 2010). The results show that lead caused an effect on the growth of *Cleome amblyocarpa* seedlings, resulting in a highly significant decrease ($P<0.001$) in the root and aerial part elongation with increasing in Pb concentrations. Heavy metals can engender damage to the root system of plants (Singh and Thakur, 2014), by causing an oxidative stress by producing free radicals (Shah *et al*., 2010), or by replacing nutrient and essential metals (Henry, 2000). Aoumeur, (2012) showed a 50% inhibition of root growth of lead-treated radish. Kranner and Colville, (2011) confirmed the inhibition of root growth in more than 15 plant species in the presence of increasing lead concentrations. Lerda, (1992) made similar observations in roots of on *Allium cepa*.Lead treatment showed greater toxic effects on root growth of *L. leucocephala* (Muhammad *et al*., 2008). The reduction in root length in metal treatments could be due to reduced mitotic cells in meristematic zone of root (Muhammad *et al*., 2008). These findings confirm that metal treatment reduced the frequency of mitotic cell in meristematic zone and are responsible for inhibition in root growth. The reason for different response of seedling and root growth to heavy metals is not known but might be due to rapid accumulation of heavy metals in root than shoot or to faster rate of detoxification in shoot than root. Generally, the roots of cleome are seen to be more affected by an increase in Pb doses than the shoots. 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). This sensibility reduced per consequence of the root/shoot ratio. El-rasafi *et al.,* 2016) reported that decrease in the root/shoot ratio may be caused due to structural and morphological changes of roots (absence of root hair, stunted and fibrous root growth and thickening or browning of roots) induced by metal.

The tolerance index examined is low at 150, 200 and 250 ppm compared to control. The increase in Pb concentrations have a significant decrease of lead toxicity tolerance of *Cleome amblyocarpa* Barr. & Murb. The reason for low tolerance against lead might be due to changes in the physiological mechanism in seed germination and seedling growth of plant (Shafik *et al*., 2008).Shafiq and Iqbal, (2005) reported similar results for low tolerance in *Cassia siamea* seedlings at 100 ppm of lead treatment as compared to control. The increase in Cd and Zn concentrations had a significant decrease of metal toxicity tolerance of bean (*Phaseolus vulgaris* L.) and wheat (*Triticum aestivum* L.) seedlings (El-rasafi *et al.,* 2016).

On the other hand, the lead phytotoxicity test indicates that the lowest concentrations of Pb induce a decrease of phytotoxicity, while high levels Pb increased the root phytotoxicity of *Cleome amblyocarpa* Barr. & Murb. El rasafi *et al.,* (2016) reported that increase of Cd levels increased significantly the percent phytotoxicity on root length of both species, the lowest percent phytotoxicity was observed at the lowest concentration (14.92 and 44.58% for wheat and bean, respectively). As regard Zn, the low doses (from 10 to 100 mg/l) were more beneficial for the growth of wheat, while the highest levels were highly toxic to roots. These finding were in concordance with the results obtained by Gang *et al.*, (2013); Habtamu *et al.,* (2013) and Shaikh *et al.,* (2013) who reported that phytotoxicity of heavy metals on roots decreased at lower concentrations and increased at higher concentrations.

Conclusion

The results of sprouting test on the different parameters studied show that leads (Pb) have a negative effect on the germination rate of *Cleome amblyocarpa* seeds. The increasing concentrations of Pb (50 to 250 ppm), leads to various phytoxicity, result in a significant reduction in the radicle and aerial elongation of seedlings relative to the control. The lead tolerance index for *Cleome amblyocarpa* Barr. & Murb decreases significantly and show a little tolerance at 200 ppm and 250 ppm. Lead treatment show significant increase of percent phytotoxicity at higher Pb concentrations. It is suggested that *Cleome amblyocarpa* be grown in lead-contaminated soils to study the tolerance stress of this plant.

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