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Research note

Toxicidad del plomo en la germinación y el crecimiento de plántulas de *Parkinsonia aculeata* L.

Lead toxicity in germination and growth of *Parkinsonia aculeata* L. seedlings

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Resumen

Las elevadas concentraciones de plomo (Pb) en el suelo limitan el establecimiento de las plantas, por lo que es importante identificar especies tolerantes. El objetivo del presente estudio consistió en determinar el efecto de $PbCl_2$ y $Pb(NO_3)_2$ en la germinación y crecimiento temprano de *Parkinsonia aculeata*. En laboratorio se imbibieron las semillas en soluciones de $PbCl_2$ y $Pb(NO_3)_2$ a concentraciones de 0.0, 0.1, 0.2, 0.5, 1.0 y 2.0 mM. Las semillas se pesaron al inicio y al final (48 h); posteriormente, se incubaron y regaron con sus respectivas soluciones (7 días). En invernadero, las semillas se sembraron en arena y se regaron (15 días) para determinar el porcentaje de germinación (laboratorio) y emergencia (invernadero); las variables consideradas fueron: longitud de vástago, radícula y peso seco (ambas condiciones). Bajo condiciones controladas, $PbCl_2$ y $Pb(NO_3)_2$ no afectaron la imbibición, pero al aumentar sus concentraciones, redujeron significativamente la germinación en 18 y 26 %, respectivamente, así como el peso seco del vástago (40 y 49 %), y radícula (40 y 57 %). En invernadero, los compuestos de Pb no redujeron la emergencia, pero inhibieron el crecimiento de radícula; mientras que el $Pb(NO_3)_2$ inhibió en mayor proporción su peso seco. El efecto del Pb puede diferir en función del compuesto de Pb utilizado. El $Pb(NO_3)_2$ presenta una mayor toxicidad.

Palabras clave: Contaminación, imbibición, leñosa, metal pesado, palo verde, tolerancia.

Abstract

The high lead (Pb) concentrations in the ground limit the establishment of plants, which makes it necessary to identify tolerant species. The objective of this study was to determine the effect of $PbCl_2$ and $Pb(NO_3)_2$ on the germination and early growth of *Parkinsonia aculeata* seedlings. In the laboratory, the seeds were soaked in solutions of $PbCl_2$ and $Pb(NO_3)_2$ at concentrations of 0.0, 0.1, 0.2, 0.5, 1.0 and 2.0 mM. The seeds were weighed at the beginning and at the end (48 h); later, they were incubated and watered with their respective solutions (7 days). In the greenhouse, the seeds were sown in sand and watered (15 days) to determine the germination percentage (laboratory) and emergence (greenhouse): stem length, radicle and dry weight (both conditions). In the laboratory, $PbCl_2$ and $Pb(NO_3)_2$ did not affect imbibition, but by increasing their concentrations, they significantly reduced germination by 18 and 26 %, respectively, as well as the dry weight of the stem (40 and 49 %), and radicle (40 and 57 %). In the greenhouse, the Pb compounds reduce emergence little, and did inhibit radicle growth; while $Pb(NO_3)_2$ inhibited in greater proportion the dry weight. The effect of Pb may differ depending on the Pb compound used, with $Pb(NO_3)_2$ presenting greater toxicity.

Key words: Pollution, imbibition, woody, heavy metal, Mexican *Palo Verde*, tolerance.

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Lead (Pb) is an element naturally present in ecosystems in low concentrations. However, alterations in geochemical cycles, caused by human activities, mainly mining, have contributed to its increase in the biosphere (Shahmoradi *et al.,* 2020). This causes a serious problem in the ecosystems of urban or agricultural areas, since Pb is toxic in high concentrations (Candido *et al.,* 2020), it is not subject to biodegradation processes and tends to accumulate in the soil (Shi *et al.,* 2019). Faced with this problem, tree species receive greater attention in the recovery of contaminated areas, due to their longevity and high biomass production (Mleczek *et al.,* 2017), which contributes to the immobilization of large amounts of metals.

Some Fabaceae species have proved tolerance to this element and are hyperaccumulators (Abbas *et al.,* 2017). Among them, *Parkinsonia aculeata* L. stands out, which is a woody species that is naturally distributed in the American continent (van Klinken *et al.,* 2009). Furthermore, it has low nutritional requirements and is tolerant to drought (Chaer *et al.,* 2011). During the germination and growth stage, plants are sensitive to pollution; therefore, these stages are used in preliminary evaluations for selecting and describing tolerant plants (Márquez-García *et al.,* 2013).

In this context, the objective of this work was to determine the effect of $PbCl_2$ and $Pb(NO_3)_2$ on the germination and early growth of *P. aculeata* seedlings in the laboratory and greenhouse.

The experiment was carried out in the facilities of the *Colegio de Postgraduados*. The seeds of *P. aculeata* were collected by the National Forestry Commission in *Delicias,* state of *Chihuahua*, Mexico (28°12'60" N, 105°27'15" W; 1 190 m altitude) and were scarified with H_2SO_4 at 98 % for 3 h (Mohnot and Chatterji, 1965). Afterwards, they were rinsed with running water for 10 min and disinfected with 10 % NaClO for 15 min. In the laboratory (*Microbiología de suelo del Colegio de Postgraduados*), the effect of $PbCl_2$ and $Pb(NO_3)_2$ on the imbibition of *P. aculeata* seeds was assessed. Each compound was applied in the concentrations: 0.1, 0.2, 0.5, 1.0 and 2.0 mM and a control treatment (distilled water), which were based on the existing literature (Muszyńska *et al.,* 2018) (Table 1). Both compounds were used to know the effect of the Pb from two sources in the early stages of the species and to rule out any influence of the accompanying anions of the compounds, mainly $(NO_3)_2$, on plant growth.

Table 1. PH and electrical conductivity (EC) values of the solutions of the Pb compounds used.

Concentration (mM)	$PbCl_2$		$Pb(NO_3)_2$	
	pH	**EC (mS)**	**pH**	**EC (mS)**
0.0	6.1	0.00	6.10	0.00
0.1	5.60	0.01	5.62	0.01
0.2	5.51	0.05	5.50	0.04
0.5	5.39	0.10	5.46	0.10
1.0	5.35	0.20	5.35	0.22
2.0	5.08	0.48	5.20	0.46

The increase in weight of the seeds was determined by difference between the initial weight (0 h) and at 48 h of imbibition (Monroy-Vázquez *et al.,* 2017) was recorded. The same seeds were deposited in polystyrene boxes (14 × 14 × 7 cm), on Whatman No.1 filter paper and 15 mL of the corresponding treatment solution were added to each box and kept in dark at 30 ± 1 °C in an incubator (GI11, Shel Lab™) for 7 days (Monroy-Vázquez *et al.,* 2017); each treatment had five replications of seven seeds.

In the greenhouse sowing, the same treatments were used as in the laboratory, with six replications and ten seeds each. Seeds were kept immersed in the solutions as described previously, to ensure homogeneous imbibition (Mohnot and Chatterji, 1965). The seeds were sown in sand, contained in pots of 0.95 L. The sand was sterilized at 126 °C for 3 h (PRESTO™, 79291), for 3 alternate days. 30 mL of solution was added to each experimental unit every third day, depending on the treatment. Fertilization was not applied for the duration of the experiment and to avoid an alteration in the solubility of the Pb compounds. The experiment was carried out with natural light, an average maximum temperature of 36 °C and a minimum of 16 °C, and a maximum relative humidity of 37 % and a minimum of 14 % (Data Logger 1000, watch dog™) for 15 days.

At the end of each experiment, the following variables were evaluated: final percentage of germination in the laboratory and final percentage of emergence in the greenhouse. Seedling growth, stem and root length (cm), in both conditions. Seedling dry weight (g) was estimated after drying in a CE5F Shel Lab^{TM} oven at 70 ° C for 72 h.

The experimental design was completely randomized and the data obtained, after testing the assumptions of normality and homogeneity of variances, were analyzed using a nested analysis of variance model (the concentrations within the source of Pb) and the mean comparison test (Tukey, $P<0.05$). For the germination percentage, the Kruskall-Wallis test and the Wilcoxon rank sum ($P \leq 0.05$) were performed using the SAS program for Windows^{TM} (SAS Institute, 1999).

The effect of both Pb compounds on the imbibition of *P. aculeata* was not significant ($P> 0.05$). According to Kranner and Colville (2011), Pb compounds reduce the germination process because they are salts, since they induce saline stress that prevents the seed to reach the critical threshold for imbibition. However, in the seeds of some species, Pb compounds do not affect the process (Ilić *et al*., 2015), as observed in the present experiment.

The utilized Pb compounds significantly inhibited germination under laboratory conditions, where the 2.0 mM concentration of $PbCl_2$ and $Pb(NO_3)_2$ inhibited germination by 18 and 26 %, respectively (Table 2). This may be due to the toxic effect of Pb ions on the embryo (Kranner and Colville, 2011). Although the seed coat may limit the entry of metal ions, some seeds may have a certain degree of permeability to Pb ions. (Wierzbicka and Obidzinsk, 1998; Ilić *et al.,* 2015). On the other hand, under greenhouse conditions, germination did not have significant differences among the concentrations of the two Pb compounds (Table 3). Wierzbicka and Obidzinsk (1998) suggest that the amount of Pb per unit mass of the seeds is an important factor and that its inhibitory effect is only possible when Pb is available in excess to the seeds.

Table 2. Effect of $PbCl_2$ and $Pb(NO_3)_2$ on seed germination and seedling growth of *Parkinsonia aculeata* L. in the laboratory.

Concentration (mM)	Germination percent (%)	Stem length (cm)	Radicle length (cm)	Stem dry weight (g)	Radicle dry weight (g)
$PbCl_2$					
0.0	100 a†	9.6 a‡	4.7a‡	206.7 a‡	20.3 a‡
0.1	97 ab	9.2 ab	4.2 a	200.8 a	19.1 ab
0.2	97 ab	8.9 abc	4.0 ab	193.3 abc	16.9 abc
0.5	97 ab	6.6 bcd	1.6 bc	158.0 bcd	14.0 cd
1.0	83 bc	6.2 de	1.3 bc	154.2 cde	12.1 cde
2.0	82 c	3.8 ef	0.7 b	124.8 e	10.5 de
$Pb(NO_3)_2$					
0.0	100 a	9.6 a	4.7 a	206.7 a	20.3 a
0.1	97 ab	9.3 a	4.2 a	200.3 ab	19.0 ab
0.2	97 ab	9.2 ab	3.0 bc	195.6 abcd	18.2 ab
0.5	92 bc	6.5 cd	1.6 bc	151.2 cde	14.5 bcd
1.0	86 abc	5.8 de	0.9 c	147.5 de	10.7 de
2.0	74 c	3.0 f	0.8 c	122.2 e	8.6 e

†Different letters in the same column indicate significant differences, Wilcoxon ($P \leq 0.05$).

‡Different letters in the same column indicate significant differences, Tukey ($P \leq 0.05$).

Table 3. Effect of $PbCl_2$ and $Pb(NO_3)_2$ on seed emergence and seedling growth of *Parkinsonia aculeata* L. in the greenhouse.

Concentration (mM)	Emergence percent (%)	Stem length (cm)	Radicle length (cm)	Stem dry weight (g)	Radicle dry weight (g)
			$PbCl_2$		
0.0	93 a†	7.1 ab‡	11.5 a‡	389.5 ab‡	92.1 a‡
0.1	90 a	7.0 ab	9.7 ab	393.3 a	90.3 ab
0.2	90 a	7.5 a	9.7 ab	380.5 ab	90.5 ab
0.5	87 a	7.5 a	8.4 b	354.0 b	86.8 ab
1.0	90 a	7.5 a	8.9 b	383.5 ab	86.0 ab
2.0	90 a	7.5 a	8.9 b	363.7 ab	88.8 ab
			$Pb(NO_3)_2$		
0.0	93 a	7.1 ab	11.5 a	389.5 ab	92.1 a
0.1	88 a	7.2 ab	9.3 ab	392.7 ab	89.0 ab
0.2	95 a	6.6 b	9.2 ab	376.0 ab	84.0 ab
0.5	90 a	6.9 ab	9.0 b	382.3 ab	78.9 ab
1.0	90 a	7.3 ab	8.8 b	384.7 ab	78.2 ab
2.0	90 a	6.9 b	8.1 b	356.0 ab	76.6 b

†Different letters in the same column indicate significant differences, Wilcoxon ($P \leq 0.05$). ‡Different letters in the same column indicate significant differences, Tukey ($P \leq 0.05$).

In the laboratory, a significant inhibition ($P \leq 0.05$) was observed in the growth of the stem and radicle of the seedlings, and in the dry matter yield of *P. aculeata* (Table 2), mainly at concentrations of 2.0 Mm. $PbCl_2$ in its highest concentration (2.0 Mm) inhibited up to 40 % the dry weight of the stem, and 49 % the radicle. The seedlings treated with $Pb(NO_3)_2$ showed an inhibition of 40 %: however, the radicle of these seedlings exhibited an inhibition of up to 57 % in their dry weight (Table 2).

In the greenhouse test, a significant inhibition ($P \leq 0.05$) in radicle growth (Table 3) by $PbCl_2$ and $Pb(NO_3)_2$ was observed in 22.6 % and 25.7 % respectively, but they affected to a lesser extent the yield in dry matter (5 %).

$Pb(NO_3)_2$ significantly inhibited ($P \leq 0.05$) radicle growth, and the dry matter yield decreased to 10 %. The inhibition of growth and dry matter yield is a well-known effect of lead at high concentrations (Iqbal *et al.,* 2017); being the root more affected than the shoot, because the root is in direct contact with Pb (Lamhamdi *et al.,* 2011). The effect of Pb on the germination and early growth of *P. aculeata* seedlings differs in terms of the Pb compounds used, thus solubility,availability and phytotoxicity of Pb compounds play an important role (Truta *et al.,* 2011; Iqbal *et al.,* 2017). Therefore, a greater inhibition was achieved with $Pb(NO_3)_2$ than with $PbCl_2$. In the laboratory, the inhibition caused by both Pb compounds is more pronounced compared to the greenhouse. The results generate information for future work on the tolerance of *P. aculeata* to soils contaminated with Pb.

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Conflict of interests

The authors declare no conflict of interest.

Contribution by author

Manuel Arturo González Villalobos: research development, statistical analysis, structure and design of the manuscript; Tomás Martínez Trinidad: supervision of the experiment, design, analysis of the results, proofreading of the manuscript; Alejandro Alarcón: supervision and proofreading of the manuscript; Francisca Ofelia Plascencia Escalante: supervision and proofreading of the manuscript.

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