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Differential effect of manganese on the germination of *Triglochin striata* (Juncaginaceae) and C*otula coronopifolia* (Asteraceae) in Laguna de Carrizal Bajo wetland, Atacama Region, Chile

Efecto diferencial de manganeso sobre la germinación de *Triglochin striata* (Juncaginaceae) y *Cotula coronopifolia* (Asteraceae) en el humedal Laguna de Carrizal Bajo, Región de Atacama, Chile

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RESUMEN

Manganeso es un metal traza cuyos efectos a nivel de semilla no han sido estudiados en profundidad. En el humedal de Carrizal Bajo se han documentado altos niveles de Mn, como aporte combinado de las actividades antrópicas y de la roca madre. En este estudio se evalua experimentalmente el efecto de distintos tratamientos de Mn (0,0; 0,001; 0,1 y 10,0 ppm) sobre la germinación de *Triglochin striata* y *Cotula coronopifolia*, dos macrófitas presentes en el humedal. Nuestros resultados mostraron que la germinación de *Cotula* disminuyó de 60 a 10 % en los tratamientos con Mn, mientras que la germinación de *Triglochin* se mantuvo en torno al 16 %, sin mostrar diferencias significativas entre los tratamientos. Estos antecedentes sugieren que Mn puede afectar diferencialmente la germinación de las especies del humedal.

Manganese (Mn) is an essential micronutrient for the metabolism of vascular plants (Broadley *et al.* 2012), but it can be toxic when pH, anaerobic conditions and/or drainage favor its bioavailability and absorption (Millaleo *et al.* 2010). Various studies have documented that Mn damages adult plants, inducing the appearance of leaf spots (Führs *et al*. 2008, Kosiada 2013), loss of biomass (Mora *et al*. 2009, Najeeb *et al*. 2009), chlorosis (Moroni *et al*. 2003, Rosas *et al*. 2007) and necrotic lesions (Moroni *et al*. 2003). In early stages (*i.e.*, seedlings), the presence of Mn decreases growth (Arya & Roy 2011, Lee *et al.* 2011) and causes alterations in stomata formation and root development (Lidon 2002), hindering the absorption of other essential nutrients (Paschke *et al.* 2005) and results in the death of seedlings (McQuattie & Schier 2000). The effect of Mn on seeds has been less studied: Santandrea *et al.* (1997) and Rajjak *et al.* (2013) showed that at concentrations above 10 ppm the germination of *Nicotiana tabacum* L. and *Triticum aestivum* L. is reduced progressively, as a result of oxidative stress and damage of the lipid component of the plasma membrane (Bewley 1986, Todorović 2008). However, there are macrophytes that can tolerate and accumulate high concentrations of trace metals, including Mn (Cooper 1984, Karlsons *et al.* 2011), thus these have been used for remediation purposes (Najeeb *et al*. 2009). In this context, to know the ability of seeds to germinate on environments with variable concentrations of Mn, could be important as a first step to ensure the establishment and persistence of remedial species in natural and artificial water systems (Kranner & Colville 2011).

In the arid ecosystems of the Northern Chile, Mn can be an important trace metal in surface watercourses, with concentrations that can reach 8.0 ppm (DGA 2004). This situation is a consequence of combined contribution of Mn from the natural erosion of the bedrock and from liquid industrial residues derived from the region's mining activity (DGA 2004). Because Mn can accumulate in coastal marshes and wetlands (Bordean *et al.* 2014), it is particularly important to evaluate a possible effect on the plants. Thus, the objective of this study was to analyze under laboratory conditions the germinative response of two macrophytes, *Triglochin striata* Ruiz & Pav., Juncaginaceae (*Triglochin* hereafter) and *Cotula coronopifolia* L., Asteraceae (*Cotula* hereafter), which grow in the Laguna de Carrizal Bajo wetland.

The Laguna de Carrizal Bajo wetland (28° 4' 60" S; 71° 8' 35'' O) is located on the coast of the Atacama Region (Fig. 1). Its climate is coastal desert, with temperatures of 10 °C - 20 °C and precipitations that vary between 10-25 mm per year (Juliá *et al.* 2008). The flora is composed mainly of species like *Distichlis spicata* (L.) Greene (Poaceae), *Sarcocornia fruticosa* (L.) A.J. Scott (Amaranthaceae) and *Typha angustifolia* L. (Typhaceae). Although they are common species, these taxa were not included in the present study because they do not have seeds available during the sampling season.

In January 2011, ripe fruits of *Triglochin* and *Cotula* were collected on the western edge of the wetland. These seeds were collected from 10 individual plants in both species. In the laboratory, were extracted manually near 1,500 seeds of *Triglochin* and 3,000 of *Cotula*; seeds collected were assigned to experimental treatments with Mn (Guerrero-Leiva 2012). For this proposal, seeds of each species were mixed in order to randomize the effects due to its maternal origin. These seeds were disposed in Petri dishes, covered with culture medium (agar 2% and Captan fungicide 1%) enriched with Mn (Guerrero-Leiva 2012). Culture media were prepared with three Mn concentrations: 0.001, 0.1 and 10.0 ppm, from a stock solution of 1,000 ppm of $MnCl_2$ in H_2O (Merck Titrisol®). These were determined based on Mn concentrations documented in several watercourses in the Northern Chile (DGA 2004). A control treatment (without addition of Mn; 0.0 ppm) was also established. Although exist evidence that usually the macrophytes require light to germinate (Naidoo & Naicker 1992, Najeeb *et al*. 2009), in our preliminary tests in culture media agar (unpublished data) we observed a higher germination of *Triglochin* and *Cotula* in darkness. In concordance to this, all the dishes were kept in the dark at temperatures between 22 °C (day) and 15 °C (night), in a culture room. According to the

Figure 1. Geographical location of the Laguna de Carrizal Bajo wetland.

Figura 1. Localización geográfica del humedal Laguna de Carrizal Bajo.

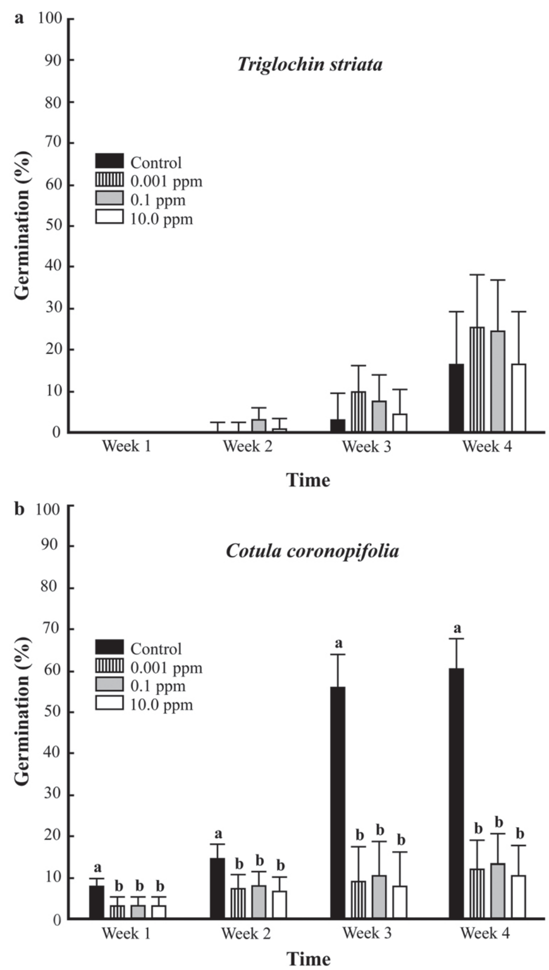

Figure 2. Percentage germination (mean ± S.D.) accumulated under different Mn concentrations and control treatment. a) *T. striata* (P = 0.36), b) *C. coronopifolia* (P < 0.05). The different letters over the bars indicate treatment groups that differ from one another (Tukey test).

Figura 2. Porcentaje de germinación (promedio ± D.E.) acumulado bajo diferentes concentraciones de Mn y tratamiento de control. a) *T. striata* (P = 0,36), b) *C. coronopifolia* (P < 0,05). Las diferentes letras sobre las barras indican los grupos de tratamiento que se diferencian entre sí (prueba de Tukey).

parameters reported by the DGA (2004), pH is around 7 on watercourses from Northern Chile, so we decided to use this condition in our experiments.

On separate dishes, 30 seeds of *Triglochin* and 50 of *Cotula* were sown. In both cases three replicates per treatment were ran. Germination in the dishes was evaluated during one month, recording the germination when the radicle was ≥ 2 mm long. Then the percentage germination accumulated each week was calculated as 100 × number of germinated seeds × (number of seeds sown)$^{-1}$. Finally, the accumulated percentage germination was analyzed with repeated measures ANOVA. The Mn concentration (with four levels: 0.0, 0.001, 0.1 and 10.0 ppm) was considered the independent factor, and the percentage germination accumulated (root-transformed) in each week was the dependent variable. When it was relevant, the Tukey test was used as a *post hoc* test.

The results showed that the presence of Mn in the culture medium generated differential responses in the germination of *Triglochin* and *Cotula*. On the one hand, the germination of *Triglochin* was not affected (Table I) because the average final germination in the control was 16.7% (± 5.8 S.D.), while it reached 16.6% (± 5.8 S.D.) in the highest concentration treatment (10.0 ppm) (Fig. 2a). On the other hand, the germination of *Cotula* was reduced significantly (Table I); in the control the average final germination was 60.7% (± 8.5 S.D.) while in the highest concentration treatment (10.0 ppm) the average final was 10.7% (± 5.5 S.D.) (Fig. 2b). Interestingly, the effect on *Cotula* was not dose-dependent because in all the Mn treatments the germination was reduced with respect to control (Fig. 2b).

In this study we performed a first approach to observe the effect of Mn upon germination of *Triglochin* and *Cotula*, under laboratory conditions that are not necessarily generalizable to the field conditions. Thus, the results should be carefully interpreted in terms of its scope and limitations. First, the pH conditions determine the oxidation state and therefore the metal bioavailability. In our trials, we only use neutral pH as condition following value documented by DGA (2004) for superficial water from Northern Chile. Second, because the seeds were collected in a site with high concentration of Mn, an important aspect to study is analyze the possible maternal uptake of this trace elements during seed formation (Kranner & Colville 2011), and how this contribution could have conditioned the germination response of species. However, our findings are a valuable first step to begin to understand how the presence and concentration of Mn affects a critical stage of the life cycle (*i.e*. the seed) of the macrophytes studied.

A question that arises from the results is: why did the germination of *Triglochin* not respond to the experimental levels of Mn? According to current studies can be suggested that genus *Triglochin* have a greater capacity to tolerate environments enriched with Mn. In fact, studies made on other species as *Triglochin maritima* L. (Cooper 1984) have shown that this taxa has the ability to germinate and grow in media enriched with Mn (> 275 ppm) without morpho-physiological damage. Additionally, Cooper (1984) and Karlsons *et al.* (2011) have reported that *Triglochin maritima* and *Triglochin palustre* L. can accumulate trace metals as Mn, Cu, Fe and Zn, while *Triglochin striata* can accumulate Mn, Cu, Fe, Zn, Cd, Cr and Pb (Chenhall *et al*. 1992, Almeida *et al.* 2011). Longer duration experiments may test the ability of seedling establishment of *Triglochin striata* under field conditions.

Table I. Repeated measures ANOVA for the germination of *Triglochin* and *Cotula* in response to the concentration of Mn in the culture medium over time.

Tabla I. ANOVA de medidas repetidas para la germinación de *Triglochin* y *Cotula* en respuesta a la concentración de Mn en el medio de cultivo, a lo largo del tiempo.

Triglochin	SS	DF	MS	F	P
Concentration	6.9	3	2.3	1.2	0.367
Time	93.4	2	46.7	96.6	< 0.001
Concentration × Time	3.8	6	0.6	1.3	0.306
Cotula	SS	DF	MS	F	P
Concentration	67.9	3	22.6	44.6	< 0.001
Time	45.6	3	15.2	94.1	< 0.001
Concentration × Time	24.8	9	2.76	17.0	< 0.001

Although Mn is an essential micronutrient (Broadley *et al.* 2012), its presence in all treatments drastically reduced the germination of *Cotula*. The experiments do not allow determining the causal mechanism of this effect, but future trials could establish whether the presence of Mn slows the germination or causes the death of seeds (Bewley 1986, Todorović *et al*. 2008, Kranner & Colville 2011). Possible responses may be related to bioavailability of manganese under pH levels, the permeability of the seed coat and possible dormant state of ungerminated seed.

In localities of northern Chile the concentration of Mn in watercourses can vary over a range of 0.01 to 8.0 ppm (DGA 2004). These levels exceed the Chilean standard NCh1333 for this metal (0.20 ppm; INN-Chile 1987), so it is very important to determine not only the sources (*i.e.*, natural *versus* anthropic), but also their effect on the conservation for the local biota (Marquet *et al.* 2012). Our results indicate that it is relevant to carry out studies to evaluate the ability of the flora associated with these ecosystems to tolerate and accumulate Mn (as well as other trace elements) as potential remediation taxa, and at the same time directing efforts to detect taxa whose local persistence is compromised as a consequence of high concentration of Mn, like those detected in the Laguna de Carrizal Bajo wetland.

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