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Observations regarding accumulation of metals in wild *Selliera radicans* Cav. in wetland environments of Central Region of Chile

Observaciones en la acumulación de metales en Cav. Selliera radicans *silvestre en ambientes de humedal de la Región Central de Chile*

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ABSTRACT

Selliera radicans Cav. is a creeping plant native to New Zealand, Australia, and Chile, found in wetland environments. Leaves of cultivated *S. radicans* plants are promising sources of food with beneficial health properties. The concentration of metals in soils and leaves of wild *S. radicans* from marshes (Vichuquén and Torca) and coastal wetlands (Putú) considered contaminated (Maule Region, Chile) was evaluated by Flame Atomic Absorption Spectroscopy. According to the results, the analyzed soils are contaminated with heavy metals (Cu, Cr, Zn, and Ni), although their pollution levels are low. Wild *S. radicans* leaves had Mn and Zn in concentrations higher than those allowed in edible plants. These results support the popular perception of considering the investigated sites as polluted. Wild *S. radicans* can colonize metalliferous soils and act as a metal bioindicator of environmental contamination in Vichuquén-Torca and Putú wetlands.

Keywords: Goodeniaceae, halophyte, heavy metals, marsh.

RESUMEN

Selliera radicans *Cav. es una planta rastrera nativa de Nueva Zelanda, Australia y Chile, que crece en humedales. Las hojas de plantas cultivadas de* S. radicans *son una fuente promisoria de alimento con propiedades benéficas para la salud. Se evaluó por espectroscopia de absorción atómica de llama la concentración de metales en suelos y hojas de* S. radicans *silvestres que crecen en pantanos (Vichuquén y Torca) y humedales costeros (Putú) considerados áreas contaminadas (Región del Maule, Chile). De acuerdo con los resultados, los suelos analizados están contaminados con metales pesados (Cu, Cr, Zn y Ni), aunque sus niveles de polución son bajos. Las hojas de plantas silvestres de* S. radicans *mostraron concentraciones de Mn y Zn superiores a las permitidas para plantas comestibles. Estos resultados contribuyen a la percepción popular de considerar los sitios investigados como contaminados. Las plantas silvestres de* S. radicans *pueden colonizar suelos metalíferos y actuar como un bioindicador de la contaminación ambiental en parte de los humedales Vichuquén - Torca y Putú.*

Palabras claves: *Goodeniaceae, halófita, metales pesados, pantano.*

Introduction

According to eHALOPH data, in Chile, there are 138 halophyte species distributed in 31 families. More than 80% of these species are herbaceous, and about 55% are exotic (Orrego *et al*., 2018). *Selliera radicans* Cav. is one of these species, native to New Zealand, Australia, and Chile (Allan, 1961). According to archaeological studies, *S. radicans* was recognized as one of the foods consumed by the oldest human settlement in America (Monte Verde, Chile) had a diet high in plant components (Dillehay, 1983). Recent studies report that cultivated *S. radicans* leaves are promising sources of foods with antioxidant capacities, and inulin and minerals that offer beneficial health properties (Soriano *et al*., 2021).

The wide range of edaphoclimatic variables that characterize Chile is reflected in its large and specific

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vegetal biodiversity. *S. radicans* grows naturally from Atacama Region to Aysén del General Carlos Ibañez del Campo Region, in riparian zones near rivers, lakes, and the sea. Local inhabitants know this creeping herbaceous plant as "hierba de las marismas". It is characterized by stolons that hold nodal fibrous roots, succulent green, shiny leaves, and small white flowers (Schiappacasse *et al.*, 2017).

Central Chile is considered abundant in coastal wetlands, including rivers or channels that reach the sea, whose waters mix with seawater constituting marshes. Halophytic plant species residing in saltwater marshes resist the changing conditions of salinity and humidity (Ramírez and Álvarez, 2012). The hydrography present in Lake Vichuquén basin crosses five terrestrial ecosystems in its way before discharging to the Pacific Ocean: i) hills and high mountains; ii) valleys; iii) Coastal Range represented by hills and piedmont; iv) lagoon and lake system, represented by Lake Vichuquén, Lagoon Torca and Lagoon Tilicura, and v) Coastal system (Briceño *et al.*, 2018). Lake Vichuquén and Lagoon Torca correspond to semi-saltwater systems, which in an earlier time had a seawater inlet, but are currently separated from it. They cover an area of about 860 ha and 186 ha, respectively, and feed mainly on waters from the Vichuquén estuary and rainwater. In addition, both environments are rich in birds, including migratory birds, and have strong anthropic pressure due to forestry, agricultural, and tourist activities (Rojas and Tavares, 2011). Pedreros *et al.* (2019) pointed out that between 2008 and 2016, the trophic level of Lake Vichuquén increased significantly, going from a mesotrophic to a eutrophic state and, in some periods, to a hypereutrophic state. Briceño *et al.* (2018) confirmed the increase in eutrophication levels in Lake Vichuquén through a water quality study.

Even though coastal wetlands are very important ecosystems for the planet, many of them are not sufficiently valued and lack environmental protection measures, to the point that worldwide has been reported a loss of approximately 25-50% of the total surface during the last 50-100 years. Restoration and rehabilitation could be important strategies to facilitate the recovery of coastal wetlands. The present decade of 2021-2030 has been declared as the "United Nations Decade for Ecosystem Restoration" (Cadier *et al.*, 2020).

Metals like lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), manganese (Mn), zinc (Zn), nickel (Ni), and copper (Cu), among others, are natural components of the soil, or products of anthropogenic activities. Some are essential for cellular functions, but they are toxic to microorganisms in the soil, plants, and animals in high concentrations. The elements Cu, Cd, Cr, Co, Zn, Fe, Ni, Hg, Pb, As, Ag, and platinum-group elements are called heavy metals. Commonly, plants absorb these metals, and when consumed by herbivores, they could be incorporated into the food chain (Rai *et al.*, 2019).

The native species *S. radicans* grows naturally in certain sites of the shores of Lake Vichuquén and Lagoon Torca, (Orrego *et al.*, 2018; Ramírez and Álvarez, 2012). It has been observed that rabbits, black-necked swans, and other animals feed on the leaves of this species. Given the relevant importance of wetlands for the ecosystem and to avoid potential damage to human health of people, it was decided to explore the ability of wild *S. radicans* to colonize metalliferous soils and its potential to become an environmental indicator in Vichuquén - Torca sites, known to be polluted. Therefore, the levels of metals and heavy metals in soil and leaves of wild S. *radicans* were assessed in 4 sites. There are no published scientific studies involving wild *S. radicans* Cav. on this topic.

Material and Methods

Study Sites and Sampling

Soil and wild *S. radicans* Cav. leaves samples were collected in March 2016 from Maule Region (ML); Site 1 and site 2: "Aeródromo" next to Torca lagoon (34º78'00" L.S.; 72º04'92" L.W.); site 3: Vichuquén lake ("Totorilla" 34º83'72" L.S.; 72º03'12" L.W.); site 4 and site 5: "Camping el Sauce" 34º78'68" L.S.; 72º07'08" L.W.); site 6 and site 7: marsh from "Costa de Putú" (35º14'47" L.S.; 72º25'60" L.W.). The soil samples were extracted with PVC tools to a depth of 10 cm. Each sample was stored in ziploc® bags, previously labeled, and taken to the laboratory (Universidad de Talca, Talca.) for further analysis. The botanist Dr. Patricio Peñailillo identified this species, and a voucher specimen (Nº 3367) was deposited at the Herbarium Universidad de Talca.

Metal Determination

The determinations of metals (Cu, Mn, Zn, Cr, Pb, and Ni) in the soil and plant leaf samples were carried out by Flame Atomic Absorption

Spectroscopy (air/acetylene), using a Unicam spectrophotometer mod. 969.

Wild *S. radicans* leaves samples were washed with bi-distilled water and dried up in a heater at 105 ºC until constant weight. The leaves were ground and homogenized, and 1.00 g of leaf tissue was weighed and calcined in a porcelain crucible at 500 ºC for 4 h; 2 mL of bi-distilled water and 10 mL of HNO_3 were added to the crucibles cooled to room temperature. The samples were then dried almost entirely under an extractor fan, with constant stirring, using a heating plate set to 120 ºC. Finally, the solutions were filtered using filters of 0.45 µm. The filtering process was performed until a final 50 mL volume with bi-distilled water (Tapia *et al*., 2014).

The soil samples were dried at 105 ºC. A representative sample of 0.50 g of soil was mixed with 50 mL of a mixture of HNO_3 - HCl (2:1), was then solubilized at 120 ºC until almost dry, with constant stirring. The resulting solution was filtered using 0.45 µm filter porosity, then washed with bi-distilled water, and made up to a final volume of 50 mL in a pre-treated volumetric flask. The analyses were done with a control solution for each sample (Tapia *et al*., 2014).

Method Validation

The reagents used were of high purity (Suprapur, Merck, Darmstadt, Germany). Standard solutions for the various metals were prepared from a concentrated solution of the metal of 1000 mg L^{-1} (Fisher Scientific International Company). Cleanliness of the material was fundamental to guarantee optimum results in analysis.

The analysis methodology for wild *S. radicans* leaves samples was validated using certified reference material (BIMEP-432), supplied by the Wageningen Evaluating Programs for Analytical Laboratories (WEPAL). The soil samples analysis methodology was validated using certified reference material (MESS-1), supplied by the National Research Council, Canada, (NRC), Division of Chemistry.

Statistical Analysis

The research methodology was based on *in vitro* experiments through triplicate analysis.

Results and discussion

Method Validation

The analytical characteristics of the methodology used to determine metals in wild *S. radicans* leaves (Table 1) and soil samples (Table 2) revealed an analysis process with accuracy and convenient design of this research.

Metal Concentrations

Table 3 shows concentrations of metals found in wild *S. radicans* leaves and soils for each of the seven sampled sites. “Camping el Sauce” had the highest concentrations of metals in leaves and soils, except for concentrations in Mn (Leaves, “Costa de Putú”), and Pb (Soil, “Totorilla”). Likewise, it was observed that the order of metal concentration in soils was Mn>Zn>Ni>Cr>Cu>Pb, while on leaves was Mn>Zn>Cu>Cr>Pb. The data reflects that the metal with the highest concentrations detected was Mn, both on leaves and soils, while the lowest was Pb. The element Ni was not detected (< 0.5 mg kg^{-1}) in leaves, even though it exists in its soil.

Table 1. Concentration (mg kg^{-1}) of Cu, Cr, Mn, Zn, Ni, and Pb in certified reference material (BIMEP - 432), from the Wageningen Evaluating Programs for Analytical Laboratories (WEPAL).

Metal	Certified Concentration*	Observed Concentration**	Relative error (%)	Recovery (%)
Cu	6.05 ± 3.50 (n = 6)	7.73 ± 0.98	+ 27.8	127.8
Cr	2.35 ± 1.65 (n = 4)	2.81 ± 0.80	+ 19.6	119.6
Mn	20.0 ± 4.00 (n = 5)	20.35 ± 0.86	+ 1.8	+ 101.8
Zn	18.5 ± 2.80 (n = 6)	17.10 ± 1.33	- 7.6	- 92.4
Ni	1.15 ± 0.50 (n = 4)	1.12 ± 0.35	- 2.6	- 97.4
Pb	1.70 ± 1.10 (n = 3)	1.58 ± 0.48	- 7.1	- 92.9

*Median ± absolute deviation; ** (n = 3).

Table 2. Concentration (mg kg^{-1}) of Cu, Cr, Mn, Zn, Ni, and Pb in certified reference material (MESS-1) from National Research Council Canada (NRC).

Metal	Certified Concentration*	Observed Concentration*,**	Relative error (%)	Recovery (%)
Cu	25.1 ± 3.8	25.9 ± 2.4	+ 3.2	103.2
Cr	71.0 ± 11	69.8 ± 3.7	- 1.7	98.3
Mn	513 ± 25	496.1 ± 18	- 3.3	96.7
Zn	191 ± 17	202.2 ± 19	+ 5.9	105.9
Ni	29.5 ± 2.7	28.7 ± 3.4	- 2.7	97.3
Pb	34.0 ± 6.1	35.2 ± 4.7	+ 3.5	103.5

*Median ± absolute deviation; **(n = 2).

Table 3. Concentrations (mg kg^{-1})* of Cu, Cr, Mn, Zn, Ni, and Pb in wild *S. radicans* leaves and soils samples from different sites of Maule Region, Chile.

	Aeródromo		Totorilla	Camping el Sauce		Costa de Putú	
Metal	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Cu Leaves	8.48	14.21	9.93	16.44	17.47	9.49	11.94
Soil	8.10	8.10	12.35	24.54	39.48	11.07	11.76
Cr Leaves	1.25	1.27	0.93	1.61	1.87	0.78	1.01
Soil	29.74	29.74	32.99	36.84	45.66	32.56	33.00
Mn Leaves	17.25	24.90	394.84	380.66	413.35	228.93	469.85
Soil	399.85	399.85	229.87	393.19	602.27	441.40	478.05
Zn Leaves	10.79	21.98	15.19	28.06	48.72	13.74	15.49
Soil	38.90	38.90	44.42	65.35	167.23	36.54	37.85
Ni Leaves	nd	nd	nd	nd	nd	nd	nd
Soil	13.06	13.06	15.57	21.87	52.25	46.54	48.33
Pb Leaves	nd	nd	nd	1.72	1.72	0.36	0.94
Soil	3.11	3.11	9.58	7.58	9.12	4.17	5.26

*In dry sample; nd – not detected (< 0.5 mg kg^{-1}).

Soil is a precious natural resource and performs multiple functions, the main one being food production. Today it is known that there are soils that have been contaminated naturally or anthropogenically. Categorizing a site as contaminated is difficult because many factors are involved. Some countries have created laws for their conservation and use, based on experimental data considering, for example, background values based on the natural geographic mineral content of native soils (Jiménez, 2017). When referring to the recommended concentrations of metals in soils, there are many differences from country to country and between regions, not only on the value itself, but also on the name used to define it, including detection value, normal value, acceptable concentration, and the target value, among others (Rodríguez-Eugenio *et al.*, 2018). Nowadays, in Chile, there are still no accepted environmental quality standards for soils, For their elaboration, it is necessary to have baselines containing the natural concentrations or background that consider the complexity and geological diversity of the country's soils. For a few years, thanks to "Plan Nacional de Geología" (National Geology Plan), a database is being generated on the multi-element chemical composition (61 elements and chemical compounds) for soils and sediments in the country (PNG, 2021). Therefore, to discuss the results of this study, data from other countries will be used.

In Table 4, values of soil concentrations in Cu, Cr, and Mn obtained in this research were within

Table 4. Comparison of Metal Concentration in soils and wild *S. radicans* leaves samples with values from other natural environments.

	Concentration in mg kg^{-1} in dry sample				
Metal	In this research	Wetland Polluted[a]	Wetland Protected[a]	Coast and Mountain[b]	Acceptable Contents
Cu Plant	9.93-16.96	332.00	10.20	1.2-62.5	5-20[c,d]
Soil	8.1-39.48	523.30	17.90	6.4-81.5	30[c,d]
Cr Plant	0.90-1.26	–	–	0.2-3.2	0.1-5[c,d]
Soil	29.74-56.33	–	–	4.6-50.4	10-50[c,d]
Mn Plant	21.08-397.00	29.19	25.80	188.6-1341.1	10-50[e]
Soil	229.87-602.27	–	–	335.6-1936.1	300-600[e]
Zn Plant	14.62-38.39	15.63	1.01	7.5-55.9	25-150[d]
Soil	36.54-167.23	–	–	15.6-65.9	40[d]
Ni Plant	< 0.5	–	–	–	0,05-10[f]
Soil	13.06-52.25	–	–	–	35[c]
Pb Plant	0.65-1.72	25.40	< 0.01	–	5-10[d]
Soil	3.11-9.58	26.70	17.80	–	14[d]

[a]Meza *et al.*, 2018; [b]Tapia *et al.*, 2019; [c]Jiménez *et al.*, 2017; [d]Barceló and Poschenrieder, 1992; [e]WHO, 2004; [f]Nieminen *et al.*, 2007.

the range recorded for other researchers that studied coastal natural sectors of Chile (Meza *et al.*, 2018; Tapia *et al.*, 2019). Considering the acceptable values of Cu and Cr concentrations in normal/natural soils, only "Camping el Sauce" had concentrations slightly higher (Barceló and Poschenrieder, 1992; Jiménez, 2017). In the case of Mn and Pb concentrations, both were within allowable levels in soils (Barceló and Poschenrieder, 1992; WHO, 2004). Nevertheless, Zn concentrations would be exceeded in "Camping el Sauce" (Barceló and Poschenrieder, 1992), a site the locals perceive as polluted. Likewise, Ni concentrations in "Camping el Sauce" and "Costa de Putú" soils would be higher than allowed (Jiménez *et al.*, 2017). Motorcycling is practiced in "Costa de Putú," which could explain the high metal content in the soil.

According to the results obtained in this investigation, some soil samples may have concentrations of metals (Cu, Cr, Zn, and Ni) that exceed the acceptable concentrations, However, it is necessary to consider the geochemical background concentrations since the Chilean soil is naturally abundant in minerals (Luzio and Casanova, 2006).

Any element deposited in the soil is not necessarily available for the plant since its uptake depends on several factors and physical-chemical characteristics of the soil (Rai *et al.*, 2019). Traditionally, halophytes have been used as food and a source of medicinal substances during the last centuries (Shamsutdinov *et al.*, 2017). In addition, some of them can be phytoremediators of heavy metal contaminated soils (Liang *et al.*, 2017; Sruthi *et al.*, 2017).

According to the results of this research, Cu, Cr, Mn, Zn, Ni, and Pb concentrations in leaves of wild *S. radicans* samples were lower than soil concentrations, except for Cu concentrations in the "Aerodromo" and "Costa de Putú," and Mn concentrations in "Totorilla". Following the literature, some halophytes species can accumulate multiple metals in their roots (Cu, Pb, Ni, Zn). Monocotyledons and dicotyledons have the peculiarity of accumulating Cu, while monocots mainly accumulate Pb (Liang *et al.*, 2017). Several factors (soil condition, metal kind, and plant species, among others) determine whether plants absorb metals by accumulating them in their roots or by translocation of metals to their aerial parts. The process of translocation of toxic elements (heavy metals, among other solutes) accumulated and excreted by the saline glands or trichomes to the surface of the leaves is called "phytoexcretion" (Manousaki and Kalogerakis, 2011). If this trend is followed, metal contents determined in wild *S. radicans* leaves would correspond to excreted metals. Thus, if Ni was not detected in leaves, it is because the roots did not exceed the concentration limits to cause translocation. This fact also occurs for Pb in three of the seven sites that were sampled.

Considering acceptable concentrations of metals in plants (Table 4), only Mn and Zn concentrations were higher in leaves of wild *S. radicans*. These results are significant since herbivores consume them. For its edible or medicinal use, plant cultivation should be promoted in systems free of these metals.

The results support the popular perception of considering the investigated sites as polluted. Wild *S. radicans* can colonize metalliferous soils and act as a metal bioindicator of environmental contamination in Vichuquén - Torca areas. Undoubtedly, after analyzing the results of this study, further research is needed to answer all the questions that arise.

Conclusions

The results revealed that the analyzed soils are contaminated with heavy metals (Cu, Cr, Zn, and Ni). However, their pollution levels were low, but geochemical background concentrations need to be considered. Mn and Zn are metals that are in concentrations higher than those allowed in plants. Marshes and coastal wetlands need to be monitored to check their metal levels and ensure that the trophic chain is not contaminated.

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